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Discovery of novel 7-membered cyclic amide derivatives that inhibit 11beta-hydroxysteroid dehydrogenase type 1

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ABSTRACT

A series of novel 5-*trans*-hydroxyadamantan-2-yl-5,6,7,8-tetrahydropyrazolo[4,3-*c*]azepin-4(1*H*)-ones that inhibit 11beta-hydroxysteroid dehydrogenase type 1 are described. We discovered these 7-membered cyclic amide derivatives by introducing a distinctive linker through pharmacophore analysis of known ligands included in X-ray co-crystal structures. Further optimization using docking studies led to highly potent inhibitors **15b** and **27**, which furthermore showed the potent efficacy in in vivo studies.

11beta-Hydroxysteroid dehydrogenase type 1 (11beta-HSD1) is an enzyme involved in glucocorticoid regulation through catalysis of the conversion of inactive cortisone to active cortisol in liver, adipose, and brain tissues.[1] An important role of cortisol is increasing gluconeogenesis in the liver and inhibiting peripheral glucose uptake in muscle and adipose tissue; hence excess cortisol or 11beta-HSD1 leads to insulin resistance and metabolic syndrome.

In genetic studies, mice overexpressing 11beta-HSD1 in liver or adipose tissue exhibited several features of metabolic syndrome and type 2 diabetes, including high active glucocorticoid levels, insulin resistance, and glucose tolerance.[2] In addition, 11beta-HSD1 knockout mice showed increased insulin sensitivity and improved glucose tolerance even when fed a high fat diet.[3] These findings suggest that 11beta-HSD1 is a novel therapeutic target for the treatment of type 2 diabetes and metabolic syndrome; therefore, researchers in the pharmaceutical industry have investigated 11beta-HSD1 inhibition as a potential therapeutic strategy for such diseases.[4] As a result, many chemical classes have been reported as potent and selective 11beta-HSD1 inhibitors (Fig. 1), for example: AMG-221,[5] PF-915275,[6] BVT-2733,[7] AZD-4017.[8] Our goal was to discover novel 11beta-HSD1 inhibitors with excellent potency and in vivo efficacy.

To facilitate the iterative design of 11beta-HSD1 inhibitors, we investigated X-ray crystal structures of the protein and selected two kinds of crystal structures (Fig. 2). One is the co-crystal of corticosterone and 11beta-HSD1 (PDB code: 1Y5R),[9] and the other is the co-crystal of an adamantane derivative prepared by Abbott (PDB code: 2IRW).[10] By examining the superposition of these ligands included in the two crystal structures, we found a common structural characteristic: two hydrophobic moieties are linked by hydrogen-bonding groups in both ligands. This observation led to the hypothesis that the combination of hydrophobic moieties and a hydrogen-bonding linker is an essential pharmacophore of 11beta-HSD1 inhibitors. To explore this hypothesis, we attempted to generate new ring scaffolds able to link two hydrophobic moieties. Following this strategy, we designed a unique 7-membered cyclic amide (Fig. 3). In this Letter, we report the discovery, structure–activity relationships, and pharmacological properties of a

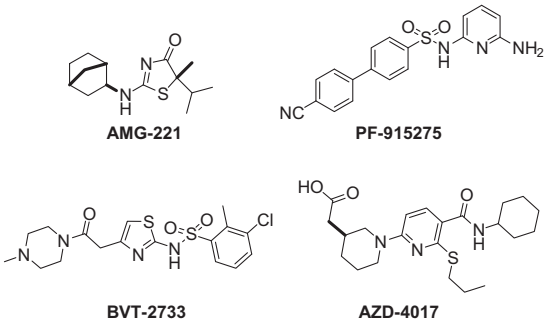

Figure 1. Previously reported 11beta-HSD1 inhibitors.

