Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2869–2873

PDE-10A inhibitors as insulin secretagogues

Louis-David Cantin,[a,*] Steven Magnuson,[a] David Gunn,[a] Nicole Barucci,[b] Marina Breuhaus,[d] William H. Bullock,[a] Jennifer Burke,[a] Thomas H. Claus,[b] Michelle Daly,[b] Lynn DeCarr,[b] Ann Gore-Willse,[b] Helana Hoover-Litty,[b] Ellalahewage S. Kumarasinghe,[a] Yaxin Li,[b] Sidney X. Liang,[a] James N. Livingston,[b] Timothy Lowinger,[a] Margit MacDougall,[b] Herbert O. Ogutu,[a] Alan Olague,[a] Ronda Ott-Morgan,[c] Robert W. Schoenleber,[a] Adrian Tersteegen,[d] Philip Wickens,[a] Zhonghua Zhang,[a] Jian Zhu,[b] Lei Zhu[a] and Laurel J. Sweet[b]

[a]*Department of Chemistry Research, Bayer Pharmaceuticals Corporation, West Haven, CT 06516, USA*
[b]*Department of Metabolic Disorders Research, Bayer Pharmaceuticals Corporation, West Haven, CT 06516, USA*
[c]*Department of Research Technologies, Bayer Pharmaceuticals Corporation, West Haven, CT 06516, USA*
[d]*Department of Cardiovascular Research and Screening, Bayer HealthCare AG, Wuppertal, Germany*

Received 29 December 2006; revised 16 February 2007; accepted 21 February 2007
Available online 25 February 2007

Abstract—Modulation of cAMP levels has been linked to insulin secretion in preclinical animal models and in humans. The high expression of PDE-10A in pancreatic islets suggested that inhibition of this enzyme may provide the necessary modulation to elicit increased insulin secretion. Using an HTS approach, we have identified quinoline-based PDE-10A inhibitors as insulin secretagogues in vitro. Optimized compounds were evaluated in vivo where improvements in glucose tolerance and increases in insulin secretion were measured.

Analogs of the incretin hormone glucagon-like peptide-1 (GLP-1) including Exenatide, Liraglutide, and Albugon have recently emerged as an important therapeutic approach for the treatment of type 2 diabetes.[1] In pancreatic β cells, these peptides activate the GLP-1 receptor, an adenylate cyclase (AC) coupled GPCR (G-protein coupled receptor), which elevates the intracellular concentration of cyclic adenosine mono-phosphate (cAMP). Signaling by this second messenger has been associated with glucose-dependent insulin secretion (GDIS), stimulation of insulin biosynthesis and increase in β cell mass. These new GLP-1 peptide agonists are not orally active but are highly effective when administered via subcutaneous injection.

Efforts to identify orally active small molecules that mimic the agonistic activity of these 30–40 amino acid peptides have not been successful. Consequently, the greatest probability to develop an oral therapy lies in identifying small molecule targets within this signaling cascade. One such example is the inhibition of DPP4 (dipeptidyl peptidase-4).[2] This protease is the key enzyme responsible for inactivating endogenously produced GLP-1 through hydrolysis of the penultimate alanine peptide bond. DPP4 inhibitors have been shown to enhance GLP-1 signaling and consequently lower blood glucose when dosed orally in both animal models of diabetes and in clinical trials.[3]

Since GLP-1 signals through AC and cAMP, an alternative approach to enhance GLP-1 signaling would be to inhibit the phosphodiesterase(s) (PDE) responsible for hydrolysis of cAMP and termination of the intracellular signal. Treatment of islets with PDE-3B inhibitors has been reported to result in glucose-dependent insulin secretion.[4] However, these PDE-3B inhibitors have been associated with concomitant liver and adipose tissue

Keywords: PDE-10A inhibition; Insulin secretagogue; Quinoline; Type 2 diabetes.
* Corresponding author. E-mail: Louis-David.Cantin@astrazeneca.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.02.061

insulin resistance, counterbalancing the effect on insulin secretion. In addition, we have recently disclosed PDE-11A inhibitors useful as insulin secretagogues.[5]

Our search of novel targets for insulin secretion led to the recent discovery of the high expression of PDE-10A in pancreatic islets. This finding prompted an extensive effort aimed at ascertaining the ability of PDE-10A inhibitors to produce GDIS.[6] Our past research efforts in the PDE-10A inhibitor field had provided a pyrrolo dihydroisoquinoline class of compounds (Fig. 1; **1** and **2**) that proved to be potent PDE-10A inhibitors (PDE-10A IC_{50} **1** = 30 nM, **2** = 33 nM) that were also highly selective over other PDEs.[7] Because the variations of cAMP levels are small and localized, the measurement of these changes in a whole cell assay proved difficult. Instead we profiled the compounds in a functional assay using dispersed islet cells (D.I. assay),[8] where compounds **1** and **2** caused significant insulin secretion when compared to control samples (D.I. fold-over-control: **1** = 1.4, **2** = 1.44).

