Human Glucagon Receptor Antagonists with Thiazole Cores. A Novel Series with Superior Pharmacokinetic Properties

Peter Madsen,* János T. Kodra, Carsten Behrens, Erica Nishimura, Claus B. Jeppesen, Lone Pridal, Birgitte Andersen, Lotte B. Knudsen, Carmen Valcarce-Aspegren, Mette Guldbrandt, Inge T. Christensen, Anker S. Jørgensen, Lars Ynddal, Christian L. Brand, Morten Aa. Bagger, and Jesper Lau

Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Maaloev, Denmark

Received December 22, 2008

The aim of the work presented here was to design and synthesize potent human glucagon receptor antagonists with improved pharmacokinetic (PK) properties for development of pharmaceuticals for the treatment of type 2 diabetes. We describe the preparation of compounds with cyclic cores (5-aminothiazoles), their binding affinities for the human glucagon and GIP receptors, as well as affinities for rat, mouse, pig, dog, and monkey glucagon receptors. Generally, the compounds had slightly less glucagon receptor affinity compared to compounds of the previous series, but this was compensated for by much improved PK profiles in both rats and dogs with high oral bioavailabilities and sustained high plasma exposures. The compounds generally showed species selectivity for glucagon receptor binding with poor affinities for the rat, mouse, rabbit, and pig receptors. However, dog and monkey glucagon receptor affinities seem to reflect the human situation. One compound of this series, **18**, was tested intravenously in an anesthetized glucagon-challenged monkey model of hyperglucagonaemia and hyperglycaemia and was shown dose-dependently to decrease glycaemia. Further, high plasma exposures and a long plasma half-life (5.2 h) were obtained.

Introduction

Glucagon, a 29-amino acid hormone, secreted from the α-cells of the pancreas, stimulates hepatic glucose output by increasing glycogenolysis and gluconeogenesis. A glucagon receptor antagonist is suggested to decrease the hepatic glucose output and thus lower hyperglycaemia in type 2 diabetic patients.[1−5] Previous studies have shown, in both acute and chronic models, that in vivo immunoneutralisation of glucagon does lead to lowered blood glucose levels in normal and STZ-induced diabetic rats, in normal and alloxan-induced diabetic rabbits, and in ob/ob mice.[6−9] Also, antidiabetic effects have been shown in db/db mice[10,11] and in ob/ob mice[11] by reduction of glucagon receptor expression by antisense oligonucleotides. Thus, the glucagon receptor is a potential target for a new drug class for treatment of type 2 diabetes. Additionally, reports of glucagon receptor gene knockout mice have been published[12,13] and these animals have lower blood glucose levels throughout the day and improved glucose tolerance compared to control animals. Moreover, the knockout mice also showed reduced adiposity and leptin levels but with normal body weight, food intake, and energy expenditure.[13] These data indicate that glucagon action is involved in regulation of whole body composition and thus that glucagon antagonism may give additional benefits to type 2 diabetic patients than just blood glucose regulation.

Several papers but only few classes of non-peptide glucagon receptor antagonists have been published, primarily by scientists from Abbott,[14] Merck,[15−20] Bayer,[21,22] Novo Nordisk/Pfizer,[23−27] and Novo Nordisk.[28] One compound, Bay 27-9955, has been tested in human phase 1 clinical trials and was shown to blunt the effect of glucagon on blood glucose in a hyperglucagonaemic clamp setting.[29] Previously we have published the discovery of a new class of β-alanine based human glucagon receptor (hGluR[a]) antagonists.[27] This class was subsequently developed into a related isoserine class of hGluR antagonists where selectivity for the hGluR over the related human glucose-dependent insulinotropic peptide receptor (hGIPR) was optimized[28] (see Figure 1). Here, we present a novel series of thiazole-based hGluR antagonists with superior pharmacokinetic properties. These compounds show species selectivities of glucagon receptor binding, and they only bind to the human, dog, and monkey receptors. Further, the present compounds also display selectivity for the hGluR over the hGIPR. A selected compound was shown to be active in a monkey model of hyperglucagonemia and hyperglycemia.

From our previous work[27,28] we have a working hypothesis that the urea linker does not form direct contacts with the receptor but merely presents the pharmacophoric elements to the receptor in a well-defined geometric orientation. The initial idea behind the strategy of changing the scaffold from a linear one (like ureas and amides) to a cyclic one as in the present publication was to rigidify the scaffold, hoping that the conformation(s) thus obtained would be receptor-active. Since this strategy results in target compounds of quite different shapes, we adopted (again) the power of numbers in combinatorial library synthesis in 96- and 384-well formats. The initial libraries were designed for maximum diversity in order to increase the chances of finding active compounds. Subsequent libraries focused more on optimizing the active compounds and including building blocks that were known from previous series to be better or optimal for binding because of steric fit and/or electrostatic properties. The initial libraries were screened at 1 μM, and later targeted libraries were screened at 100 nM.

As affinity for the hGIPR has been demonstrated in the previous series of β-alanine urea compounds[27] and the selectivity

* To whom correspondence should be addressed. Phone: +4544434894, +4530754894. Fax: +4544663450. E-mail: pem@novonordisk.com.

[a] Abbreviations: hGluR, human glucagon receptor; GIP, glucose-dependent insulinotropic peptide; hGIPR, human glucose-dependent insulinotropic peptide receptor; GLP-1, glucagon-like peptide-1; hGLP-1R, human glucagon-like peptide-1 receptor; SPA, scintillation proximity assay.

10.1021/jm8016249 CCC: $40.75
Published on Web 04/22/2009

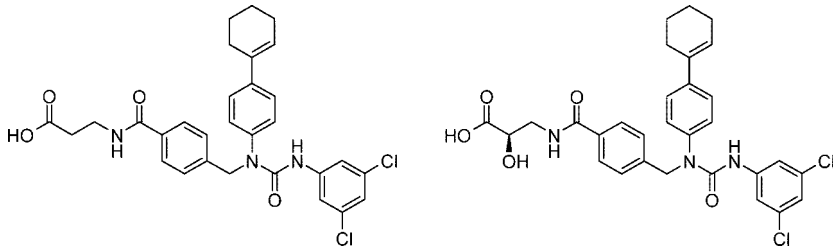
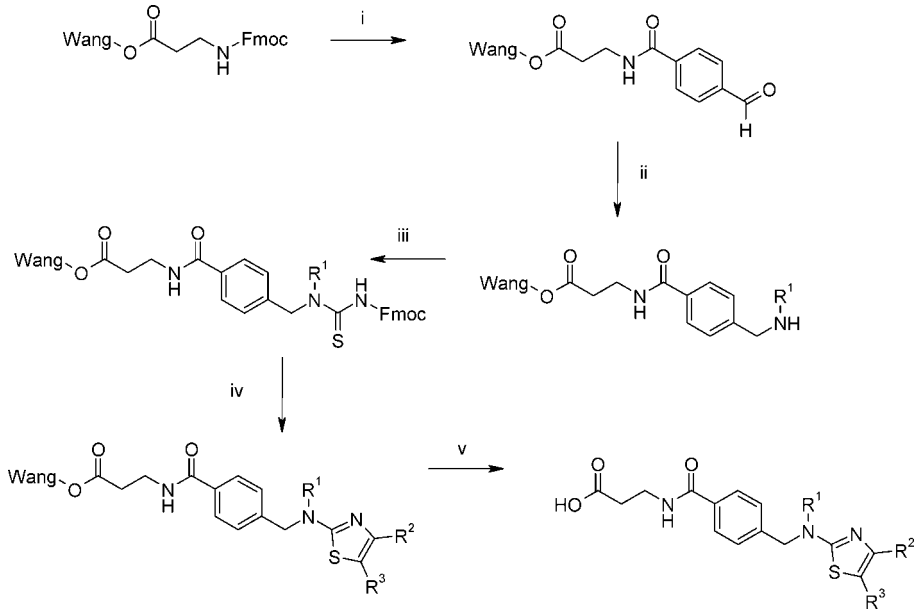


Figure 1. First examples of β-alanine and isoserine urea-based benzoylaminopropionic acids as hGluR antagonists.

Scheme 1. Preparation Method A[a]



[a] Reagents: (i) (1) piperidine, DMF, (2) 4-CHO-PhCO$_2$H, HOBt, DIC; (ii) R^1-NH2, NaCNBH$_3$; (iii) Fmoc-NCS; (iv) (1) piperidine, (2) R^{3}CHBrCOR^2; (v) TFA.

for glucagon receptor binding subsequently was optimized[28] by changing the β-alanine motif to an (*R*)-isoserine motif, the affinity for the hGIPR was monitored throughout this work. Selected compounds were further tested for affinity for the related human glucagon-like-1 peptide receptor (hGLP-1R).

The primary assay used for screening was the SPA (scintillation proximity assay) hGluR assay, and selected hits and resynthesised compounds were assayed in SPA assays for hGluR and hGIPR binding (IC_{50}) in parallel. Selected compounds were further assayed for hGluR and hGIPR binding (IC_{50}) in the previously described membrane filter assays[26] so that comparisons of receptor and species selectivities could be done in similar assays. Usually, but not always, the binding IC_{50} values were better (i.e., lower) in the filter assay than in the SPA assay.

The goal of this work was to find new potent, orally available hGluR selective antagonists that were efficacious in animal models of type 2 diabetes and that had both high plasma exposure and long half-life.

Selected compounds (usually those better than 50 nM for the hGluR) were tested for rat PK properties. Selected compounds were assayed also for species selectivities, and it became evident that these compounds had poor affinities for the rodent glucagon receptors. This was important when deciding for the animal model in which to show pharmacodynamic effects.

