Optimization of Alkylidene Hydrazide Based Human Glucagon Receptor Antagonists. Discovery of the Highly Potent and Orally Available 3-Cyano-4-hydroxybenzoic Acid [1-(2,3,5,6-Tetramethylbenzyl)-1*H*-indol-4-ylmethylene]hydrazide

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Received February 25, 2002

Highly potent human glucagon receptor (hGluR) antagonists have been prepared employing both medicinal chemistry and targeted libraries based on modification of the core (proximal) dimethoxyphenyl group, the benzyl ether linkage, as well as the (distal) benzylic aryl group of the lead **2**, 3-cyano-4-hydroxybenzoic acid (3,5-dimethoxy-4-isopropylbenzyloxybenzylidene)-hydrazide. Electron-rich proximal aryl moieties such as mono- and dimethoxy benzenes, naphthalenes, and indoles were found to be active. The SAR was found to be quite insensitive regarding the linkage to the distal aryl group, since long and short as well as polar and apolar linkers gave highly potent compounds. The presence of a distal aryl group was not crucial for obtaining high binding affinity to the hGluR. In many cases, however, the affinity could be further optimized with substituted distal aryl groups. Representative compounds have been tested for in vitro metabolism, and structure−metabolism relationships are described. These efforts lead to the discovery of **74**, NNC 25-2504, 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6-tetramethylbenzyl)-1*H*-indol-4-ylmethylene]hydrazide, with low in vitro metabolic turnover. **74** was a highly potent noncompetitive antagonist of the human glucagon receptor (IC_{50} = 2.3 nM, K_B = 760 pM) and of the isolated rat receptor (IC_{50} = 430 pM, K_B = 380 pM). Glucagon-stimulated glucose production from isolated primary rat hepatocytes was inhibited competitively by **74** (K_i = 14 nM). This compound was orally available in dogs (F_{po} = 15%) and was active in a glucagon-challenged rat model of hyperglucagonemia and hyperglycemia.

Introduction

Glucagon, a 29 amino acid hormone, secreted from the α-cells of the pancreas, stimulates hepatic glucose output via increasing glycogenolysis and gluconeogenesis. The human glucagon receptor was cloned in 1993,[1] and the use of the receptor has been patented.[2] A glucagon receptor antagonist is suggested to decrease the hepatic glucose output and thus lower hyperglycemia in type 2 diabetic patients.[3−7] Previous studies have shown, in both acute and chronic models, that in vivo immunoneutralization of glucagon does lead to lowered blood glucose levels in normal and STZ-induced diabetic rats, in normal and alloxan-induced diabetic rabbits, and in ob/ob-mice.[8−11] Thus, the glucagon receptor is a potential target for a new drug class for treatment of type 2 diabetes. Concerns have been raised that glucagon antagonism poses a risk of hypoglycemia. However, hypoglycemia was never observed in any of the immunoneutralization studies.[8−11] Furthermore, a patient with hyperglucagonemia has been described[12] to have a nonfunctional glucagon receptor and yet has no symptoms of either the glucagonoma syndrome or hypoglycemia. Very recently, glucagon receptor gene knock-out mice have been published[13] and their phenotype was strikingly similar to that of this patient.[12]

A few classes of non-peptide glucagon receptor antagonists have recently been published, primarily by scientists from Merck, Bayer, and Novo Nordisk.[14−17] Previously we have published[18] the discovery of a new class of human glucagon receptor (hGluR) antagonists, alkylidene hydrazides (**1**), and the subsequent optimization leading to **2**[19] (see Figure 1). In the present publication, further optimization of these two compounds are reported leading to series of high-affinity hGluR antagonists. Among these series, one promising compound that is orally available in dogs has been

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× Department of Computational Chemistry, Pfizer Global Research and Development.

10.1021/jm0208572 CCC: $22.00
Published on Web 11/20/2002

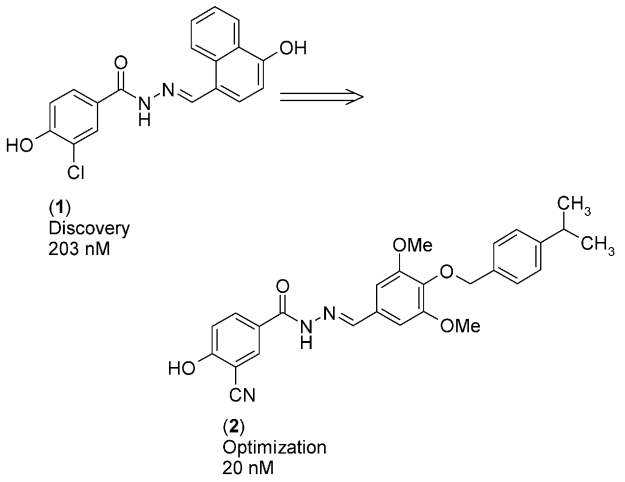


Figure 1. First examples of alkylidene hydrazide hGluR antagonists.

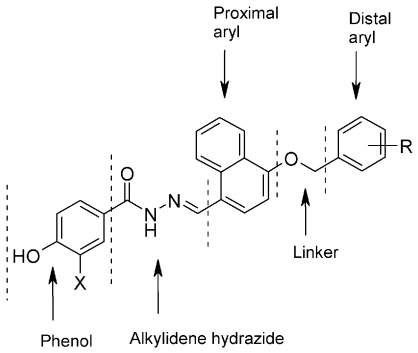


Figure 2. Definition of structural components of alkylidene hydrazide hGluR antagonists.

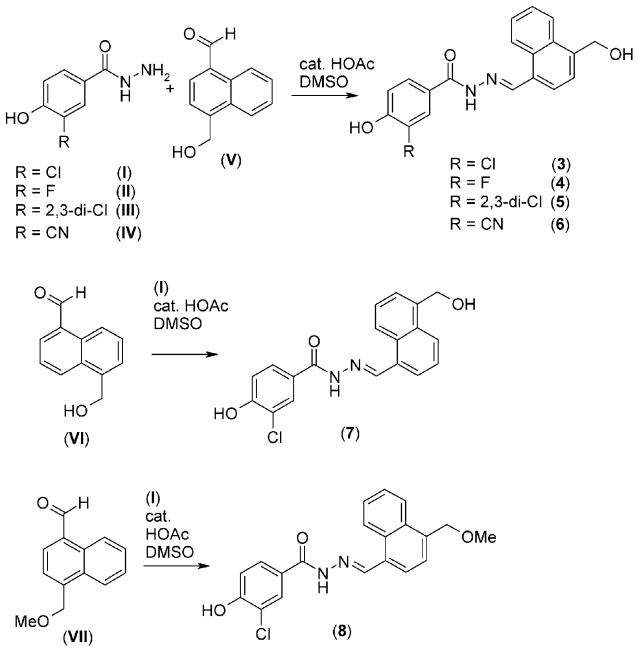
tested further and was found to be active in vivo in a glucagon-challenged rat model of hyperglucagonemia and hyperglycemia.

During the discovery[18] and optimization[19] of **1**, the most important SAR and hypothesis of receptor binding interactions became apparent (see Figure 2). An acidic phenol group in the benzoic acid part of the molecule was found to be an important pharmacophore, and electronegative ortho substituents (such as chloro, nitro, and cyano) gave access to very potent compounds. All attempts to replace the alkylidene hydrazide moiety resulted in compounds with low affinity, and it was found that the electron-rich proximal aryl moieties (such as 3,5-dimethoxyphenyl, indolyl, and naphthyl) gave compounds with high affinity to the hGluR.

