Expedited Articles

Discovery and Structure−Activity Relationship of the First Non-Peptide Competitive Human Glucagon Receptor Antagonists

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The first non-peptide competitive human glucagon receptor antagonist, 2-(benzimidazol-2-ylthio)-1-(3,4-dihydroxyphenyl)-1-ethanone, NNC 92-1687 (**2**), is described. This antagonist has a binding affinity of 20 μM (IC_{50}) and a functional $K_i = 9.1$ μM at the human glucagon receptor. A structure−activity relationship (SAR) was obtained on this compound, and the results show that only the benzimidazole part can be changed without complete loss of affinity. Analogues with *tert*-butyl or benzyloxy groups in the 5-position of the benzimidazole moiety were found to be equipotent or slightly more potent, all displaying binding affinities around 5−20 μM. Most of the changes to the catechol and the linker gave compounds without any affinity toward the human glucagon receptor. The 3-hydroxy group could, however, in the presence of a 4-hydroxy group be changed to a methoxy or a chloro group while retaining affinity.

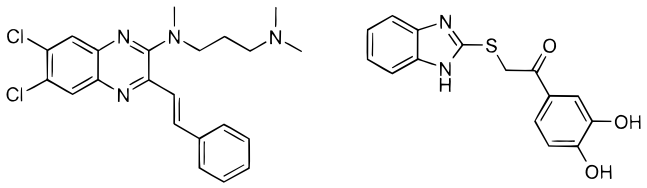
Introduction

Type 2 diabetes mellitus (NIDDM) is a prevalent metabolic disorder of heterologous origin, which is characterized by fasting hyperglycemia consequential to impaired pancreatic function and elevated peripheral insulin resistance (PIR). A major consequence of raised PIR is that hepatic glucose production fails to be fully suppressed despite the hyperinsulinemia which is characteristic of early stages of NIDDM.[1,2] There is evidence to support the idea that diabetic hyperglycemia is a bihormonal disorder where lack of insulin and excess of glucagon combine to increase hepatic glucose production.[1,3−5]

Glucagon, a peptide hormone consisting of 29 amino acid residues produced in the α-cells of the pancreas, exerts its action on the liver by stimulation of both glycogenolysis and gluconeogenesis. Recent studies have shown that immunoneutralization of endogenous glucagon with a monoclonal glucagon antibody normalizes hyperglycemia in moderately streptozosin-diabetic rats[6] as well as in normal and alloxan-induced diabetic rabbits,[7] thus supporting the above hypothesis and suggesting that an effective glucagon receptor antagonist may provide effective drug therapy of NIDDM. Indirect clinical support for the concept is provided by the fact that exogenously infused Glucagon-Like Peptide-1 can, concurrently, lower hyperglycemia and glucagon levels in IDDM patients rendered temporarily hyperglycemic by withdrawal of insulin.[8]

The glucagon receptor is a seven-transmembrane G-protein-coupled receptor belonging to the secretin family. The receptor was cloned in 1993. It couples to G_s and the adenylyl cyclase second messenger system.[9] Over the years, some evidence has suggested involvement of the inositol phosphate pathway and perhaps also Ca^{2+} signaling.

Chart 1



Several publications describe peptide antagonists. Of these, the most thoroughly characterized antagonist is DesHis[1][Glu[9]]-glucagon amide,[10,11] and others reported are DesHis[1],Phe[6][Glu[9]]-glucagon amide,[12] DesHis[1],-DesPhe[6][Glu[9]]-glucagon amide,[13] and NLeu[9],Ala[11,16]-glucagon amide.[14] However, being peptides, these antagonists are unlikely to be either orally available or metabolically stable, prompting us to search for non-peptide glucagon receptor antagonists.

The existence of a non-peptide glucagon receptor antagonist, CP-99,711 (**1**) (Chart 1), has been reported previously,[15] binding to the rat receptor with an IC_{50} of 4 ± 1 μM. Functionally, it inhibited glucagon stimulated formation of cAMP with an IC_{50} of 7 ± 1 μM, although the compound lacked specificity and it was suggested[15] that there was an interaction with a common motif in G-protein-linked receptors which is most likely distinct from the glucagon-binding domain.

Thus, competitive glucagon receptor antagonists are highly desired as probes, biological tools, and drugs, but compounds of this type are not currently described in

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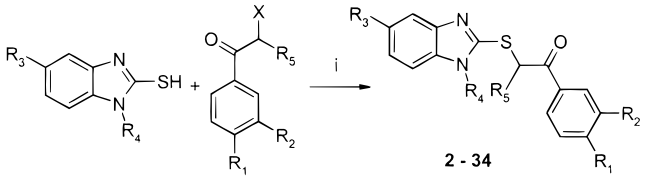
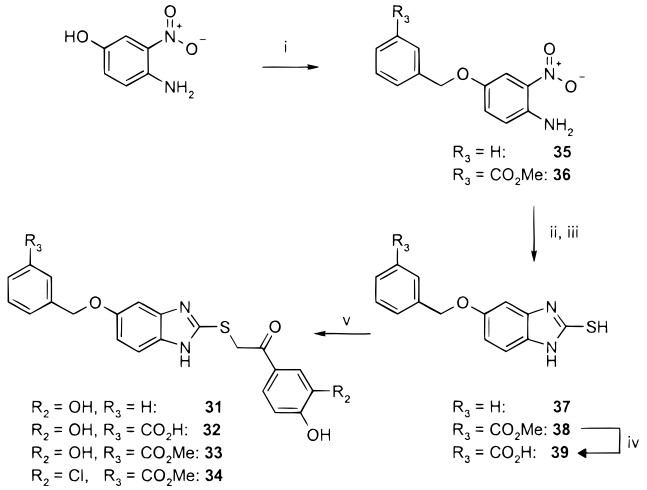
Scheme 1[a]



[a] Reagents and conditions: (i) for X = Cl, MeCN, reflux 3−16 h; for X = Br, MeCN, rt, 16 h.