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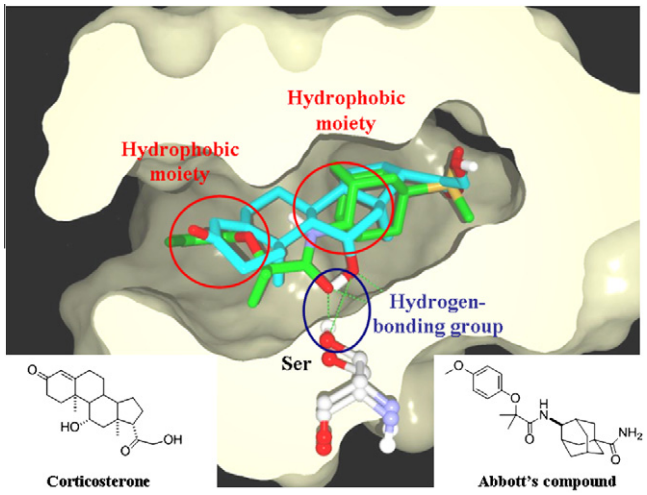
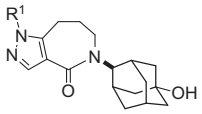


Figure 2. Superposition of two X-ray co-crystal structures. corticosterone (PDB code: 1Y5R) and adamantane derivative (by Abbot; PDB code: 2IRW) with 11beta-HSD1.

series of novel 5-*trans*-hydroxyadamantan-2-yl-5,6,7,8-tetrahydropyrazolo[4,3-*c*]azepin-4(1*H*)-ones as potent 11beta-HSD1 inhibitors.

Cyclic amide analogues were prepared according to Scheme 1. Commercially available 4-benzyloxy butanoic acid **1** was converted to ketoester **2**[11] through reaction with potassium 3-ethoxy-3-oxopropanoate in the presence of CDI. Treatment of **2** with DMF–DMA gave the enamine, which was then reacted with a series of hydrazines or hydrazine hydrochlorides to yield the corresponding pyrazoles **3**.[12] The protecting benzyl ether was removed by hydrogenolysis, and then aldehyde **4** was obtained by Dess–Martin oxidation. Reductive amination of **4** with *trans*-5-hydroxy-2-adamantylamine[13] followed by hydrolysis of the ethyl ester gave the corresponding carboxylic acid. Lastly, intramolecular cyclization using HBTU gave the desired 7-membered cyclic amide derivatives **5a–g**.

Table 1
11beta-HSD1 inhibitory activity of **5a–g**



Compound	R^1	Human 11beta-HSD1 IC_{50} (nM)	Mouse 11beta-HSD1 IC_{50} (nM)
5a	Me	>300	>300
5b	Et	111	>300
5c	*tert*-Bu	39.3	104
5d	Cyclohexyl	54.4	24.3
5e	Cycloheptyl	73.9	N.D.
5f	Ph	127	27.4
5g	2-Pyridyl	83.1	59.3

The synthesized compounds were evaluated for inhibition of human and mouse 11beta-HSD1 enzymes. The results are shown in Table 1. In regard to substituent R^1, the derivatives with methyl or ethyl groups (**5a** and **5b**) were less potent. In the case of *tert*-butyl, cycloalkyl, or aryl groups (**5c**–**5g**), higher potency was observed. These results suggested that the steric factor of substituent R^1 has a considerable impact on 11beta-HSD1 inhibitory activity.

Next, to further investigate the design of other inhibitors, we carried out docking studies of the cyclic amide **5f** with the X-ray crystal structure of 11beta-HSD1 (2IRW) (Fig. 4). The results