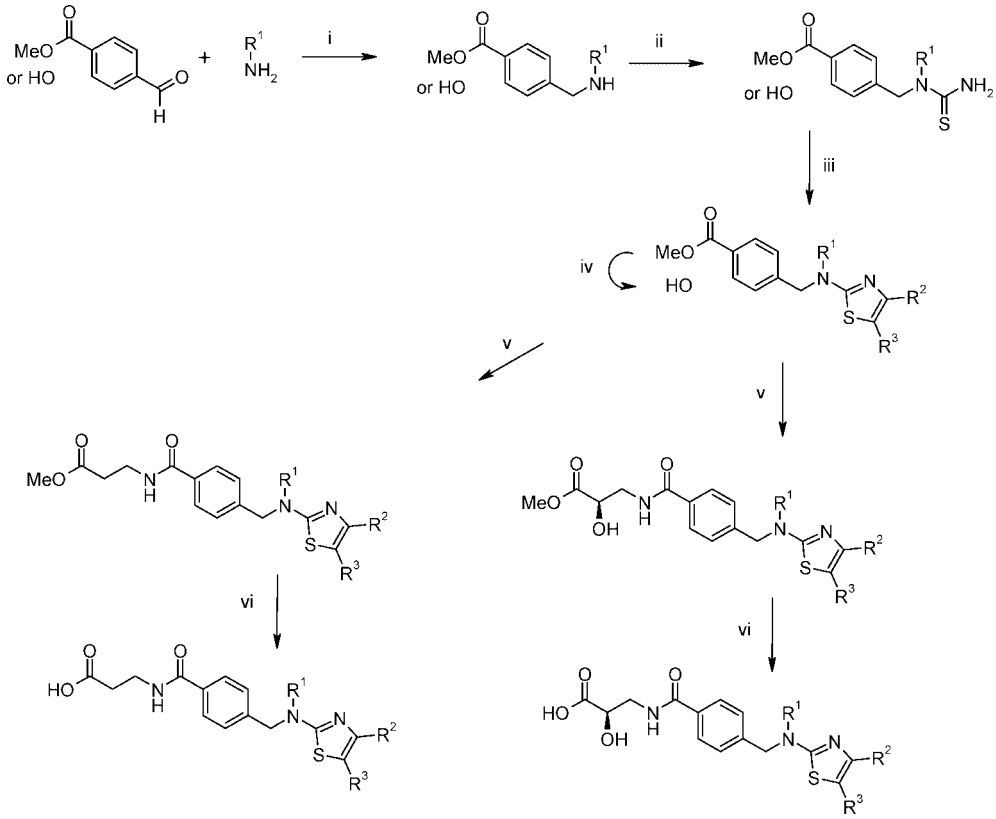
Chemistry

The aminothiazoles were first prepared on solid phase in a library format (preparation method A, Scheme 1, on a 50 μmol scale), and resyntheses of hits were conveniently done by utilizing the same procedure employing more resin. Commercially available Fmoc-β-Ala-Wang resin was deprotected and coupled with 4-formylbenzoic acid using HOBt/DIC activation. The amine was prepared through reductive amination of the aldehyde functionality, and the benzylic amine was subsequently treated with Fmoc-NCS.[31,32] Deprotection, reaction with α-bromoketones,[32] and cleavage from the resin furnished the desired products in both high yields and good purities. By use of this procedure, several focused libraries comprising more than 800 analogues were prepared.

Preparation of compounds (including resynthesis of library hits) was also done using solution phase chemistry as shown below in Scheme 2, preparation method B. 4-Formylbenzoic acid (or the corresponding methyl ester) was reductively aminated, and the benzylic amine was converted to the thiourea. This conversion was in certain cases problematic, and various methods were employed depending on the reactivity of the secondary amine in question (see Experimental Section). Formation of the aminothiazole was done easily with α-bromoketones in acetic acid. The benzoic acids were subsequently coupled with either β-alanine or (*R*)-isoserine methyl esters followed by alkaline hydrolysis to furnish the desired substituted β-alanines or (*R*)-isoserines. Alternatively, the benzoic acids were coupled with 5-aminotetrazole to afford the corresponding tetrazolylbenzamides.

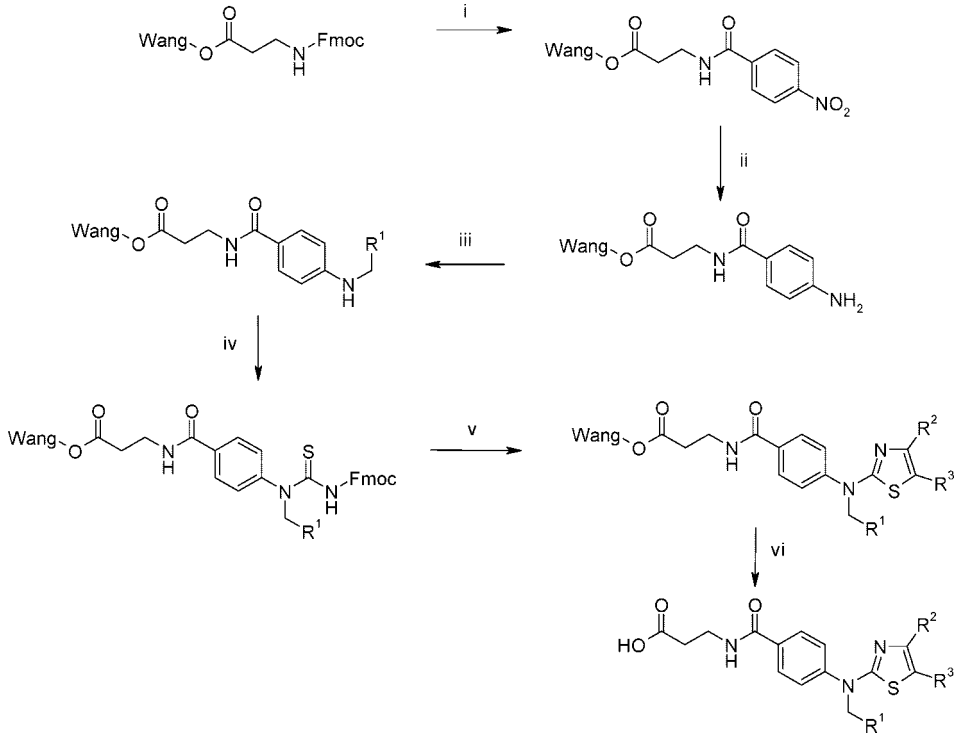
Analogues with altered connectivities were prepared as described in Schemes 3 and 4, on solid support and in solution,

Scheme 2. Preparation Method B[a]



[a] Reagents and conditions: (i) $NaCNBH_3$, HOAc; (ii) KSCN, HOAc, Δ or (1) EtO_2CNCS, (2) NaOH or (1) AcNCS, (2) NaOH; (iii) $R^3CHBrCOR^2$, DMF, HOAc; (iv) 1 N NaOH, EtOH; (v) (1) EDAC, HOBt, Et_3N, (2) H-β-Ala-OMe·HCl or H-(*R*)-iso-Ser-OMe·HCl; (vi) 1 N NaOH, EtOH.

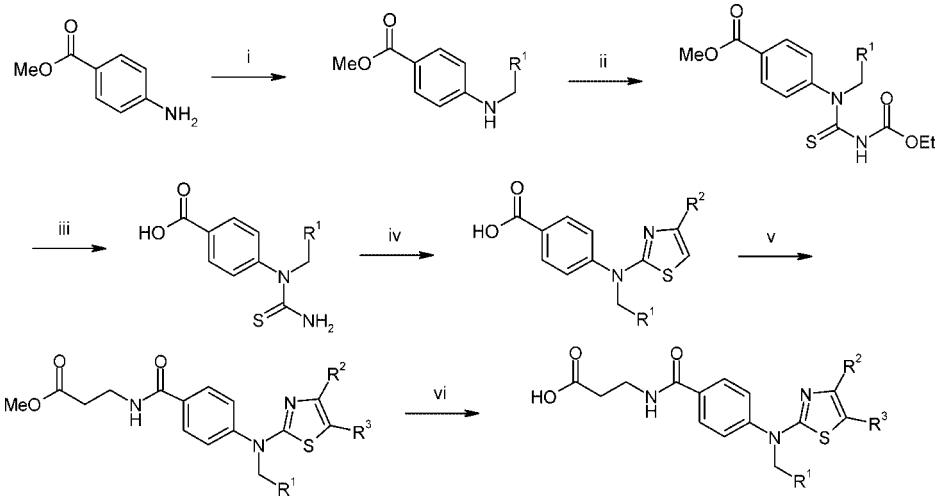
Scheme 3. Preparation Method C[a]



[a] Reagents: (i) (1) piperidine, (2) 4-NO_2-Ph-COCl, DIPEA; (ii) $SnCl_2·2H_2O$; (iii) R^1-CHO, $NaCNBH_3$; (iv) Fmoc-NCS; (v) (1) piperidine, (2) $R^3CHBrCOR^2$; (vi) TFA.