Scheme 2[a]



[a] Reagents and conditions: (i) 3-R_3-$C_6H_4CH_2Br$, K_2CO_3, DMF; (ii) $SnCl_2$, EtOH; (iii) $EtOS_2^-K^+$, MeOH, reflux; (iv) 1 N NaOH dioxane; (v) $ClCH_2COC_6H_4$-3,4-OH or $BrCH_2COC_6H_4$-3-Cl-4-OH, MeCN.

the literature. This prompted us to report our identification of the first competitive glucagon receptor antagonist, NNC 92-1687 (**2**) (Chart 1), and its associated structure−activity relationship (SAR).

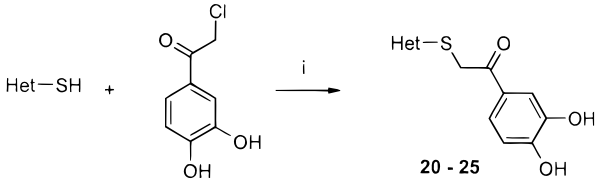
Chemistry

Compounds **2**−**34** were prepared via alkylation of mercapto heterocycles with α-haloacetophenones (Scheme 1). When using α-chloro-3,4-dihydroxyacetophenone, equimolar amounts of reagents were refluxed overnight in MeCN, and when using α-bromoacetophenones, the reaction mixture was stirred overnight at room temperature. In all cases except **20** and **21**, the products precipitated as HCl or HBr salts, respectively. **20** and **21** crystallized from the reaction mixture as free bases. In some cases the products were washed with refluxing MeCN, ethanol, or acetone. α-Bromoacetophenones were, in turn, prepared by α-bromination[16] of acetophenones with $CuBr_2$ and were used without further purification.

14 was prepared by alkylation of 2-mercaptobenzimidazole with 2-bromo-1-(3,4-dihydroxyphenyl)pentan-1-one in MeCN similarly as shown in Scheme 1. **15** was similarly prepared by alkylation of 2-mercaptobenzimidazole with 4-chloro-1-(3,4-dihydroxyphenyl)butan-1-one in refluxing MeCN according to Scheme 1.

Compounds **16**−**19** were prepared via O-silylated **7** as shown in Scheme 4. Thus, 3-chloro-4-hydroxyacetophenone was protected as the *tert*-butyldimethylsilyl ether. Subsequent bromination[16] and reaction with **35** afforded the O-silylated **7**, which turned out to be partly desilylated. Oximations of O-silylated **7** afforded **16**−**18**. Reduction of O-silylated **7** using KBH_4 gave **19**.

Scheme 3[a]



[a] Reagents and conditions: (i) MeCN, reflux 3−16 h.

Compounds **31**−**34** were prepared as shown in Scheme 2. O-Benzylations of 3-nitro-4-aminophenol afforded the corresponding 4-*O*-benzyl-2-nitroanilines **35** and **36**. These were converted to the corresponding 2-mercaptobenzimidazoles **37** and **38** by reduction ($SnCl_2$) followed by cyclization with potassium ethylxanthogenate.[17] Hydrolysis of **38** afforded **39**. The 2-mercaptobenzimidazoles **37**−**39** were then S-alkylated to give **31**−**34**.

Discussion

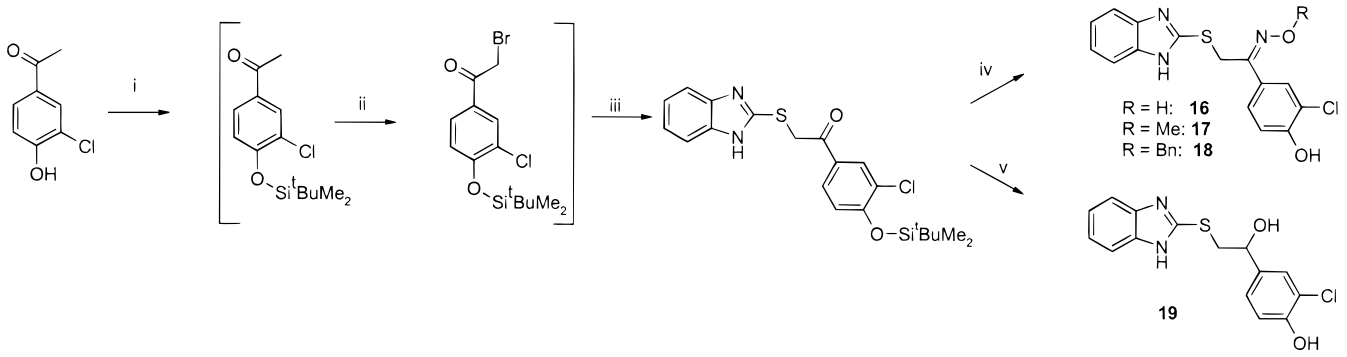
2 was discovered in HT screening in our glucagon antagonist program. As shown in Figure 1, **2** was found to be a competitive glucagon receptor antagonist with a functional K_i of 9.1 μM, the first such compound to be described. It seems specific as it does not antagonize forskolin-stimulated cAMP formation (data not shown). As shown in Figure 2, it has not been possible to show that CP-99,711 (**1**) is a competitive antagonist, in agreement with the earlier published data.[15]

Consequently, **2** was the basis for investigations to determine the pharmacophoric requirements of the receptor toward this ligand and to improve the binding affinity. **2** possesses three motifs which were analyzed independently: (A) the catechol, (B) the linker, and (C) the heterocycle (see Figure 3).

The activity of the different compounds has been assessed by measuring the binding affinity for the recombinant human hepatic glucagon receptor. Selected compounds have been measured functionally (data not shown), and there was generally agreement between these data and the binding affinities. **7** with a binding affinity of 12 μM had a functional K_i of 4.8 μM, and **30** with a binding affinity of 5 μM had a functional K_i of 1.9 μM. All compounds tested functionally were competitive antagonists.