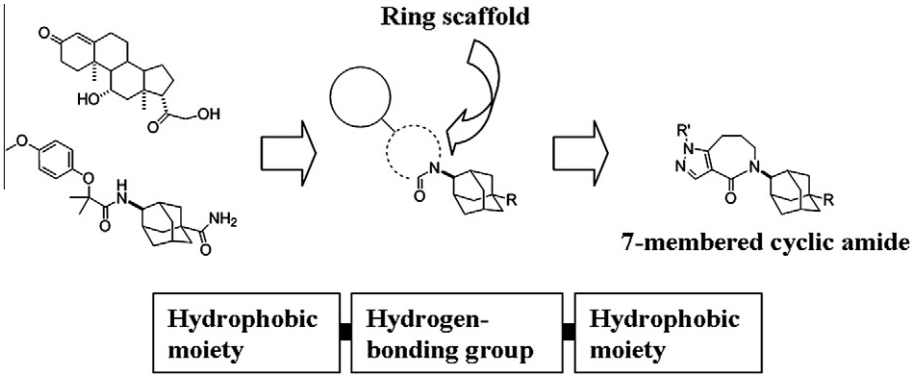
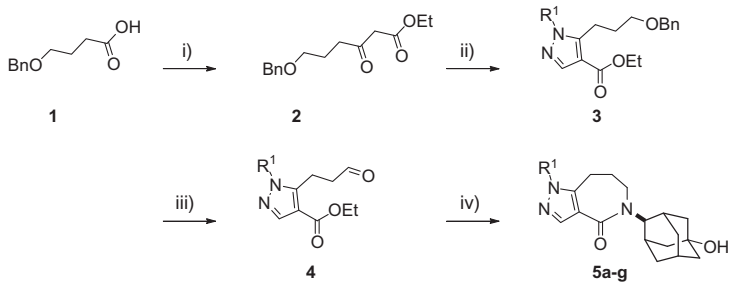


Figure 3. Our strategy for designing a new scaffold.



Scheme 1. Reagents and conditions: (i) potassium 3-ethoxy-3-oxopropanoate, CDI, $MgCl_2$, Et_3N, CH_3CN, 90 °C, 86%; (ii) (a) DMF–DMA, 80 °C, (b) R^1-$NHNH_2$, EtOH, 80 °C; or R^1-$NHNH_2$·HCl, Et_3N, EtOH, 80 °C, 35–98%; (iii) (a) H_2, Pd/C, EtOH, rt; or H_2, $Pd(OH)_2$/C, EtOH, rt, (b) DMP, CH_2Cl_2, rt, 21–59%; (iv) (a) *trans*-5-hydroxy-2-adamantylamine, $NaBH(OAc)_3$, AcOH, $CHCl_3$, rt, (b) 1 N NaOH, MeOH, rt, (c) HBTU, DIPEA, $CHCl_3$, rt, 9–56%.