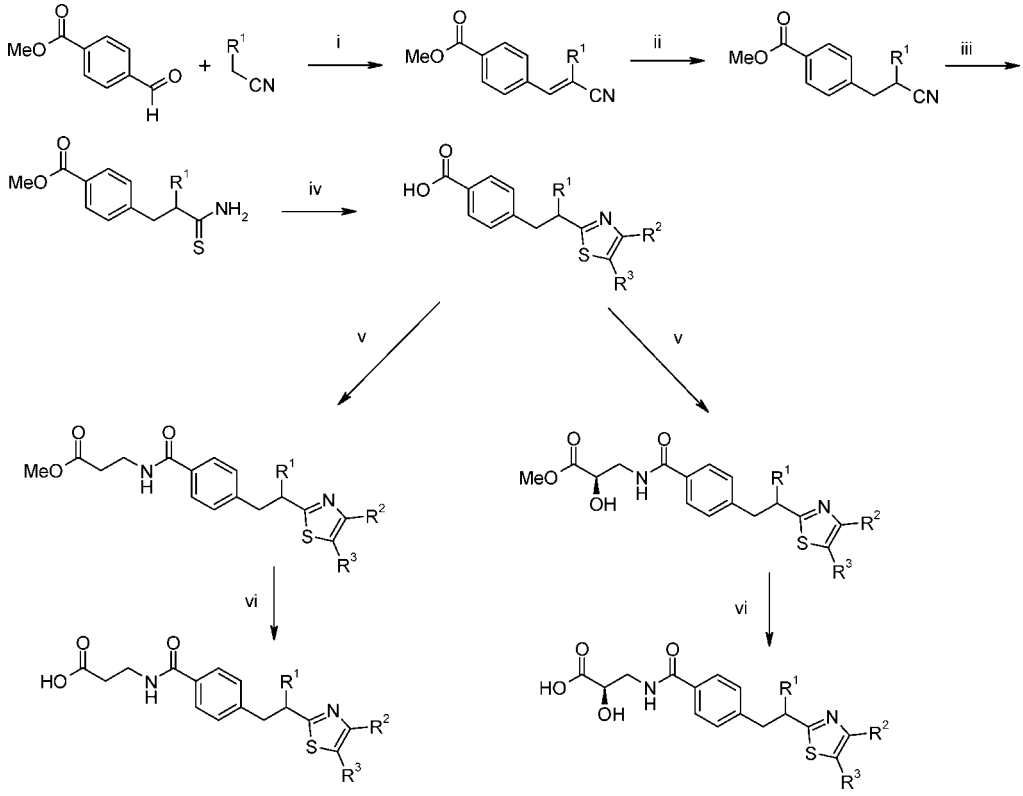
respectively. The solid supported route (preparation method C) is very similar to method A; 4-nitrobenzoyl chloride was coupled to resin-bound β-alanine, the nitro group serving as a protection for an amine function, being unmasked with $SnCl_2$ reduction.

Scheme 4. Preparation Method D[a]



[a] Reagents: (i) R^1-CHO, $NaCNBH_3$; (ii) EtO_2C-NCS; (iii) NaOH; (iv) $R^3CHBrCOR^2$; (v) (1) EDAC; HOBt, (2) H-β-Ala-OMe, HCl; (vi) NaOH.

Scheme 5. Preparation Method E[a]



[a] Reagents and conditions: (i) K_2CO_3, Δ, MeOH; (ii) $NaBH_4$, THF; (iii) $(EtO)_2P(S)SH$; (iv) $R^3CHBrCOR^2$; (v) (1) EDAC, HOBt, (2) H-β-Ala-OMe, HCl or H-iso-Ser-OMe, HCl; (vi) NaOH.

The amine was reductively alkylated, and the thiourea functionality was introduced with Fmoc isothiocyanate followed by treatment with piperidine. Cyclization with α-bromoketones afforded the desired products in high yields and purities.

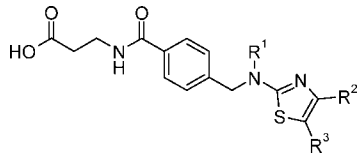
The solution phase method (preparation method D) is very similar to method B and is shown in Scheme 4.

Synthesis of analogues wherein the core 5-aminothiazole functionality has been changed to a 5-alkylthiazole core is shown in Scheme 5 (preparation method E). Knoevenagel condensation[33] of methyl 4-formylbenzoate with phenylacetonitriles afforded the corresponding acrylonitriles. Reduction[34] of the double bond ($NaBH_4$/THF) followed by treatment of the saturated nitrile with dithiophosphoric acid *O,O*-diethyl ester[35] afforded the saturated thioamide. Subsequently, Hantzsch thiazole formation[36] as before using α-bromoketones followed by coupling with β-alanine methyl ester (or (*R*)-isoserine methyl ester) and hydrolysis afforded the desired compounds.

Discussion

Early on it became evident that in order to confer hGluR binding affinity, compounds of the present aminothiazole series with aliphatic R^1 groups were not well tolerated. In Table 1, statistics for representative aliphatic R^1 groups are listed and, for comparison, two entries with aromatic R^1 groups. Numerous

Table 1. Representative Aliphatic R^1 Groups and Their Statistics from Library Screening



R^1	# Cpds. prepared	# Hits at 1 μM screening	Best screening IC_{50}
	27	2	250-390 nM
	27	0	
	8	0	
	25	0	
	33	4	575-1250 nM
	25	10 (@ 100 nM)	70 nM
	16	8 (@ 100 nM)	30 nM

R^2 and R^3 groups were identical or similar in these libraries, indicating the strong preference for aromatic R^1 groups.

Instead, focus was put on compounds with aromatic R^1 groups, and in particular compounds with cyclohex(en)ylphenyl groups were pursued. The SAR (see Table 2) proved to be quite insensitive regarding R^2 and R^3, and even the very bulky **5** with two phenyl substituents on the thiazole ring was among the compounds with the highest affinities in this class.

Although compounds like **1** and **2** had reasonable affinities (around 120 nM; see Table 2), they suffered from very poor rat PK properties with short plasma half-lives ($T_{1/2}$ of around 30−40 min) high clearances, and poor F_{po} values (2−5%) (see Table 4). **3** and **4** with higher binding affinities (IC_{50} = 30−50 nM) were equally poor on the PK parameters. The fluorene analogue **6** also had better hGluR affinity (50 nM), showed improved $T_{1/2}$ (148 min), and very low clearance but still associated with an unacceptable low F_{po} (12%).

Compounds with smaller aliphatically substituted phenyl groups as R^1 substituents, such as annelated cyclopentyl- and cyclohexylphenyl groups proved to be much better. The first two in this class, **7** and **8**, had only moderate hGluR affinities but had significantly improved rat PK profiles with full oral bioavailabilities (89% and 87%, respectively) and very high plasma exposures (C_{max} 920 and 704 ng/mL, respectively). Consequently with these encouraging data, more focus was put into elaborating this class further. We tried to increase the size of the annelated ring, from a five-membered to a six-membered, and affinities were similar; e.g., compare **11** with **7** (77 vs 86 nM) and **10** with **9** (79 vs 99 nM). With respect to rat PK parameters, **11** and **10** were as good as **7** and **8**, although oral availabilities were lower (27% and 36% versus 89% and 87%, respectively) and clearances were significantly improved (7 and 5 (mL/min)/kg versus 13 and 15 (mL/min)/kg, respectively). It is noteworthy that **10** had an extremely high plasma exposure, C_{max} = 1180 ng/mL.

We also investigated other aromatic carbon-substituted R^1 groups, e.g., 3-methyl-4-isopropylphenyl (**12** and **13**), 4-*tert*-butylphenyl (**14**), and 1,2,3,4-terahydronaphthalen-1-yl (**15**), but they had not increased hGluR affinities.

Aromatic R^1 groups with (pseudo)halogen substituents were similarly investigated, and several promising compounds were discovered. Of compounds with the R^1-group 4-chlorophenyl, **16** and **17** were particularly interesting. They had reasonable affinities for the hGluR, especially in the membrane filter assay (both 53 nM) but showed poor bioavailabilities following oral administration to rats (13% and 16%, respectively).

R^1 groups 4-CF_3-, 4-OCF_3-, and 4-SCF_3-phenyl did confer both good binding affinities (e.g., **18**, **19**, and **21**) and superior rat PK properties. Especially **18** had an encouraging profile with high oral bioavailability (58%), low clearance (1 (mL/min)/kg), long plasma $T_{1/2}$ (228 min after iv administration), and extremely high plasma exposure (C_{max} = 2100 ng/mL).

In Figure 2, the rat PK profile of **18** is shown. The compound may enter enterohepatic circulation, as the po profile indicates.

10, **16**, **17**, **18**, and **19** were tested for pharmacokinetic properties in dogs, and all showed encouraging profiles, as can be seen in Table 5. The high (>100%) bioavailability of **18** indicates that this compound in dogs might enter enterohepatic circulation. Clearances were generally very low (<5 (mL/min)/kg), and peroral bioavailabilities were generally very high.

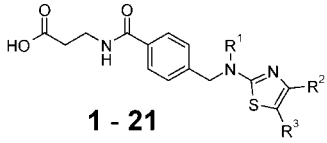
The necessity of the R^2 group not being electron rich, as expected from previous series,[27,28] was evident from library screenings results, i.e., no or few hits were found among those compounds. This was further substantiated by the low affinity of **20** (420 nM).

A few compounds were made to investigate the influence of changing the *β*-alanine moiety to an (*R*)-isoserine moiety, a modification that in the urea series of glucagon receptor antagonists proved to enhance selectivity toward GIP receptor affinity[28] while retaining glucagon receptor affinity. This modification did not give the desired effects, as in the present series of aminothiazoles glucagon receptor affinity was damaged substantially by introduction of a (*R*)-isoserine-moiety (**22** and **23**), all with affinities >200 nM.

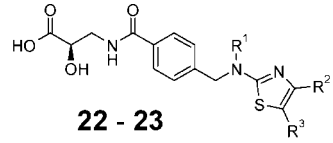
We also investigated the possibility of changing the connectivities going from a 4-(thiazol-2-ylaminomethyl)benzamide scaffold (as in **1**) to a 3- or 4-(thiazol-2-ylamino)benzamide scaffold (as in **24**). Screening results from libraries indicated that meta-substitution on the benzamide was not tolerated, as no hits were found. We found that compounds of equal hGluR affinity could be obtained from the series of 4-(thiazol-2-ylamino)benzamides, as **24** had IC_{50} = 34 nM in the hGluR membrane filter assay. The rat PK parameters of **25** and **26** were, however, not encouraging when compared to those of **6**.

The possibility of changing the exocyclic nitrogen in the aminothiazoles to a (racemic) sp^3-carbon was investigated. This change results in an altered geometry around the tripod in question, going from a planar nitrogen atom to a tetrahedral carbon atom. This change did not result in dramatically different binding affinities; e.g., compare **30** with **19** (119 versus 92 nM). A compound of this class, **27**, had, however, a very poor PK-profile with high clearance and low exposure after po dosing, and consequently this class was abandoned.