(A) The Catechol. It was found (Table 1) that the phenol group (R_1) para to the carbonyl functionality, when it possessed an electronegative ortho substituent, was crucial for activity. The absence of hydroxyl groups (**3**), or the presence of only one hydroxy group (**8** and **9**), resulted in total loss of all binding affinity. Substituting R_2 for a chlorine (**7**) or a methoxy group (**6**) resulted in activity being slightly increased or decreased, respectively. It was not possible to change this catechol-like motif in other ways without damaging binding affinity. Thus, for 3,4-dimethoxy (**4**), as well as for 3,4-dichloro (**5**), all affinity was lost. Furthermore, the 3,4-substitution pattern could not be replaced by a 3,5-substitution pattern (**10**). It was then investigated whether a carboxylic acid functionality could replace the hydroxy groups of the catechol (**11**−**13**), and this was also found not to be possible.

Scheme 4[a]



[a] Reagents and conditions: (i) TBDMS-Cl, imidazole, DMF; (ii) $CuBr_2$, EtOAc, Δ; (iii) **35**, MeCN, rt; (iv) (a) NH_2OR, (b) TBAF; (v) (a) KBH_4, MeOH, rt, (b) TBAF.

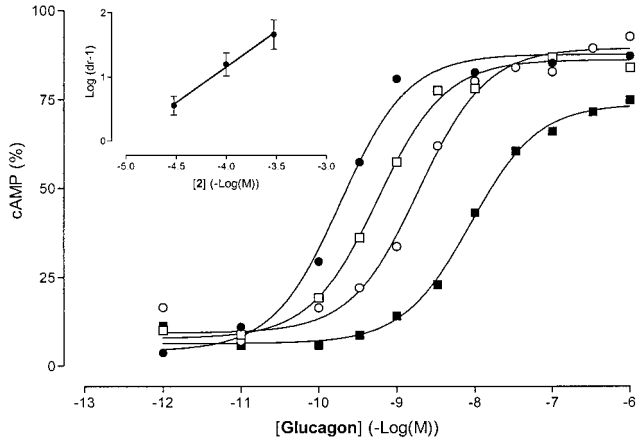


Figure 1. Inhibition of glucagon-stimulated cAMP by **2** using the recombinant human glucagon receptor in BHK cells. Shown is the observed shift in the glucagon dose−response curve by addition of **2**: glucagon (●), glucagon + 30 μM **2** (□), glucagon + 100 μM **2** (○), glucagon + 300 μM **2** (■). Data are means from three separate experiments. Inserted is the Schild plot. dr-1 was calculated individually from the three experiments and is shown as mean ± SD. K_i was determined to be 9.1 μM; the slope was 1.1.

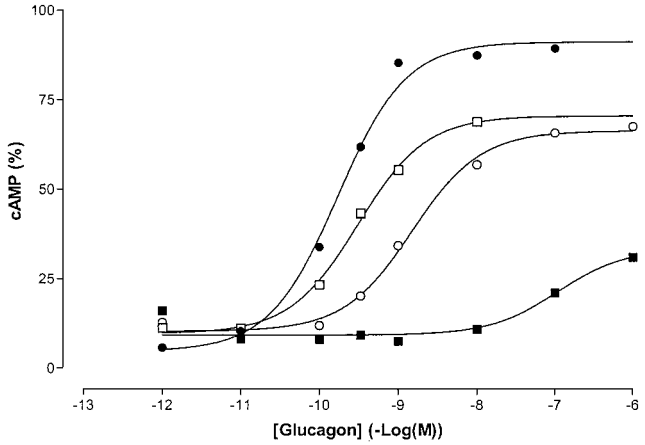


Figure 2. Inhibition of glucagon-stimulated cAMP by CP-99,711 using the recombinant human glucagon receptor in BHK cells. Shown is the observed shift in the glucagon dose−response curve by addition of CP-99,711: glucagon (●), glucagon + 0.3 μM CP-99,711 (□), glucagon + 1 μM CP-99,711 (○), glucagon + 3 μM CP-99,711 (■). Data are means from three separate experiments.

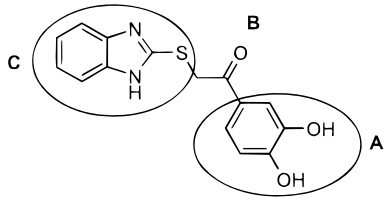


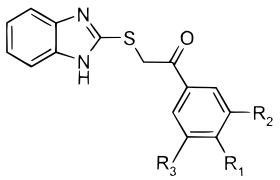
Figure 3. Three motifs studied: the catechol (A), the linker (B), and the heterocycle (C).

(B) The Linker. We then investigated the importance of the linker moiety (Table 2). Increasing the distance between the sulfur atom and the carbonyl group to three methylene groups (**15**) resulted in the total loss of binding affinity. Branching of the linker with a *n*-propyl group (**14**) also gave an inactive compound. To investigate the importance of the carbonyl group, four compounds were made. The hybridization of the carbonyl carbon was changed from sp^2 to sp^3 (**19**). This compound was found not to bind to the receptor. Further, the carbonyl function was transformed into oximes, and both the parent oxime (**16**), the *O*-methyloxime (**17**), and the *O*-benzyloxime (**18**) were found not to bind to the receptor.

(C) The Heterocycle. Investigations on the heterocyclic system have revealed the following (Scheme 3 and Table 3): Replacement of benzimidazole by benzothiazole (**20**) or benzoxazole (**21**) gave compounds with less affinity, although **20** was still somewhat active (73 μM). Deletion of the "benzo" part of benzimidazole (**22**) resulted in total loss of all binding affinity. However, binding affinity was regained, in part, on introduction of two phenyl rings on the imidazole (**23**, 180 μM), as well as changing to a 5-phenyl-1,3,4-triazolyl ring system (**24**, 55 μM). Replacing benzimidazole for quinoline gave a compound with substantially reduced binding affinity (**25**, 150 μM).