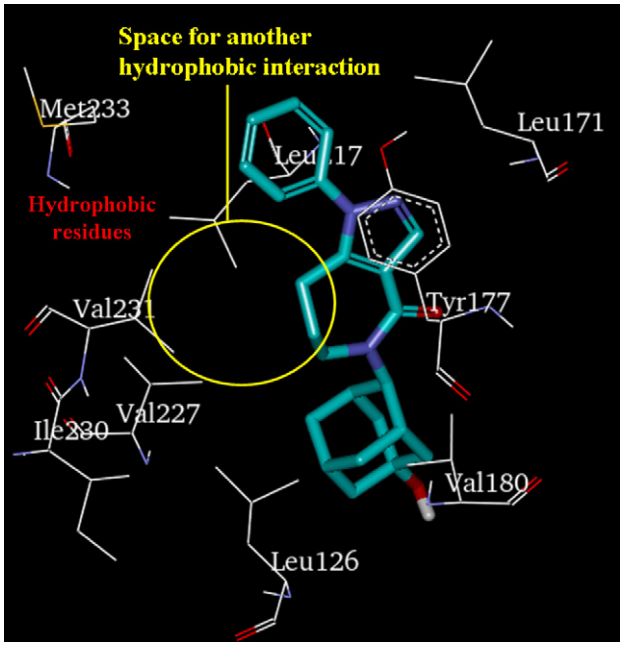
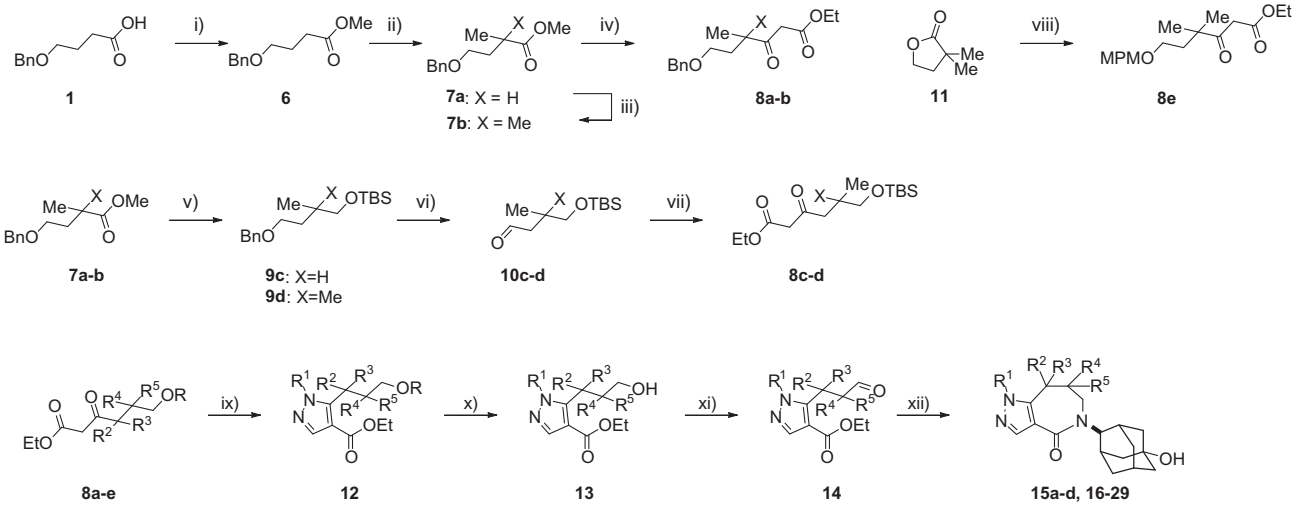


Figure 4. Binding site of the putative structure of the complex of 11beta-HSD1 and **5f**.

revealed a hydrophobic void composed of hydrophobic amino acid residues, such as Val231 and Val227, around the methylene linker of the 7-memberd ring. Thus, we hypothesized that this space was likely available for an additional ligand–protein interaction. To test this hypothesis, we attempted to introduce substituents capable of suitably occupying this space.

The monomethyl- or dimethyl-substituted cyclic amide derivatives were prepared according to Scheme 2. Compounds **15a–d** and **16–25** were synthesized from ketoesters **8a–d**, and **26–29** were synthesized from ketoester **8e**. The preparation of **8a–b** was as follows: Esterification of carboxylic acid **1** with concentrated H_2SO_4 in EtOH, followed by methylation of the carbonyl alpha-carbon using LDA and MeI led to monomethylated ester **7a**. The dimethylated ester 7**b** was prepared by the same procedure but with **7a** as the starting material. The desired ketoesters were prepared by hydrolysis of these compounds followed by reaction with potassium 3-ethoxy-3-oxopropanoate and CDI. The preparation of **8c–d** was as follows: Reduction of methylesters **7a–b** with $LiAlH_4$, and subsequent protection of the alcohol using TBSCl, gave the silyl-protected alcohol. Deprotection of the benzyl ether and Dess–Martin oxidation afforded aldehydes **10c–d**. Oxidation of aldehydes to carboxylic acids progressed under Pinnick oxidation conditions,[14] and further reaction to form ketoester was performed as described above. Compound **8e** was prepared as follows: Hydrolysis of commercially available 3,3-dimethyldihydrofuranone with KOH, and protection of the alcohol with MPM led to the desired ketoester. Ketoesters **8a–e** were converted to enamines by treatment with DMF–DMA, and cyclized by reaction with a series of hydrazines or hydrazine hydrochlorides to give pyrazole **12**. The alcohol was deprotected by hydrogenolysis or treatment with TBAF or CAN, and then oxidized to aldehyde **14**. Further steps to the substituted cyclic amides followed the approach shown in Scheme 1.