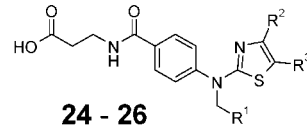
Table 2. Structures, Binding Affinities, and Synthetic Methods of Synthesized Compounds



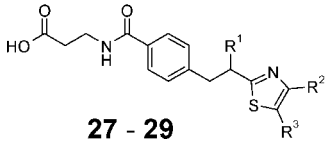
1 - 21



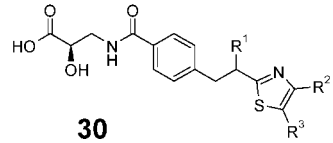
22 - 23



24 - 26



27 - 29



30

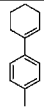
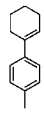
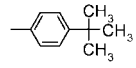
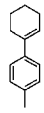
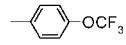
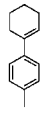
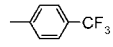
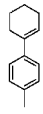
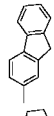
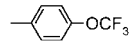
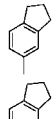
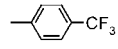
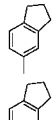
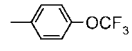
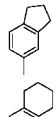
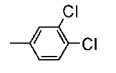
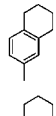
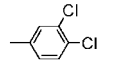
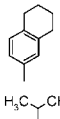
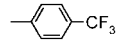
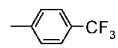
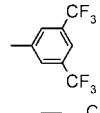
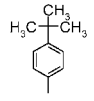
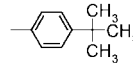
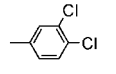
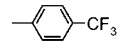
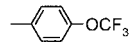
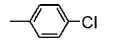
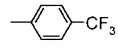
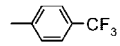
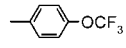
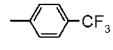
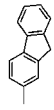
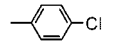
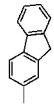
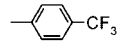
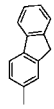
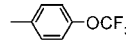
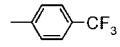
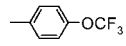
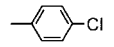
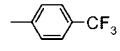
Compd	R^1	R^2	R^3	SPA hGluR binding affinity[a] (nM)	SPA hGlPR binding affinity[a] (nM)	hGluR binding affinity[b] (nM)	hGlPR binding affinity[b] (nM)	Prep. method
1			H	130	389			A
2			H	118	721			A
3			H	36	207			A
4			H	48	368			A
5			Ph	86	1002			A
6			H	50	488	59	290	A, B
7			H	86	1095	64	510	A
8			H	85	950	96	770	A
9			H	99	638			A
10			H	79	830	54	390	A, B
11			H	77	564	48	390	A
12			H	119	633	93	293	A
13			H	131	584			A
14			H	315	729			A
15			H	531	1113			A

Table 2. Continued

Compd	R^1	R^2	R^3	SPA hGluR binding affinity[a] (nM)	SPA hGIPR binding affinity[a] (nM)	hGluR binding affinity[b] (nM)	hGIPR binding affinity[b] (nM)	Prep. method
16			H	76	649	53	410	A
17			H	82	869	53	460	A, B
18			H	92	990	93	1100	A, B
19			H	92	833	51	480	A, B
20			H	420	>1000			A
21			H	34	777			A
22			H	225	>1000			B
23			H	261	>1000			B
24			H	93	555	34	320	D
25			H	78	645			C
26			H	81	732			C, D
27			H	96	381	38	211	E
28			H	153	429	25		E
29			H	98	316			E
30			H	119	565			E

[a] Binding affinity of compounds binding to the recombinant human glucagon and GIP receptors on membranes from BHK cells in a scintillation proximity assay (SPA). Data are expressed as mean IC_{50}. SD was typically within 10−15% of the reported mean, $n = 2-6$. [b] Binding affinity of compounds binding to the recombinant human glucagon and GIP receptors on membranes from BHK cells in a filtration assay. Data are expressed as mean IC_{50}. SD was typically within 10−15% of the reported mean, $n = 2-6$.

In summary, the SAR regarding hGluR affinities is as follows: R^1 being lipophilic, large, and bulky (like 4-(cyclohex-1-enyl)phenyls **1**−**5** or fluorenyl **6**) is conferring the highest hGluR binding affinities. Making R^1 smaller (like annelated cyclopen-

Table 3. Binding Affinities (IC_{50} Values in nM) to Isolated Glucagon Receptors from Various Species and Inhibition of Glucose Production from Cultures Primary Hepatocytes (IC_{50} Values in μM)

compd	human[a] (nM)	rat (nM)	mouse (nM)	rabbit (nM)	pig (nM)	dog (nM)	monkey (nM)	human hepatocytes (μM)
6	59	1800	218				62	0.7
7	64	5400	830				81	<20
8	96	4400	680				94	8.6
10	54	1600	452	1900	3300	81	55	4.0
11	48						3.0	
16	53	1400	372	943	4000	68	51	2.7
17	53	1700	418	1200	5700	78	60	2.8
18	93	2700	648	1600	7000	155	114	6.7
19	51	2000	552		5300	96	72	3.6

[a] The assay for the human receptor is the same as "hGlu binding affinity" from Table 2.

Table 4. Rat pharmacokinetic properties of selected compounds

compd	$T_{1/2}$, iv (min)	F_{po} (%)	Cl ((mL/min)/kg)	C_{max} (ng/mL)	T_{max} (min)	iv dose (mg/kg)	po dose (mg/kg)
1	37	5	35	94	90	1.9	3.7
2	30	2	39	13	90	1.6	3.15
3	43	3	34	47	90	1.9	3.7
4	35	3	44	36	30	1.9	3.9
6	148	12	5	471	300	1.1	2.1
7	131	89	13	920	60	1.66	3.34
8	61	87	15	704	240	1.66	3.34
10	104	36	5	1180	60	1.6	3.15
11	74	27	7	615	90	1.62	3.25
16	135	13	5	216	90	1.60	3.25
17	91	16	6	521	60	1.59	3.20
18	228	58	1	2100	85	1.62	3.04
19	454	29	2	638	>360	1.49	3.19
25	40	13	31	93	90	1.66	3.0
26	46	13	31	82	90	1.66	3.0
27	24	52	28	117	30	1.58	3.16

tylphenyls **7**−**9** or 4-(pseudo)halophenyls **16**−**21**) causes a drop in affinity of about 2-fold. The SAR for R^2 (and R^3) is of minor importance as long as the substituents on R^2 are lipophilic and present in the meta or para position of the phenyl ring.

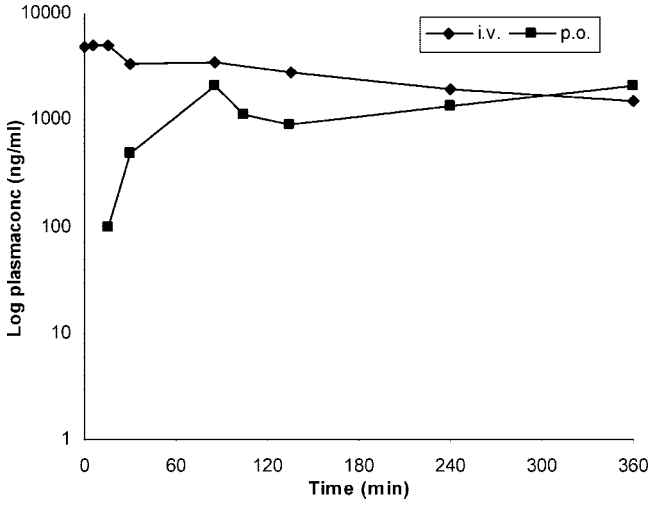


Figure 2. Rat pharmacokinetic profile of **18**. Plasma concentration vs time plots of **18** after intravenous (◆) and peroral (●) dosings to rats of 1.65 and 3.06 mg/kg, respectively. Selected pharmacokinetic parameters are given in Table 4.

Table 5. Dog Pharmacokinetic Properties[a] of Selected Compounds

compd	$T_{1/2}$, iv (min)	F_{po} (%)	Cl ((mL/min)/kg)	C_{max} (ng/mL)	T_{max} (min)
10	124	92	1.44	1190	51
16	80	16	4.66	120	105
17	66	71	4.64	554	90
18	104	141	2.23	1253	90
19	235	55	0.78	1000	88

[a] Doses: iv, 0.25 mg/kg; po, 0.5 mg/kg.

Compounds of the present series were generally quite selective for the hGluR over the hGIPR by 5- to 10-fold irrespective of the substitution patterns and even more selective over the hGLP-1R by >50-fold (data not shown), so receptor selectivity was not found to be an issue in the present series. It is not possible to conclude whether or not the isoserine analogues **22** and **23** are as selective as the isoserines were in the urea series[28] compared to corresponding β-alanines[27] (10-fold) because of the low hGluR affinity.

The SAR regarding rat PK properties is slightly different (opposite) from the SAR for hGluR binding. The poorest properties (very low F_{po} and high clearance rates) are obtained with R^1 being lipophilic, large, and bulky (like 4-(cyclohex-1-enyl)phenyls **1**−**5** or fluorenyl **6**). The highest F_{po} (88%) is obtained with R^1 being annelated cyclopentylphenyl (**7**, **8**) but still associated with relatively high clearance rates (14 (mL/min)/kg). With R^1 being annelated cyclohexylphenyl (**10**, **11**), clearance rates are improved (6 (mL/kg)/min), but F_{po} is lower (∼30%). With R^1 being 4-chlorophenyl (**16**, **17**), clearance rates are 6 (mL/kg)/min, but F_{po} is further lowered (to ∼15%). When 4-chlorophenyl is changed to 4-CF_3- or 4-CF_3O-phenyl (**18**, **19**), clearance rates are further lowered (to ∼1 (mL/kg)/min) and F_{po} is increased substantially (58% and 29%, respectively). Changing the connectivity around the aminothiazole (**25**, **26** compared with **6**) was neither beneficial for binding affinity nor for PK properties with much higher clearance rates (31 vs 5 (mL/kg)/min).