Utilizing a HTS approach, we identified multiple chemical subclasses of selective PDE-10A inhibitors capable of causing insulin secretion in vitro, among which quinoline scaffold **3** proved to be of high interest (Fig. 2). Indeed, Kyowa researchers[9] had also determined that similar compounds were PDE-10A inhibitors, targeting oncology applications. Initial studies allowed us to determine that a nitrogen atom handle at C4 could be used to further homologate the carboxylic acid group.

Previous reports[9] indicated that an ortho-substituent on the distal aryl ring was beneficial. Our synthesis of compounds[11] **4**–**8** (Table 1) confirmed the preference for ortho-substituents with our quinoline amino-acid inhibitors, and also revealed a very high tolerance to various steric and electronic properties. From this initial set of analogs, compound **8** became the starting point of our chemical optimization effort aimed at improving the aqueous solubility and CYP inhibition profile, while achieving good oral exposure.

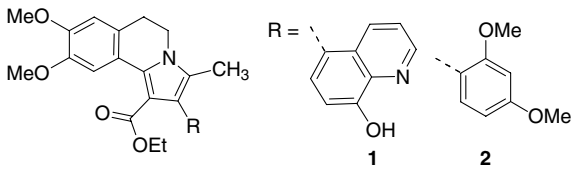


Figure 1. Pyrrolodihydroisoquinoline PDE-10A inhibitors.

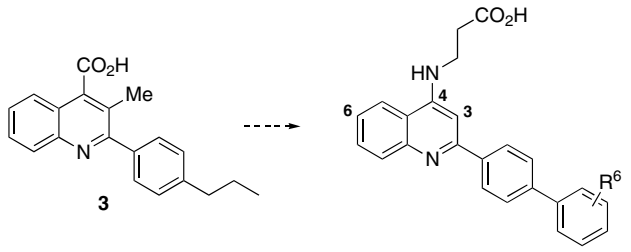


Figure 2. Quinoline-based PDE-10A inhibitors from HTS.

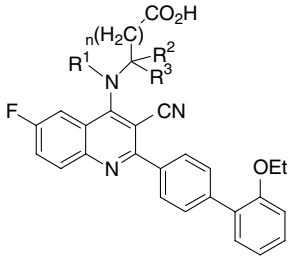
Table 1. SAR of substituents of the biphenyl moiety

Compound	R^6	PDE-10[10] IC_{50} (nM)
3	n/a	400
4	4-Me	140
5	2-CH_2-morpholine	45
6	2-CF_3	30
7	2-Et	35
8	2-OEt	35

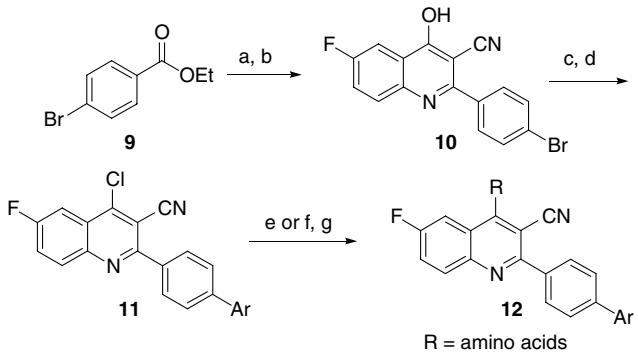
Before starting our formal optimization work, we decided to incorporate a fluorine atom at C6 and a nitrile at C3 (see Table 2, compound **14**), which were found to be favorable in a parallel chemical series. These changes provided some improvements in the potency, physicochemical properties, and oral exposure of the compound class. In addition, the presence of at electron withdrawing group at C-3 provided the synthetic flexibility of introducing the amino-acid head-group as the last step of the synthesis.