The effects of methyl substitution at the methylene linker (i.e., the 7- or 8-positon of the tetrahydropyrazoloazepin ring) were examined (Table 2). The results suggest that methyl substitution significantly enhanced in vitro inhibitory activity, and that hydrophobicity at the 7- or 8-position was advantageous for inhibition of 11beta-HSD1. Next, the inhibitory activities of the 7- and 8-substituted compounds were compared between human and mouse 11beta-HSD1. The potency of the compounds was in the following order: no substitution (**5f**) < 7-monomethyl (**15c**) < 7,7-dimethyl (**15d**) < 8-monomethyl (**15a**) < 8,8-dimethyl (**15b**). Notably, methyl substitution at the 8-position achieved an approximately 15-fold improvement in potency over the non-substituted compound for inhibition of human 11beta-HSD1. Therefore, this strategy led to more potent compounds. Additionally, further derivatization of R^1 was performed. For this series, an interesting



Scheme 2. Reagents and conditions: (i) H_2SO_4, MeOH, rt, 99%; (ii) LDA, THF, then MeI, 0 °C, 94%; (iii) LDA, THF, then MeI, 0 °C, 94%; (iv) (a) 1 N NaOH, MeOH, rt to 50 °C, (b) potassium 3-ethoxy-3-oxopropanoate, CDI, $MgCl_2$, Et_3N, CH_3CN, 90 °C, 95%; (v) (a) $LiAlH_4$, THF, rt, (b) TBSCl, imidazole, DMF, rt, 86–94%; (vi) (a) H_2, Pd/C, rt, (b) DMP, CH_2Cl_2, rt, 60–79%; (vii) (a) $NaClO_2$, NaH_2PO_4, *t*BuOH, H_2O, rt, (b) potassium 3-ethoxy-3-oxopropanoate, CDI, $MgCl_2$, Et_3N, CH_3CN, 90 °C, 46–54%; (viii) (a) KOH, MPMCl, toluene, 110 °C, (b) potassium 3-ethoxy-3-oxopropanoate, CDI, $MgCl_2$, Et_3N, CH_3CN, 90 °C, 53%; (ix) (a) DMF–DMA, 80 °C, (b) R^1-$NHNH_2$, EtOH, 80 °C; or R^1-$NHNH_2$·HCl, Et_3N, EtOH, 80 °C, 81–86%; (x) H_2, Pd/C, EtOH, rt to 50 °C; or H_2, $Pd(OH)_2$/C, EtOH, rt to 50 °C; or TBAF, THF, rt; or CAN, MeCN, H_2O, rt, 17–83%, (xi) DMP, CH_2Cl_2, *t*BuOH, rt, 58–81%, (xii) (a) *trans*-5-hydroxy-2-adamantylamine, $NaBH(OAc)_3$, AcOH, $CHCl_3$, rt, (b) 1 N NaOH, MeOH, rt to 60 °C, (c) HBTU, DIPEA, $CHCl_3$, rt, 41–60%.

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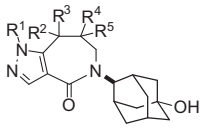
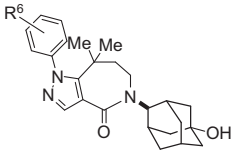
Table 2
11beta-HSD1 inhibitory activity of **5f**, **15a–d** and **16–18**