The SAR of the series of alkylidene hydrazides was investigated further, and here, we describe the influence of combinations of various substituted distal aromatic groups through several linkages with the proximal aryl groups for binding to the hGluR. The improvement of the ADME properties was performed in parallel to the optimization of the binding affinity to the hGluR.

Compounds **1** and **2** were found to possess inadequate pharmacokinetic properties in rats characterized by very short in vivo half-lives and no oral bioavailabilities. In vitro investigations in rat liver microsomes suggested that the inadequate PK properties primarily were due to a high metabolic turnover, oxidative metabolism in the distal aromatic ring (phase I metabolism), and glucuronidation of the phenol moiety (phase II metabolism).

Scheme 1. Preparation Method A



The objective for the studies presented here was consequently to optimize the pharmacological/pharmacokinetic profile of this alkylidene hydrazide class of glucagon receptor antagonists. It was speculated that the inadequate pharmacokinetic profiles primarily were due to the physicochemical properties of the compounds, their high lipophilic character, and low aqueous solubilities. The strategy adopted was consequently to improve on these two parameters through incorporation of more polar motifs in the molecule, guided by in vitro metabolism screening in rat liver microsomes (i.e., to generate structure−metabolism relationships (SMRs). Thus, in parallel to assaying the compounds for receptor binding, the compounds were assayed for in vitro metabolism in rat liver microsomes or primary rat hepatocytes, and for selected compounds the metabolic profile (i.e., the structures of metabolites formed in vitro) was determined. These data provided structural feedback for the design of new types of compounds and targeted libraries.

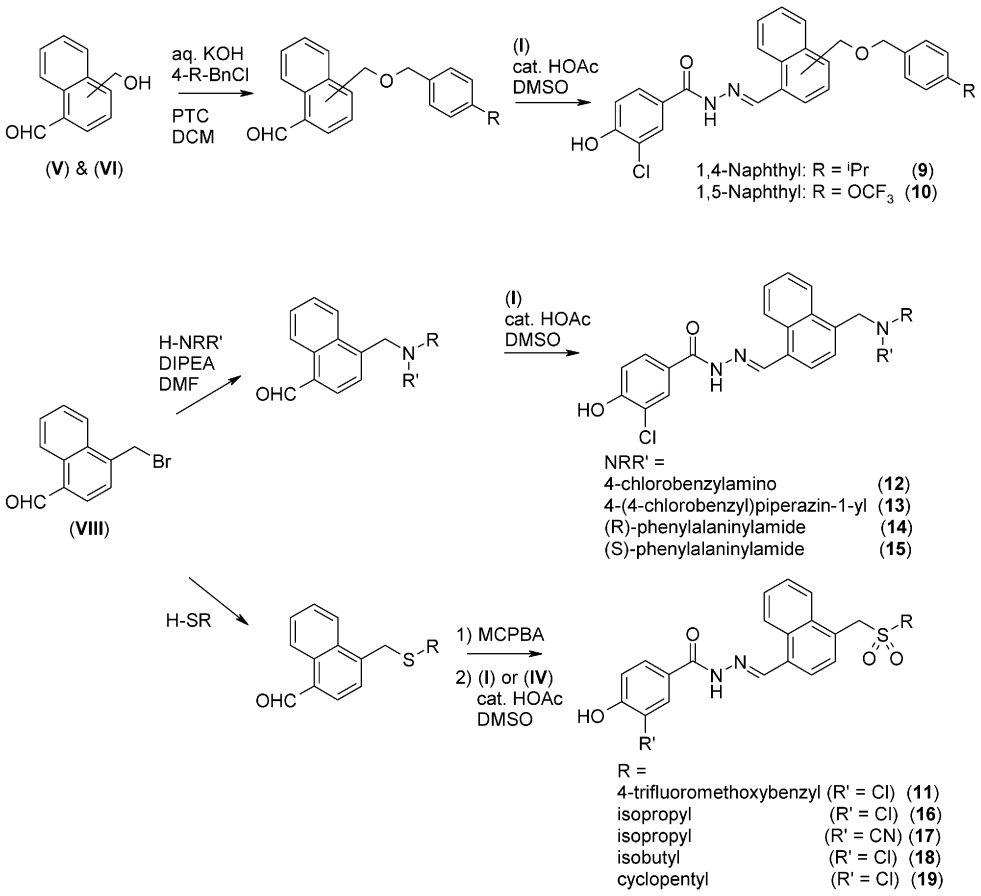
Primarily high-throughput synthesis of targeted libraries and also traditional medicinal synthesis of single compounds were employed to obtain ligands with the desired properties. All data presented herein are based on either purified library hits or resynthesized pure compounds. Consequential to the utilization of combinatorial chemistry, the inactive compounds were neither analyzed nor resynthesized and the SAR was primarily developed around the positively identified hGluR antagonists.

Chemistry

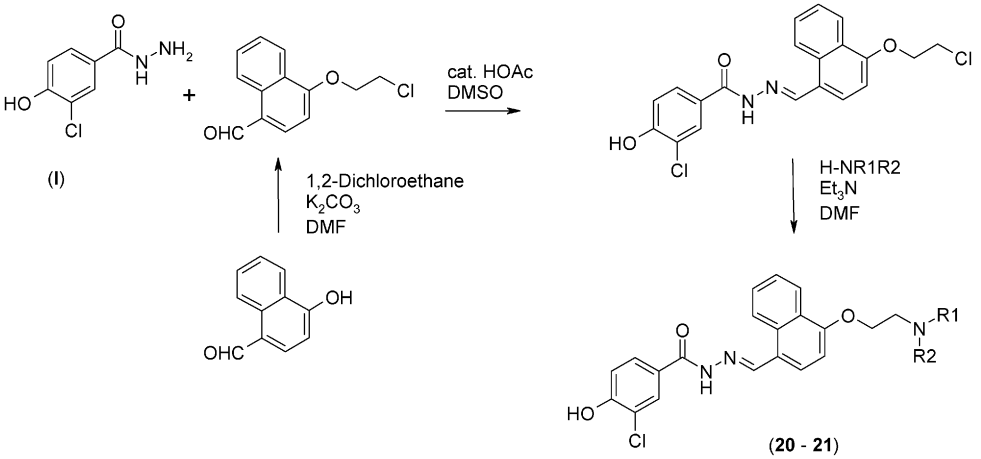
The hydroxymethyl analogues (**3**−**6** and **8**) and the naphthalene 1,5-regioisomer **7** were prepared through condensation of the corresponding hydrazides (**I**−**IV**) with the naphthaldehydes (**V**−**VII**) as shown in Scheme 1 (preparation method A).

Compounds that were further derivatized through the hydroxymethyl group were prepared as shown in Scheme 2, preparation method B. Benzylation of the hydroxy-