The general synthetic route allowed for the independent optimization of the different subunits of the analogs; Scheme 1 illustrates the late-stage introduction of the amino acid moiety. It should be noted that the Suzuki and chlorination–displacement steps can be inverted to allow for an efficient exploration of the SAR of the biphenyl fragment. The addition of the sodium anion of acetonitrile to a bromo benzoate, followed by reaction of the incipient enolate with 5-fluoroisatoic anhy-

Table 2. SAR of the amino acid head-group



Compound	R^1	R^2	R^3	n	PDE-10 IC_{50} (nM)
13	H	H	H	0	7
14	H	H	H	1	2
15	H	H	H	2	3
16	Me	H	H	0	4
17	Et	H	H	0	7
18	Ph	H	H	0	120
19	H	Me	H	0	6
20	H	Et	H	0	5
21	H	-CH_2-CF_3	H	0	2
22	H	-CH_2-Ph	H	0	3
23	H	-CH_2-(3-Pyr)	H	0	0.3
24	Me	Me	H	0	10
25	H	-CH_2-CH_2-		0	1
26	H	-CH_2-CH_2-CH_2-CH_2-		0	16
27	H	*R*-Me		0	6
28	H	*S*-Me		0	4
29	H	*R*-Bn		0	22
30	H	*S*-Bn		0	2



Scheme 1. Reagents and conditions: (a) NaH, MeCN, THF; **9**, THF; (b) 5-fluoroisatoic anhydride, THF; 70% yield (two steps); (c) Ar–$B(OH)_2$, $PdCl_2$(dppb), Na_2CO_3, DMF, H_2O, 120 °C, 80–90% yield; (d) $POCl_3$, 120 °C, 95% yield; (e) amino acid, Et_3N, DMA, 120 °C, 10–50% yield; (f) amino ester, Et_3N, DMSO; (g) LiOH, EtOH, H_2O, THF, 30–75% yield (two steps).

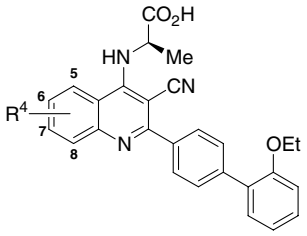
dride, produced the desired quinoline **10** in a one-pot process (Scheme 1). Subsequent Suzuki-type cross-coupling and chlorination produced advanced intermediate **11**. Treatment of **11** with amino acids or to amino esters followed by hydrolysis, provided target compounds **12**.

SAR of the amino acid group revealed a general tolerance for groups of various sizes and electronic properties (Table 2). The length of the spacer between the amino and acid groups has little effect on the potency of the compounds (**13**–**15**). Because of concerns with the potential formation of reactive metabolites with the β-amino acid moiety, we focused on α-amino acids. Further alkylation or arylation of the nitrogen (**16**–**18**) decreases the potency, and also decreased the stability of the compounds in aqueous acids. Branching of substituents on the α-carbon is also well tolerated (**19**–**23**). However, we found that the introduction of large lipophilic groups at the R^2/R^3 positions generally leads to poor CYP inhibition profile. Disubstitution is also well tolerated, as the gem-dimethyl (**24**), spiro-cyclopropyl (**25**), and spiro-cyclopentyl (**26**) analogs are potent PDE-10A.

Consistent with these findings, we did not measure significant eudismic ratios with small substituents (Table 2; **27** and **28**), and larger substituents (**29** and **30**) display only a slight preference for the (*S*)-enantiomer. Upon further profiling of compounds **27** and **28**, we measured a significant difference in the CYP inhibition profile between the two enantiomers (CYP 2C9 (IC_{50}) **27**, 2000 nM; **28**, 66 nM). The SAR exploration was continued using the *R*-enantiomer of alanine at C4.

Having better defined the SAR at the C4 position, the corresponding O-linked and CH_2-linked analogs of compound **13** were synthesized.[11] In both cases the modification caused a significant decrease in potency (100- and 10-fold, respectively). We then turned our attention to the other positions of the quinoline, keeping the C2–C4 positions constant (Table 3).[11] The C6 position of the quinoline proved tolerant to both electron-withdrawing and -donating groups (**27**, **32**, and **33**) similarly

Table 3. SAR of the quinoline ring substituents



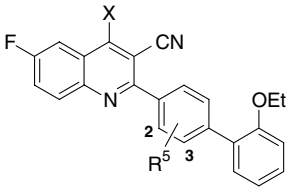
Compound	R^4	PDE-10A IC_{50} (nM)
31	H	2
27	6-F	6
32	6-Cl	18
33	6-MeO	3
34	7-Me	6
35	7-Cl	21
36	8-Me	1900
37	5-F,6-F	>4000
38	6-F,7-F	100

to the C7 position (**34** and **35**) and to 6,7-disubstituted compounds (**38**). However, introduction of small groups at either C5 or C8 (**36** and**37**), as well as 6,7-disubstitution (**38**), resulted in significant decreases in potency.

