New β-Alanine Derivatives Are Orally Available Glucagon Receptor Antagonists

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A weak human glucagon receptor antagonist with an IC_{50} of 7 μM was initially found by screening of libraries originally targeted to mimic the binding of the glucagon-like peptide (GLP-1) hormone to its receptor. Optimization of this hit for binding affinity for the glucagon receptor led to ligands with affinity in the nanomolar range. In addition to receptor binding, optimization efforts were made to stabilize the molecules against fast metabolic turnover. A potent antagonist of the human human glucagon receptor was obtained that had 17% oral availability in rats with a plasma half-life of 90 min. The major metabolites of this lead were identified and used to further optimize this series with respect to pharmacokinetic properties. This final optimization led to a potent glucagon antagonist that was orally available in rats and dogs and was efficacious in lowering blood glucose levels in a diabetic animal model.

Introduction

Glucagon, a 29 amino acid hormone, is the counter-regulatory hormone to insulin such that when blood glucose levels are low, the pancreatic α-cells secrete glucagon, which acts to promote glucose production from the liver by stimulating both glycogenolysis (breakdown of glycogen) and gluconeogenesis (the de novo synthesis of glucose from metabolic precursors). One of the defects in type 2 diabetes is the apparent α-cell "blindness" to elevated blood glucose, resulting in glucagon secretion despite relatively high blood glucose and insulin levels. This relative hyperglucagonemia leads to increased hepatic glucose output and contributes to the insulin resistance that is central to the etiology of diabetes.[1−6] Human glucagon receptor antagonists are thus expected to lower blood glucose by alleviating one of the pathophysiological condition of type 2 diabetes, namely, excessive, uncontrolled glucagon action. Furthermore, since this relative hyperglucagonemia is observed during all stages of the disease, from the prediabetic impaired glucose tolerance stage to late-stage type 2 diabetes, human glucagon receptor (hGluR) antagonists are a new class of compounds that could offer benefit to type 2 diabetic patients.

Preclinical proof of concept comes from a number of published studies demonstrating the blood glucose lowering effects of glucagon immunoneutralizing monoclonal antibodies when administered to diabetic rats,[7,8] rabbits,[9] and mice.[10] Immunoneutralization of glucagon in these diabetic animals resulted in an acute lowering of blood glucose, while in diabetic ob/ob mice treated for 14 days a reduction in glycosylated hemoglobin A_{1c} (HbA1c) and triglyceride levels was observed.[10] Thus, it has clearly been demonstrated that by preventing the actions of glucagon in diabetic animals, it is possible to alleviate their hyperglycemia. One glucagon antagonist is reported to be effective and safe in human volunteers. During a hyperglucagonemic period the effect of glucagon on blood glucose concentration and rate of hepatic glucose production was blunted in the group dosed with (+)-3,5-diisopropyl-2-(1-hydroxyethyl)-6-propyl-4′-flouro-1,1′-biphenyl (Bay 27-9955).[11]

Non-peptide hGluR antagonists have been published, primarily by scientists from the industry.[12−30]

The synthesis and biological activities from structure−function studies with a focus on optimizing binding affinity for the hGluR and the drug metabolism and pharmacokinetics (DMPK) properties in parallel are reported here. This led to the identification of potent hGluR antagonists with IC_{50} values in the nanomolar range that were orally available and efficacious in lowering blood glucose levels in diabetic animals.

Chemistry

Most compounds were prepared as single entities in parallel libraries using either solid-phase or solution-phase synthesis protocols. The initial libraries were prepared (method A) on a polystyrene Wang resin (Scheme 1). An Fmoc protected amino acid such as 3-aminopropionic acid **1** was attached to the resin using symmetric anhydride mediated esterification followed by deprotection. Subsequently *p*-bromomethylbenzoic acid was coupled to the resin-bound β-alanine using standard peptide chemistry. Introduction of the first variable proximal R1 group was obtained by nucleophilic substitution with a variety of primary amines. The reaction sequence was continued by acylating the secondary amine with a set of carboxylic acids **6** in order to introduce the second set of distal R2 groups into the library. This method was used for either semiautomated or fully automated synthesis of single compounds in library format and subsequently screened for binding to the human glucagon receptor. An example of a library produced using the described

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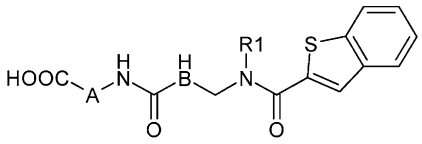
Amino acids	16 BB
Bromo-acids	8 BB
Amines	3 BB
Acids	1 BB
384 Compounds	



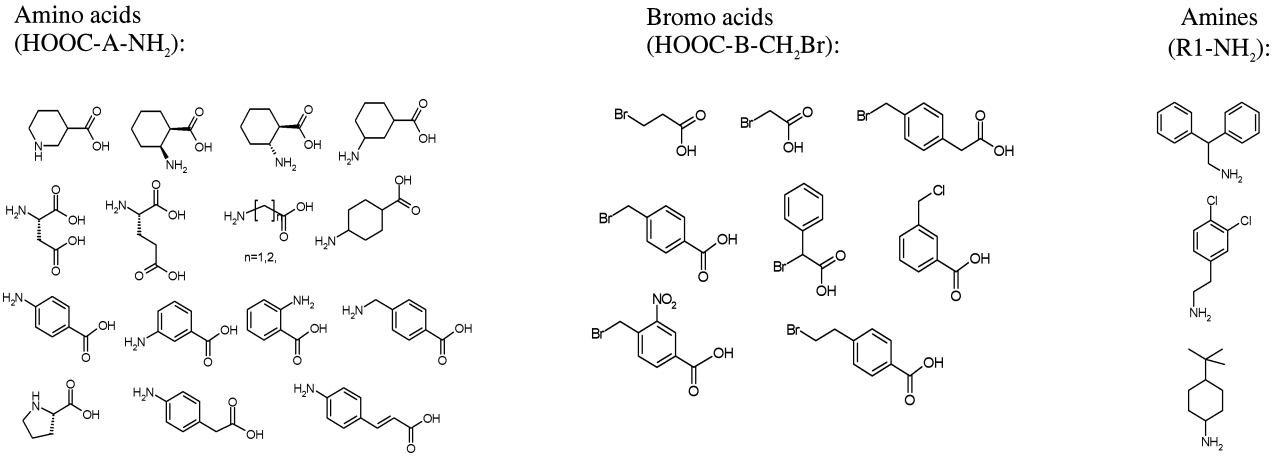
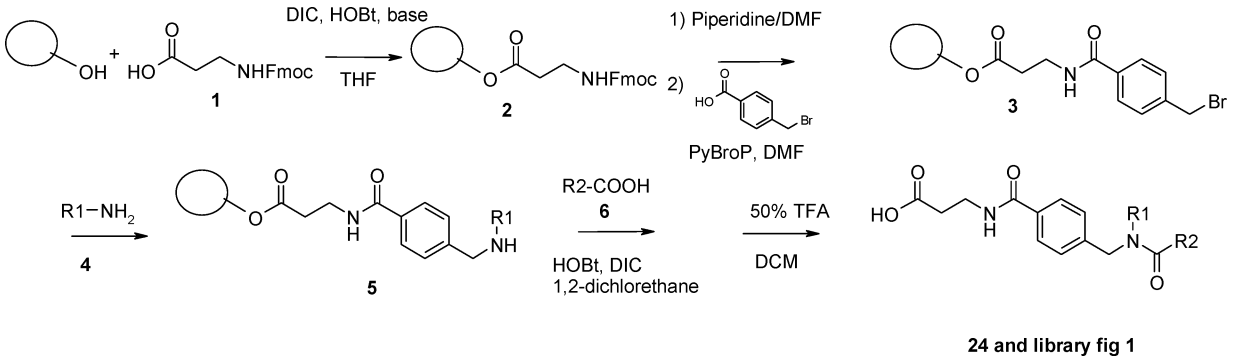
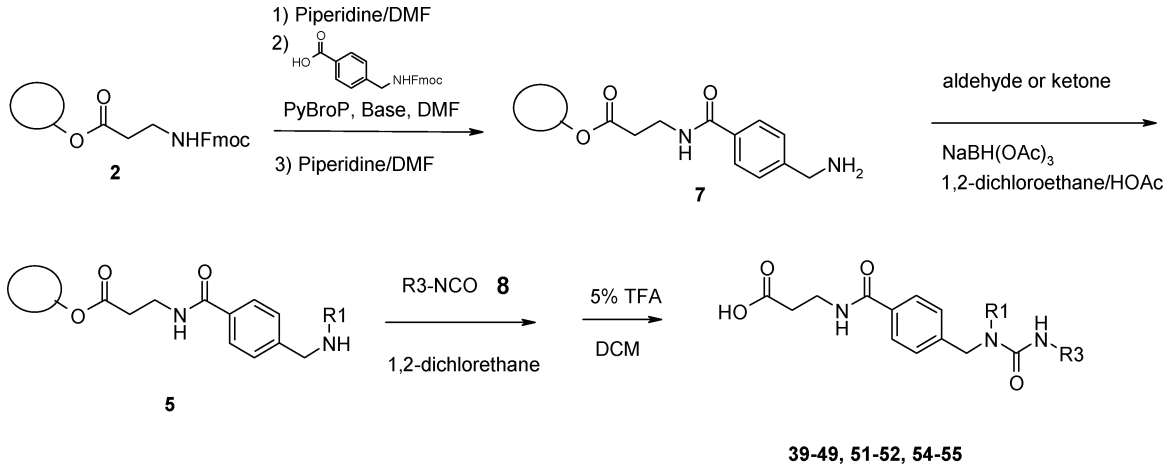


Figure 1. Design of exploration library.

Scheme 1. Preparation Method A



Scheme 2. Preparation Method B

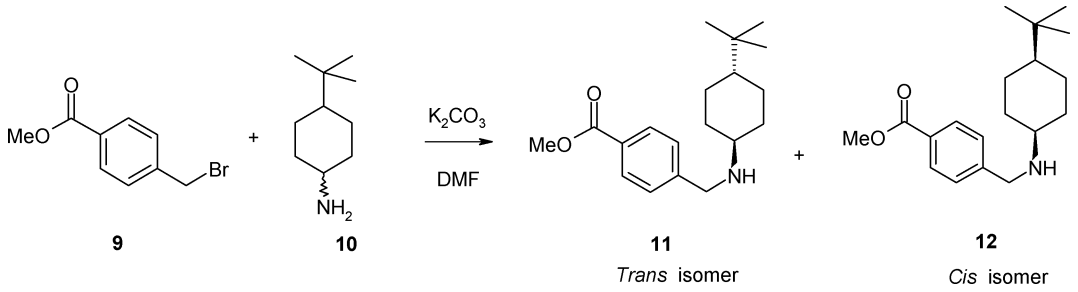


solid-phase synthesis applying other amino acids is shown in Figure 1. In general, only selected compounds that showed significant binding affinity to hGluR were purified and characterized in more details.