Scheme 2. Preparation Method B



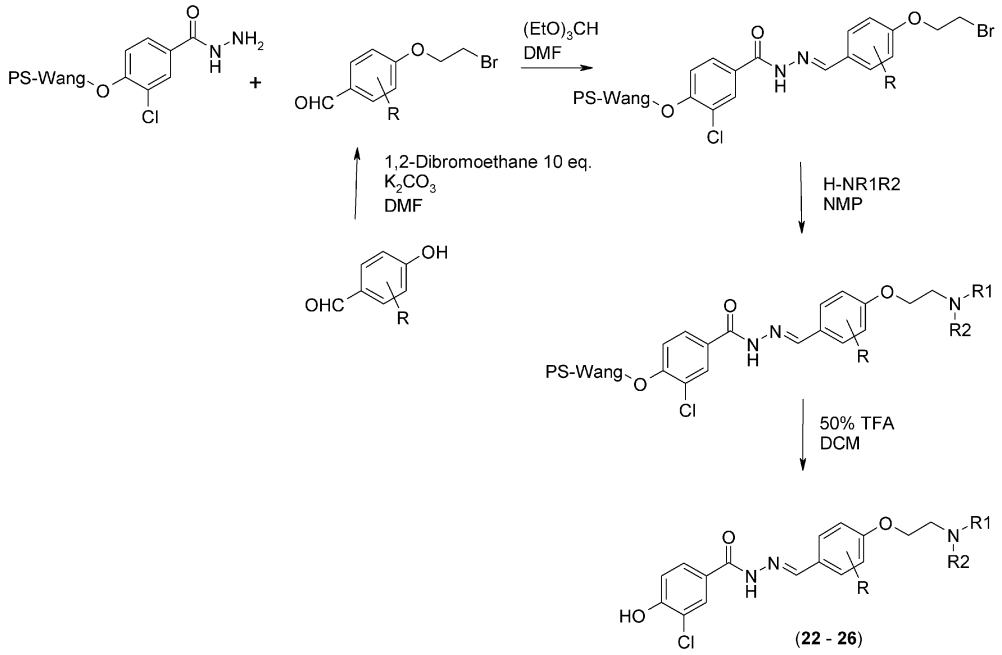
Scheme 3. Preparation Method C



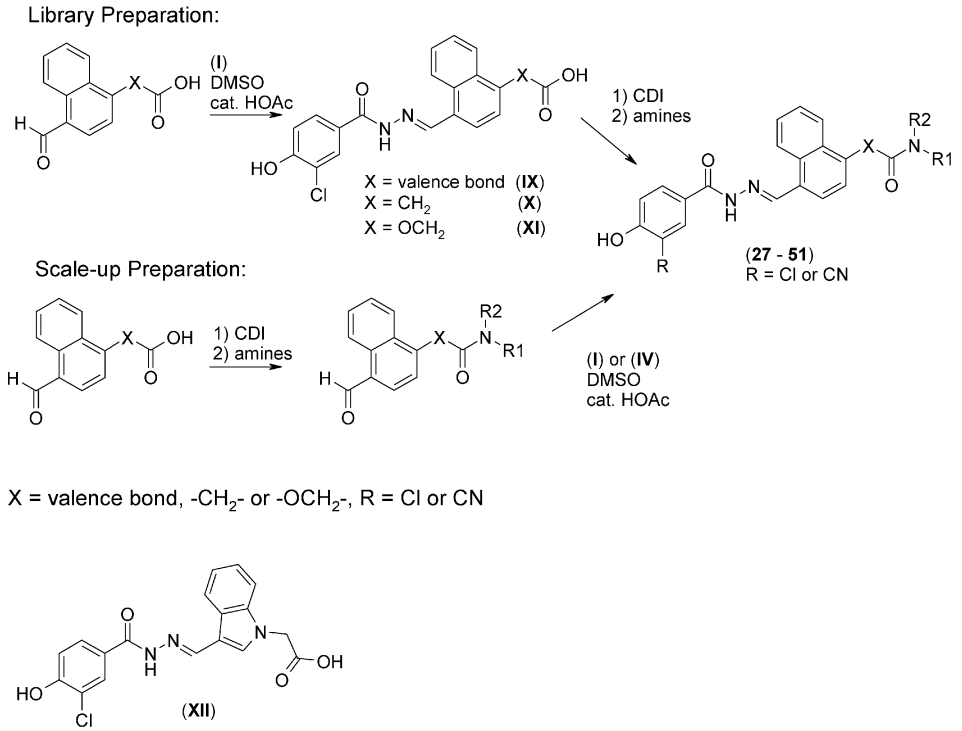
methyl naphthaldehydes **V** and **VI** with benzyl halides followed by condensation with **I** afforded **9** and **10**. The amines (**12**−**15**) were made from the bromomethylnaphthalene (**VIII**) using amines as nucleophiles followed by condensation with **I**. The sulfones (**11** and **16**−**19**) were similarly made using the corresponding thiols as nucleophiles, and the resulting sulfides were oxidized to sulfones prior to hydrazone formation.

Compounds with a 2-aminoethoxy linker between the proximal and the distal aromatic rings were prepared either using a solution-phase route as shown in Scheme 3 (preparation method C) or using a solid-phase approach as shown in Scheme 4 (preparation method D). In the solution-phase route, the 4-hydroxynaphthalene-1-carbaldehyde was O-alkylated with 1,2-dichloroethane and the 2-chloroethoxynaphthaldehyde was then condensed with the hydrazide (**I**). Subsequent nucleophilic displacement of the chloro group with amines furnished the desired products (**20** and **21**). In the solid-phase method, preparation method D, resin-bound hydrazide (**I**) was prepared by attachment of 3-chloro-4-hydroxybenzoic acid methyl ester to a PS−Wang resin via nucleophilic substitution of PS−Wang mesylate. The resulting resin-bound ester was subsequently hydrolyzed using potassium trimethylsilanolate followed by acetic acid. Activation of the resin-bound carboxylic acid using PyBoP followed by reaction with hydrazine afforded the resin-bound hydrazide (**I**). Methoxy-substi-

Scheme 4. Preparation Method D



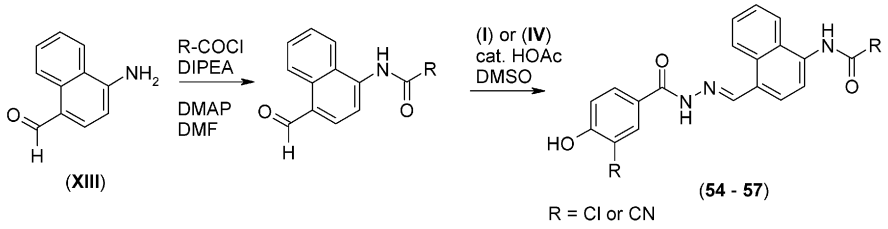
Scheme 5. Preparation Method E



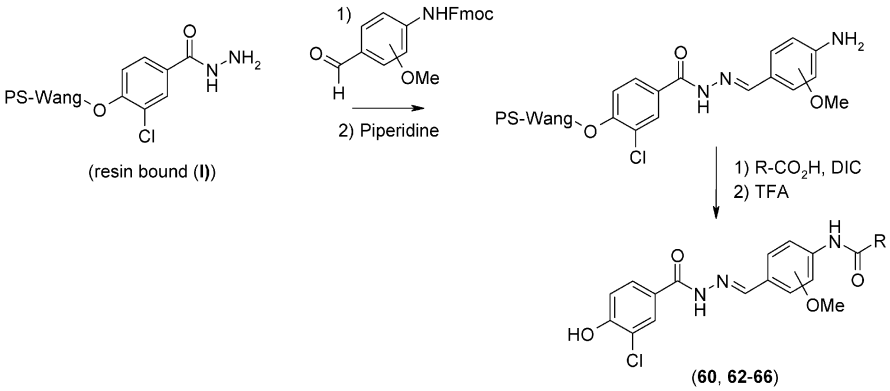
tuted 4-(2-bromoethoxy)benzaldehydes or 4-(2-bromoethoxy)-1-naphthaldehydes were then condensed with the resin-bound hydrazide (**I**), and subsequent nucleophilic substitution with amines gave, after TFA cleavage, the desired compounds. This procedure was used in a library approach on a 50 mg scale (of resin-bound hydrazide **I**) for preparation of a targeted library (eight different 2-bromoethoxy-substituted aldehydes × 48 amines, 384 compounds) as well as for scale-up of representative hits (**23** and **24**, using 3 g of resin-bound hydrazide **I**). The solid-phase method was found to be better than the solution-phase method because in solution products coming from overalkylation of the employed amines with the 2-chloroethoxy-substituted hydrazide were difficult to remove by chromatography. This problem was not encountered on solid support (i.e., no cross-linking was observed).