Consequently, as benzimidazole seemed to be the best choice for the heterocyclic system, a SAR was sought for this part of the molecule (Table 4). *N*-Methylation (**26**) was found to result in total loss of binding affinity. However, it was found that introduction of substituents in the 5-position was allowed. We found that substituents in the 5-position having increased bulk and lipophilicity resulted in unchanged or increased binding affinities, (**31**, 18 μM; **33**, 16 μM; and **30**, 5 μM). The 5,6-dichlorobenzimidazole (**27**) was equipotent and the 5-methoxybenzimidazole (**29**) had reduced binding affinity as compared to the parent benzimidazole (**2**). Introduction of a carboxylic acid substituent in the

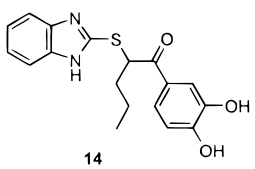
Table 1. Structures, Physical Properties, and Binding Affinity of Synthesized Compounds



Compd	R_1	R_2	R_3	Yield	M.p. (°C)	Formula	Analysis	Binding Affinity[a] (μM)
2	OH	OH	H	84%	> 250	$C_{15}H_{12}N_2SO_3$.HCl	CHN	20 ± 2.8
3	H	H	H	75%	233-234	$C_{15}H_{12}N_2SO$.HBr	CHN	> 500
4	OMe	OMe	H	69 %	> 250	$C_{17}H_{16}N_2SO_3$.HBr.¼H_2O	CHN	> 500
5	Cl	Cl	H	69 %	> 230	$C_{15}H_{10}N_2Cl_2SO$.HBr	CHN	> 500
6	OH	OMe	H	62 %	234	$C_{16}H_{14}N_2SO_3$.HBr. ¼H_2O	CHN	41 ± 9.9
7	OH	Cl	H	21 %	> 250	$C_{15}H_{11}N_2ClSO_2$.HBr. ¼H_2O	CHN	12 ± 0.0
8	OH	H	H	57 %	248-250	$C_{15}H_{12}N_2SO_2$.HBr	CHN	> 60
9	H	OH	H	62 %	224-227	$C_{15}H_{12}N_2SO_3$.HBr. ¼H_2O	CHN	380 ± 180
10	OH	H	OH	82 %	238-240	$C_{15}H_{12}N_2SO_3$.HBr. ¼H_2O	CHN	> 500
11	OH	CO_2H	H	66 %	233-234	$C_{16}H_{11}N_2SO_4$.HBr.0.75H_2O	CHN	200 ± 45
12	H	CO_2H	H	49 %	243-245	$C_{16}H_{12}N_2SO_3$.HBr.H_2O	CHN	> 500
13	CO_2H	H	H	81 %	> 250	$C_{16}H_{12}N_2SO_3$.HBr	CHN	> 500

[a] Binding affinity of compounds binding to the recombinant human glucagon receptor in BHK cells. Data are expressed as mean IC_{50} ± SD; $n = 2$ unless otherwise indicated.

Table 2. Structures, Physical Properties, and Binding Affinity of Synthesized Compounds



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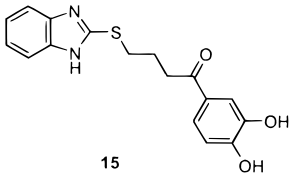

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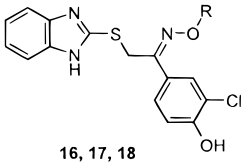
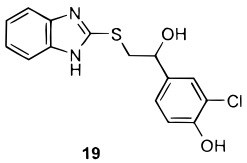


16, 17, 18



19

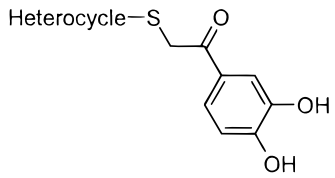
Compd	R	Yield	M.p. (°C)	Formula	Analysis	Binding affinity[a] (μM)
14		85 %	88 (dec.)	$C_{18}H_{18}N_2SO_3$.HBr.1¼H_2O	CHN	> 500
15		54 %	211-212	$C_{17}H_{16}N_2SO_3$.HCl	CHN	> 500
16	H	6 %	193-194	$C_{15}H_{12}N_3O_2SCl$	HR-MS	> 500
17	Me	22 %	130-131	$C_{16}H_{14}N_3O_2SCl$	HR-MS	> 500
18	CH_2Ph	22 %	180-181	$C_{22}H_{18}N_3ClSO_2$	CHN	> 500
19		28 %	158-160	$C_{15}H_{13}ClSO_2$	CHN	> 500

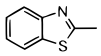
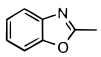
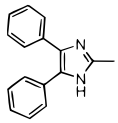
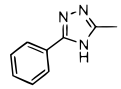
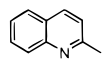
[a] Binding affinity of compounds binding to the recombinant human glucagon receptor in BHK cells. Data are expressed as mean IC_{50} ± SD; $n = 2$ unless otherwise indicated.

benzyloxy moiety as well as a nitro substituent in the 5-position gave compounds with reduced binding affinities (**32**, 95 μM; and **28**, 55 μM, respectively). Changing the catechol moiety of **33** to a 3-chloro-4-hydroxyphenyl moiety, **34**, resulted in a slightly improved binding affinity, 9 μM.