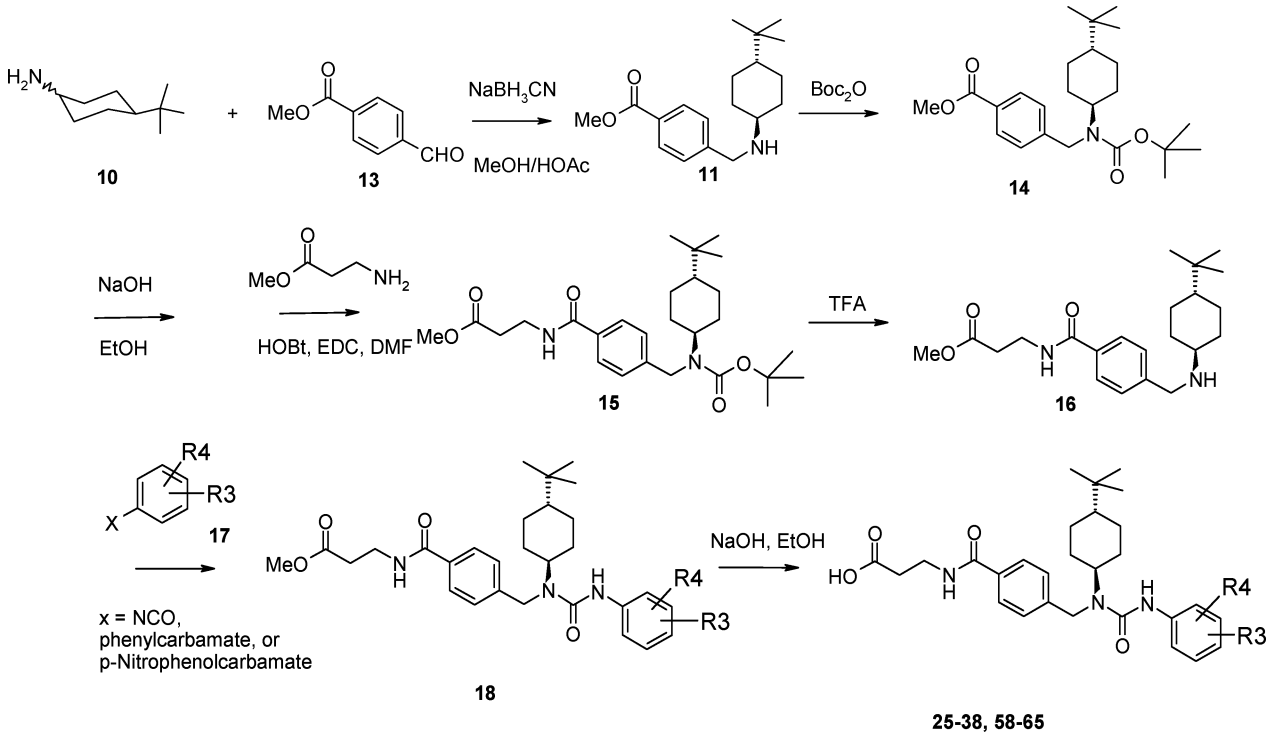
An alternative protocol was developed in order to explore the importance of the proximal R1 groups while keeping the distal R3 group constant with urea connectivity (method B, Scheme 2). In this procedure intermediate primary amine **7** was reductively alkylated with a set of aldehydes or ketones. The reaction sequence was finalized by reaction of the secondary amine **5** with a phenyl isocyanate **8**, and finally the compounds were cleaved from the resin.

On the basis of the initial libraries that were prepared and screened for hGluR binding, it was evident that *tert*-butylcycloalkyl was an important proximal substituent in the urea series. It was accordingly decided to develop a general solution-phase

Scheme 3. Preparation Method C



Scheme 4. Preparation Method D



synthesis method diverting from key intermediate **16** with trans stereochemistry, thereby allowing for preparation of larger batches of interesting compounds to be used for further biological characterization (Schemes 3 and 4). First, an attempt was made to adjust the solid-phase procedure (method A) to a solution-phase protocol (method C, Scheme 3). 4-Bromomethylbenzoic acid methyl ester **9** was reacted with a cis/trans mixture of 4-*tert*-butylcyclohexylamine **10**. The formed mixture of cis and trans isomers of 4-[(4-*tert*-butylcyclohexylamino)-methyl]benzoic acid methyl ester (**11** and **12**) was separated by chromatography on silica gel. These two isomers were used to prepare the final trans isomer **25** as well as the corresponding cis isomer **26**. Though the cis and trans isomers had similar binding affinity to hGluR, it turned out that the trans isomer was metabolically more stable, and it was thus decided to improve the synthesis of this particular isomer (method D, Scheme 4). The advantage of this method was that intermediate trans isomer of the imine 4-[(4-*tert*-butylcyclohexylimino)-methyl]benzoic acid methyl crystallized out of the solution and could be isolated by simple filtration. Subsequently it was reduced to give *trans*-4-[(4-*tert*-butylcyclohexylamino)methyl]-benzoic acid methyl ester **11**. The remaining transformations were simple functional group chemistries applicable for the synthesis of a range of compounds to be used for generating SAR information at the distal aryl group. Thus, **16** was reacted with a variety of aryl isocyanates **17**, and after final saponification, a series of potential hGluR antagonists (**25**−**38** and **58**−**65**) were obtained.

In order to scale up compounds having diverse proximal and distal groups, a modified and efficient solution-phase method (method E, Scheme 5) was developed in which β-alanine methyl ester **19** was coupled to 4-formylbenzoic acid **20** to give the key intermediate aldehyde **21**. The aldehyde was reductively aminated with primary amines and subsequently condensed with isocyanates **8** and deprotected to give the final hGluR antagonists in only four steps.

Biological Methods

The biological methods used are described in detail in the Supporting Information.

Receptor assays were carried out using plasma membranes from BHK cells expressing the cloned human glucagon receptor as earlier described.[19] Metabolic rates were estimated from incubations with rat liver microsomes in order to rank compounds in terms of their metabolic stability. Analysis was performed by means of LC−MS. Selected compounds were profiled with respect to the major metabolites formed in rat liver microsomes. Metabolic profiles were analyzed by means of LC−NMR and LC−MS−MS. The acute in vivo efficacy of the compounds was studied in a glucagon challenged rat model and in db/db mouse.

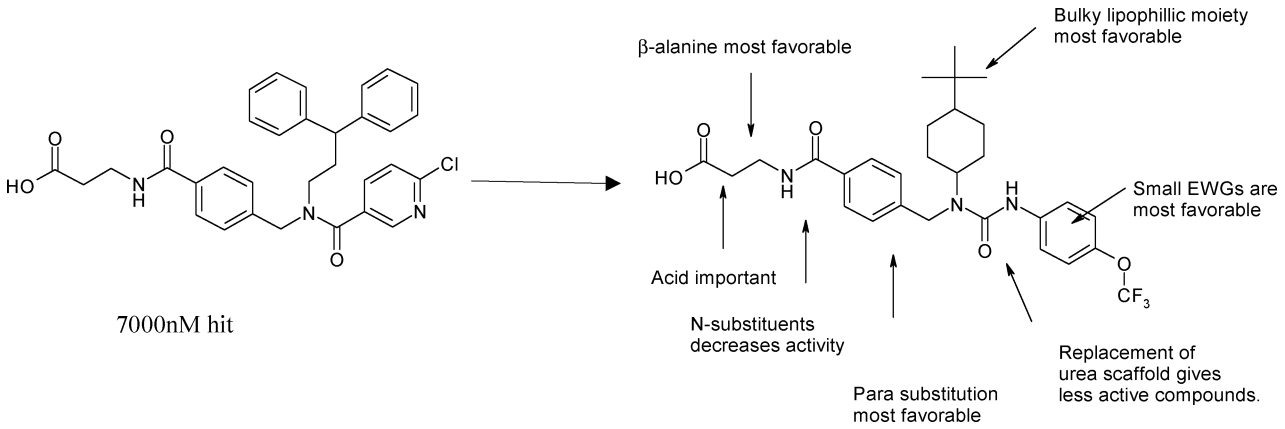


Figure 2. HTS hit and structure−activity overview.

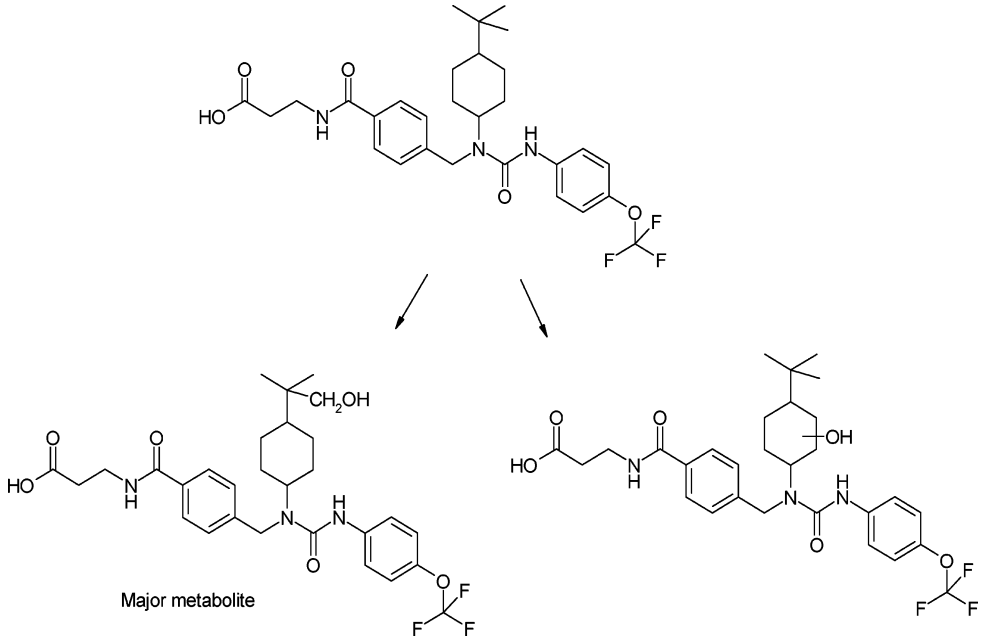
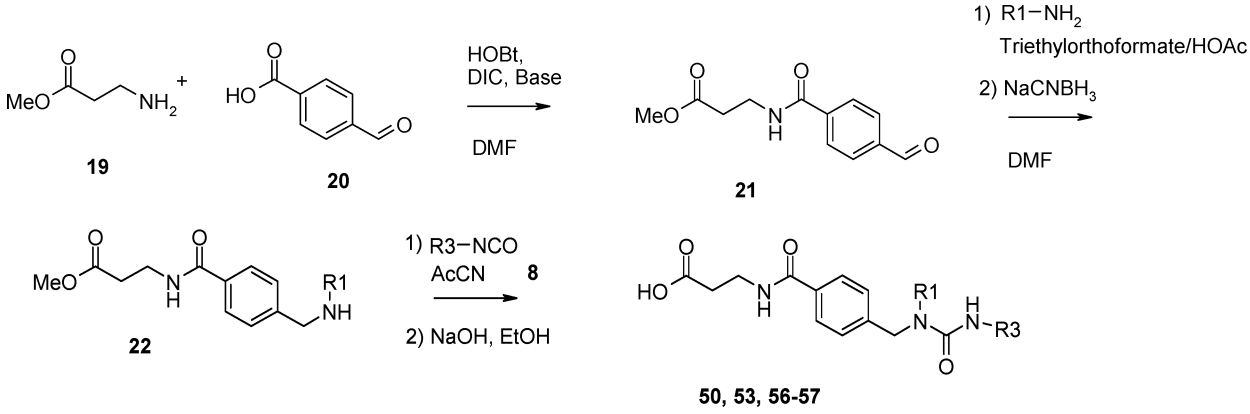


Figure 3. Major metabolites of compound **25**.