Large libraries of amides were prepared as shown in Scheme 5 (preparation method E). Compounds **27**−**51** also including heteroaromatic proximal rings such as indoles were made. Targeted libraries were made by activation of the carboxylic acids (such as **IX**−**XI** and the corresponding indole, **XII**, eight carboxylic acids in total) by carbonyldiimidazole followed by reaction with a large set of amines. In all, >2300 amides were made and >50 hits were found when screened at 100 nM

Scheme 6. Preparation Method F



Scheme 7. Preparation Method G



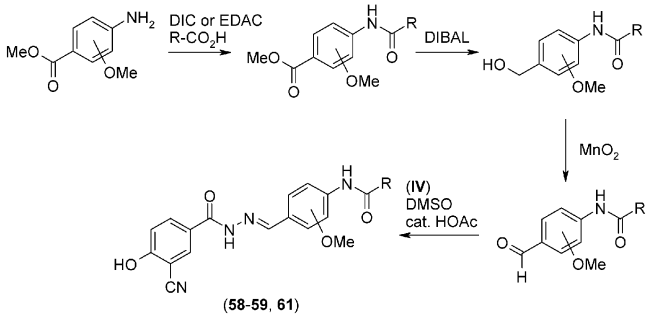
concentration. Scale-up of selected compounds was done according to Scheme 5. Here, the naphthaldehydes, appended with a carboxylic acid functionality, were converted into the corresponding amides prior to hydrazone formation.

Compounds where the amide was reversed compared to the amides described in Scheme 5 were prepared as described in Scheme 6 (preparation method F) by reacting 4-amino-1-naphthaldehyde (**XIII**) (or indole carboxaldehydes, not shown in scheme) with carboxylic acid chlorides. The resulting amides were in turn condensed with either **I** or **IV** to afford the corresponding amides (**52**−**57**).

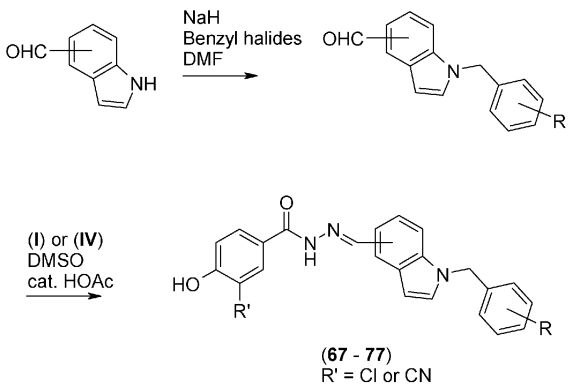
Another series of amides with a methoxy-substituted phenyl ring as the proximal aryl group (**58**−**66**) was made in library format on solid support and as single compounds in solution. In Scheme 7 (preparation method G), the solid-phase synthesis is described. The resin-bound hydrazide **I** was condensed with methoxy-substituted Fmoc-protected 4-aminobenzaldehydes. After deprotection of the amine, coupling of carboxylic acids to afford the desired amides was done using symmetric anhydrides. The targeted libraries were prepared from 50 mg of resin-bound hydrazide **I**. In all, eight different Fmoc-protected aminobenzaldehydes were used and >250 compounds were made. The Fmoc-protected 4-aminobenzaldehydes were prepared from the corresponding 4-aminobenzoic acid esters via Fmoc protection, DIBAL reduction, and MnO_2 oxidation. Resynthesis of hits (scale-up) was done either on solid support by employing more resin or in solution as shown in Scheme 8 using preparation method H.

Here, coupling of the desired carboxylic acid (using the symmetric anhydride) with methoxy-substituted 4-aminobenzoic acid methyl esters followed by DIBAL reduction of the ester and MnO_2 oxidation furnished the appropriately substituted benzaldehydes. These were condensed with the 3-chloro- or 3-cyano-4-hydroxybenzoic acid hydrazides (**I** and **IV**, respectively) to afford the desired compounds.

Scheme 8. Preparation Method H



Scheme 9. Preparation Method I



Finally, a library of benzylated indoles (**73**−**77**) was prepared as described in Scheme 9 (preparation method I). Various substituted indolecarbaldehydes were deprotonated with NaH in DMF, and the corresponding anion was benzylated using benzyl chlorides or bromides. The resulting aldehydes were then condensed with hydrazide **I** or **IV** essentially as described earlier for phenol alkylation.[19]

Biological Methods

The biological methods used are described in detail in the Experimental Section.

Receptor Assays. Receptor assays were carried out using plasma membranes from BHK cells expressing the cloned human glucagon receptor. The functional assays were carried out as earlier described,[20] whereas the binding assay was slightly modified.

Primary Hepatocyte Assays. As a model system for hepatic glucose production, rat hepatocytes were isolated and cultured as described earlier.[21] The hepatocytes were loaded with glycogen, and the effect of the glucagon antagonists on glucagon-induced glycogenolysis was studied.

In Vitro Drug Metabolism. It had initially been found that the present series of compounds exhibited fast metabolic turnover with resulting poor in vivo PK profiles; therefore, optimization with respect to binding potency and DMPK properties was performed in parallel. Metabolic rates were estimated from incubations with rat liver microsomes in order to rank compounds in terms of their metabolic stability in the liver. Analysis was performed by means of LC−MS. Selected compounds were profiled with respect to the major metabolites formed in rat liver microsomes. In this manner, potential weak spots (from a metabolic point of view) in the molecules could be identified. Metabolic profiles were analyzed by means of LC−MS−MS.

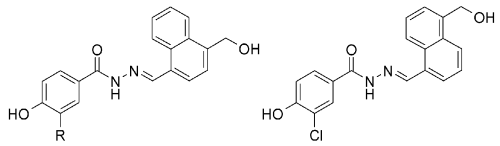
Pharmacokinetics. For pharmacokinetic experiments, four purpose-bred male beagle dogs weighing 11−19 kg and that were 15−18 months of age were used. Blood sampling was done at predetermined time points from predose to 4 h postdose. The blood samples were analyzed using LC−MS−MS.

Glucagon-Challenged Rat. To demonstrate acute in vivo efficacy, the effect of the compounds on glucagon-stimulated hyperglycemia was studied in rats. Nonfasted male Sprague−Dawley rats (200 g) were maintained in the anesthetized state during the test by subcutaneous administration of a 1:1 mixture of hypnorm (fentanyl, 0.05 mg/mL, and fluanizone, 2.5 mg/mL, Janssen Pharma Ltd., Copenhagen, Denmark) and dormicum (midazolam, 1.25 mg/mL, Roche, Basel, Switzerland). A catheter was inserted in a jugular vein for administration of compounds. Approximately 60 min after initiation of anesthesia, test compounds (0, 3, and 10 mg/kg) and glucagon (3 μg/kg) were each administered in a 5 min interval. Samples for determination of blood glucose concentrations were taken from the tail tip 25 and 5 min prior to administration of the compound to represent average basal values and again 10 min after administration of glucagon (time for peak response to glucagon). The results were expressed as the change (Δ) in values, which is the value obtained 10 min after glucagon administration minus the average of the two basal values. This method was adapted from the method previously published.[8]

Discussion

The original lead compound **1** contained both a phenol and a naphthol functionality, and it was speculated that these groups were causing the fast metabolic turnover due to glucoronidation. The phenolic group was earlier shown to be important for binding.[18] The naphthol group was, however, not the optimal moiety because the homologous hydroxymethylnaphthalene resulted in an increased binding affinity, 41 nM for **3** as shown in Table 1. The substitution pattern of the naphthalene moiety had influence on the affinity because the 1,5-substituted naphthalene (**7**) was less potent than the 1,4-analogues. Replacing the 3-chloro substituent of **3** with other electron-withdrawing groups such as fluorine (**4**) and cyano (**6**) did not alter the binding affinity significantly, but an impact on in vitro metabolism was observed. The microsomal turnover of **6** with a 3-cyano group was reduced compared to **3** with a 3-chloro substituent, 37 vs 75 pmol min^{-1} mg^{-1} protein, respectively. This trend was quite general for this and other subclasses described in the present publication and was the result of a diminished rate of glucuronidation of the phenolic group when the chloro substituent was replaced with a cyano group. Interestingly, it was possible to diminish the in vitro metabolic turnover in parallel to optimization of the hGluR affinity because by a change in the substitution pattern in the naphthalene moiety, e.g., the less potent 1,5 analogue, **7** also had the fastest metabolic turnover within this series, 225 pmol min^{-1} mg^{-1} protein, whereas the 1,4-analogue **6** was much more potent and had lower metabolic turnover compared to **7**.