The SAR for this compound class is very steep for most of the structures. Thus, only the "meta" hydroxy functionality of the catechol can be replaced with a methoxy or chlorine substituent. All other modifications of the catechol were detrimental to the binding affinity. Further, we have not been able to modify the linker

Table 3. Structures, Physical Properties, and Binding Affinity of Synthesized Compounds



Compd	Heterocycle	Yield	M.p. (°C)	Formula	Analysis	Binding affinity[a] (μM)
20		35 %	171-174	$C_{15}H_{11}NS_2O_3.0.75H_2O$	CHN	73 ± 7.1
21		56 %	162-164	$C_{15}H_{11}NSO_4.½H_2O$	CHN	250 ± 42
22		95 %	231-232	$C_{11}H_{10}N_2SO_3.HCl$	CHN	> 500
23		99 %	222-223	$C_{23}H_{18}N_2SO_3.HCl$	CHN	180 ± 14
24		91 %	201-202	$C_{16}H_{13}N_3SO_3.HCl$	CHN	55 ± 0.71
25		83 %	210-213	$C_{17}H_{13}NSO_3.HCl$	CHN	160 ± 21

[a] Binding affinity of compounds binding to the recombinant human glucagon receptor in BHK cells. Data are expressed as mean IC_{50} ± SD; $n = 2$ unless otherwise indicated.

while retaining binding affinity, as all modifications in this part of **2** have resulted in total loss of binding affinity. The carbonyl group was found to be essential for binding affinity, since both reduction of the π-system and oximations are not allowed. Benzimidazole is by far the best of the tested heterocyclic ring systems. The benzimidazole H-bond donor system is required for binding affinity since N-methylation results in loss of all binding affinity. Only modifications on the benzo part of the benzimidazole part of **2** have proven to be allowed, and increased binding affinity was found for the lipophilic and bulky substituent in the 5-position of benzimidazole, *tert*-butyl (**30**).

Conclusions

We have described the first competitive non-peptide glucagon receptor antagonists. The best of these compounds have K_i and IC_{50} values in the low micromolar range and are thus not potent enough for development as drugs for NIDDM, but our results indicate that small molecule competitive antagonists for the glucagon receptor can be found. Further optimization of binding affinity is required before compounds of this type could be considered as satisfactory drug therapy.

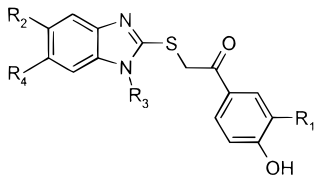
Experimental Section

General. ^{1}H NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ at 200 or 400 MHz (DRX 200 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 at the microanalytical laboratory, Novo Nordisk A/S. Column chromatography[18] was performed on silica gel 60 (40−63 μm). Melting points were determined on a Büchi 535 apparatus and are uncorrected. Chemicals and solvents used were commercially available and were used without further purification. Yields refers to analytically pure materials and are not optimized. 3-Chloro-4-hydroxyacetophenone[19] and 4-chloro-1-(3,4-dihydroxyphenyl)butan-1-one[20] were prepared by published procedures. 2-Bromo-1-(3,4-dihydroxyphenyl)pentan-1-one was prepared using the general procedure.[20] CP-99,711 (**1**) was kindly provided by Pfizer, Inc.

Alkylations Using 3,4-Dihydroxy-α-chloroacetophenone. General Procedure: 2-Mercaptobenzimidazole (**35**) (3.0 g, 20 mmol) and 3,4-dihydroxy-α-chloroacetophenone (3.9 g) were mixed in MeCN (30 mL), and the mixture was heated at reflux temperature for 16 h. After cooling, the mixture was filtered and washed with MeCN. The crude product (6.3 g, 94%) was suspended in EtOH (or MeCN) (100 mL), refluxed for 30 min, cooled, and filtered to give 5.7 g (84%) of 2-(1*H*-benzimidazol-2-ylsulfanyl)-1-(3,4-dihydroxyphenyl)ethanone (**2**) as a solid, mp > 250 °C. ^{1}H NMR: δ 5.39 (2H, s), 6.91 (1H, d), 7.40−7.47 (3H, m), 7.50 (1H, dd), 7.63−7.7 (2H, m). Anal. ($C_{15}H_{12}N_2SO_3$·HCl) CHN.

Alkylations Using α-Bromoacetophenones. General Procedure: 4-Hydroxyacetophenone (6.1 g, 45 mmol) was dissolved in EtOAc (30 mL), and finely ground $CuBr_2$ (17 g) was added. The mixture was heated at reflux temperature for 2.5 h, filtered through active carbon and evaporated in vacuo. The residue was dissolved in MeCN (60 mL), 2-mercaptobenz-

Table 4. Structures, Physical Properties, and Binding Affinity of Synthesized Compounds



Compd	R_1	R_2	R_3	Yield	M.p. (°C)	Formula	Analysis	Binding affinity[a] (μM)
26	OH	H	Me	51 %	> 230	$C_{16}H_{14}N_2ClSO_3.HCl$	CHN	> 500
27	OH	Cl (R_4 = Cl)	H	96 %	> 230	$C_{15}H_9N_2Cl_2SO_3.HCl.¼H_2O.½MeCN$	CHN	20 ± 4.8
28	OH	NO_2	H	58 %	> 230	$C_{15}H_{11}N_3SO_5.HCl$	CHN	55 ± 9.3
29	OH	OMe	H	87 %	> 240	$C_{16}H_{14}N_2SO_4.HCl$	CHN	42 ± 11
30	OH	t-Bu	H	74 %	> 230	$C_{19}H_{20}N_2SO_3.HCl$	CHN	5.4 ± 2.1
31	OH	OCH_2Ph	H	73 %	> 230	$C_{22}H_{18}N_2SO_3.HCl.H_2O$	CHN	18 ± 2.6
32	OH	OCH_2-C_6H_4-m-CO_2H	H	91 %	> 230	$C_{23}H_{18}N_2SO_6.HCl$	CHN	95 ± 6.9
33	OH	OCH_2-C_6H_4-m-CO_2Me	H	54 %	> 230	$C_{24}H_{10}N_2SO_6.HCl$	CHN	16 ± 4.3
34	Cl	OCH_2-C_6H_4-m-CO_2Me	H	83 %	> 230	$C_{24}H_{19}N_2ClSO_5.HCl$	CHN	9.2 ± 2.9

[a] Binding affinity of compounds binding to the recombinant human glucagon receptor in BHK cells. Data are expressed as mean IC_{50} ± SD; $n = 2$ unless otherwise indicated.

imidazole (2.7 g, 18 mmol) was added, and the resulting mixture was stirred at room temperature for 16 h. The crude product was filtered and washed with MeCN, suspended in acetone (100 mL), refluxed for 30 min, cooled, and filtered to give 3.7 g (57%) of 2-(1*H*-benzimidazol-2-ylsulfanyl)-1-(4-hydroxyphenyl)ethanone (**8**) as a solid, mp > 250 °C. ^{1}H NMR: δ 5.41 (2H, s), 6.96 (2H, d), 7.50 (2H, m), 7.72 (2H, m), 7.98 (2H, d). Anal. ($C_{15}H_{12}N_2SO_2$·HBr) CHN.