Scheme 5. Preparation Method E



Results and Discussion

A weak hGluR antagonist (Figure 2) with an IC_{50} of 7 μM was found through high-throughput screening (HTS) of compound libraries prepared on solid support and biased toward unpublished hypotheses of the binding structure of the glucagon-like peptide (GLP-1) hormone to its receptor. This initial hit turned out to be a ligand for the GLP-1 receptor (IC_{50} = 100 μM) as well as for the hGluR. It was accordingly decided to target the hGluR in the design of new libraries. On the basis of the structure of the initial hit, a series of followup libraries were designed. The initial hit was considered potentially toxic because of the 2-chloropyridyl moiety. In order to obtain initial SAR information and to replace the undesirable 2-chloropyridyl moiety, two initial libraries (96 and 384 members) were produced. In the first 96-membered library the biphenylpropyl of the original hit was replaced with 12 other lipophilic groups

and the 2-chloropyridyl was replaced with 8 other aromatic groups. Screening of this library showed that the 2-chlorpyridyl group could be replaced with several other aromatic groups, such as benzothiophenes, to give hits with affinity in the micromolar range. It was also found that the large lipophilic biphenylpropyl group could be replaced with smaller moieties such as 4-*tert*-butylbenzyl. In order to obtain ligands with affinity in the nanomolar range for the hGluR, a second-generation 384-membered library was designed (Figure 1). This library was designed to explore the importance of the β-alanine and the core benzoyl of the original hit.

Several potent hits were found in this library, and the first indications of structural elements important for affinity to hGluR were obtained. The aliphatic *tert*-butylcyclohexyl moiety was included in several hits (R1 group in Figure 1). Because of the smaller size and lower lipophilicity compared to the diphenylpropyl group in the initial hit, this group was among the selected amines to be used in the further optimization. The preliminary conclusions were based on screening of crude libraries, and it was decided to include purified single compounds in the future efforts to obtain a reliable SAR (Tables 1−4). Consideration of the metabolic properties of this series was included in the optimization that guided us to the lead structure **25** (Table 1) that was shown to be orally available in dogs and to decrease blood glucose levels in diabetic animals. In total, 4 libraries (1056 compounds) were produced to find the lead structure **25**. Refinements of this structure with focus on the pharmacokinetics (PK) and metabolic properties ultimately resulted in compound **57** (Table 3), which had attractive pharmacodynamic and pharmacokinetic properties (Figures 5 and 6 and Table 5).

One of the initial libraries was biased to β-alanine analogues containing two lipophilic moieties (a distal benzothiazole and a proximal aromatic or aliphatic group) at 8−11 Å distance from an acidic group connected through a tertiary amide backbone. One of the hits obtained **24**, was resynthesized, and found to be the most potent hit within this series of amide libraries.

On the basis of this promising result, it was decided to enlarge the structural scope of this new class of hGluR antagonists. Both solid support libraries and solution-phase single-compound approaches were included in a structure−activity guided optimization.

In order to optimize the geometrical spacing of the binding moieties, it was decided to change the central amide connectivity to the urea functionality. From the first urea-based libraries, it became evident that compounds with 4-*tert*-butylcyclohexyl as a proximal substituent consistently proved to give high-affinity ligands when screened against the hGluR. Tables 1−4 show the binding properties of hGluR antagonists that were selected and purified from a series of libraries in order to obtain information of the pharmacophores necessary for binding.

In Table 1, the influence of the substitution pattern of the distal phenyl moiety on the binding to hGluR is shown. On the basis of the series of trans isomers, the following structure−activity relationship was observed: The electronic properties of the substituents had a significant influence on the binding properties. Selected small electron-withdrawing groups such as 4-OCF_3 in **25**, 3,5-di-CF_3 in **30**, 3-CN and 4-OCF_3 in **37**, and 3,4-di-Cl in **38** gave compounds with IC_{50} below 100 nM, whereas the unsubstituted compound **35** had binding affinity of around 1 μM. However, certain small electron withdrawing groups do not provide high affinity, as the 4-CN substituent in **27** did not increase the binding affinity when compared to the unsubstituted compound **35**. In contrast to the effect observed with selected small electron-withdrawing substituents, no hits were found in the libraries where the phenyl group was substituted with small electron-donating groups such as OMe, OH, and aminoalkyl. Also, compounds that contained larger electron-withdrawing substituents with heteroatoms in the proximity of the phenyl group like $CON(Et)_2$ in **31** had weaker binding properties compared to the unsubstitued analogue **35**. Larger lipophilic substituents in the 4-position, e.g., 4-isopropyl in **29** or 4-OCH_2-cyclopropyl in **32**, had a positive effect on the binding properties when compared to the unsubstituted analogue **35**.

Compounds with two substituents in the phenyl group (R2 in Table 1) were also prepared. The SAR of these compounds was more complex, and more compounds are needed to make general conclusions. There are, however, examples with high affinity among the disubstituted compounds such as **30**, **37**, and **38**.

On the basis of these observations and the considerable data obtained from the screening of crude libraries, it was concluded that compounds containing either small electron-withdrawing groups or larger lipophilic groups in the para position of the distal phenyl moiety gave very potent antagonists with affinity below 100 nM (Figure 2).

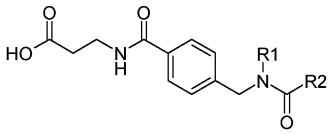
The nature of the proximal urea constituents was also investigated. As exemplified with compounds **25** and **26**, the cis and trans isomers of the *tert*-butylcyclohexyl were equipotent, and the overall structure−activity relationship of the series was not markably influenced by the cis/trans geometry of this group. The equipotency of the cis and trans isomers may be explained by the conformational preferences of the two isomeric compounds. In the cis isomer the chair conformation of the cyclohexane ring is destabilized, shifting the equilibrium toward a twist boat conformation, and the latter can easily be superimposed on the chair conformation of the trans isomer.

The importance of the 4-substituent of the cyclohexyl moiety was investigated further. When *tert*-butyl was exchanged with isopropyl as in **46**, the binding affinity was not significantly changed, whereas compounds with *n*-propyl in **47** were slightly less potent. However, substituents containing heteroatoms like CONH-alkyl in **40** and **41**, COOH in **42**, or OMe in **43** led to inactive compounds. Derivatives with $CON(alkyl)_2$ as in **44** or OPh in **48** gave only weak ligands compared to the lead **25**.

Among the various alternatives to the 4-(*tert*-butyl)cyclohexyl moiety that were investigated, most were found to give inactive compounds. One exception is using 4-cyclohexylphenyl instead of 4-(*tert*-butyl)cyclohexyl as the proximal liphophilic moiety exemplified in **56**, which has similar binding affinity as the lead **25**. In vitro metabolic studies gave important structural insight on how to combine high in vitro affinity with acceptable in vivo pharmacokinetic and dynamic properties. Incubation of compounds containing the 4-cyclohexylphenyl in rat liver microsomes showed that this moiety in general was metabolized to the unsaturated 4-cyclohex-1-enylphenyl as the major metabolite. This inspired us to introduce the 4-cyclohex-1-enylphenyl, thereby eliminating a metabolic liability while retaining affinity equivalent to that of the similar 4-cyclohexylphenyl containing derivatives. Among the compounds synthesized, the 3,5-dichloro derivative **57** was found to be an optimal compound combining high receptor affinity with an acceptable pharmacokinetics profile. The overall conclusion was that the proximal part of the molecule had to be bulky and lipophilic and that the size and shape were both crucial in order to obtain highly active compounds (Figure 2).

The importance of the β-alanine moiety was also investigated (Table 4). The significance of an acidic group was investigated

Table 1



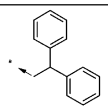
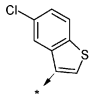
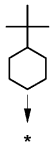
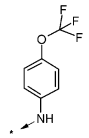
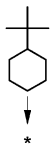
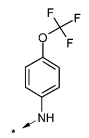
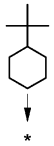
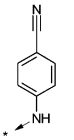
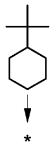
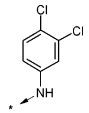
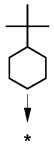
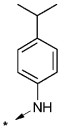
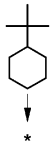
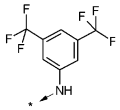
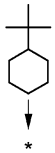
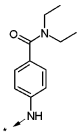
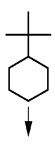
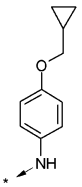
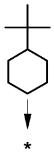
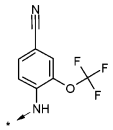
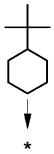
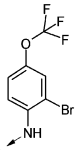
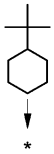
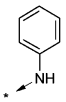
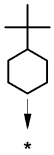
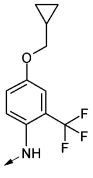
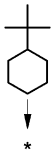
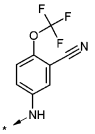
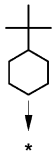
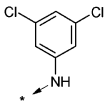
Compound	R1	Configuration	R2	hGluR binding affinity IC_{50} (nM)	Mw	Prep. Method	Formula	Analysis	m.p.
24		-		116	597.14	A	$C_{34}H_{29}ClN_2O_4S.1.5H_2O$	CHN	n.d.
25		trans		55	563.62	D	$C_{29}H_{36}F_3N_3O_5$	CHN	152-154 °C
26		cis		47	563.62	D	$C_{29}H_{36}F_3N_3O_5$ 0.25 EtOAc	CHN	n.d.
27		trans		1045	504.63	D	$C_{29}H_{36}N_4O_4$ 0.75 H_2O	CHN	n.d.
28		trans		226	548.51	D	$C_{28}H_{35}Cl_2N_3O_4$	CHN	n.d.
29		trans		35	521.71	D	$C_{31}H_{43}N_3O_4$	CHN*	n.d.
30		trans		56	615.62	D	$C_{30}H_{35}F_6N_3O_4$	CHN	n.d.
31		trans		2429	578.76	D	$C_{33}H_{46}N_4O_5$ 0.5H_2O	CHN	n.d.
32		trans		140	549.72	D	$C_{32}H_{43}N_3O_5$ 0.25H_2O	CHN*	n.d.

Table 1 (Continued)

Compound	R1	Con-figuration	R2	hGluR binding affinity IC_{50} (nM)	Mw	Prep. Method	Formula	Analysis	m.p.
33		trans		215	588.63	D	$C_{30}H_{35}F_3N_4O_5$	CHN	n.d.
34		trans		153	642.52	D	$C_{29}H_{35}BrF_3N_3O_5$	CHN	n.d.
35		trans		1088	479.62	D	$C_{28}H_{37}N_3O_4$	CHN	n.d.
36		trans		182	617.71	D	$C_{33}H_{42}F_3N_3O_5$. 0.25 H_2O	CHN	99-104 ^{0}C
37		trans		56	588.63	D	$C_{30}H_{35}F_3N_4O_5$ .0.5 H_2O	CHN	n.d.
38		trans		80	548.51	D	$C_{28}H_{35}Cl_2N_3O_4$	CHN	240-1^{0}C

* **29** Calc: 71.37%C, Found: 70.95%C. **32** Calc: 7.91%H,; Found: 7.47%H,

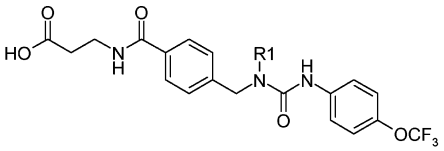
by preparing the corresponding primary amide **63** and the methyl ether **59**, which were both inactive. The GABA analogue **60** was also inactive, whereas the glycine analogue **62** and the corresponding hydroxamic acid **65** had weak binding affinity to the hGluR.