The SAR of the middle aromatic ring of the biphenyl group was also explored, which revealed an interesting interplay with the amino acid moiety. Indeed, introduction of small substituents on the proximal aryl ring is well tolerated when combined with a γ-amino acid at C4 (**39**–**42**), whereas the same substituents caused a significant decrease in potency when combined with the alanine at C4 (**43**–**46**) (Table 4).

After further profiling of compounds in functional physiochemical and CYP inhibition assays, two compounds (**27** and **40**) were progressed toward in vivo evaluation. Both compounds are potent and selective PDE-10A inhibitors, and show strong insulin secretion in the

Table 4. SAR of the amino acid head-group with modifications to the biphenyl substituents



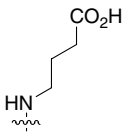
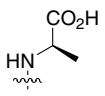
Compound	X	R^5	PDE-10 IC_{50} (nM)
39		3-F	18
40		2-F	4
41		2-Me	120
42		3-Me	34
43		3-F	160
44		2-F	79
45		2-Me	4000
46		3-Me	1300

Table 5. In vitro profile of compounds **27** and **40**

	Compound **27**	Compound **40**
PDE-10A (nM)	6	4
PDE selectivity[12]	>1000-fold	>100-fold
Disp. Islets (F.O.C.)	1.4	1.9

F.O.C., fold over control.

dispersed islet cell assay (Table 5).[8] The rat oral exposure of both compounds was evaluated at a dose of 1 mpk, and sustained plasma levels were measured well past 12 h.

Having identified compounds with suitable in vitro pharmacology and in vivo pharmacokinetic profiles, the in vivo efficacy of these PDE-10A inhibitors was assessed using a rat intraperitoneal glucose tolerance test (IPGTT),[13] where both compounds significantly reduced glucose levels at various doses (e.g., Figs. 3 and 4, **27**: 20.4% glucose AUC reduction, **40**: 22.8% glucose AUC reduction) following a glucose challenge. Further, these compounds were shown not to cause hypoglycemia in rats. In addition, compound **27** provided measurable increases in insulin secretion in vivo.[14] Taken together, these results suggest that PDE-10A inhibitors may prove useful agents in the treatment of type 2 diabetes through improvements in the insulin secretion function of β-cells in a glucose-dependent manner.

In summary, we have shown that selective PDE-10A inhibitors from multiple chemical series are potent insulin secretagogues in vitro. Further optimization allowed us to demonstrate that this activity also translated into glucose lowering effects in vivo, without signs of hypoglycemia.

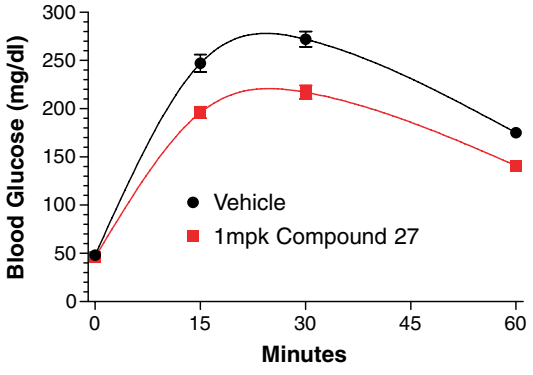


Figure 3. Evaluation of compound **27** in the rat IPGTT.

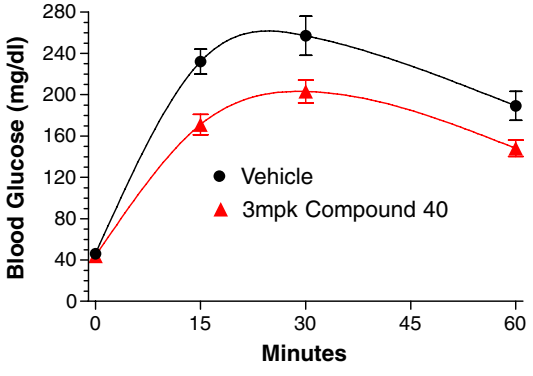


Figure 4. Evaluation of compound **40** in the rat IPGTT.