4-(1*H*-Benzimidazol-2-ylsulfanyl)-1-(3,4-dihydroxyphenyl)butan-1-one (15). 2-Mercaptobenzimidazole (2.2 g, 15 mmol) and 4-chloro-1-(3,4-dihydroxyphenyl)butan-1-one (3.5 g) were mixed in MeCN (40 mL), and the mixture was heated at reflux temperature for 4 h. After cooling, the mixture was filtered, and washed with MeCN. The crude product was suspended in acetone (50 mL), refluxed for 15 min, cooled, and filtered to give 2.87 g (54%) of 4-(1*H*-benzimidazol-2-ylsulfanyl)-1-(3,4-dihydroxyphenyl)butan-1-one (**15**) as a solid, mp 211−212 °C. ^{1}H NMR: δ 2.03 (2H, p), 3.10 (2H, t), 3.56 (2H, t), 6.82 (1H, d), 7.37 (2H, m), 7.43 (2H, m), 7.69 (2H, m). Anal. ($C_{17}H_{16}N_2SO_3$·HCl) CHN.

4-(Benzyloxy)-2-nitroaniline (35). 4-Amino-3-nitrophenol (10.8 g, 70 mmol) was dissolved in DMF (100 mL), and KI (100 mg), K_2CO_3 (30.5 g, 220 mmol), and benzyl chloride (8.9 mL, 77 mmol) were added. The mixture was stirred at 40 °C for 3 h, cooled, and partitioned between EtOAc (400 mL) and water (400 mL). The organic phase was washed with water (300 mL). The organic extract was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography eluting with a mixture of EtOAc and heptane (1:4). Fractions with R_f = 0.39 were evaporated to afford 4.3 g (25%) of 4-(benzyloxy)-2-nitroaniline (**35**), mp 141 °C. ^{1}H NMR ($CDCl_3$): δ 5.03 (2H, s), 5.9 (2H, bs), 6.77 (2H, d), 7.14 (1H, dd), 7.30−7.45 (5H, m), 7.67 (1H, d). Anal. ($C_{13}H_{12}N_2O_3$) CHN.

5-(Benzyloxy)-2-mercaptobenzimidazole (37). 4-(Benzyloxy)-2-nitroaniline (**35**) (4.0 g, 16 mmol) was suspended in EtOH (40 mL). The mixture was cooled, and a solution of $SnCl_2$ (12.8 g, 57 mmol) in 5 N HCl (200 mL) was added. The mixture was heated at 50 °C for 16 h, cooled, and evaporated in vacuo. Water (100 mL) was added to the residue, and 50% KOH was added until pH reached 14. Extraction with EtOAc (3 × 300 mL), drying of the combined organic extracts ($MgSO_4$), and evaporation in vacuo afforded 3.15 g (91%) of crude 4-(benzyloxy)-1,2-phenylenediamine, ^{1}H NMR ($CDCl_3$): δ 5.10 (2H, s), 6.78 (1H, d), 6.80 (1H, dd), 7.05 (1H, d), 7.34−7.46 (5H, m).

This crude 4-(benzyloxy)-1,2-phenylenediamine (3.1 g, 14 mmol) was added to EtOH (30 mL), water (5 mL), and potassium ethylxanthogenate (2.5 g, 16 mmol), and the mixture was refluxed for 4 h. Active carbon was added, and the mixture was refluxed for 10 min and filtered while hot. Water (30 mL) was added, the mixture was heated to reflux temperature, and acetic acid (2.5 mL) and water (5 mL) were added; then the mixture was cooled. Filtration and washing with water and EtOH afforded, after drying, 2.3 g (67%) of 5-(benzyloxy)-2-mercaptobenzimidazole (**37**), mp 228−230 °C. ^{1}H NMR (DMSO-d_6): δ 5.10 (2H, s), 6.8 (2H, m), 7.06 (1H, d), 7.35−7.46 (5H, m). Anal. ($C_{14}H_{12}N_2SO$) CHN.

Methyl 3-[(4-Amino-3-nitrophenyloxy)methyl]benzoate (36). 4-Amino-3-nitrophenol (14.3 g, 93 mmol) was dissolved in DMF (200 mL), and KI (100 mg), K_2CO_3 (30.5 g, 0.28 mol), and methyl 3-(bromomethyl)benzoate (23.4 g, 0.1 mol) were added. The mixture was stirred at 40 °C for 2 h, cooled, and partitioned between EtOAc (250 mL) and water (250 mL). The aqueous phase was extracted with EtOAc (2 × 250 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was washed successively with a 1:2 mixture of EtOAc and heptane, MeOH, and CH_2Cl_2. Filtration and washing with a 1:2 mixture of EtOAc and heptane followed by drying in vacuo afforded 11.4 g (43%) of methyl 3-[(4-amino-3-nitrophenyloxy)methyl]benzoate (**36**), mp 159−160 °C. ^{1}H NMR: δ 3.93 (3H, s), 5.09 (2H, s), 5.9 (2H, bs), 6.78 (1H, d), 7.15 (1H, dd), 7.49 (1H, t), 7.64 (1H, bd), 7.66 (1H, d), 8.00 (1H, bd), 8.12 (1H, bs). Anal. ($C_{15}H_{13}N_2O_5$) CHN.