Aiming for more active compounds, a set of constrained analogues of **25** were prepared. The nipecotic acid analogue **61** had weak binding affinity, and the geminal dimethyl substitued β-alanine **64** was inactive. On the basis of these limited data, it is not obvious whether it is geometry or the substitution of the secondary amide that caused the decrease in binding affinity of **61**. In conclusion, the acidic properties and the geometry and position of this group were found to be very crucial for binding of this series of hGluR antagonists.

The original hit **24** contained a tertiary amide core moiety, but several attempts to optimize such compounds have so far not resulted in more potent compounds compared to the corresponding urea series. Replacement of the urea moiety with tertiary amines turned out to give active ligands but with weaker binding affinity compared to **25**. On the basis of these observations, it was hypothesized that the urea moiety does not directly have contact with the receptor cavity, but it is more considered as a scaffold that keeps the important binding moieties together in a geometry that is important for the binding of the ligand to the receptor. The summary of the obtained SAR is depicted in Figure 2. Further studies on the role of the central urea core will be published separately. The original hit had a weak affinity toward the GLP-1 receptor, and it was accordingly interesting to test the selectivity of the optimized compounds. **25** and **57** were tested for GLP-1 receptor affinity in a GLP-1 binding assay. Both compounds had weak binding affinity to the GLP-1 receptor (IC_{50} = 1 and 4.9 μM, respectively), giving a selectivity of 180-fold for **57** in favor of GluR.

Drug Metabolism and Pharmacokinetics. As mentioned above, the in vitro metabolism was used together with the receptor affinity studies to give combined structural information

Table 2



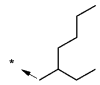
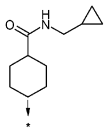
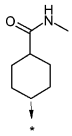
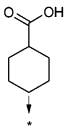
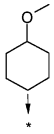
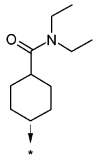
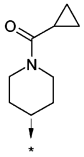
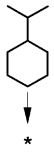
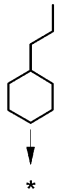
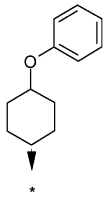
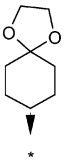
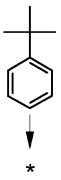
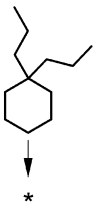
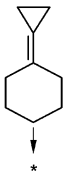
Compound	R1	hGluR binding affinity IC_{50} (nM)	Mw	Prep. Method	Formula	Analysis	m.p.
39		141	537.58	B	$C_{27}H_{34}F_3N_3O_5$	CHN	123.5-125.5 °C
40		>10000	604.63	B	$C_{30}H_{35}F_3N_4O_6$	CHN	n.d.
41		>10000	564.57	B	$C_{27}H_{31}F_3N_4O_6$	CHN	n.d.
42		>10000	551.52	B	$C_{26}H_{28}F_3N_3O_7$	CHN	n.d.
43		>10000	537.54	B	$C_{26}H_{30}F_3N_3O_6$ 0.5H_2O	CHN	205-6°C
44		782	606.65	B	$C_{30}H_{37}F_3N_4O_6$ 1.0 H_2O	CHN	n.d.
45		1984	576.58	B	$C_{28}H_{31}F_3N_4O_6$ 0.75 H_2O	CHN	n.d.
46		59	549.60	B	$C_{28}H_{34}F_3N_3O_5$	CHN	n.d.
47		122	549.60	B	$C_{28}H_{34}F_3N_3O_5$ 0.5H_2O	CHN	n.d.
48		528	599.61	B	$C_{31}H_{32}F_3N_3O_6$ 0.5 H_2O	CHN	97-110 ^{0}C

Table 2 (Continued)

Compound	R1	hGluR binding affinity IC_{50} (nM)	Mw	Prep. Method	Formula	Analysis	m.p.
49		1207	565.55	B	$C_{27}H_{30}F_3N_3O_7$ $0.5H_2O$	CHN	n.d.
50		96	557.57	E	$C_{29}H_{30}F_3N_3O_5$ $0.5H_2O$	CHN	n.d.
51		137	591.68	B	$C_{31}H_{40}F_3N_3O_5$ $0.5H_2O$	CHN	n.d.
52		571	545.56	B	$C_{28}H_{30}F_3N_3O_5$	CHN	n.d.

on how to optimize this series of glucagon receptor antagonists to orally available drug candidates. Whereas the initial hits had very low oral availability and short plasma half-lives in rats, the lead **25** was the first compound tested to have some oral availability in rats and dogs (Table 5). The oral availability in rats was found to be 17%, and the in vivo $t_{1/2}$ value (after iv dosing) was 90 min. The pharmacokinetic parameters in the dog were similar (F_{po} = 10−20%, $t_{1/2}$ = 101 min (iv)). The compound had a small volume of distribution in both rats and dogs, and the half-life in plasma was accordingly expected to be determined by the metabolic stability of the molecule. Metabolite profiling of **25** was performed by LC−NMR and LC−MS after incubation in rat liver microsomes in order to unambiguously identify the major metabolites. The main metabolism for **25** as well as for other similar compounds tested was oxidation of the proximal *tert*-butylcyclohexyl moiety. As mentioned earlier, these studies also revealed that compounds containing a 4-cyclohexylphenyl moiety were oxidized to 4-cyclohexenylphenyl as the major metabolite.

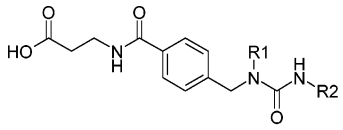
The metabolic studies showed that the rate of metabolism could be controlled by proper substitution of the distal aryl group and the effect on metabolic stability of the distal substituents as shown in Figure 4. The rate of metabolism of **29** having a 4-isopropyl substituent was much faster than the metabolism of, for example, **25** and **37** that both contain a 4-OCF_3 substituent. Structural investigations of the metabolism revealed that in some of the compounds metabolism took place not only in the proximal *tert*-butylcyclohexyl moiety but also in the distal aryl moiety, which was the case for, **29** containing an isopropyl group on the distal phenyl. The metabolic rates were reflected in the in vivo PK experiments where short half-lives were observed for the compounds with the higher metabolic rates (i.e., **25** gave a half-life of 90 min and oral availability of 17%, whereas **29** gave a half-life of 20 min and only 2% oral availability).

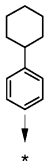
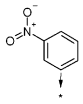
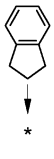
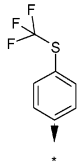
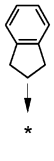
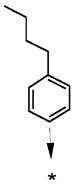
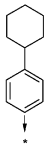
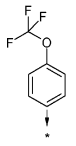
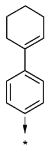
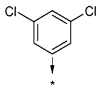
The plasma half-lives and the oral availabilities for the cyclohexylphenyl and cylohexenylphenyl derivatives **56** and **57** were the same order of magnitude, and the oral bioavailabilities were improved compared to **25**. **57** was shown to be the compound with the highest oral availability within this series, and this compound was also found to have acceptable pharmacokinetic properties in dogs (F_{po} = 65%, $t_{1/2}$ = 92 min).

Pharmacodynamics. To demonstrate in vivo efficacy, the lead compound **25** and the optimized derivative **57** were tested in the glucagon challenged rat model in a dose-response dosing regime. Both compounds inhibited the glucagon-stimulated rise in blood glucose in a dose-dependent manner (Figure 5). In this model **25** at a dose of 3 mg/kg iv significantly reduced blood glucose caused by exogenous administration of glucagon. The optimized compound **57** only showed significant effect at doses above 3 mg/kg iv. Even though **57** has a higher affinity to hGluR than **25**, it had reduced in vivo potency relative to **25** in this rat model. This may partly be explained by the species differences. **25** had similar affinity to the rat and human glucagon receptors (IC_{50} = 56 and 55 nM, respectively), whereas **57** had lower affinity to the rat receptor compared to the human glucagon receptor (IC_{50} = 122 and 27 nM respectively).

The conclusion from these acute glucagon-challenged studies in rats was that both **25** and **57** are able to inhibit the rise in blood glucose levels elicited by exogenous administered glucagon, most likely because of the direct inhibition of glucagon stimulated hepatic glucose output.

Table 3



Compound	R1	R2	hGluR binding affinity IC_{50} (nM)	Mw	Prep. Method	Formula	Analysis	m.p.
53			167	544.61	E	$C_{30}H_{32}N_4O_6$ $0.5H_2O$	CHN	n.d.
54			262	557.60	B	$C_{28}H_{26}F_3N_3O_4S$	CHN*	n.d.
55			179	513.64	B	$C_{31}H_{35}N_3O_4$	CHN*	n.d.
56			61	683.6	E	$C_{31}H_{32}F_3N_3O_5$ $1.5H_2O$	CHN	n.d.
57			27	566.48	E	$C_{30}H_{29}Cl_2N_3O_4$	CHN	n.d.

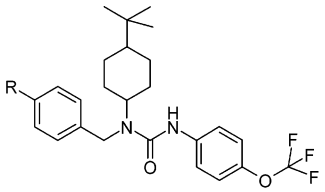
* **54** Calc: 7.54%N; Found: 7.13%N. **55** Calc: 8.18%N; Found:, 7.70%N. **56** Calc: 6.88%N; Found: 6.98%N

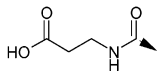
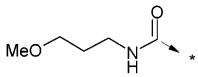
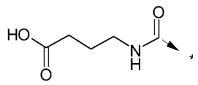
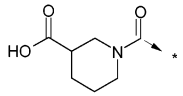
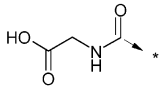
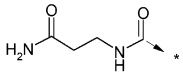
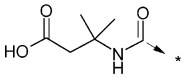
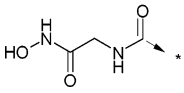
The leptin-deficient obese model of type 2 diabetes, the ob/ob mouse, was selected for further studies of **57**. Predose blood glucose levels were 17.1 ± 1.8 (n = 6) and 18.3 ± 0.9 mM (n = 6) in the vehicle and **57**-treated mice, respectively. In vehicle-treated mice, blood glucose levels changed by 0.9 ± 1.4, −0.2 ± 2.2, −2.0 ± 1.8, and −1.9 ± 2.0 mM at 2, 4, 6, and 24 h postdose, whereas blood glucose in the **57**-treated mice changed by −6.8 ± 1.0 (p = 0.001), −8.4 ± 1.4 (p = 0.010), −9.5 ± 1.7 (p = 0.012), and −1.8 ± 1.6 mM (p = 0.964) at the corresponding time points (Figure 6). Cumulative food intake from 6 to 24 h postdose was 10.2 and 8.4 g/mouse in the vehicle and **57**-treated groups, respectively. From this study it is concluded that **57** reduced the hyperglycemia for at least 6 h after dosing with a maximum blood glucose reduction of 9.5 mM (Figure 6).