Compound	R^1	R^2	R^3	R^4	R^5	Human 11beta-HSD1 IC_{50} (nM)	Mouse 11beta-HSD1 IC_{50} (nM)
5f	Ph	H	H	H	H	127	27.4
15a	Ph	Me	H	H	H	11.9	12.2
15b	Ph	Me	Me	H	H	8.9	9.8
15c	Ph	H	H	Me	H	25.3	71.8
15d	Ph	H	H	Me	Me	3.5	39.3
16	Me	Me	Me	H	H	29.3	>100
17	Cyclohexyl	Me	Me	H	H	36.1	23.7
18	2-Pyridyl	Me	Me	H	H	10.3	>100

Table 3
Optimization of phenyl substitution: 11beta-HSD1 inhibitory activity of **15b** and **19–29**



Compound	R^6	Human 11beta-HSD1 IC_{50} (nM)	Mouse 11beta-HSD1 IC_{50} (nM)
15b	H	8.9	9.8
19	2-Me	<10	8.0
20	3-Me	13	7.9
21	4-Me	5.4	31.5
22	2-OMe	11.1	18.4
23	4-OMe	11.1	>100
24	3-F	6.7	28.7
25	4-F	3.8	34.7
26	3-Cl	9.8	17.0
27	4-Cl	<3	35.0
28	3-CF_3	8.0	25.5
29	4-CF_3	19.8	28.5

finding was that the most appropriate substituent proved to be phenyl.

Next, we introduced substituents on the phenyl ring to optimize **15b** (Table 3). A variety of substituents were well-tolerated. Although **19** bearing a 2-methyl substituent gave high in vitro inhibitory activity, this compound was found to have low stability against human cytochrome P450 (CYP) metabolism (CL_{int} = 0.041 mL/min/mg). Among assessed compounds, **15b** and **27** showed excellent stability against CYP metabolism (**15b**: CL_{int} = 0.002 mL/min/mg; **27**: CL_{int} = 0.008 mL/min/mg). For these two derivatives, in vivo efficacy was tested in a disease mouse model. Diet-induced obesity (DIO) mice were orally administered 30 mg/kg of **15b**, **27**, or pioglitazone, which is among the most commonly used first-line drugs for type 2 diabetes treatment.[15] Then blood glucose excursion was examined. Some levels of the reduction in fasting glucose level were observed in mice administered **15b** or **27** (Fig. 5). These results showed that **15b** and **27** were orally active and they showed the same tendency as pioglitazone in DIO mice.

In conclusion, we have discovered novel 7-membered cyclic amide derivatives that are potent 11beta-HSD1 inhibitors, through pharmacophore analysis of known ligands included in X-ray co-crystal structures. Our initial hit compound showed relatively weak inhibitory activity; we then investigated the putative structure of the complex of 11beta-HSD1 protein and **5f** in a docking study. From this study, we found a hydrophobic void in the binding site of the protein, which was available for another ligand–protein interaction. In line with our hypothesis, significant improvements in potency were achieved by introducing methyl substitution at the methylene linker. Compound **15b** and **27** were orally active and also showed potent in vivo efficacy similar to that of the standard therapeutic agent in DIO mice model as well as the excellent inhibition of 11beta-HSD1. Further optimization of the derivatives is now being investigated.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.090.

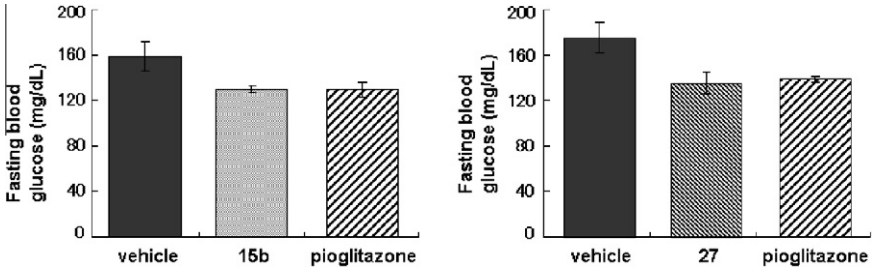


Figure 5. In vivo effects of **15b** and **27** and pioglitazone on fasting blood glucose in DIO mice.