Table 1. Structures, Binding Affinities, Rates of in Vitro Metabolism, and Physical Properties of Synthesized Compounds



3 - 6 **7**

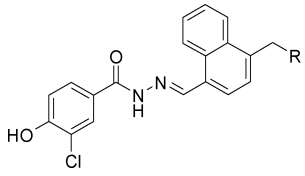
Compd	R	hGluR binding affinity[a] (nM)	*In vitro* metabolism (pmol/min/mg)	Prep. method	M.p. (°C)	Formula	Anal.
3	Cl	41.1 ± 10.1	75	A	>250	$C_{19}H_{15}ClN_2O_3$	CHN[b]
4	F	29.4 ± 10.4	89	A	>250	$C_{19}H_{15}FN_2O_3$	HR-MS
5	2,3-di-Cl	53.8 ± 16.6	103	A	195-198.5	$C_{19}H_{14}Cl_2N_2O_3$	HR-MS
6	CN	29.8 ± 8.3	37	A	>250	$C_{20}H_{15}N_3O_3.0.25H_2O$	CHN
7		200 ± 55	225	A	>250	$C_{19}H_{15}ClN_2O_3.0.25H_2O$	CHN

[a] Binding affinity of compounds bound to the recombinant human glucagon receptor in BHK cells. Data are expressed as the mean IC_{50} ± SD, $n = 3$. [b] N: calcd, 7.90; found, 7.22.

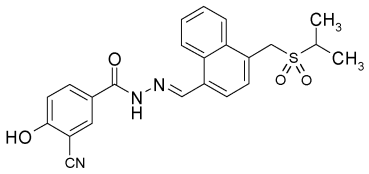
The encouraging results obtained for the hydroxymethylnaphthalenes prompted further exploration and optimization of this region by alkylation of the hydroxymethyl group. The hydrogen bond donor property of the hydroxymethyl group of **3** was not essential for hGluR binding because the corresponding methoxy analogue **8** was only about 2-fold less potent, as can be seen in Table 2. As experienced in the former naphthol series,[18] it was found that extension of the hydroxymethylnaphthalenes with lipophilic moieties (O-benzylation, **9** and **10**) resulted in improvement of the binding affinity of the compounds. Interestingly, the extension with lipophilic moieties improved the potency for both the 1,4- and the 1,5-substituted naphthalenes, and the effect on binding of this extra lipophilic moiety was more pronounced for the 1,5-arrangement (**10**) than for to 1,4-arrangement (**9**).

The importance of the ether linkage between the proximal naphthyl group and the distal aryl group was also investigated, and it was found that other functional

Table 2. Structures, Binding Affinities, Rates of in Vitro Metabolism, and Physical Properties of Synthesized Compounds



8 - 9
11 - 16
18 - 19



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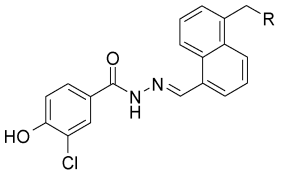

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Compd	R	hGluR binding affinity[a] (nM)	*In vitro* metabolism (pmol/min/mg)	Prep. method	M.p. (°C)	Formula	Anal.
8	MeO-	75.5 ± 21.4	ND	A	204-206.5	$C_{20}H_{17}ClN_2O_3.0.25H_2O$	CHN
9	4-^{i}PrBnO-	4.3 ± 0.5	ND	B	100-105	$C_{29}H_{27}ClN_2O_3$	HR-MS
10	4-CF_3OBnO-	12.9 ± 0.8	ND	B	183-185	$C_{27}H_{20}ClF_3N_2O_4$	CHN
11	4-CF_3OBnSO_2-	6.9 ± 0.9	14	B	240-241	$C_{27}H_{20}ClF_3N_2O_5S$	HR-MS
12	4-ClBnNH-	8.3 ± 2.0	304	B	158-163	$C_{26}H_{21}Cl_2N_3O_2$	HR-MS
13	4-(4-ClBn)piperazin-1-yl-	96.7 ± 11.7	51	B	149-152	$C_{30}H_{28}Cl_2N_4O_2.0.75H_2O$	CHN[b]
14	(R)-Bn-CH($CONH_2$)NH	7.4 ± 1.1	153	B	154-161	$C_{28}H_{25}ClN_4O_3.H_2O$	CHN
15	(S)-Bn-CH($CONH_2$)NH-	61.8 ± 5.4	178	B	157-161	$C_{28}H_{25}ClN_4O_3.1.5H_2O$	CHN
16	$^{i}PrSO_2$-	357 ± 11.5	110	B	267-269	$C_{22}H_{21}ClN_2O_4S$	HR-MS
17		163 ± 15.6	31	B	294-297	$C_{23}H_{21}N_3O_4S$	HR-MS
18	$^{i}BuSO_2$-	181 ± 20.4	110	B	240-242	$C_{23}H_{23}ClN_2O_4S.1.25H_2O$	CHN[c]
19	$^{c}PentylSO_2$-	221 ± 31.5	ND	B	245-247	$C_{24}H_{23}ClN_2O_4S.0.25H_2O$	CHN

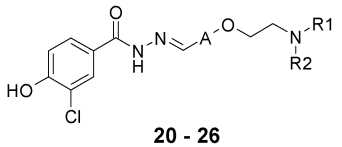
[a] Binding affinity of compounds bound to the recombinant human glucagon receptor in BHK cells. Data are expressed as the mean IC_{50} ± SD, $n = 3$. ND: not determined. [b] N: calcd, 9.99; found, 9.32. [c] N: calcd, 5.82; found, 5.38.

groups in the linker could give access to very potent compounds. Thus, a strong hydrogen bond acceptor (a sulfone, **11**) and a positively charged group (a secondary amine, **12**) in this linker were both well tolerated, indicating that these linkers did not form direct contacts with the receptor. It was also concluded that compounds that were lacking the distal aryl group had much less affinity to hGluR as exemplified with compounds **16**−**19**. On the basis of these results, it was hypothesized that the role of the linker was to orientate the distal lipophilic moiety in a correct position relative to the proximal naphthyl group, thereby adding extra lipophilc receptor interactions. This hypothesis was further confirmed by the preparation of two chiral compounds **14** and **15** with (*R*)- and (*S*)-phenylalanine carboxamide as distal moieties, respectively. The (*R*) and (*S*) stereochemistry of these two compounds controls the orientation of the distal liphophilic benzylic moiety, which resulted in significant impact on the binding affinities, since the (*R*)-isomer (**14**) was almost 10-fold more potent than the (*S*)-isomer (**15**). It is noteworthy that these highly polar and charged linkers without any loss of affinity could replace the lipophilic benzyl ether linkages in **2** and in **9**.