Conclusion

On the basis of the extensive examination of the SAR of this new series of hGluR antagonists, it was concluded that it is crucial for receptor recognition that the compounds of this series contain a carboxylic acid together with a proximal and a distal lipophilic moiety positioned in a well-defined geometric orientation. A very steep SAR was demonstrated around the β-alanine moiety, and it was crucial for potency that the proximal lipophilic moiety did not contain heteroatoms. The largest tolerance for structural variation was observed around the distal aromatic group, where optimal binding was obtained when selected electron-withdrawing substituents were attached. The metabolic turnover was optimized in parallel to the binding affinity in order to obtain compounds with higher possibilities for appropriate pharmacokinetics and duration of action in whole animals. **25** was shown to have an optimal lead profile for a hGluR antagonist, and further in vivo studies confirmed this. Further optimization of this series of compounds guided by in vitro metabolism and structural characterization of metabolites was crucial, leading to the cyclohexylphenyl and cyclohexenylphenyl derivatives **56** and **57** with high in vitro hGluR binding affinity and acceptable PK properties. Furthermore, it was demonstrated that **57** lowered blood glucose in a murine type 2 diabetes model.

Table 4



Compound	R	hGluR binding affinity IC_{50} (nM)	Mw	Prep. method	Formula	Analysis	m.p.
58			492.54	D	$C_{26}H_{31}F_3N_2O_4$	CHN	n.d.
25		55	563.62	D	$C_{29}H_{36}F_3N_3O_5$	CHN	152-4 °C
59		>10000	563.67	D	$C_{30}H_{40}F_3N_3O_4$	CHN	151-2 °C
60		>10000	577.65	D	$C_{30}H_{38}F_3N_3O_5$ 0.75 H_2O	CHN	131-3 °C
61		1000	603.69	D	$C_{32}H_{40}F_3N_3O_5$	CHN	206-9 °C
62		1700	549.60	D	$C_{28}H_{34}F_3N_3O_5$ $0.5H_2O$	CHN	n.d.
63		>10000	562.64	D	$C_{29}H_{37}F_3N_3O_4$ $0.25CH_2Cl_2$	CHN	164-8°C
64		>10000	591.68	D	$C_{31}H_{40}F_3N_3O_5$	CHN	180-4 °C
65		2960	564.61	D	$C_{28}H_{35}F_3N_4O_5$ 0.75 H_2O	CHN	154-6°C

Experimental Section

The preparation methods are illustrated by single representative experimental procedures. Further experimental details can be found in the patent literature.[32]

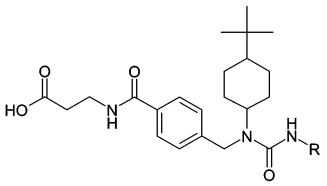
Method A: 3-(4-{[*N*-(5-Chlorobenzo[*b*]thiophen-3-carbonyl)-*N*-(2,2-diphenylethyl)amino]methyl}benzoylamino)propionic Acid 24. Polystyrene resin derived with the Wang linker (1.07 mmol/g, 25.0 g, 26.75 mmol) was treated overnight at 25 °C with a solution of *N*-Fmoc-3-aminopropionic acid (33.3 g, 107 mmol) activated with DIC (8.5 mL, 54 mmol) in the presence of 4-dimethylaminopyridine (0.2 g) in 100 mL of THF. Excess of reagents was removed by filtration. The resin-bound intermediate was successively washed with 3 × 100 mL of THF, 3 × 100 mL of DMF, and 3 × 100 mL of MeOH. The resin was dried in vacuo at 50 °C for 16 h to afford 31.54 g of resin-bound Fmoc-3-aminopropionic acid.

The above resin-bound Fmoc-3-aminopropionic acid (3.95 g, 2.4 mmol) was treated with 40 mL of 50% piperidine in DMF for 15 min. The reagent was removed by filtration. The resin was successively washed with 3 × 20 mL of DMF and 20 mL of a 1 M solution of HOBt in DMF. The resulting resin-bound intermediate was treated with a solution of 4-bromomethylbenzoic acid (2.15 g, 10 mmol) and HOBt (1.52 g, 10 mmol) in 25 mL of THF activated by DIC (1.57 mL, 10 mmol). The reaction was performed at 25 °C for 12 h. Excess of reagents was removed by filtration. The resin-bound intermediate was successively washed with 3 × 20 mL of THF, 3 × 20 mL of DMF, and 3 × 20 mL of MeOH. The resin was dried in vacuo at 50 °C for 16 h to afford 3.77 g of resin-bound 3-[4-(bromomethyl)benzoyl]aminopropionic acid.

The above resin-bound 3-[4-(bromomethyl)benzoyl]aminopropionic acid (1.0 g, 0.64 mmol) was treated with 2,2-diphenylethylamine (1.26 g, 6.4 mmol) in 4 mL of DMSO. The mixture was stirred at 80 °C for 12 h. Excess of reagents was removed by filtration. The resin was successively washed with 3 × 10 mL of DMSO and 3 × 10 mL of MeOH and dried in vacuo at 50 °C for 16 h to afford 1.07 g of resin-bound 3-(4-{[(*N*-{2,2-diphenylethyl}-amino)methyl]benzoyl}amino)propionic acid.

The above resin-bound 3-(4-{[(*N*-{2,2-diphenylethyl}amino)-methyl]benzoyl}amino)propionic acid (1.02 g, 0.61 mmol) was suspended in THF and successively washed with 2 × 10 mL of THF, 2 × 10 mL of 5% DIPEA in THF, and 5 × 10 mL of THF. The resin slurry was then treated with with 5-chlorobenzo[*b*]-thiophen-3-carboxylic acid (0.51 g, 2.4 mmol) in 4 mL of THF, 4 mL of pyridine, DIC (0.19 mL, 1.2 mmol), and 4-dimethylaminopyridine (24 mg, 0.12 mmol). The reaction mixture was stirred at 25 °C for 12 h. The resin was drained and successively washed with 3 × 10 mL of THF, 3 × 10 mL of DMF, and 5 × 10 mL of DCM.

The resin-bound 3-(4-{[*N*-(5-chlorobenzo[*b*]thiophen-3-carbonyl)-*N*-(2,2-diphenylethyl)amino]methyl}benzoylamino)propionic



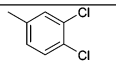
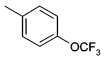
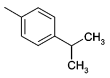
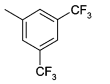
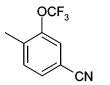
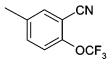
Compound	R	Metabolic turnover in rat liver microsomes [pmol/min/mg protein]
25		100
28		232
29		283
30		139
33		130
37		63

Figure 4. Metabolic turnover of compounds in rat liver microsomes.

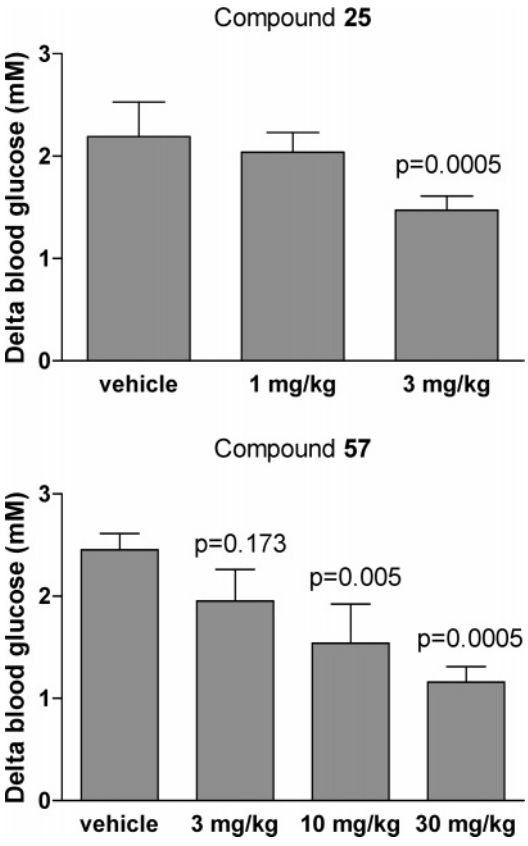


Figure 5. Dynamic effect of compounds **25** and **57** in glucagon challenged rats. Anesthetized animals were given an intravenous dose of compounds **5** min prior to a 3 μg/kg glucagon load.

acid **24** was treated with a 50% solution of TFA in DCM (10 mL). The cleavage mixture was stirred for 45 min at 25 °C. The resin was drained and washed with DCM several times. The combined filtrates were concentrated in vacuo. The residue was dissolved in a 1:1 mixture of MeOH and DCM (1 mL) and concentrated in vacuo to afford 0.359 g of crude **24**. The crude product (50 mg) was purified by column chromatography on RP-C18 silica gel (Sep-Pak, Waters), eluting with a mixture of MeCN and water. Pure

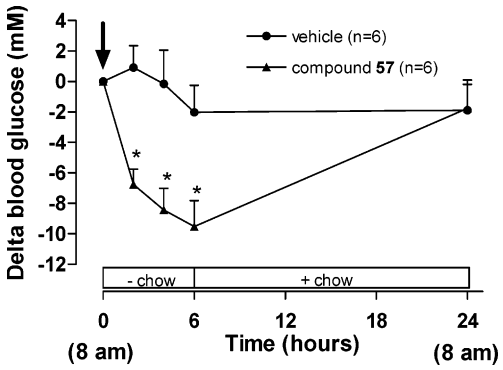


Figure 6. Dynamic effect of compound **57** (100 mg/kg po) in ob/ob mice.

Table 5. Pharmacokinetic Properties of Compounds **25**, **29**, **56**, and **57** in Rats Dosed with 4 mg/kg po and 2 mg/kg iv

compound	C_{max} (ng mL^{-1})	T_{max} (min)	AUC (ng min^{-1} mL^{-1})	$t_{1/2}$ (min, iv)	F_{po} (%)
25	106	60	13045	90	17
29	40	15	2597	20	2
56	316	240	95764	64	54
57	275	60	178226	82	69

fractions were pooled and evaporated *in vacuo* to afford 16.7 mg of 3-(4-{[*N*-(5-chlorobenzo[*b*]thiophen-3-carbonyl)-*N*-(2,2-diphenylethyl)amino]methyl}benzoylamino)propionic acid **24**. Anal. ($C_{34}H_{29}ClN_2O_4S\cdot1.5H_2O$) C, H, N.

Method B: 3-{4-[1-(4-Propylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic Acid 47. *N*-Fmoc-3-aminopropionic acid (47 mg, 150 μmol) was dissolved in a mixture of 250 μL of DCM, 250 μL of DMF, and 100 μL of DIPEA and added to 50 mg of polystyrene resin functionalized with a 2-chlorotrityl chloride linker. After the suspension was shaken for 4 h at 25 °C, the resin was isolated by filtration and washed with 2 × 1 mL of DCM/MeOH/DIPEA 17:2:1 and 2 × 1 mL of DMF.