References and notes

1. (a) Tomlinson, J. W.; Walker, E. A.; Bujalska, I. J.; Draper, N.; Lavery, G. G.; Cooper, M. S.; Hewison, M.; Stewart, P. M. *Endocr. Rev.* **2004**, *25*, 831; (b) Seckl, J. R.; Walker, E. A. *Trends Endocrinol. Metab.* **2004**, *15*, 418; (c) Stulnig, T. M.; Waldhausl, W. *Diabetologia* **2004**, *47*, 1; (d) Seckl, J. S.; Walker, B. R. *Endocrinology* **2001**, *142*, 1371.
2. (a) Masuzaki, H.; Paterson, J.; Shinyama, H.; Morton, N. M.; Mullins, J. J.; Seckl, J. R.; Flier, J. S. *Science* **2001**, *294*, 2166; (b) Masuzaki, H.; Yamamoto, H.; Kenyon, C. J.; Elmwuist, J. K.; Morton, N. M.; Paterson, M. M.; Shinyama, H.; Sharp, M. G.; Fleming, S.; Mullins, J. J.; Seckl, J. R.; Flier, J. S. *J. Clin. Invest.* **2003**, *112*, 83; (c) Paterson, J. M.; Morton, N. M.; Fievet, C.; Kenyon, C. J.; Holmes, M. C.; Staels, B.; Seckl, J. R.; Mullins, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7088.
3. (a) Kotelevtsev, Y.; Holmes, M. C.; Burchell, A.; Houston, P. M.; Schmoll, D.; Jamieson, P.; Best, R.; Brown, R.; Edwards, C. R. W.; Seckl, J. R.; Mullins, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14924; (b) Morton, N. M.; Holmes, M. C.; Fievet, C.; Staels, B.; Tailleux, A.; Mullins, J. J.; Seckl, J. R. *J. Biol. Chem.* **2001**, *276*, 41293; (c) Morton, N. M.; Paterson, J. M.; Masuzaki, H.; Holmes, M. C.; Staels, B.; Fievet, C.; Walker, B. R.; Filler, J. S.; Mullins, J. J.; Seckl, J. R. *Diabetes* **2004**, *53*, 931.
4. Recent reports and references contained within the following: (a) St. Jean, D. J., Jr.; Yuan, C.; Bercot, E. A.; Cupples, R.; Chen, M.; Fretland, J.; Hale, C.; Hungate, R. W.; Komorowski, R.; Vaniant, M.; Wang, M.; Zhang, X.; Fotsch, C. *J. Med. Chem.* **2007**, *50*, 429; (b) Sun, D.; Di, Y.; Jaen, J. C.; Labelle, M.; Ma, J.; Miao, S.; Sudom, A.; Tang, L.; Tomooka, C. S.; Ursu, S.; Wang, Z.; Yan, X.; Ye, Q.; Tu, H.; Powers, J. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3513; (c) Olson, S.; Aster, S. D.; Brown, K.; Carbin, I.; Graham, D. W.; Hermanowski-Vosatka, A.; LeGrand, C. B.; Mundt, S. S.; Shah, K.; Springer, M. S.; Tan, E. Y.; Thieringer, R.; Hermanowski-Vosatka, A.; Zokian, H. J.; Balkovec, J. M.; Waddell, S. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4359; (d) Gu, X.; Dragovic, J.; Koo, G. C.; Koprak, S. I.; LeGrand, C.; Mundt, S. S.; Shah, K.; Springer, M. S.; Tan, E. Y.; Thieringer, R.; Hermanowski-Vosatka, A.; Zokian, H. J.; Balkovec, J. M.; Waddell, S. T.; Sun, D.; Di, Y.; Jaen, J. C.; Labelle, M.; Ma, J.; Miao, S.; Sudom, A.; Tang, L.; Tomooka, C. S.; Ursu, S.; Wang, Z.; Yan, X.; Ye, Q.; Tu, H.; Powers, J. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5266; (e) McMinn, D. L.; Rew, Y.; Sudom, A.; Caille, S.; DeGraffenreid, M.; He, X.; Hungate, R.; Jiang, B.; Jaen, J.; Julian, L. D.; Kaizerman, J.; Novak, P.; Sun, D.; Tu, H.; Ursu, S.; Walker, N. P. C.; Yan, X.; Ye, Q.; Wang, Z.; Powers, J. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1446.