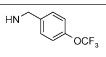
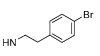
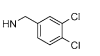
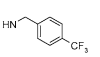
The in vitro metabolic turnovers of some of the compounds from this series were investigated. Most of the compounds tested had a very fast metabolic turnover, and **12** with a basic secondary amine as linker was found to be metabolically very labile with a turnover of 304 pmol min^{-1} mg^{-1} protein. Interestingly, the other basic analogue **13** had a much lower metabolic turnover (51 pmol min^{-1} mg^{-1} protein). It was found that this was due to the fact that the piperazine linker was more stable toward oxidative N-dealkylation than the secondary amine in **12**. As in the series of substituted hydroxymethylnaphthalenes (Table 1), in this series of compounds it was also possible to optimize the receptor affinity in parallel with the in vitro metabolic stability. The best compound (**11**) had exceptional low metabolic turnover (14 pmol min^{-1} mg^{-1} protein) and was also a high-affinity ligand (6.8 nM). Unfortunately, further in vivo studies with this compound showed that the PK profile was still inappropriate with a fast in vivo clearance, which was concluded not to be driven by biotransformations.

On the basis of these results, it was decided to continue with the strategy of improving binding affinity and the rate of in vitro metabolism in parallel by further modifications of the substitution pattern of the distal aryl group and to combine these with other types of linkers to the proximal aryl moiety. Again, it was hypothesized that the inappropriate PK profile of the compounds could be improved by an increased aqueous solubility, and accordingly, it was decided to prepare a series of compounds wherein a polar 2-aminoethoxy linker (Table 3) was examined with respect to SAR and SMR. The binding affinities of the substituted benzylamines prepared showed a weak preference for para substitution as seen by comparison of the affinities of **20** and **21**. No tight distance constraints were observed, since **20** and **22** were almost equipotent (28 and 20 nM, respectively). For further improvement of the hydro-

Table 3. Structures, Binding Affinities, Rates of in Vitro Metabolism, and Physical Properties of Synthesized Compounds



20 - 26

Compd	-A-	-NR1R2	hGluR binding affinity[a] (nM)	*In vitro* metabolism (pmol/min/mg)	Prep. method	M.p. (°C)	Formula	Anal.
20			27.7 ± 4.0	167	C	125-127	$C_{28}H_{23}ClF_3N_3O_4.1.75H_2O$	CHN[b]
21			54.3 ± 0.6	233	C	ND	$C_{28}H_{23}ClF_3N_3O_4$	HR-MS
22			19.7 ± 2.1	250	D	163.1-164.5	$C_{28}H_{25}BrClN_3O_3.1.5H_2O$	CHN[d]
23			14.7 ± 1.4	ND	D	132.2-136.0	$C_{27}H_{22}Cl_3N_3O_3$	HR-MS
24			120 ± 10	322	D	170.5-171.9	$C_{26}H_{26}ClN_3O_4$	HR-MS
25			133 ± 25	ND	D	124-126.3	$C_{25}H_{23}ClF_3N_3O_4.H_2O$	CHN[c]
26			485 ± 44	ND	D	161.4-163.6	$C_{26}H_{25}Cl_2N_3O_4.H_2O$	CHN

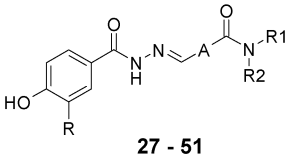
[a] Binding affinity of compounds bound to the recombinant human glucagon receptor in BHK cells. Data are expressed as the mean IC_{50} ± SD, $n = 3$. ND: not determined. [b] H: calcd, 4.53; found 3.97. [c] N: calcd, 7.78; found 8.35. [d] H: calcd. 4.75; found. 5.17. N: calcd, 7.08; found, 7.77.

philicity of the compounds, the proximal lipophilic naphthyl moiety was replaced with monocyclic aryl groups. Replacing the proximal naphthalene moiety with substituted 1,4-phenylene systems, such as 2-methoxyphenylene (**24** and **25**) and 3-chloro-5-methoxyphenylene (**26**) resulted, however, in less potent compounds. The SMR of this series of compounds was also investigated. The general trend of this subclass of compounds was that the in vitro metabolic turnover was very high. This was primarily due to phase I metabolism, oxidative N-dealkylations in the linker, or in the case of **24** and **25**, oxidations leading to dihydroisoquinolines and isoquinolinium salts.

The conclusion from these results confirmed the hypothesis of the large tolerance of the receptor toward the linker. Also, the previous finding[19] that the proximal aromatic ring needs to be electron-rich was substantiated. However, compounds with highly polar linkers, such as the charged amines (e.g., **12** and **23**) were generally rapidly metabolized (due to oxidative N-dealkylations) and were thus not considered to be worth pursuing further.

For a functionalized linkage that could be metabolically more stable, it was decided to follow up on the previous finding[19] that a compound with a diethyl amide in the linker region was quite active. The amide group is not charged at physiological pH and has a different geometry and polarity compared to amines. It was hypothesized that the metabolically driven N-dealkylation of the compounds containing an amine linkage might be avoided using an amide linker and thereby give access to potent compounds with reduced metabolic turnover. Among the compounds prepared, it was found that a distal aromatic ring was not needed in order to obtain high-affinity compounds as seen, for example, for the series of diethyl amides **27**−**30** (Table 4). The importance of the proximal naphthalene ring was emphasized by the low affinity of the corresponding indole **31**. The potency of **28** could be further optimized to a single digit nanomolar range by a combination of constraint of the flexibility of the alkyl groups in a piperidine ring and by an increase of the lipophilic size of the piperidine ring with alkyl groups (**32**−**34**). It was also possible to obtain compounds with similar high affinity by appending an aromatic residue to the piperidine ring (e.g., as phenylpiperidine (**35**−**37**) or as a 1,2,3,4-tetrahydroisoquinoline (**38**)). The affinities were, however, not further increased compared to the affinities of the methyl- and ethylpiperidine systems in **32**−**34**. Exchange of the neutral piperidine amide system with a basic methylpiperazine moiety in **39** resulted in a substantial loss in affinity. Increasing the distal lipohilicity of the piperazine compounds with aromatic groups such as benzyl groups (**40** and **41)** or with phenyl groups (**42** and **43**) gave compounds with regained binding affinity. The presence of a distal aromatic residue was similarly important for potency in the much more flexible ring-opened analogues as seen by comparison of, for example, the methyl analogue **44** (441 nM) with the corresponding benzyl analogue **45** (6.2 nM). The in vitro metabolic stability of this series of amides was in general low, and the high in vitro metabolic turnover was mainly due to oxidative N-dealkylations as observed in the previous amine series. In a few cases it was, however, possible to improve the metabolic stability by a combination of substitutions

Table 4. Structures, Binding Affinities, Rates of in Vitro Metabolism, and Physical Properties of Synthesized Compounds