To the above resin-bound *N*-Fmoc-3-aminopropionic acid was added 500 μL of a 20% solution of piperidine in DMF. Upon shaking for 30 min, the resin was drained and washed with 1 mL of DMF containing HOBt (50 mg/mL) and DMF (2 × 1 mL). Then 4-[(9*H*-fluoren-9-ylmethoxycarbonylamino)methyl]benzoic acid (74.2 mg, 200 μmol) dissolved in a mixture of 430 μL of DMF and 70 μL of diethylisopropylamine was added followed by bromo-tris-pyrrolidinophosphonium hexafluorophosphate (PyBrOP, 93 mg, 200 μmol) dissolved in 500 μL of DMF. The mixture was shaken for 4 h at 25 °C followed by filtration and washing of the resin with 3 × 1 mL of DMF.

The Fmoc protecting group was removed from the above resin-bound 3-{4-[(9*H*-fluoren-9-ylmethoxycarbonylamino)methyl]benzoylamino}propionic acid using 500 μL of a 20% solution of piperidine in DMF. Upon shaking for 30 min, the resin was drained and washed with 1 mL of a solution containing HOBt (50 mg/mL) and DMF (2 × 1 mL), 2 × 1 mL of DCE, and 20 μL of acetic acid dissolved in 1 mL of DCE.

The resulting resin-bound 3-(4-[aminomethyl]benzoylamino)-propionic acid was treated with 4-propylcyclohexanone (98 mg, 700 μmol) dissolved in 500 μL of DCE, 50 μL of acetic acid, and a slurry of $NaBH(OAc)_3$ (148 mg, 700 μmol) in 1 mL of DCE. Overnight shaking at 25 °C followed by filtration and washing with 2 × 1 mL of DCM, 2 × 1 mL of 1:1 CH_3OH/DMF and 3 × 1 mL of DMF afforded resin-bound 3-{4-[(4-propylcyclohexylamino)-methyl]benzoylamino}propionic acid.

An amount of 200 μmol of 4-trifluoromethoxyphenyl isocyanate dissolved in 500 μL of DCE was added to the above resin-bound 3-{4-[(4-propylcyclohexylamino)methyl]benzoylamino}propionic acid. Shaking the mixture for 5 h at 25 °C followed by filtration and washing of the resin with 2 × 1 mL of DCM, 4 × 1 mL of

DMF, 2 × 1 mL of H_2O, 3 × 1 mL of THF, and 3 × 1 mL of DCM afforded the resin-bound **47**.

The above resin-bound **47** was treated with 1 mL of 5% TFA in DCM for 1 h at 25 °C. The product was filtered off, and the resin was washed with 1 mL of DCM. The combined extracts were concentrated in vacuo. The residue was dissolved in 50 μL of DMSO + 500 μL of CH_3CN and purified by preparative HPLC using a Supelcosil ABZ+ 25 cm × 10 mm, 5 μm column. The starting eluent composition was 5% CH_3CN in H_2O, changing over 30 min to 90% CH_3CN in H_2O, which was then kept constant for 5 min before going back to the starting composition over 10 min. The flow rate was kept constant at 8 mL/min, collecting one fraction per minute. The process was monitored using a UV detector operating at 214 nm. The fractions containing the desired products were combined and evaporated in vacuo to afford 3-{4-[1-(4-propylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic acid **47**. Anal. ($C_{28}H_{34}F_3N_3O_5 \cdot 0.5H_2O$) C, H, N.

Method C: *trans*-3-{4-[1-(4-*tert*-Butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic Acid 25. 4-(Bromomethyl)benzoic acid methyl ester (5.0 g, 22 mmol) and 4-*tert*-butylcyclohexylamine (as a 17/83% cis/trans mixture) (3.4 g, 22 mmol) were dissolved in DMF, and K_2CO_3 (6.1 g, 44 mmol) was added. The reaction mixture was stirred at 100 °C for 7 h and for 16 h at 20 °C. Water (100 mL) and EtOAc (200 mL) were added to the reaction mixture. The organic phase was isolated and washed with water (2 × 100 mL) and brine (2 × 100 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to give a cis/trans mixture of 4-[(4-*tert*-butylcyclohexylamino)methyl]benzoic acid methyl ester as a crude product. The two isomers were separated on silica (110 g) using a mixture of EtOAc and DCM (7:3) as eluent.

Trans Isomer 11. Anal. ($C_{19}H_{29}NO_2$) C, H, N. ^{1}H NMR (DMSO-d_6), 400 MHz: δ 7.90 (d, 2H), 7.48 (d, 2H), 3.82 (s, 3H), 3.78 (s, 2H), 2.30−2.20 (m, 1H), 2.05−1.90 (m, 3H), 1.73−1.65 (m, 2H), 1.10−0.90 (m, 4H), 0.80 (s, 9H).

Cis Isomer 12. ^{1}H NMR (DMSO-d_6), 400 MHz: δ 7.92 (d, 2H), 7.58 (d, 2H), 3.90 (dd, 1H), 3.85 (s, 3H), 3.80 (dd, 1H), 2.50−2.35 (m, 1H), 2.00−1.85 (m, 2H), 1.80−1.70 (m, 2H), 1.70−1.45 (m, 2H), 1.00−0.80 (m, 1H), 0.80 (s, 9H).

A solution of **12** (2.6 g, 8.6 mmol) and 4-(trifluoromethoxy)-phenyl isocyanate (1.7 g, 8.6 mmol) in MeCN (40 mL) was stirred at 20 °C for 16 h. The reaction mixture was concentrated in vacuo, and the crude product was purified on silica (100 g) using heptane and EtOAc (3:1) as eluent to give *trans*-4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoic acid methyl ester. The product was suspended in EtOH (80 mL), and NaOH (4 N, 17 mL) was added. The reaction mixture was stirred at 50 °C for 3 h and then concentrated in vacuo until all ethanol was removed. The reaction mixture was diluted with water (100 mL) and adjusted to pH 2 with 4 N HCl. The aqueous phase was extracted with EtOAc (3 × 75 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated in vacuo to give *trans*-4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoic acid. ^{1}H NMR (DMSO-d_6), 400 MHz: δ 12.80 (s, 1H), 8.55 (s, 1H), 7.90 (d, 2H), 7.55 (d, 2H), 7.35 (d, 2H), 7.21 (d, 2H), 4.62 (s, 2H), 4.10−4.00 (m, 1H), 2.00 (s, 2H), 1.80−1.60 (m, 4H), 1.48−1.38 (m, 2H), 1.20−1.00 (m, 2H), 1.00−0.88 (m, 1H), 0.80 (s, 9H).

trans-4-[1-(4-*tert*-Butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoic acid (2.0 g, 4.1 mmol), HOBt (0.6 g, 4.3 mmol), and DIPEA (0.8 g, 4.3 mmol) were dissolved in DMF (40 mL). A solution of DIPEA (0.5 g, 4.1 mmol) and 3-aminopropionic acid ethyl ester hydrochloride (0.4 g, 4.3 mmol) in DMF (10 mL) was added, and the reaction mixture was stirred for 16 h at 20 °C. EtOAc (150 mL) and water (100 mL) were added, and the organic phase was isolated. The aqueous phase was extracted with EtOAc (50 mL), and the organic phases were combined, dried, ($MgSO_4$) and concentrated in vacuo. The crude product was purified on silica (80 g) using heptane and EtOAc (1:1) as eluent to give *trans*-3-{4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic acid ethyl ester. Mp = 108−111 °C. ^{1}H NMR (DMSO-d_6), 400 MHz: δ 8.55 (s, 1H), 8.50 (t, 1H), 7.75 (d, 2H), 7.55 (d, 2H), 7.31 (d, 2H), 7.22 (d, 2H), 4.60 (s, 2H), 4.10−4.00 (m, 1H), 3.60 (s, 3H), 3.45 (dd, 2H), 2.55 (t, 2H), 1.80−1.60 (m, 4H), 1.50−0.80 (m, 5H), 0.80 (s, 9H).

trans-3-{4-[1-(4-*tert*-Butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic acid ethyl ester (1.4 g, 2.4 mmol) was suspended in ethanol (50 mL), and NaOH (4 N, 6 mL) was added. The reaction mixture was stirred for 2 h at 50 °C and then concentrated in vacuo until all EtOH was removed. The reaction mixture was diluted with water (100 mL) and adjusted to pH 2 with 4 N HCl, and *trans*-3-{4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic acid **25** was isolated by filtration. Mp = 152−154 °C. ^{1}H NMR (DMSO-d_6), 400 MHz: δ 12.20 (s, 1H), 8.55 (s, 1H), 7.95 (t, 1H), 7.75 (d, 2H), 7.55 (d, 2H), 7.31 (d, 2H), 7.22 (d, 2H), 4.60 (s, 2H), 4.10−4.00 (m, 1H), 3.45 (dd, 2H), 2.50 (2H), 1.80−1.60 (m, 4H), 1.45−1.35 (m, 2H), 1.15−1.05 (m, 2H), 0.95−0.85 (m, 1H), 0.80 (s, 9H). Anal. ($C_{29}H_{36}F_3N_3O_5$) C, H, N.

Method D: *trans*-3-{4-[3-(3,5-Bistrifluoromethylphenyl)-1-(4-*tert*-butylcyclohexyl)ureidomethyl]benzoylamino}propionic Acid 30. 4-Formylbenzoic acid methyl ester (10.6 g, 64.4 mmol) was dissolved in MeOH (200 mL). A 17:83 cis/trans mixture of 4-*tert*-butylcyclohexylamine (10.0 g, 64.4 mmol, Aldrich) was added, leading to immediate precipitation of white crystals. The mixture was heated to reflux for 30 min to complete imine formation and cooled to 0 °C on an ice bath. The crystalline pure *trans*-4-[(4-*tert*-butylcyclohexylimino)methyl]benzoic acid methyl ester was then collected by filtration and dried overnight in vacuo to afford 15.3 g (78%). ^{1}H NMR ($CDCl_3$), 300 MHz: δ 8.37 (s, 1H), 8.06 (d, 2H), 7.77 (d, 2H), 3.92 (s, 3H), 3.17 (m, 1H), 1.83 (m, 4H), 1.60 (m, 2H), 1.09 (m, 3H), 0.87 (s, 9H). Anal. ($C_{19}H_{27}NO_2$) C, H, N.

The mother liquor was evaporated to dryness in vacuo to leave 4.2 g (22%) of a white solid, which according to the NMR spectrum consisted mainly of the imino cis isomer. ^{1}H NMR ($CDCl_3$), 300 MHz: δ 8.36 (s, 1H), 8.07 (d, 2H), 7.81 (d, 2H), 3.92 (s, 3H), 3.54 (m, 1H), 1.55−1.92 (m, 8H), 1.14 (m, 1H), 0.90 (s, 9H).

trans-4-[(4-*tert*-Butylcyclohexylimino)methyl]benzoic acid methyl ester (21.0 g, 69.2 mmol) was suspended in MeOH (300 mL), and acetic acid (50 mL) was added. To the resulting clear solution was added $NaCNBH_3$ (3.5 g, 55.5 mmol), and the mixture was stirred at ambient temperature for 30 min. The reaction volume was then reduced to one-third by rotary evaporation, and EtOAc (500 mL) was added. The mixture was washed with sodium carbonate solution (5%, 500 mL) and dried (Na_2SO_4). The solvent was removed in vacuo to afford 21.1 g (100%) of **11** as a white crystalline solid sufficiently pure for further reactions. ^{1}H NMR ($CDCl_3$), 300 MHz: δ 7.98 (d, 2H), 7.38 (d, 2H), 3.90 (s, 3H), 3.86 (s, 2H), 2.39 (m, 1H), 2.01 (m, 2H), 1.77 (m, 2H), 1.51 (bs, 1H), 0.93−1.18 (m, 5H), 0.82 (s, 9H).