Selected compounds were assayed for binding to isolated glucagon receptors from various species to select an animal model to show pharmacodynamic effects. As can be seen from Table 3, the present compounds bound poorly to most of the receptors of the species panel. Thus, rodents (mice, rats, and rabbits) and pigs were not adequate as animal models. On the contrary, only dog and monkey receptors recognized the tested compounds with affinities similar to those of the human

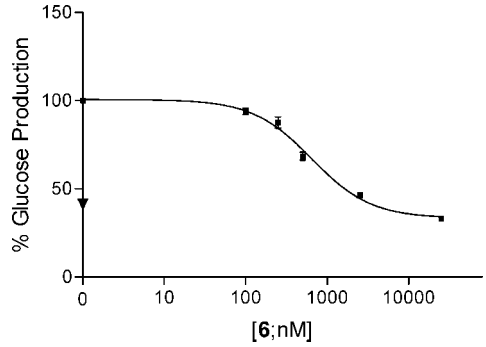


Figure 3. Example of inhibition of glucagon-stimulated glucose production in cultured primary human hepatocytes. The hepatocytes were cultured as described in the methods section. The hepatocytes were preincubated with **6** for 10 min, and then the glycogenolysis was induced with 5 nM glucagon for 60 min. Glucose in the medium was measured (glucagon-induced glycogenolysis (■) and basal glycogenolysis (▲)). Results are means values ± SEM for four experiments. The IC_{50} was calculated to 0.7 μM.

glucagon receptor, suggesting that dogs or monkeys would be relevant species in which to show pharmacodynamic effects.

6, **7**, **8**, **10**, **16**, **17**, **18**, and **19** were tested for inhibition of glucagon stimulated glucose production in human hepatocytes,[30] and as can be seen in Figure 3 and Table 3, these compounds inhibited glucose production in a dose-dependent manner. The IC_{50} values were in the range from 0.7 to 10 μM. The same compounds were also tested in freshly isolated rat hepatocytes, and none of them showed any activity (data not shown), in agreement with the rat receptor binding data. It is noted that the IC_{50} values in this hepatocyte assay were generally much higher than in the receptor binding assays. This was at least partly due to the fact that the compounds bind to albumin that was present in the hepatocyte assays and not in the binding assays.

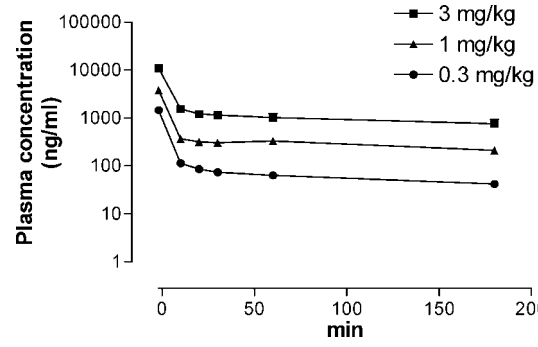


Figure 5. Plasma concentration of **18** vs time plots after intravenous dosings to monkeys.

In order to demonstrate acute in vivo efficacy in a nonhuman primate model, **18** was chosen to be tested in an efficacy model of glucagon antagonism: intravenous administration in an anesthetized glucagon-challenged monkey model of hyperglucagonaemia and hyperglycaemia. We would have preferred to test the compound orally, but because of animal handling issues, we had to do the dosing to anesthetized animals and, consequently, intravenously. **18** was chosen on the basis of rat PK data (Table 4) as the best compromise between low clearance, long $T_{1/2}$ (iv), high F_{po}, and high C_{max}. In monkeys **18** was shown (Figure 4) dose-dependently to decrease glucagon stimulated glycaemia. At doses of 1 and 3 mg/kg the hyperglycaemic effect of exogenous administered glucagon was completely abolished. Further, blood samples were assayed for presence of compound, and also in the monkey, high plasma exposure was obtained (Figure 5). Additionally, a long $T_{1/2}$ was obtained, 314 min.

Conclusion

The discovery of and library-assisted optimization of aminothiazole-based glucagon receptor antagonists have been

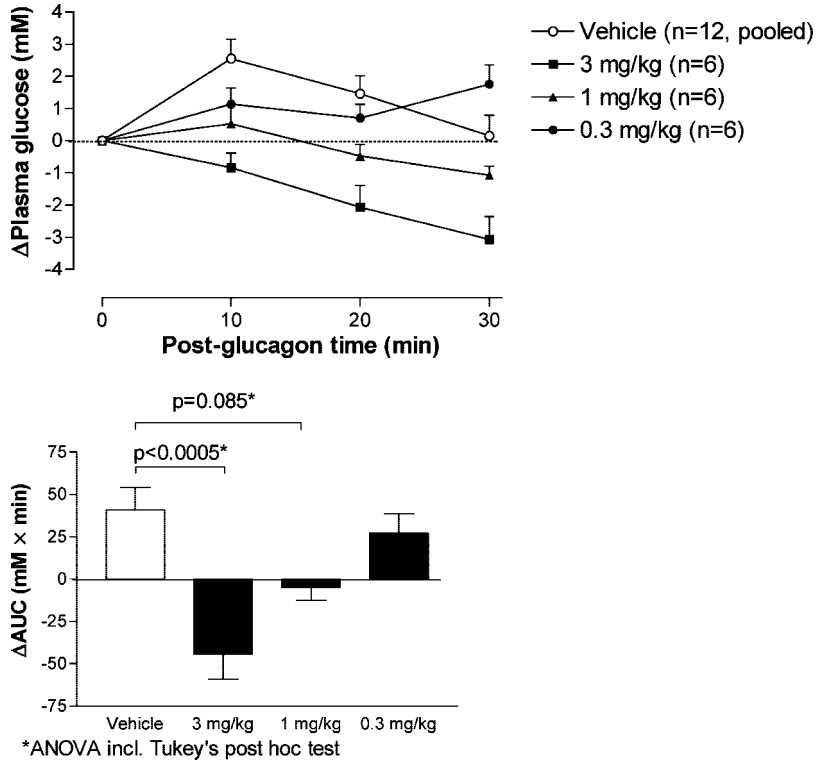


Figure 4. Plasma glucose concentration vs time plot (upper panel) of intravenous administration of **18** to anesthetized glucagon-challenged monkeys. The lower panel shows the AUCs (areas under the curves) and statistical *p*-values.

described. These compounds are species selective; binding to human receptors was only paralleled by the dog and monkey receptors, whereas affinities for the rat, mouse, and rabbit receptors were low. The compounds displayed selectivity for the hGluR over both the hGIPR and hGLP-1R. Efficacy of selected compounds in human hepatocytes was demonstrated. These compounds also generally showed superior pharmacokinetic properties in both rats and dogs with high plasma exposures and long plasma half-lives. One compound, **18**, was tested in a glucagon-challenged monkey model of hyperglucagonemia and hyperglycemia and was found to be active. In addition, this compound was shown also in the monkey to have high plasma exposure and long plasma half-life.

Experimental Section

General. ^{1}H NMR spectra were recorded in deuterated solvents at 200, 300, or 400 MHz (DRX 200, DRX 300, and AMX2 400 from Bruker Instruments, respectively). Chemical shifts are reported as δ values (ppm) relative to internal tetramethylsilane ($\delta = 0$ ppm). Elemental analyses were performed by the microanalytical laboratories at Novo Nordisk A/S, Denmark. High resolution mass spectra (MALDI-TOF) were recorded at the University of Southern Denmark, Odense. Column chromatography was performed on silica gel 60 (40−63 μm). Chemicals and solvents used were commercially available and were used without further purification. Yields refer to pure materials and are not optimized. The preparation methods are illustrated by single representative experimental procedures. The purities of the key target compounds (>95%) were verified by combustion analyses.

Preparation Method A. 3-[4-({(4-Chlorophenyl)-[4-(4-trifluoromethylphenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic Acid (16). Fmoc-β-Ala-Wang resin (0.29 mmol/g, 2 g, 0.58 mmol) was treated with piperidine (20% in NMP, 20 mL) for 40 min, and the resin was drained. This was repeated once. The resin was washed with NMP (6 × 20 mL), 4-formylbenzoic acid (0.348 g, 2.3 mmol), and HOBt (0.35 g, 2.6 mmol) in NMP (20 mL). DIC (0.36 mL, 2.3 mmol) in MeCN (5 mL) was added, and the resulting mixture was shaken for 16 h at room temperature. The resin was drained and washed with DMF (5 × 20 mL). To the resulting resin-bound aldehyde was added 4-chloroaniline (0.37 g, 2.9 mmol) dissolved in a mixture of NMP and trimethyl orthoformate (1:1, 20 mL). HOAc (2 mL) was added, and the mixture was shaken for 4 h at room temperature. $NaCNBH_3$ (0.36 g, 5.8 mmol) in a mixture of NMP and methanol (1:1, 6 mL) was added, and the resulting mixture was shaken for 16 h at room temperature, drained, and washed successively with NMP/MeOH (1:1, 2 × 20 mL), NMP (3 × 20 mL), DCP/DIPEA (7:1, 3 × 20 mL), and DCP (3 × 20 mL). To the resulting resin-bound amine was added Fmoc isothiocyanate (1.79 g, 6.38 mmol) in DCP (20 mL), and the mixture was shaken for 4 h at room temperature and washed with DCM (3 × 20 mL) and NMP (3 × 20 mL). The resin was treated with piperidine (20% in NMP, 20 mL) for 1 h, and the resin was drained. The resin was washed with NMP (6 × 20 mL). 2′-Bromo-4-trifluoromethylacetophenone (1.60 g, 5.8 mmol) in NMP (15 mL) and acetic acid (4 mL) were added to the resin, and the mixture was shaken for 16 h at room temperature and washed with NMP (3 × 20 mL), MeOH (3 × 20 mL), and DCM (10 × 20 mL). The product was cleaved from the resin by treatment with 50% TFA in DCM (20 mL) for 30 min. Concentration in vacuo and purification of the residue by column chromatography afforded 0.32 g (98%) of 3-[4-({(4-chlorophenyl)-[4-(4-trifluoromethylphenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic acid. ^{1}H NMR (DMSO-d_6): $\delta = 2.49$ ("t", below DMSO), 3.43 (2H, q), 5.33 (2H, s), 7.43 (2H, d), 7.52 (4H, m), 7.75 (4H, m), 8.07 (2H, d), 8.46 (1H, t), 12.2 (1H, bs). Anal. ($C_{27}H_{21}N_3ClF_3O_3S$) C, H, N.