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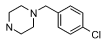
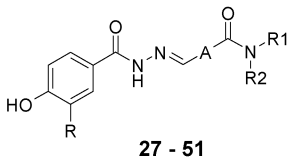
Compd	R	A	NR1R2	hGluR binding affinity[a](nM)	*In vitro* metabolism (pmol/min/mg)	Prep. method	M.p. (°C)	Formula	Anal.
27	Cl			35.4 ± 6.0	124	E	ND	$C_{23}H_{22}ClN_3O_3$	HR-MS
28	Cl			22.6 ± 4.8	323	E	ND	$C_{24}H_{24}ClN_3O_3$	HR-MS
29	CN			16.5 ± 1.8	130	E	265-268	$C_{25}H_{24}N_4O_3.0.5H_2O$	CHN
30	Cl			49.7 ± 8.9	302	E	244-246	$C_{24}H_{24}ClN_3O_4$	CHN
31	Cl			2540 ± 240	165	E	226-228	$C_{22}H_{23}ClN_4O_3$	HR-MS
32	Cl			8.3 ± 0.9	267	E	ND	$C_{26}H_{26}ClN_3O_3$	HR-MS
33	CN			11.6 ± 1.7	65	E	>250	$C_{27}H_{26}N_4O_3.0.5H_2O$	CHN
34	Cl			7.4 ± 0.9	279	E	164-165	$C_{27}H_{28}ClN_3O_3.H_2O$	CHN
35	Cl			11.4 ± 2.6	ND	E	ISv	$C_{31}H_{27}BrClN_3O_5$	CHN
36	Cl			6.6 ± 0.9	44	E	ND	$C_{31}H_{25}BrClN_3O_4$	HR-MS
37	CN			6.1 ± 1.6	50	E	219 (dec.)	$C_{32}H_{25}BrN_4O_4.2.25H_2O$	CHN
38	Cl			13.8 ± 3.4	254	E	>250	$C_{29}H_{24}ClN_3O_4.0.5H_2O$	CHN
39	Cl			268 ± 10	283	E	148-151	$C_{25}H_{25}ClN_4O_3.2H_2O$	CHN
40	Cl			9.2 ± 2.5	359	E	ND	$C_{31}H_{29}ClN_4O_3$	HR-MS
41	Cl			4.6 ± 0.6	101	E	ND	$C_{31}H_{28}Cl_2N_4O_3$	HR-MS
42	Cl			7.9 ± 1.9	178	E	ND	$C_{30}H_{26}ClFN_4O_3$	HR-MS
43	Cl			12.3 ± 2.4	160	E	ND	$C_{30}H_{26}ClFN_4O_3$	HR-MS
44	Cl			441 ± 13	ND	E	120-150	$C_{25}H_{27}ClN_4O_3.HCl.H_2O$	CHN
45	Cl			6.2 ± 0.5	ND	E	160-164	$C_{31}H_{31}ClN_4O_3$	HR-MS

Table 4 (Continued)



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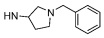
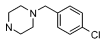
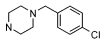
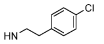
Compd	R	A	NR1R2	hGluR binding affinity[a](nM)	*In vitro* metabolism (pmol/min/mg)	Prep. method	M.p. (°C)	Formula	Anal.
46	CN			10.4 ± 3.0	240	E	168-172	$C_{32}H_{31}N_5O_3.2.5H_2O$	CHN
47	Cl			51.7 ± 7.5	ND	E	233-234	$C_{26}H_{19}Cl_2N_3O_4.1.5H_2O$	CHN
48	Cl			89.3 ± 8.2	187	E	216.8-217.1	$C_{31}H_{29}ClN_4O_4.2H_2O$	CHN
49	Cl			11.3 ± 1.5	77	E	ND	$C_{30}H_{26}Cl_2N_4O_3$	HR-MS
50	CN			4.9 ± 1.4	53	E	>250	$C_{31}H_{26}ClN_5O_3.0.75H_2O$	CHN
51	Cl			5.9 ± 1.6	32	E	>250	$C_{27}H_{21}Cl_2N_3O_3$	HR-MS

[a] Binding affinity of compounds bound to the recombinant human glucagon receptor in BHK cells. Data are expressed as mean IC_{50} ± SD, $n = 3$. ND: not determined.

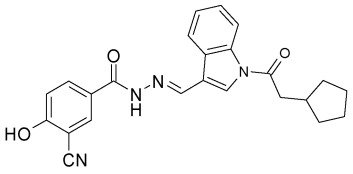
that gave compounds with diminished metabolic turnover without losing potency. Thus, the in vitro metabolic turnover of **32** was quite high but was markedly reduced by exchanging the 3-chloro substituent with a 3-cyano (**33**) without loosing potency (267 vs 65 pmol min^{-1} mg^{-1} protein). Further investigations showed that the reduced metabolic turnover of **33** was mainly due to decreased glucuronidation of the phenol moiety. This example demonstrates the importance of improving potency and metabolic stability in parallel in order to get access to druglike compounds.

Another series of amide compounds where the amide linker was reversed were investigated (Table 5), since it was hypothesized that reversing the amide functionality might improve the metabolic stability because *N*-acylindoles and anilides cannot be oxidatively metabolized by the same mechanism as the previous amides. Also, in this series of reversed amides, high-affinity ligands could be obtained without aromatic distal groups. The aliphatic amides **54** and **57** had moderate affinities (39 and 33 nM, respectively) and increasing the lipophilicity and size of the distal moiety by an extra *N*-acyl substituent improved the affinity to low nanomolar levels for **55** and **56** (3.4 and 2.8 nM, respectively).

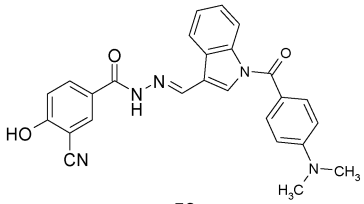
During this optimization of the compounds, it was decided to replace the lipophilic naphthalene core for improvement of the aqueous solubility, thereby decreasing the oxidative metabolic turnover. Previously, it was found that this core must be very electron-rich. Consequently, incorporation of monomethoxyaniline systems as alternatives to the aminonaphthalene system was investigated, and such compounds were found to possess good binding properties; e.g., the phenylacetanilide **58** had $IC_{50} = 29$ nM. Homologation to the 3-phenylpropionanilides **60** and **62** and to the phenylthioacetanilide **63** gave similarly potent compounds (21, 34, and 15 nM, respectively). Compounds included in the libraries with methyl substituents on the proximal phenyl ring or with a 1,3-branched substitution pattern did not result in hits when screened in the binding assay. These results indicated the preference for electron-rich 1,4-branched proximal aromatic rings. Additionally, it was found that for these reversed amides an indole could replace the proximal naphthalene moiety to give high-affinity compounds, **52** and **53** with $IC_{50} = 6$ and 19 nM, respectively. Unfortunately, none of these amides had suitable pharmacokinetic properties because of high metabolic turnovers (oxidative metabolisms).

As mentioned above, an indole was a good naphthalene replacement in the series of "reversed amides" (**52**) and not in the amide series (**31**). This was intriguing and prompted a reinvestigation of compounds with indole as the proximal aromatic ring and lipophilic linkers tethering the distal aromatic ring (*N*-benzylindoles, Table 6). It was found that substituted 1,4-branched indoles gave potent compounds and that compounds with multiple methyl substituents on the distal phenyl group resulted in very potent compounds (**73**, 5.3 nM; **72**, 5.8 nM). It was also found that the branching of the indole ring was very important because the 1,4- was much more potent than the 1,5- and 1,3-branched analogues (**71**, **75**, and **76**, respectively). The importance of the substituents in the benzyl part of the molecules regarding affinity was evidenced by the reduced potency of the unsubstituted **77**. As observed in the other series of compounds in this publication, the ortho chlorophenol moiety could be replaced by an ortho

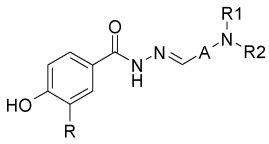
Table 5. Structures, Binding Affinities, Rates of in Vitro Metabolism, and Physical Properties of Synthesized Compounds