Compound **11** (20.0 g, 65.9 mmol) was dissolved in THF (300 mL). Di-*tert*-butyl pyrocarbonate (16.0 g, 73.4 mmol) and DIPEA (12.0 g, 92.9 mmol) were added, and the clear solution was stirred overnight at ambient temperature. The solvent was removed in vacuo, and crude **14** was redissolved in EtOH (200 mL), and aqueous NaOH solution (100 mL, 4 N) was added whereafter the mixture was heated to 70 °C for 4 h. After the mixture was cooled, the reaction volume was reduced to one-third by rotary evaporation and water (300 mL) was added. The mixture was extracted with diethyl ether (2 × 200 mL) to remove traces of nonhydrolyzed material. The aqueous phase was then acidified to pH 3.0 by addition of aqueous 4 N HCl. The solid formed was isolated by filtration, and the crystals were washed once with water and dried overnight in vacuo (40 °C) to afford 24.3 g (93%) of *trans*-4-{-[*N*-(*tert*-butoxycarbonyl)-*N*-(4-*tert*-butylcyclohexyl)amino]methyl}-benzoic acid **14**. ^{1}H NMR ($CDCl_3$), 300 MHz: δ 8.04 (d, 2H), 7.31 (d, 2H), 4.39 (bs, 2H), 4.05 (bs, 1H), 1.78 (bd, 4H), 0.95−1.65 (m, 14 H), 0.83 (s, 9H). The signals were broad because of the presence of cis/trans carbamate isomers. Anal. ($C_{23}H_{35}NO_4$) C, H, N.

trans-4-{[*N*-(*tert*-Butoxycarbonyl)-*N*-(4-*tert*-butylcyclohexyl)-amino]methyl}benzoic acid (22.0 g, 56.5 mmol) and HOBt (8.6 g, 57.0 mmol, containing 12% w/w water) were dissolved in DMF (300 mL). *N*-Dimethylaminopropyl-*N*′-ethylcarbodiimide hydrochloride (10.9 g, 56.8 mmol), *β*-alanine methyl ester hydrochloride (8.4 g, 60 mmol), and DIPEA (25 mL, 142 mmol) were added, and the clear solution was stirred at 20 °C for 16 h. The solvent was removed in vacuo, and the residual oil was redissolved in EtOAc (500 mL). The organic phase was washed with 5% aqueous sodium hydrogen carbonate solution (2 × 500 mL), 0.5 M citric acid (2 × 250 mL), and brine before being dried (Na_2SO_4). The solvent was evaporated in vacuo, and the residual oil was evaporated twice from MeCN to afford 24.0 g (89%) of **15**. ^{1}H NMR ($CDCl_3$), 300 MHz: *δ* 7.69 (d, 2H), 7.28 (d, 2H), 6.81 (t, 1H), 4.38 (bs, 2H), 3.23 (s, 3H), 3.21 (t, 2H), 2.66 (t, 2H), 1.75 (bd, 4H), 0.95−1.65 (m, 14 H), 0.80 (s, 9H).

Intermediate **15** (19.5 g, 41.1 mmol) was dissolved in a mixture of TFA (200 mL) and DCM (200 mL), while cooling in an ice bath. The ice bath was removed, and the mixture was allowed to stir at room temperature for 30 min. The solvent was evaporated in vacuo, and the residue was crystallized from EtOAc/heptane to afford 14.8 g (74%) of the trifluoroacetate of **16**. ^{1}H NMR ($CDCl_3$), 300 MHz: *δ* 9.98 (trace of TFA), 8.06 (bs, 2H), 7.73 (d, 2H), 7.41 (d, 2H), 7.34 (t, 1H), 4.21 (t, 2H), 3.75 (s, 3H), 3.74 (t, 2H), 3.04 (m, 1H), 2.70 (t, 2H), 2.17 (bd, 2H), 1.95 (bd, 2H), 1.50 (m, 2H), 0.92−1.15 (m, 3H), 0.85 (s, 9H). Anal. ($C_{22}H_{34}N_2O_3 \cdot C_2HF_3O_2$) C, H, N.

3,5-Bis(trifluoromethyl)aniline (2.0 g, 8.7 mmol) was dissolved in DCM (80 mL), and *N*,*N*-DIPEA (1.6 mL, 9.6 mmol) was added followed by slow addition of phenyl chloroformate (1.1 mL, 8.7 mmol). The resulting mixture was stirred at room temperature for 16 h. The mixture was successively washed with 1 N hydrochloric acid (3 × 100 mL), water (3 × 100 mL), a mixture of water and saturated aqueous sodium hydrogen carbonate (1:1, 2 × 100 mL), and a mixture of water and brine (1:1, 3 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was crystallized from heptane containing a little diethyl ether to afford 1.3 g (43%) of (3,5-bistrifluoromethylphenyl)carbamic acid phenyl ester as a solid. Anal. ($C_{15}H_9F_6NO_2$) C, H, N.

The above carbamic acid phenyl ester (1.0 g, 2.9 mmol) was mixed with **16** (1.07 g, 2.9 mmol), prepared as described above, and triethylamine (1.2 mL, 8.6 mmol) in DCM (55 mL). The resulting mixture was refluxed for 4 h. The cooled reaction mixture was diluted with toluene (50 mL) and washed with water (3 × 50 mL) followed by a mixture of water and saturated aqueous sodium chloride (1:1, 3 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo to afford 1.2 g (67%) of 3-{4-[3-(3,5-bistrifluoromethylphenyl)-1-(4-*tert*-butylcyclohexyl)ureidomethyl]benzoylamino}propionic acid methyl ester as a solid. ^{1}H NMR ($CDCl_3$): *δ* 7.80 (s, 2H), 7.77 (d, 2H), 7.46 (s, 1H), 7.38 (d, 2H), 6.90 (t, 1H), 6.80 (s, 1H), 4.57 (s, 2H), 4.18 (bt, 1H), 3.72 (m, 5H), 7.62 (t, 1H), 1.88 (bt, 4H), 0.9−1.5 (m, 5H), 0.83 (s, 9H).

The above propionic acid methyl ester (1.0 g, 1.6 mmol) was dissolved in warm EtOH (50 mL), and after the mixture was cooled to room-temperature, 4 N NaOH (6 mL) was added. The reaction mixture was stirred at room temperature for 1 h. Glacial acetic acid (10 mL) was added, and the mixture was concentrated in vacuo. The residue was partitioned between water (50 mL) and EtOAc (2 × 50 mL). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo to afford 0.88 g (93%) of *trans*-3-{4-[3-(3,5-bistrifluoromethylphenyl)-1-(4-*tert*-butylcyclohexyl)ureidomethyl]-benzoylamino}propionic acid **30**. ^{1}H NMR (DMSO-d_6): *δ* 12 (bs, 1H), 9.00 (s, 1H), 8.45 (t, 1H), 8.24 (s, 2H), 7.60 (d, 2H), 7.32 (d, 2H), 4.62 (s, 2H), 4.05 (bt, 1H), 3.45 (q, below water, 2H), 2.50 (t, below DMSO, 2H), 1.70 (bt, 4H), 0.9−1.5 (m, 5H), 0.83 (s, 9H). Anal. ($C_{30}H_{35}F_6N_3O_4$) C, H, N.

***trans*-3-{4-[1-(4-*tert*-Butylcyclohexyl)-3-(4-cyclopropylmethoxyphenyl)ureidomethyl]benzoylamino}propionic Acid 32.** 4-Nitrophenol (2.0 g, 14.4 mmol) was dissolved in DMF (50 mL). K_2CO_3 (6.0 g, 43 mmol) and bromomethylcyclopropane (1.51 mL, 16 mmol) were added, and the resulting mixture was stirred at room temperature for 16 h. The mixture was diluted with EtOAc (50 mL) and washed with a saturated aqueous solution of sodium chloride (2 × 50 mL). Drying ($MgSO_4$) and concentration afforded 2.23 g (75%) of 1-cyclopropylmethoxy-4-nitrobenzene as an oil. ^{1}H NMR ($CDCl_3$): *δ* 8.12 (2H, d), 6.94 (2H, d), 3.89 (2H, d), 1.30 (1H, m), 0.58 (2H, m), 0.40 (2H, m).

The above 1-cyclopropylmethoxy-4-nitrobenzene (2.0 g, 9.5 mmol) was dissolved in EtOH (50 mL), and tin(II) chloride dihydrate (10.7 g, 48 mmol) was added. The mixture was refluxed for 24 h. After cooling, the mixture was concentrated in vacuo. The residue was dissolved in EtOAc (40 mL) and 1 N NaOH (180 mL). This mixture was filtered through Celite. The aqueous phase was extracted with EtOAc (2 × 50 mL), and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to afford 0.88 g (52%) of 4-cyclopropylmethoxyaniline. ^{1}H NMR (DMSO-d_6): *δ* 6.62 (2H, d), 6.4−6.5 (3H, m), 4.55 (2H, s), 3.63 (2H, d), 1.15 (1H, m), 0.53 (2H, m), 0.25 (2H, m).

32 was prepared from the above aniline and **16**, similar to method E for **30**. ^{1}H NMR (DMSO-d_6): *δ* 7.76 (2H, d), 7.40 (2H, d), 7.10 (2H, d), 6.88 (1H, t), 6.77 (2H, d), 6.08 (1H, s), 4.52 (2H, s), 4.14 (1H, t), 2.70 (4H, m), 1.9 (4H, bt), 1.1−1.4 (5H, m), 0.92 (1H, m), 0.85 (9H, s), 0.62 (2H, m), 0.42 (2H, m).

***trans*-3-{4-[3-(2-Bromo-4-trifluoromethoxyphenyl)-1-(4-*tert*-butylcyclohexyl)ureidomethyl]benzoylamino}propionic Acid 34.** 4-Trifluoromethoxyaniline (1.0 g, 5.6 mmol) was dissolved in HOAc (10 mL). Bromine (585 *μ*L, 11 mmol) dissolved in HOAc (2 mL) was added with stirring for 10 min at room temperature. The resulting mixture was stirred at room temperature for 2 h and then poured into water (100 mL). The solid formed (2,5-dibromo-4-trifluoromethoxyaniline) was filtered off. The filtrate was made alkaline with solid NaOH and extracted with DCM (100 mL), dried ($MgSO_4$), and concentrated (30 °C, 200 mbar) to afford 0.57 g (40%) of 2-bromo-4-trifluoromethoxyaniline. ^{1}H NMR (DMSO-d_6): *δ* 7.37 (1H, d), 7.10 (1H, dd), 6.85 (1H, d), 5.55 (2H, bs).