Methyl 3-[(2-Mercaptobenzimidazol-5-yloxy)methyl]-benzoate (38). Methyl 3-[(4-amino-3-nitrophenyloxy)methyl]-benzoate (**36**) (10.5 g, 36 mmol) was dissolved in ethanol (200 mL), and anhydrous $SnCl_2$ (34.4 g, 5 equiv) was added. The resulting mixture was stirred at reflux temperature for 24 h. After cooling, the mixture was concentrated in vacuo. The residue was dissolved in EtOAc (200 mL), and 1 N KOH (800 mL) was added. The aqueous suspension was extracted with EtOAc (2 × 200 mL), and the combined organic extracts were washed with water, dried ($MgSO_4$), and evaporated in vacuo to give 9.8 g (99%) of crude methyl 3-[(3,4-diaminophenyloxy)-methyl]benzoate, ^{1}H NMR ($CDCl_3$): δ 3.90 (3H, s), 4.97 (2H,

s), 6.30 (1H, dd), 6.35 (1H, d), 6.59 (1H, d), 7.41 (1H, t), 7.59 (1H, d), 7.94 (1H, d), 8.06 (1H, s).

This crude methyl 3-[(3,4-diaminophenyloxy)methyl]benzoate (9.6 g, 35 mmol) was dissolved in MeOH (100 mL), potassium ethylxanthogenate (6.2 g, 39 mmol) was added, and the resulting mixture was stirred at reflux temperature for 4 h, cooled, and filtered. Acetic acid (5 mL) and water (100 mL) were added to the filtrate, and the resulting suspension was stirred for 15 min, filtered, washed with water, and dried in vacuo to afford 4.23 g (38%) of methyl 3-[(2-mercaptobenzimidazol-5-yloxy)methyl]benzoate (**38**), mp 204−206 °C. ^{1}H NMR (DMSO-d_6): δ 3.92 (3H, s), 5.08 (2H, s), 6.75−6.81 (2H, m), 7.06 (1H, dd), 7.47 (1H, t), 7.64 (1H, dt), 7.98 (1H, dt), 8.09 (1H, t). Anal. ($C_{16}H_{14}N_2SO_3 \cdot 0.25H_2O$) CHN.

3-[(2-Mercaptobenzimidazol-5-yloxy)methyl]benzoic Acid (39). Methyl 3-[(2-mercaptobenzimidazol-5-yloxy)methyl]-benzoate (**38**) (1.0 g, 3.2 mmol) was dissolved in dioxane (100 mL), and 1 N NaOH (10.5 mL) was added. The resulting mixture was stirred at room temperature for 16 h and concentrated in vacuo, and the residue was dissolved in water (25 mL). With stirring, acetic acid (5 mL) was added, and the resulting suspension was stirred at rom temperature for 1 h. Filtration and washing with water afforded, after drying, 0.90 g (95%) of 3-[(2-mercaptobenzimidazol-5-yloxy)methyl]benzoic acid (**39**), mp > 230 °C. ^{1}H NMR: δ 5.18 (2H, s), 6.77−6.95 (2H, m), 7.04 (1H, d), 7.53 (1H, t), 7.72 (1H, dt), 7.88 (1H, dt), 8.02 (1H, bs). Anal. ($C_{15}H_{12}N_2SO_3 \cdot 0.25H_2O$) CHN.

5-*tert*-Butyl-2-mercaptobenzimidazole. 4-*tert*-Butyl-1,2-phenylenediamine (2.5 g, 15 mmol) was dissolved in ethanol (25 mL). Water (2.5 mL) and potassium ethylxanthogenate (2.64 g, 16.5 mmol) were added, and the mixture was heated at reflux temperature for 3 h. Active carbon was added, and the mixture was refluxed for 10 min and filtered. Water (15 mL) and acetic acid (2.5 mL) were added, and the mixture was concentrated in vacuo to half volume. Filtration and washing with water afforded, after drying, 0.90 g (29%) of *5-tert*-butyl-2-mercaptobenzimidazole, mp > 230 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (9H, s), 7.05−7.10 (2H, m), 7.20 (1H, dd).

Oxiamation. General Procedure for 16−18: 3-Chloro-4-hydroxyacetophenone (27.5 g, 0.16 mol) and imidazole (27.6 g, 0.41 mol) were dissolved in DMF (600 mL), *tert*-butyldimethylsilyl chloride (31.35 g, 0.21 mol) was added in portions, and the resulting mixture was stirred at room temperature for 16 h. Water (500 mL) was added, and the mixture was extracted with EtOAc (600 mL). The organic phase was washed with water (2 × 500 mL), dried ($MgSO_4$), and evaporated. The residue (45.6 g) was dissolved in EtOAc (300 mL), and the solution was added to a refluxing suspension of $CuBr_2$ in EtOAc (300 mL). The mixture was refluxed for 5 h, cooled, filtered and concentrated in vacuo. The residue (52.4 g) was dissolved in MeCN (500 mL), 2-mercaptobenzimidazole (19.7 g, 0.13 mol) was added, and the mixture was refluxed for 4 h. The solid was filtered and resuspended in MeCN (500 mL), and the mixture was refluxed for 5 min, filtered, and dried in vacuo at 50 °C for 1 day to afford 58.7 g of crude O-protected **7** which turned out to be 50% desilylated.

The above partly desilylated crude O-protected **7** (1 equiv) was dissolved in EtOH (10 mL/g), Na_2CO_3 (1.1 equiv) and the appropriate hydroxylamine hydrochloride (1.1 equiv) were added, and the mixture was refluxed for 4 h. EtOAc was added, and the mixture was washed with water (3×). The organic extract was dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography eluting with mixtures of EtOAc and heptane. The crude protected oxime was dissolved in THF (15 mL/g), TBAF (1.5 equiv) was added, and the mixture was stirred at room temperature for 18 h. EtOAc was added, and the mixture was washed with water. After drying ($MgSO_4$), the product was purified by column chromatography eluting with mixtures of EtOAc and heptane.