5. Veniant, M. M.; Hale, C.; Hungate, R. W.; Gahm, K.; Emery, M. G.; Jona, J.; Joseph, S.; Adams, J.; Hague, A.; Moniz, G.; Zhang, J.; Bartberger, M. D.; Li, V.; Syed, R.; Jordan, S.; Komorowski, R.; Chen, M. M.; Cupples, R.; Kim, K. W.; St. Jean, D. J., Jr.; Johansson, L.; Henriksson, M. A.; Williams, M.; Vallgarda, J.; Fotsch, C.; Wang, M. *J. Med. Chem.* **2010**, *53*, 4481.
6. Siu, M.; Johnson, T. O.; Wang, Y.; Nair, S. K.; Taylor, W. D.; Cripps, S. J.; Matthews, J. J.; Edwards, M. P.; Pauly, T. A.; Ermolieff, J.; Castro, A.; Hosea, N. A.; LaPaglia, A.; Fanjul, A. N.; Vogel, J. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3493.
7. Barf, T.; Vallgarda, J.; Emond, R.; Haggstrom, C.; Kurz, G.; Nygren, A.; Larwood, V.; Mosialou, E.; Axelsson, X.; Olsson, R.; Engblom, L.; Edling, N.; Ronquist-Nii, Y.; Ohman, B.; Allberts, P.; Abrahmsen, L. *J. Med. Chem.* **2002**, *45*, 3813.
8. Scott, J. S.; Deschoolmeester, J.; Gerhardt, S.; Hargreaves, D.; Kilgour, E.; Lloyd, A.; Mayers, R. M.; McCoull, W.; Newcombe, N. J.; Ogg, D.; Packer, M. J.; Rees, A.; Revill, J.; Schofield, P.; Selmi, N.; Swales, J. G.; Whittamore, P. R. O. *J. Med. Chem.* **2012**, *55*, 5951.
9. Zhang, J.; Osslund, T. D.; Plant, M. H.; Clogston, C. L.; Nybo, R. E.; Xiong, F.; Delaney, J. M.; Jordan, S. R. *Biochemistry* **2005**, *44*, 6948. PDB code: 1Y5R.
10. Patel, J. R.; Shuai, Q.; Dinges, J.; Winn, M.; Pliushcher, M.; Fung, S.; Monzon, K.; Chiou, W.; Wang, J.; Pan, L.; Wagaw, S.; Engstrom, K.; Kerdesky, F. A.; Longenecker, K.; Judge, R.; Qin, W.; Imade, H. M.; Stolarik, D.; Beno, D. W. A.; Brune, M.; Chovan, L. E.; Sham, H. L.; Link, J. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 750. PDB code: 2IRW.
11. Schuda, P. F.; Greenlee, W. J.; Chakravarty, P. K.; Eskola, P. *J. Org. Chem.* **1988**, *53*, 873.
12. Menozzi, G.; Morello, L.; Fossa, P.; Schenone, S.; Ranise, A.; Mosti, L.; Bondavalli, F.; Loddo, R.; Murgioni, C.; Mascia, V.; La Colla, P.; Tamburini, E. *Bioorg. Med. Chem.* **2004**, *12*, 5465.
13. Jaroskova, L.; Van der Veken, L.; de Belser, P.; Diels, G.; de Groot, A.; Linders, J. T. M. *Tetrahedron Lett.* **2006**, *47*, 8063.
14. Bal, B. S.; Childers, W. E., Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.
15. Reuter, T. Y. *Drug Discovery Today: Disease Models* **2007**, *4*, 3.