From this and **16**, *trans*-3-{4-[3-(2-bromo-4-trifluoromethoxyphenyl)-1-(4-*tert*-butylcyclohexyl)ureidomethyl]benzoylamino}-propionic acid **34** was similarly prepared as described for **30**. ^{1}H NMR (DMSO-d_6): *δ* 8.26 (2H, d), 7.76 (2H, d), 7.43 (2H, d), 7.31 (1H, d), 7.16 (1H, dd), 6.88 (1H, s), 6.85 (1H, t), 4.60 (2H, s), 4.16 (1H, t), 3.75 (2H, q), 2.74 (2H, t), 1.9 (4H, m), 1.4 (2H, m), 1.2 (2H, m), 0.95 (1H, m), 0.86 (9H, s).

***trans*-3-{4-[1-(4-*tert*-Butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic Acid 25.** Intermediate **16** (10.0 g 24 mmol) was suspended in MeCN (300 mL), and DIPEA (4.14 mL, 24 mmol) was added. To this suspension 4-trifluoromethoxyphenyl isocyanate (3.75 mL, 24 mmol) was added. Stirring at room temperature was continued for 4 h, and then the mixture was left at 5 °C for 16 h. Filtration and washing with cold MeCN afforded 11.9 g (85%) of *trans*-3-{4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic acid methyl ester. Hydrolysis of this ester afforded 11 g (94%) of *trans*-3-{4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic acid **25**. Mp 152−154 °C. Anal. ($C_{29}H_{36}F_3N_3O_5$) C, H, N.

General Procedure for Preparing Isocyanates from (Substituted) Anilines and Diphosgene. To a solution of the aniline in anhydrous toluene was added dry 1 N HCl in diethyl ether (5 equiv). A precipitate was formed. The toluene was evaporated. To the solid was added more anhydrous toluene, and the toluene was evaporated again to remove excess HCl. This procedure was repeated 2−3 times. To a mixture of the solid in toluene (about 10 g in 300 mL) was added diphosgene (trichloromethyl chloroformate) (10 equiv or more) or phosgene. The mixture was refluxed under nitrogen overnight. A clear solution was obtained. If more solid was present, more diphosgene was added and reflux continued. When a clear solution was obtained, the reaction mixture was concentrated in vacuo at 60−90 °C to remove the toluene and excess diphosgene until the theoretical weight was obtained. To the crude isocyanate was added more anhydrous toluene, and the mixture was concentrated again to remove excess HCl. The crude isocyanate was used

without further purification. This method gives the isocyanates in pure form, which may be used in the next step.

***cis*-3-{4-[1-(4-*tert*-Butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic Acid 26.** 4-[(*cis*-4-*tert*-Butylcyclohexylamino)methyl]benzoic acid methyl ester (0.36 g, 1.2 mmol) and 4-(trifluoromethoxy)phenyl isocyanate (0.24 g, 1.2 mmol) were dissolved in MeCN (10 mL) and stirred at 20 °C for 16 h. The reaction mixture was concentrated in vacuo, and the crude product was purified on silica (25 g) using heptane and EtOAc (9:1) as eluent to give *cis*-4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoic acid methyl ester. The product was suspended in EtOH (10 mL), and NaOH (4 N, 1.1 mL) was added. The reaction mixture was stirred at 50 °C for 3 h and then concentrated in vacuo until all EtOH was removed. The reaction mixture was diluted with water (50 mL) and adjusted to pH 2 with hydrochloric acid (4 N). The aqueous phase was extracted with EtOAc (75 mL), and the organic phase was dried ($MgSO_4$) and concentrated in vacuo to give *cis*-4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoic acid **26**. ^{1}H NMR (DMSO-d_6), 400 MHz: δ 12.80 (s, 1H), 8.61 (s, 1H), 7.90 (d, 2H), 7.55 (d, 2H), 7.35 (d, 2H), 7.22 (d, 2H), 4.72 (s, 2H), 4.32−4.22 (m, 1H), 1.85−1.70 (m, 2H), 1.65−1.45 (m, 4H), 1.40−1.10 (m, 3H), 0.80 (s, 9H). Anal. ($C_{26}H_{31}F_3N_2O_4$) C, H, N.

cis-4-[1-(4-*tert*-Butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)-ureidomethyl]benzoic acid (0.3 g, 0.6 mmol), HOBt (0.1 g, 0.7 mmol), and DIPEA (0.13 g, 0.7 mmol) were dissolved in DMF (10 mL). DIPEA (0.1 g, 0.7 mmol) and 3-aminopropionic acid ethyl ester hydrochloride (0.07 g, 0.7 mmol) in DMF was added, and the reaction mixture was stirred for 16 h at 20 °C. EtOAc (80 mL) and water (50 mL) were added, and the organic phase was isolated. The aqueous phase was extracted with EtOAc (50 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated in vacuo. The residue was crystallized from heptane and EtOAc (4:1) to give 3-{4-[1-(*cis*-4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic acid ethyl ester. Mp = 87−90 °C. Anal. ($C_{30}H_{38}F_3N_3O_5$) C, H, N. ^{1}H NMR (DMSO-d_6), 400 MHz: δ 8.60 (s, 1H), 8.48 (t, 1H), 7.75 (d, 2H), 7.52 (d, 2H), 7.28 (d, 2H), 7.21 (d, 2H), 4.70 (s, 2H), 4.30−4.20 (m, 1H), 3.60 (s, 3H), 3.48 (dd, 2H), 2.55 (t, 2H), 1.82−1.70 (m, 2H), 1.60−1.45 (m, 4H), 1.40−1.10 (m, 3H), 0.80 (s, 9H).

cis-3-{4-[1-(4-*tert*-Butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)-ureidomethyl]benzoylamino}propionic acid ethyl ester (0.2 g, 0.3 mmol) was suspended in EtOH (8 mL), and NaOH (4 N, 0.6 mL) was added. The reaction mixture was stirred for 16 h at 20 °C and then concentrated in vacuo until all EtOH was removed. The reaction mixture was diluted with water (50 mL) and adjusted to pH 2 with hydrochloric acid (4 N). The aqueous phase was extracted with EtOAc (80 mL), and the organic phase was dried ($MgSO_4$) and concentrated in vacuo to give *cis*-3-{4-[1-(4-*tert*-butylcyclohexyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}-propionic acid **26**. Anal. ($C_{29}H_{36}F_3N_3O_5 \cdot 0.25H_2O$) C, H, N. ^{1}H NMR (DMSO-d_6), 400 MHz: δ 12.20 (s, 1H), 8.60 (s, 1H), 8.45 (t, 1H), 7.77 (d, 2H), 7.53 (d, 2H), 7.28 (d, 2H), 7.20 (d, 2H), 4.70 (s, 2H), 4.30−4.20 (m, 1H), 3.45 (dd, 2H), 2.50 (2H), 1.82−1.70 (m, 2H), 1.60−1.45 (m, 4H), 1.40−1.30 (m, 2H), 1.20−1.10 (m, 1H), 0.80 (s, 9H).

Method E: 3-{4-[1-(4-*tert*-Butylphenyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic Acid 50. 4-Formylbenzoic acid (15 g, 100 mmol) was dissolved in DMF (250 mL). HOBt (14.9 g, 110 mmol) and DIPEA (21.1 g, 110 mmol) were added, and the resulting mixture was stirred at room temperature for 30 min. Triethylamine (34.8 mL, 250 mmol) and β-alanine methyl ester hydrochloride (15.4 g, 110 mmol) were added, and the resulting mixture was stirred at room temperature for 1 h. More triethylamine (17.4 mL) and β-alanine methyl ester hydrochloride (7.7 g) were added, and the mixture was stirred at room temperature for 16 h. The mixture was concentrated in vacuo, and the residue was partitioned between EtOAc (200 mL) and water (200 mL). The organic phase was dried ($MgSO_4$) and concentrated to afford 16.2 g (70%) of 3-(4-formylbenzoylamino)propionic acid methyl ester as an oil. ^{1}H NMR ($CDCl_3$): δ 2.70 (2H, t), 3.69 (3H, s), 3.70 (2H, q), 7.68 (1H, bt), 7.9−8.0 (4H, m), 10.1 (1H, s).

The above compound **21** (2.0 g, 8.5 mmol) was dissolved in DMF (20 mL). Triethyl orthoformate (10 mL), glacial acetic acid (1 mL), $NaCNBH_3$ (0.81 g, 12.8 mmol), and 4-*tert*-butylaniline (1.27 g, 8.5 mmol) were added, and the resulting mixture was stirred at room temperature for 16 h. To the mixture was added saturated aqueous sodium chloride (100 mL), and the mixture was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with saturated aqueous sodium chloride (3 × 100 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel, eluting with a mixture of EtOAc and heptane (1:1) to afford 0.87 g (30%) of 3-{4-[(4-*tert*-butylphenylamino)methyl]benzoylamino}-propionic acid methyl ester as an oil. ^{1}H NMR ($CDCl_3$): δ 7.73 (2H, d), 7.44 (2H, d), 7.19 (2H, d), 6.83 (2H, d), 6.57 (2H, d,) 4.38 (2H, s), 4.04 (1H, s), 3.73 (5H, m), 2.67 (2H, t), 1.28 (9H, s).

The above 3-{4-[(4-*tert*-butylphenylamino)methyl]benzoylamino}-propionic acid methyl ester (0.82 g, 2.2 mmol) was dissolved in MeCN (15 mL). *N*,*N*-DIPEA (378 μL, 2.2 mmol) and 4-(trifluoromethoxy)phenyl isocyanate (500 μL, 3.3 mmol) were added. The resulting mixture was stirred at room temperature for 5 h and was refluxed for 16 h. The cooled reaction mixture was concentrated in vacuo, and the residue was dissolved in EtOAc (50 mL) and washed with water (2 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was crystallized from a mixture of EtOAc and heptane (1:1) containing 1% glacial acetic acid to afford 0.40 g (32%) of 3-{4-[1-(4-*tert*-butylphenyl)-3-(4-trifluoromethoxyphenyl)-ureidomethyl]benzoylamino}propionic acid methyl ester as a solid. ^{1}H NMR ($CDCl_3$): δ 7.70 (2H, d), 7.43 (2H, d), 7.3−7.4 (4H, m), 7.1 (4H, m), 6.80 (1H, t), 6.30 (1H, s), 4.94 (2H, s), 3.72 (5H, m), 2.67 (2H, t), 1.34 (9H, s),

The above propionic acid methyl ester (0.25 g, 0.44 mmol) was dissolved in 1,4-dioxane (25 mL), and then 4 N NaOH (6 mL) was added. The mixture was stirred at room temperature for 16 h, and then 36% aqueous hydrochloric acid (10 mL) was added. The mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was crystallized from a mixture of diethyl ether and heptane to afford 0.10 g (42%) of 3-{4-[1-(4-*tert*-butylphenyl)-3-(4-trifluoromethoxyphenyl)ureidomethyl]benzoylamino}propionic acid **50**. Anal. ($C_{29}H_{30}F_3N_3O_5 \cdot 0.5H_2O$) C, H, N. ^{1}H NMR ($CDCl_3$): δ 7.68 (2H, d), 7.43 (2H), 7.35 (2H, d), 7.28 (2H, d), 7.1 (4H, m), 6.88 (1H, t), 6.32 (1H, s), 4.95 (2H, s), 3.71 (2H, q), 2.70 (2H, t), 1.33 (9H, s).

Supporting Information Available: Details of biological methods that were used and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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