Acknowledgment

We thank Jefferson Chin, László Musza, and Anthony Paiva for NMR and LC-MS support.

References and notes

1. Drucker, D. J. *Cell Metab*. **2006**, *3*, 153.
2. (a) Mest, H. J. *Curr. Opin. Investig. Drugs* **2006**, *7*, 338; (b) Ahren, B. *Expert Opin. Investig. Drugs* **2006**, *15*, 431.
3. (a) Weber, A. *J. Med. Chem.* **2004**, *48*, 4135; (b) Drucker, D. *J. Exp. Opin. Investig. Drugs* **2003**, *12*, 87; (c) Wiedeman, P. E.; Trevillyan, J. M. *Curr. Opin. Investig. Drugs* **2003**, *4*, 412; (d) Miller, S. A.; St. Onge, E. L. Sitagliptin: A Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type 2 Diabetes. *Ann. Pharmacother.* **2006**, *40*, 1336; (e) Ahren, B. *Expert Opin. Investig. Drugs* **2006**, *15*, 431.
4. (a) Shafiee-Nick, R.; Pyne, N. J.; Furman, B. L. *Br. J. Pharmacol.* **1995**, *115*, 1486; (b) Han, P.; Werber, J.; Surana, M.; Fleischer, N.; Michaeli, T. *J. Biol. Chem.* **1999**, *274*, 22337.
5. (a) Bullock, W.; Chen, L.; Barucci, N.; Breuhaus, M.; Claus, T.; Daly, M.; Gunn, D.; Lemoine, L.; Li, Y.; Liu, Q.; Livingston, J.; Sweet, L.; Tersteegen, A.; Town, C.; Wang, H.; Zhu, J.; Heurich, R.; Wang, Y. *Abstracts of Papers*, 232nd ACS National Meeting, San Francisco, CA, United States, Sept 10–14, 2006; (b) Wang, Y.; Bear, T.; Bullock, W.; Chen, L.; Guernon, L.; Gunn, D.; Hunyadi, L.; Kramss, R.; Li, T.; Liang, S.; Liu, Q.; Liu, D.; Magnuson, S.; Mannelly, G.; Miller, D.; Mull, E.; Natero, R.; O'Keefe, D.; Qi, N.; Wood, J.; Barucci, N.; Breuhaus, M.; Claus, T.; Daly, M.; Lemoine, L.; Li, Y.; Livingston, J.; Sweet, L.; Tersteegen, A.; Wang, H.; Zhu, J.; Heurich, R. *Abstracts of Papers*, 232nd ACS National Meeting, San Francisco, CA, United States, Sept 10–14, 2006.
6. Sweet, L. PDE10a inhibitors for treating diabetes and related disorders. PCT WO 2005012485.
7. Niewoehner, U.; Bauser, M.; Ergueden, J.-K.; Flubacher, D.; Naab, P.; Repp, T.-O.; Stoltefuss, J.; Burkhardt, N.; Sewing, A.; Schauer, M.; Schlemmer, K.-H.; Weber, O.; Boyer, S.J.; Miglarese, M. Preparation of pyrrolo[2,1-a]isoquinolines as phosphodiesterase 10a inhibitors. WO 2002048144.
8. Dispersed islet cells assay.*Dispersed pancreatic islet isolation*. Lean rats (male Sprague–Dawley, 200–250 g) are anesthetized with CO_2 (60 mg/kg, ip) and the abdomen opened to expose the liver and pancreas. The pancreas is distended by injection of Hanks' solution into the bile duct, and then the pancreas is excised and minced with scissors while in Hanks' solution. After rinsing the tissue with buffer, the pancreas is digested for 10 min with collagenase, rinsed, and the islets separated from debris on a Ficoll gradient. The isolated islet fraction is then rinsed with buffer, incubated with EDTA for 8 min, followed by incubation with trypsin and DNAase I for an additional 10 min. The dispersed islets are transferred to culture media containing 8 mM glucose, seeded in 'V-bottom' 96-well plates (2500 cells per well), and cultured overnight.For assaying insulinotropic compounds, the

dispersed islets are pre-incubated in 3 mM glucose for 30 min. The islets are then transferred to media containing 8 mM glucose, test compounds, and 0.3 μM forskolin, and incubated for an additional 30 min. The media are then assayed for insulin content using scintillation proximity assay (SPA). Data are expressed as a fold increase over the response of a 8-mM glucose and 0.3 μM forskolin solution. Values reported are means of at least two experiments.