By using the above general procedure, the following compounds were prepared. **16**, 2-(1*H*-benzimidazol-2-ylsulfanyl)-1-(3-chloro-4-hydroxyphenyl)ethanone oxime, ^{1}H NMR (DMSO-d_6): δ 4.57 (2H, s), 6.93 (1H, d), 7.12 (2H, m), 7.44 (2H, m), 7.54 (2H, dd), 7.81 (1H, d). HR-MS: calcd for $C_{15}H_{12}N_3O_2SCl$, 333.03396; found, 333.03388.

17, 2-(1*H*-benzimidazol-2-ylsulfanyl)-1-(3-chloro-4-hydroxyphenyl)ethanone *O*-methyloxime, ^{1}H NMR (DMSO-d_6): δ 3.93 (3H, s), 4.57 (2H, s), 6.94 (1H, d), 7.15 (2H, m), 7.36 (1H, m), 7.55 (2H, m), 7.84 (1H, d). HR-MS: calcd for $C_{16}H_{14}N_3O_2SCl$, 347.04962; found, 347.04953.

18, 2-(1*H*-benzimidazol-2-ylsulfanyl)-1-(3-chloro-4-hydroxyphenyl)ethanone *O*-benzyloxime, ^{1}H NMR (DMSO-d_6): δ 4.60 (2H, s), 5.22 (2H, s), 6.94 (1H, d), 7.13 (2H, m), 7.30−7.40 (6H, m), 7.55 (2H, m), 7.84 (1H, d). Anal. ($C_{22}H_{18}N_3ClSO_2$) CHN.

4-[2-(1*H*-Benzimidazol-2-ylsulfanyl)-1-hydroxyethyl]-2-chlorophenol (19). The above partly desilylated crude O-protected **7** (0.54 g, 1 mmol) was dissolved in MeOH (20 mL), KBH_4 (62 mg, 1.2 mmol) was added, and the mixture was stirred at room temperature for 45 min. Additional KBH_4 (62 mg, 1.2 mmol) was added, and the mixture was stirred at room temperature for 45 min. EtOAc (50 mL) was added, and the mixture was washed with water (2 × 40 mL). The organic phase was dried ($MgSO_4$) and evaporated. The residue was dissolved in THF (15 mL), and a solution of TBAF (0.36 g, 1.4 mmol) in THF (2 mL) was added dropwise. The mixture was stirred at room temperature for 2.5 h. EtOAc (30 mL) was added, and the mixture was washed with saturated $NaHCO_3$ (20 mL), dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography, eluting with EtOAc/heptane (1:1) to give 90 mg (28%) of 4-[2-(1*H*-benzimidazol-2-ylsulfanyl)-1-hydroxyethyl]-2-chlorophenol (**19**), mp 158−160 °C. ^{1}H NMR (DMSO-d_6): δ 3.48 (1H, dd), 3.57 (1H, dd), 4.81 (1H, dt), 5.39 (≤1H, d, OH), 6.94 (1H, dd), 7.10 (2H, m), 7.18 (1H, d), 7.35 (1H, m), 7.40 (1H, d), 7.51 (1H, m). Anal. ($C_{15}H_{13}ClSO_2$) CHN.

Receptor Pharmacology. Receptor data was obtained using the cloned human receptor.[21] The receptor, inserted in the pLJ6′ expression vector using *Eco*RI/*SSt*1 restriction sites,[21] was expressed in a baby hamster kidney cell line (A3 BHK 570-25). Clones were selected in the presence of 0.5 mg/mL G-418 and were shown to be stable for more than 40 passages. The K_d was shown to be 0.1 nM for glucagon. Cells were grown in DMEM with the addition of 10% fetal calf serum (FCS). Plasma membranes were prepared as earlier described for the GLP-1 receptor.[22] Glucagon was iodinated according to the chloramine T method[23] and purified using anion exchange chromatography.[24] The specific activity was ca. 460 μCi/μg on the day of iodination. The tracer was stored at −18 °C in aliquots and was used immediately after thawing.

Binding assays were carried out in filter microtiter plates (MADV N65, Millipore). The buffer used in this assay was 25 mM HEPES, 2.5 mM $CaCl_2$, 1.0 mM $MgSO_4$, 0.005% bacitracin (Sigma), 0.003% Tween 20, 0.1% casein (BDH Laboratories Supplies, England), and 0.1% bovine serum albumin (BSA) (Sigma, RIA grade), pH 7.4. Glucagon was dissolved in 0.05 M HCl with equal amounts (w/w) of human serum albumin (HSA) and freeze-dried. On the day of use, it was dissolved in water and diluted in buffer to the desired concentrations. Test compounds were diluted in buffer containing 10% DMSO.

Sample (175 μL) (glucagon or test compound) was added to each well. Tracer (50.000 cpm) was diluted in buffer, and 25 μL was added to each well; 0.5 μg of freshly thawed plasma membrane protein diluted in buffer was then added in 25 μL to each well, and plates were incubated at 25 °C for 2 h. Nonspecific binding was determined with 10^{-6} M glucagon. Bound and unbound tracer were then separated by vacuum filtration (Millipore vacuum manifold). The plates were washed once with 150 μL of buffer/well and air-dried for a couple of hours, whereafter filters were separated from the plates using a Millipore puncher. The filters were counted in a γ-scintillation counter.

The functional assay was carried out in 96-well microtiter plates (tissue culture plates, Nunc) in a total volume of 140 μL. The buffer was 50 mM Tris/HCl, 1 mM EGTA, 1.5 mM $MgSO_4$, 1.7 mM ATP, 20 μM GTP, 2 mM IBMX, 0.02% Tween-20, and 0.1% HSA. pH was 7.4.

The antagonist was diluted in DMSO and added in 10 μL; 5 μg of plasma membrane protein was used. The assay was incubated for 2 h at 37 °C with continuous shaking, after which the reaction was terminated by addition of 25 μL of 0.5 M HCl. cAMP was measured by the use of a scintillation proximity kit (Amersham).

For functional characterization, antagonism was measured as the ability of the compounds to right-shift the glucagon dose−response curve. Using three different antagonist concentrations, the K_i was calculated from a Schild plot.

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