Preparation Method B. 3-[4-({(4-Chlorophenyl)-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic Acid (17). 4-Formylbenzoic acid (15 g, 100 mmol) was suspended in methanol (500 mL), and 4-chloroaniline (12.7 g, 100 mmol) was added. The resulting suspension was heated at reflux temperature for 30 min and subsequently cooled to approximately 35 °C. Acetic acid glacial (50 mL) was added followed by $NaCNBH_3$ (4.96 g, 80 mmol) in portions. The mixture was stirred at room temperature for 30 min. The mixture was concentrated in vacuo to approximately 150 mL by rotary evaporation. Water (150 mL) was added, and the resulting suspension was stirred at room temperature for 16 h. Filtration, washing with water, and drying afforded 26.8 g (100%) of 4-[(4-chlorophenylamino)methyl]benzoic acid as a solid. ^{1}H NMR (DMSO-d_6): $\delta = 4.31$ (2H, d), 6.54 (4H, "d"), 7.05 (2H, d), 7.45 (2H, d), 7.89 (2H, d), 12.8 (1H, bs).

A mixture of 4-[(4-chlorophenylamino)methyl]benzoic acid (33.3 g, 127 mmol) and KSCN (37 g, 382 mmol) in 1 N HCl (500 mL) was refluxed for 16 h. The suspension was filtered and washed with water. The solid was mixed with KSCN (37 g, 382 mmol) and 1 N HCl (500 mL), and the mixture was again refluxed for 16 h. Filtration, washing with water, and resubmission to the reaction conditions above afforded 30.2 g (70%) of 4-[1-(4-chlorophenyl)thioureidomethyl]benzoic acid as a solid. ^{1}H NMR (DMSO-d_6): $\delta = 5.44$ (2H, s), 7.14 (2H, d), 7.39 (4H, "t"), 7.85 (2H, d), 12.9 (1H, bs).

4-[1-(4-Chlorophenyl)thioureidomethyl]benzoic acid (29 g, 88.8 mmol) was dissolved in DMF (300 mL), and acetic acid (40 mL) and 2′-bromo-4-trifluoromethoxyacetophenone (25.1 g, 88.8 mmol) were added. The resulting mixture was stirred at room temperature for 16 h. EtOAc (500 mL) was added, and the mixture was washed with water (2 × 500 mL), saturated aqueous NaCl (500 mL), and saturated aqueous NH_4Cl (500 mL). Drying ($MgSO_4$) and concentration in vacuo afforded 48 g (quant) of 4-({(4-chlorophenyl)-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoic acid as an oil. ^{1}H NMR (DMSO-d_6): $\delta = 5.35$ (2H, s), 7.36 (1H, s), 7.39 (2H, d), 7.47−7.57 (6H, m), 7.90 (2H, d), 7.97 (2H, d), 12.7 (1H, bs).

4-({(4-Chlorophenyl)-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoic acid (47 g, 93 mmol) was dissolved in DMF (500 mL), and HOBt (18.9 g, 140 mmol) and EDAC·HCl (26.7 g, 140 mmol) were added. The mixture was stirred at room temperature for 1.5 h. DIPEA (19.1 mL, 111 mmol) and 3-aminopropionic acid methyl ester hydrochloride (15.5 g, 111 mmol) were added to the mixture, and the resulting mixture was stirred at room temperature for 16 h. EtOAc (500 mL) was added, and the mixture was washed with water (500 mL). The aqueous phase was extracted with EtOAc (500 mL). The combined organic phases were washed with water (2 × 500 mL) and saturated aqueous NH_4Cl (2 × 300 mL), dried (Na_2SO_4), and concentrated in vacuo to afford 60 g (quant) of crude product. Recrystallization from ethanol afforded 31.2 g (55%) of pure 3-[4-({(4-chlorophenyl)-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoylamino]-propionic acid methyl ester as a solid. ^{1}H NMR (DMSO-d_6): $\delta =$ 2.57 (3H, t), 3.46 (2H, q), 3.59 (3H, s), 5.32 (2H, d), 7.36 (1H, s), 7.41 (4H, m), 7.41 (4H, m), 7.76 (2H, d), 7.97 (2H, d), 8.51 (1H, t).

3-[4-({(4-Chlorophenyl)-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic acid methyl ester (18.7 g, 31.7 mmol) was dissolved in ethanol (500 mL), and 1 N sodium hydroxide (63 mL, 63 mmol) was added. The resulting mixture was stirred at room temperature for 16 h. The mixture was filtered, washed with ethanol, and dried in vacuo at 40 °C for 16 h to afford 8.0 g (42%) of 3-[4-({(4-chlorophenyl)-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic acid as the sodium salt, mp 134−139 °C. ^{1}H NMR (DMSO-d_6): $\delta = 2.13$ (2H, t), 3.37 (m, below water), 5.31 (2H, s), 7.34−7.39 (5H, m), 7.50 (4H, m), 7.74 (2H, d), 7.97 (2H, d), 8.78 (1H, bs). Anal. ($C_{27}H_{20}N_3Cl_1F_3O_4SNa\cdot 2H_2O$) C, H. N calcd, 6.63%; found, 7.30%.

The above filtrate was added to 1 N HCl (80 mL) and water (200 mL), and the mixture was extracted with ethyl acetate (300 mL). The organic phase was washed with water (300 mL), dried, and concentrated in vacuo to afford 6.63 g (36%) of 3-[4-({(4-chlorophenyl)-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic acid as a solid. ^{1}H NMR (DMSO-d_6): $\delta = 2.47$ (m, below DMSO), 3.43 (2H, q), 5.32 (2H, s), 7.36

(1H, s), 7.41 (4H, "t"), 7.39 (5H, m), 7.51 (4H, m), 7.76 (2H, d), 7.97 (2H, d), 8.48 (1H, t), 12.2 (1H, bs). Anal. ($C_{27}H_{21}N_3Cl_1F_3O_4S$) C, H, N.

2(*R*)-Hydroxy-3-[4-({indan-5-yl-[4-(4-trifluoromethoxyphenyl)-thiazol-2-yl]amino}methyl)benzoylamino]propionic Acid (22). 4-({Indan-5-yl-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoic acid (0.44 g, 0.86 mmol) was dissolved in DMF (10 mL), and HOBt (0.18 g, 1.29 mmol) and EDAC HCl (0.25 g, 1.29 mmol) were added. The resulting mixture was stirred at room temperature for 1.5 h. 3-Amino-2(*R*)-hydroxypropionic acid methyl ester hydrochloride ((*R*)-isoserine methyl ester hydrochloride), prepared as previously described[28] (0.16 g, 1.03 mmol), and DIPEA (225 μL, 1.29 mmol) were added, and the resulting mixture was stirred at room temperature for 16 h. EtOAc (100 mL) was added, and the mixture was washed with water (2 × 80 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue (0.35 g) was purified by column chromatography on silica gel, eluting with a mixture of EtOAc and heptane (1:1). This afforded 0.10 g (19%) of 2(*R*)-hydroxy-3-[4-({indan-5-yl-[4-(4-trifluoromethoxy-phenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic acid methyl ester. ^{1}H NMR ($CDCl_3$): δ = 2.10 (2H, p), 2.90 (4H, m), 3.81 (5H, m), 4.39 (1H, bs), 5.26 (2H, s), 6.50 (1H, t), 6.66 (1H, s), 7.04 (1H, dd), 7.14 (1H, s), 7.21 (3H, "d"), 7.46 (2H, d), 7.69 (2H, d), 7.84 (2H, d).

2(*R*)-Hydroxy-3-[4-({indan-5-yl-[4-(4-trifluoromethoxyphenyl)-thiazol-2-yl]amino}methyl)benzoylamino]propionic acid methyl ester (0.10 g, 0.16 mmol) was dissolved in MeOH (5 mL), and 1 N NaOH (0.16 mL, 0.16 mmol) was added. The resulting mixture was stirred at room temperature for 16 h. Then 1 N HCl (0.17 mL) and water (50 mL) were added, and the mixture was extracted with EtOAc (50 mL). The organic phase was washed with water (50 mL), dried ($MgSO_4$), and concentrated in vacuo. This afforded 0.06 g (61%) of 2(*R*)-hydroxy-3-[4-({indan-5-yl-[4-(4-trifluoromethoxy-phenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic acid. ^{1}H NMR ($CDCl_3$): δ = 2.09 (2H, m), 2.88 (4H, m), 3.78 (1H, m), 3.87 (1H, m), 4.37 (1H, t), 5.24 (2H, s, 6.65 (1H, s), 7.00 (1H, t), 7.02 (1H, d), 7.13 (1H, s), 7.19 (3H, "d"), 7.45 (2H, d), 7.68 (2H, d), 7.82 (2H, d). HR-MS calcd for $C_{30}H_{26}F_3N_3O_5S$: 598.1618 (M + 1). Found: 598.1647.

In the following, details as to how the thiourea functionality has been prepared are given.

3-(4-{[[4-(4-Chlorophenyl)thiazol-2-yl]-(4-trifluoromethylphenyl)-amino]methyl}benzoylamino)propionic Acid (18). 4-[(4-Trifluoromethylphenylamino)methyl]benzoic acid methyl ester (1.00 g; 3.23 mmol) was dissolved in DCM (10 mL), and Fmoc isothiocyanate (0.91 g; 3.23 mmol) was added. The resulting mixture was stirred at room temperature for 16 h. The mixture was concentrated in vacuo, and the residue was purified by flash chromatography using DCM as eluent. This afforded 1.79 g (94%) of [(4-trifluoromethylphenyl)-(4-mehoxycarbonylbenzyl)thiocarbamoyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester. ^{1}H NMR ($CDCl_3$): δ = 3.88 ppm (s, 3H), 4.05 (t, 1H), 4.30 (d, 2H), 5.52 (s, 2H), 7.15−7.30 (m, 5H), 7.30−7.45 (m, 7H), 7.53 (d, 2H), 7.72 (d, 2H), 7.95 (d, 2H).