9. Osakada, N.; Haruoka, M.; Ikeda, K.; Toki, S.; Miyaji, H.; Shimada, J. Preparation of quinoline derivatives as phosphodiesterase 10A inhibitors. PCT WO 2004002484.
10. PDE inhibition assays.*Preparation of PDE isoenzymes.*Human PDEs 11A, 10A, 9A, 8A, 7B, 4B, 3B, and 2A were recombinantly expressed in Sf9 insect cells. Cell-free extracts were prepared and stored at −80 °C. PDE5 was purified from human platelets by sonication followed by centrifugation and ion exchange chromatography of the supernatant on a Mono Q 10/10 column. PDE6 was purified from rod outer segments (ROS) of bovine retinae, activated by mild trypsination, and further purified by ion exchange chromatography on a Mono Q column by Dr. Körschen, Forschungszentrum Jülich, Germany, as described in Tejada et al. *Int. J. Impot. Res.* **2001**, *13*, 282–290. Bovine PDE1 was purchased from Sigma–Aldrich (P 9529, Taufkirchen, Germany).*Enzyme inhibition.*The commercially available ^{3}H-cAMP and ^{3}H-cGMP Scintillation Proximity Assay (GE Healthcare, Little Chalfont, Buckinghamshire, UK) system was used for PDE enzyme inhibition studies as detailed in Wunder, F.; Tersteegen, A.; Rebmann, A.; Erb, C.; Fahrig, T.; Hendrix M. *Mol. Pharmacol*. **2005**, 68(6), 1775–1781. IC_{50} values were determined from sigmoidal curves by plotting the percentage of PDE activity versus log compound concentration. Values reported are means of at least two experiments. A reference compound was used on every plate to ensure reproducibility and a typical standard deviation was ±30%.
11. (a) All compounds described gave consistent ^{1}H NMR and LC/MS data. For more details on their synthetic preparations, see: (a) Cantin, D.; Magnuson, S.; Gunn, D.; Bullock, W.; Burke, J.; Fu, W.; Kumarasinghe, E.S.; Liang, S.X.; Newcom, J.; Ogutu, H.; Wickens, P.; Zhang, Z.; Bierer, D. Phenyl substituted quinoline and quinazoline compounds for the treatment of diabetes. WO2006034491; (b) Cantin, D.; Magnuson, S.; Gunn, D.; Bullock, W.; Burke, J.; Fu, W.; Kumarasinghe, E.S.; Liang, S.X.; Newcom, J.; Ogutu, H.t; Olague, A.; Wang, M.; Wickens, P.; Zhang, Z.; Bierer, D. Phenyl-substituted quinoline and quinazoline compounds for the treatment of diabetes. WO2006034512.
12. PDE selectivity assays were performed with PDE-2, PDE-3, PDE-4, PDE-5, PDE-7, PDE-8, PDE-9, and PDE-11.
13. *In vivo studies.* All animals were purchased at 6 weeks of age and were maintained on standard laboratory rodent chow ad libitum. Lean rats (male Wistar, 250–300 g) are fasted overnight and divided into two groups: vehicle and compound treatment (eight rats per group). Vehicle or compound is administered via oral gavage (1.5 mL/rat). Three hours later, a glucose solution (30%, 2 g/kg body weight) is injected intraperitoneally. Tail blood samples are collected at 0, 15, 30, and 60-min time points after the glucose injection to measure blood glucose using Glucometer (Bayer Diagnostics, Mishawaka, IN). FA-GLP-1 was used as a positive control in this assay, proving ∼20% decrease in glucose AUC.*Statistical analysis.* All results are expressed as means ± SEM for the number of animals indicated in the figure legends. ANOVA was used to evaluate the effects of compounds **27** and **40** using InStat (GraphPad Software Inc., San Diego, CA). The Tukey–Kramer multiple comparisons test was used for the parametric ANOVA. Whenever a nonparametric ANOVA was required, the Kruskal–Wallis Test was used. Results were considered significant at $p < 0.05$.
14. Compound **40** was not tested in the in vivo insulin secretion assay.