[(4-Trifluoromethylphenyl)-(4-mehoxycarbonylbenzyl)thiocarbamoyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester (1.70 g, 2.87 mmol) was dissolved in a mixture of piperidine and DCM (1:4, 20 mL). The mixture was stirred at room temperature for 30 min and then evaporated to dryness in vacuo. The residue was purified by column chromatography, eluting with dichloromethane to give an oil, which was subsequently crystallized from ethyl acetate/heptane to afford 495 mg (46%) of 4-[1-(4-trifluoromethylphenyl)thioureidomethyl]benzoic acid methyl ester. ^{1}H NMR ($CDCl_3$): δ = 3.88 ppm (s, 3H), 5.62 (s, 2H), 5.65 (bs, 2H), 7.20 (d, 2H), 7.40 (d, 2H), 7.65 (d, 2H), 7.96 (d, 2H).

3-[4-({(9*H*-Fluoren-2-yl)-[4-(4-trifluoromethoxyphenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic Acid (6). 4-[(9*H*-Fluoren-2-ylamino)methyl]benzoic acid methyl ester (10 g, 30.36 mmol) was dissolved in dichloromethane (160 mL), and ethoxycarbonyl isothiocyanate (4.65 mL, 39.47 mmol) was added. The resulting mixture was stirred at room temperature for 16 h. The mixture was concentrated in vacuo to afford 14 g (100%) of [(9*H*-fluoren-2-yl)-(4-mehoxycarbonylbenzyl)thiocarbamoyl]carbamic acid ethyl ester. ^{1}H NMR (DMSO-d_6): δ = 10.18 (s, 1H), 7.90−7.80 (m, 4H), 7.56−7.50 (m, 3H), 7.42 (s, 1H), 7.38−7.30 (m, 3H), 7.17 (d, 1H), 5.64 (s, 2H), 3.88−3.80 (m, 5H), 0.92 (t, 3H).

[(9*H*-Fluoren-2-yl)-(4-mehoxycarbonylbenzyl)thiocarbamoyl]carbamic acid ethyl ester (14 g, 30.36 mmol) was dissolved in warm EtOH (96%, 160 mL), and 4 N NaOH (76 mL, 304 mmol) was added to the solution. The resulting mixture was refluxed for 16 h. The mixture was concentrated in vacuo to remove ethanol. The residue was suspended in water (150 mL), and 4 N HCl (76 mL, 304 mmol) was carefully added. The suspension was stirred for $^1/_2$ h at 25 °C and filtered, washed with water, and dried in vacuo at 40 °C for 16 h to afford 11.4 g (100%) of 4-{[1-(9*H*-fluoren-2-yl)thioureido]methyl}benzoic acid. ^{1}H NMR (DMSO-d_6): δ = 12.88 (broad, 1H), 7.88−7.84 (m, 4H), 7.58 (d, 1H), 7.46−7.38 (m, 5H), 7.09 (d, 1H), 5.53 (s, 2H), 3.87 (s, 2H).

3-[4-({(4-Trifluoromethoxyphenyl)-[4-(4-trifluoromethylphenyl)thiazol-2-yl]amino}methyl)benzoylamino]propionic Acid (19). 4-[(4-Trifluoromethoxyphenylamino)methyl]benzoic acid methyl ester (15 g, 46.11 mmol) was dissolved in DCM (220 mL), and ethoxycarbonyl isothiocyanate (7.05 mL, 59.95 mmol) was added. The resulting mixture was stirred at room temperature for 16 h. The mixture was concentrated in vacuo to afford 21 g (100%) of [(4-trifluoromethoxyphenyl)-(4-mehoxycarbonylbenzyl)thiocarbamoyl]carbamic acid ethyl ester. ^{1}H NMR (DMSO-d_6): δ = 10.44 (s, 1H), 7.89 (dd, 2H), 7.51 (dd, 2H), 7.36−7.28 (m, 4H), 5.61 (s, 2H), 3.88−3.80 (m, 5H), 0.91 (t, 3H).

[(4-Trifluoromethoxyphenyl)(4-mehoxycarbonylbenzyl)thiocarbamoyl]carbamic acid ethyl ester (21 g, 46.11 mmol) was dissolved in warm EtOH (96%, 250 mL), and 4 N NaOH (115 mL, 460 mmol) was added. The resulting mixture was refluxed for 16 h. After cooling, the mixture was evaporated in vacuo to remove ethanol, the residue was suspended in water (400 mL), and 4 N HCl (115 mL, 460 mmol) was carefully added. The water was decanted from the oil, and the residual oil was dissolved in DCM, dried ($MgSO_4$), and evaporated. The amorphous residue was partly dissolved in EtOAc, filtered, and evaporated to afford 17 g (100%) of 4-[1-(4-trifluoromethoxyphenyl)thioureidomethyl]benzoic acid. ^{1}H NMR (DMSO-d_6): δ = 12.9 (s, 1H), 7.87 (d, 2H), 7.40 (d, 2H), 7.33 (d, 2H), 7.26 (d, 2H), 5.45 (s, 2H).

Acknowledgment. The excellent technical assistance of Dorthe Egholm Jensen, Annette Nielsen, Paw Bloch, Jette Bolding Lenstrup, Bent Høgh, Poul Enrico Nielsen, Helle Bach, Janni Kaja Kastrup Sørensen, Brian S. Hansen, Jeanette Hansen, Mette Winther, Annette Hansen, Lone Honoré, Pia Justesen, and Karin Hamborg Albrechtsen is gratefully acknowledged. The library production/purification team (Dr. Helle Demuth, Dr. Leif Christensen, Vibeke Rhode, Anne-Mette Jacobsen, and Lone Riisegaard) was instrumental for the work described here, and their efforts are highly acknowledged. Dr. Vladimíra Panajotova is acknowledged for supervising the monkey studies.

Supporting Information Available: Experimental procedures for the preparation of compounds according to preparation methods C, D, and E; purity information for target compounds; in vitro and in vivo protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) DeFronzo, R. A.; Bonadonna, R. C.; Ferrannini, E. Pathogenesis of NIDDM, a balanced overview. *Diabetes Care* **1992**, *15*, 318–368.
(2) Consoli, A.; Nurjhan, N.; Capiani, F.; Gerich, J. Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. *Diabetes* **1989**, *38*, 550–557.
(3) Unger, R. H.; Orci, L. The essential role of glucagon in the pathogenesis of diabetes mellitus. *Lancet* **1975**, *1*, 14–16.
(4) Dobbs, R. E.; Sakurai, H.; Sasaki, H.; Faloona, G.; Valverde, I.; Baetens, D.; Orici, L.; Unger, R. Glucagon: role in the hyperglycemia of diabetes mellitus. *Science* **1975**, *187*, 544–547.

(5) Gerich, J. E.; Lorenzi, M.; Bier, D. M.; Schneider, V.; Tsalikian, E.; Karam, J. H.; Forsham, P. H. Prevention of human diabetic ketoacidosis by somatostatin. *N. Engl. J. Med.* **1975**, *292*, 985–989.
(6) Brand, C. L.; Rolin, B.; Jørgensen, P. N.; Svendsen, I.; Kristensen, J. S.; Holst, J. J. Immunoneutralisation of endogenous glucagon with monoclonal glucagon antibody normalizes hyperglycaemia in moderately streptozotocin-diabetic rats. *Diabetologia* **1994**, *37*, 985–993.
(7) Brand, C. L.; Jørgensen, P. N.; Knigge, U.; Warberg, J.; Svendsen, I.; Kristensen, J. S.; Holst, J. J. Role of glucagon in maintanence of euglycemia in fed and fasted rats. *Am. J. Physiol.* **1995**, *32*, E469–E477.
(8) Brand, C. L.; Joergensen, P. N.; Svendsen, I.; Holst, J. J. Evidence for a major role for glucagon in regulation of plasma glucose in conscious, nondiabetic, and alloxan-induced diabetic rabbits. *Diabetes* **1996**, *45*, 1076–1083.
(9) Brand, C. L.; Hansen, B.; Gronemann, S.; Moysen, M.; Holst, J. J. Subchronic glucagon neutralisation improves diabetes in ob/ob mice. *Diabetes* **2000**, *49* (S1), A81.
(10) Liang, Y.; Osborne, M. C.; Monia, B. P.; Bhanot, S.; Gaarde, W. A.; Reed, C.; She, P.; Jetton, T. L.; Demarest, K. T. Reduction in glucagon receptor expression by an antisense oligonucleotide ameliorates diabetic syndrome in db/db mice. *Diabetes* **2004**, *53*, 410–417.
(11) Sloop, K. W.; Cao, J. X.-C.; Siesky, A. M.; Zhang, H. Y.; Bodenmiller, D. M.; Cox, A. L.; Jacobs, S. J.; Moyers, J. S.; Owens, R. A.; Showalter, A. D.; Brenner, M. B.; Raap, A.; Gromada, J.; Berridge, B. R.; Monteith, D. K. B.; Porksen, N.; McKay, R. A.; Monia, B. P.; Bhanot, S.; Watts, L. M.; Dodson, M. Hepatic and glucagon-like peptide-1-mediated reversal of diabetes by glucagon receptor antisense oligonucleotide inhibitors. *J. Clin. Invest.* **2004**, *113*, 1571–1581.
(12) Parker, J. C.; Andrews, K. M.; Allen, M. R.; Stock, J. L.; McNeish, J. D. Glycemic control in mice with targeted disruption of the glucagon receptor gene. *Biochem. Biophys. Res. Commun.* **2002**, *290*, 839–843.
(13) Gelling, R. W.; Du, X. Q.; Dichmann, D. S.; Rømer, J.; Huang, H.; Cui, L.; Obici, S.; Tang, B.; Holst, J. J.; Fledelius, C.; Johansen, P. B.; Rossetti, L.; Jelicks, L. A.; Serup, P.; Nishimura, E.; Charron, M. J. Lower blood glucose, hyperglucagonemia, and pancreatic α cell hyperplasia in glucagon receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1438–1443.
(14) Kurukulasuriya, R.; Sorensen, B. K.; Link, J. T.; Patel, J. R.; Jae, H. S.; Winn, M. X.; Rohde, J. R.; Grihalde, N. D.; Lin, C. W.; Ogiela, C. A.; Adler, A. L.; Collins, C. A. Biaryl amide glucagon receptor antagonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2047–2050.
(15) Chang, L. L.; Sidler, K. L.; Cascieri, M. A.; de Laszlo, S.; Koch, G.; Li, B.; MacCoss, M.; Mantlo, N.; O'Keefe, S.; Pang, M.; Rolando, A.; Hagmann, W. K. Substituted imidazoles as glucagon receptor antagonists. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2549–2553.
(16) Qureshi, S. A.; Candelore, M. R.; Xie, D.; Yang, X.; Tota, L. M.; Ding, V. D. H.; Li, Z.; Bansal, A.; Miller, C.; Cohen, S. M.; Jiang, G.; Brady, E.; Saperstein, R.; Duffy, J. L.; Tata, J. R.; Capman, K. T.; Moller, D. E.; Zhang, B. B. A novel glucagon receptor antagonist inhibits glucagon-mediated biological effects. *Diabetes* **2004**, *53*, 3267–3273.
(17) Duffy, J. L.; Kirk, B. A.; Konteatis, Z.; Campbell, E. L.; Liang, R.; Brady, E. J.; Candelore, M. R.; Ding, V. D. H.; Jiang, G.; Liu, F.; Qureshi, S. A.; Saperstein, R.; Szalkowski, D.; Tong, S.; Tota, L. M.; Xie, D.; Yang, X.; Zafian, P.; Zheng, S.; Chapman, K. T.; Zhang, B. B.; Tata, J. R. Discovery and investigation of a novel class of thiophene-derived antagonists of the human glucagon receptor. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1401–1405.
(18) Shen, D. M.; Zhang, F.; Brady, E. J.; Candelore, M. R.; Yang, Q. D.; Ding, V. D. H.; Dragovic, J.; Feeny, W. P.; Jiang, G.; McCann, P.; Mock, S.; Quereshi, S. A.; Saperstein, R.; Shen, X.; Tamvakopoulos, C.; Tong, X.; Tota, L. M.; Wright, M. J.; Yang, X.; Zheng, S.; Chapman, K. T.; Zhang, B. B.; Tata, J. R.; Parmee, E. R. Discovery of novel, potent, and orally active spiro-urea human glucagon receptor antagonist. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4564–4569.
(19) Liang, R.; Abrado, L.; Brady, E. J.; Candelore, M. R.; Ding, V.; Saperstein, R.; Tota, L. M.; Wright, M.; Mock, S.; Tamvakopoulos, C.; Tong, S.; Zheng, S.; Zhang, B. B.; Tata, J. R.; Parmee, E. R. Design and synthesis of conformatiuonally constrained trisubstituted ureas as potent antagonists of the human glucagon receptor. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 587–592.
(20) Kim, R. M.; Chang, J.; Lins, A. R.; Brady, E.; Candelore, M. R.; Dallas-Yang, Q.; Ding, V.; Dragovic, J.; Iliff, S.; Jiang, G.; Mock, S.; Quereshi, S.; Saperstein, R.; Szalkowski, D.; Tamvakopoulos, C.; Tota, L.; Wright, M.; Yang, X.; Tata, J. R.; Chapman, K.; Zhang, B. B.; Parmee, E. R. Discovery of potent, orally active benzimidazole glucagon receptor antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3701–3705.
(21) Ladouceur, G. H.; Cook, J. H.; Doherty, E. M.; Schoen, W. R.; MacDougall, M. L.; Livingston, J. N. Discovery of 5-Hydroxyalkyl-4-phenylpyridines as a new class of glucagon receptor antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 461–464.
(22) Smith, R. A.; Hertzog, D. L.; Osterhout, M. H.; Ladouceur, G. H.; Korpusik, M.; Bobko, M. A.; Howard Jones, J.; Phelan, K.; Romero, R. H.; Hundertmark, T.; MacDougall, M. L.; Livingston, J. N.; Schoen, W. R. Optimization of the 4-aryl group of 4-aryl-pyridine glucagon antagonists: development of an efficient, alternative synthesis. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1303–1306.
(23) Madsen, P.; Knudsen, L. B.; Wiberg, F. C.; Carr, R. D. Discovery and structure−activity relationship of the first non-peptide competitive human glucagon receptor antagonists. *J. Med. Chem.* **1998**, *41*, 5150–5157.
(24) Ling, A.; Hong, Y.; Gonzalez, J.; Gregor, V.; Polinsky, A.; Kuki, A.; Shi, S.; Teston, K.; Porter, J.; Kiel, D.; Lakis, J.; Anderes, K.; May, J.; Knudsen, L. B.; Lau, J. Identification of alkylidene hydrazides as glucagon receptor antagonists. *J. Med. Chem.* **2001**, *44*, 3141–3149.
(25) Ling, A.; Plewe, M.; Gonzalez, J.; Madsen, P.; Sams, C. K.; Lau, J.; Gregor, V.; Murphy, D.; Teston, K.; Kuki, A.; Shi, S.; Truesdale, L.; Kiel, D.; May, J.; Lakis, J.; Anderes, K.; Iatsimirskaia, E.; Sidelmann, U. G.; Knudsen, L. B.; Brand, C. L.; Polinsky, A. Human glucagon receptor antagonists based on alkylidene hydrazides. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 663–666.
(26) Madsen, P.; Ling, A.; Sams, C. K.; Knudsen, L. B.; Sidelmann, U. G.; Ynddal, L.; Brand, C. L.; Plewe, M.; Murphy, D.; Teng, M.; Truesdale, L.; Kiel, D.; May, J.; Kuki, A.; Shi, S.; Feng, J.; Johnson, M. D.; Teston, K. A.; Lakis, J.; Anderes, K.; Gregor, V.; Lau, J. Optimization of alkylidene hydrazide based human glucagon receptor antagonists. Discovery of the highly potent and orally available 3-cyano-4-hydroxybenzoic acid (1-(2,3,5,6-tetramethylbenzyl)-1*H*-indol-4-ylmethylene-hydrazide. *J. Med. Chem.* **2002**, *45*, 5755–5775.
(27) Lau, J.; Behrens, C.; Sidelmann, U. G.; Knudsen, L. B.; Lundt, B.; Sams, C.; Ynddal, L.; Brand, C. L.; Pridal, L.; Ling, A.; Kiel, D.; Plewe, M.; Shi, S.; Madsen, P. New *β*-alanine derivatives are orally available glucagon receptor antagonists. *J. Med. Chem.* **2007**, *50*, 113–128.
(28) Kodra, J. T.; Jørgensen, A. S.; Andersen, B.; Behrens, C.; Brand, C. L.; Christensen, I. T.; Gulbrandt, M.; Jeppesen, C. B.; Knudsen, L. B.; Madsen, P.; Nishimura, E.; Sams, C. K.; Sidelmann, U. G.; Pedersen, R. A.; Lynn, F. C.; Lau, J. Novel glucagon receptor antagonists with improved selectivity over the glucose-dependent insulinotropic polypeptide receptor. *J. Med. Chem.* **2008**, *51*, 5387–5396.
(29) Petersen, K. F.; Sullivan, J. T. Effects of a novel glucagon receptor antagonist (Bay 27-9955) on glucagon-stimulated glucose production in humans. *Diabetologia* **2001**, *44*, 2018–2024.
(30) Parker, J. C.; McPherson, R. K.; Andrews, K. M.; Levy, C. B.; Dubins, J. S.; Chin, J. E.; Perry, P. V.; Hulin, B.; Perry, D. A.; Inagaki, T.; Dekker, K. A.; Tachikawa, K.; Sugie, Y.; Treadway, J. L. Effects of skyrin, a receptor-selective glucagon antagonist, in rat and human hepatocytes. *Diabetes* **2000**, *49*, 2079–2086.
(31) Kearney, P.; Fernandez, M.; Flygare, J. A. Solid-phase synthesis of 2-aminothiazoles. *J. Org. Chem.* **1998**, *63*, 196–200.
(32) Grimstrup, M.; Zaragoza, F. Solid-phase synthesis of 2-amino-5-sulfanylthiazoles. *Eur. J. Org. Chem.* **2002**, 2953–2960.
(33) Ladhar, F.; El Gharbi, R. Direct synthesis of α,*β*-unsaturated nitriles in solid/liquid heterogenous medium. *Synth. Commun.* **1991**, *21*, 413–417.
(34) Kulp, S. S.; Caldwell, C. B. Reduction of α,*β*-diarylacrylonitriles by sodium borohydride. *J. Org. Chem.* **1980**, *45*, 171–173.
(35) Shabana, S.; Meyer, H. J.; Lawesson, S.-O. Studies on organophosphorous compounds part 55. The transformation of nitriles to thioamides with *O*,*O*-dialkyldithiophosphoric acid. *Phosphorus Sulfur Silicon* **1985**, *25*, 297–306.
(36) Reddy Sastry, C. V.; Bensal, O. P.; Srinivasa Rao, K.; Pulla Rao, P.; Jain, M. L.; Shridhar, D. R. Synthesis and biological acitivity of some new 6-isothiocyanato-6-*N*-[*N*,*N*-bis(methoxycarbonyl)guanidine]-, and 6-(2-aryl/2-arylaminothiazol-4-yl)-*2H*-1,4-benzoxazin-3(4*H*)-ones. *Indian J. Chem.* **1987**, *B26*, 662–665.
(37) King, L. C.; Kostrum, G. Selective bromination with copper(II) bromide. *J. Org. Chem.* **1964**, *29*, 3459–3461.

JM8016249