52



53



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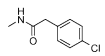
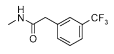
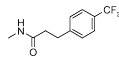
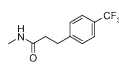
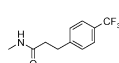
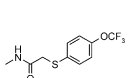
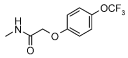
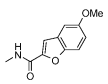
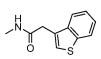
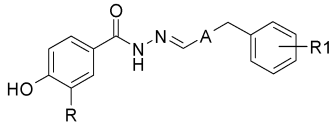
Compd	R	-A-	-NR1R2	hGluR binding affinity[a] (nM)	*In vitro* metabolism (pmol/min/mg)	Prep. method	M.p. (°C)	Formula	Anal.
52				6.4 ± 1.3	136	F	235-237	$C_{24}H_{22}N_4O_3.0.5H_2O$	CHN
53				19.1 ± 4.7	606	F	ND	$C_{27}H_{23}N_5O_3$	HR-MS
54	Cl			38.8 ± 9.4	134	F	>250	$C_{24}H_{22}ClN_3O_3$	CHN
55	Cl			3.4 ± 0.6	ND	F	>250	$C_{30}H_{30}ClN_3O_4$	CHN
56	CN			2.8 ± 0.2	250	F	>250	$C_{31}H_{30}N_4O_4.0.25H_2O$	CHN
57	Cl			33.0 ± 7.3	45	F	ND	$C_{23}H_{22}ClN_3O_3$	HR-MS
58	CN			28.7 ± 1.1	ND	H	193.7-197.8	$C_{24}H_{19}ClN_4O_4.2.5H_2O$	CHN[b]
59	CN			47.8 ± 8.9	51	H	257-260	$C_{25}H_{19}F_3N_4O_4.1.25H_2O$	CHN
60	Cl			21.2 ± 3.6	107	G	233-235	$C_{25}H_{21}ClF_3N_3O_4$ ¼ H_2O	CHN
61	CN			15.7 ± 4.0	ND	H	>250	$C_{26}H_{21}F_3N_4O_4.1.75H_2O$	CHN[c]
62	Cl			33.5 ± 4.1	ND	G	235-236.5	$C_{24}H_{19}ClF_3N_3O_6$	CHN
63	Cl			14.5 ± 2.6	23	G	223-225	$C_{24}H_{19}ClF_3N_3O_4S.0.75H_2O$	CHN
64	Cl			73.0 ± 10.9	136	G	223-224.9	$C_{24}H_{19}ClF_3N_3O_6.1.75H_2O$	CHN
65	Cl			90.6 ± 15.7	31	G	250-253	$C_{25}H_{20}ClN_3O_6$ ¼ H_2O	CHN
66	Cl			63.2 ± 7.1	74	G	>160 (dec)	$C_{25}H_{20}ClN_3O_4S$ 1.5 H_2O	CHN

[a] Binding affinity of compounds bound to the recombinant human glucagon receptor in BHK cells. Data are expressed as the mean IC_{50} ± SD, $n = 3$. ND: not determined. [b] N: calcd, 11.03; found, 10.09. [c] N: calcd, 10.34; found, 9.81.

cyanophenol moiety without a decrease in receptor affinity, and by this minor structural change, the rate of in vitro metabolism was significantly reduced (**73** vs **74** (105 vs 34 pmol min^{-1} mg^{-1} protein) and **67** vs **68** (116 vs 59 pmol min^{-1} mg^{-1} protein)). The metabolic profile obtained in rat hepatocytes of **74**, with IC_{50} = 2.3 ± 0.6 nM, the most potent and in vitro metabolically stable compound from this indole series, was investigated (see Figure 3). The major metabolic route was oxidation of the methyl groups on the distal aromatic ring; also observed was conjugation of the parent compound, resulting in both the glucuronide and the sulfate. N-Debenzylation of the indole ring was a minor metabolic route. The high binding affinity and low rate of in vitro metabolism of **74** prompted a more detailed in vitro and in vivo evaluation of this compound.

In Vitro Pharmacology. A functional in vitro characterization of **74** was carried out (see Figures 4 and

Table 6. Structures, Binding Affinities, Rates of in Vitro Metabolism, and Physical Properties of Synthesized Compounds



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Compd	R	-A-	R1	hGluR binding affinity[a] (nM)	*In vitro* metabolism (pmol/min/mg)	Prep. method	M.p. (°C)	Formula	Anal.
67	Cl		4-CF_3	33.8 ± 4.3	116	I	155-156	$C_{24}H_{17}ClF_3N_3O_2$	CHN
68	CN		4-CF_3	22.3 ± 1.8	59	I	222-223	$C_{25}H_{17}F_3N_4O_2.H_2O$	CHN
69	Cl		3,4-Cl_2	27.5 ± 4.2	190	I	167-168	$C_{23}H_{16}Cl_3N_3O_2.0.25H_2O$	CHN
70	Cl		4-^{i}Pr	23.0 ± 3.4	48	I	188.5-192.3	$C_{26}H_{24}ClN_3O_2$	CHN
71	Cl		4-OCF_3	22.9 ± 3.2	ND	I	163-165.7	$C_{24}H_{17}ClF_3N_3O_3$	CHN
72	Cl		2,4,6-Me_3	5.8 ± 1.0	ND	I	221.9-222.5	$C_{26}H_{24}ClN_3O_2.H_2O$	CHN
73	Cl		2,3,5,6-Me_4	5.3 ± 0.9	105	I	>250	$C_{27}H_{26}ClN_3O_2.1.25H_2O$	CHN
74	CN		2,3,5,6-Me_4	2.3 ± 0.6	34	I	>250	$C_{28}H_{26}N_4O_2.0.25H_2O$	CHN
75	Cl		4-OCF_3	286 ± 51	ND	I	159.1-162.7	$C_{24}H_{17}ClF_3N_3O_3.0.25H_2O$	CHN
76	Cl		4-$OCHF_2$	305 ± 52	ND	I	203-205.6	$C_{24}H_{18}ClF_2N_3O_3$	HR-MS
77	Cl		(unsubst.)	615 ± 114	ND	I	>250	$C_{23}H_{18}ClN_3O_2.0.25H_2O$	CHN

[a] Binding affinity of compounds bound to the recombinant human glucagon receptor in BHK cells. Data are expressed as the mean IC_{50} ± SD, $n = 3$. ND: not determined.

5). It was shown to be a mixed competitive and noncompetitive antagonist of the human receptor because it right-shifted the glucagon dose−response curve and also lowered the maximal response (Figure 4). K_B was calculated[22] for the individual experiments and found to be 0.76 ± 0.18 nM. Similarly, by use of isolated rat liver membranes, **74** was shown to be a highly potent, truly noncompetitive antagonist of the rat receptor with IC_{50} = 0.43 ± 0.1 nM and K_B = 0.38 ± 0.26 nM. The maximal response in the glucagon dose−response assay was lowered with only a slight right-shift (Figure 5). This species difference may be explained by the fact that there are more spare receptors in the BHK cells expressing the human receptor than in the isolated rat liver.

Primary Hepatocytes. The effect of **74** on glucagon-induced glycogenolysis was studied in primary cultured rat hepatocytes. **74** inhibited glucagon-induced (0.5 nM) glycogenolysis (glycogen breakdown) dose-dependently with an IC_{50} value of 160 ± 90 nM (data not shown). Likewise, the glucose production (glycogenolysis) from hepatocytes was inhibited with an IC_{50} value of 120 ± 20 nM (data not shown). These two IC_{50} values are not significantly different, and therefore, glucose production was used as a measure of hepatic glycogenolysis in the following study. To characterize the mode of inhibition in rat hepatocytes, a functional K_i was determined (Figure 6). The mode of inhibition was found to be competitive with a right-shift of the dose−response curve and no effect on the maximal response. K_i was calculated from four individual experiments and found to be 14.1 ± 3.9 nM. The higher K_i value in this experiment compared to the functional data on the isolated rat liver glucagon receptor is possibly due to

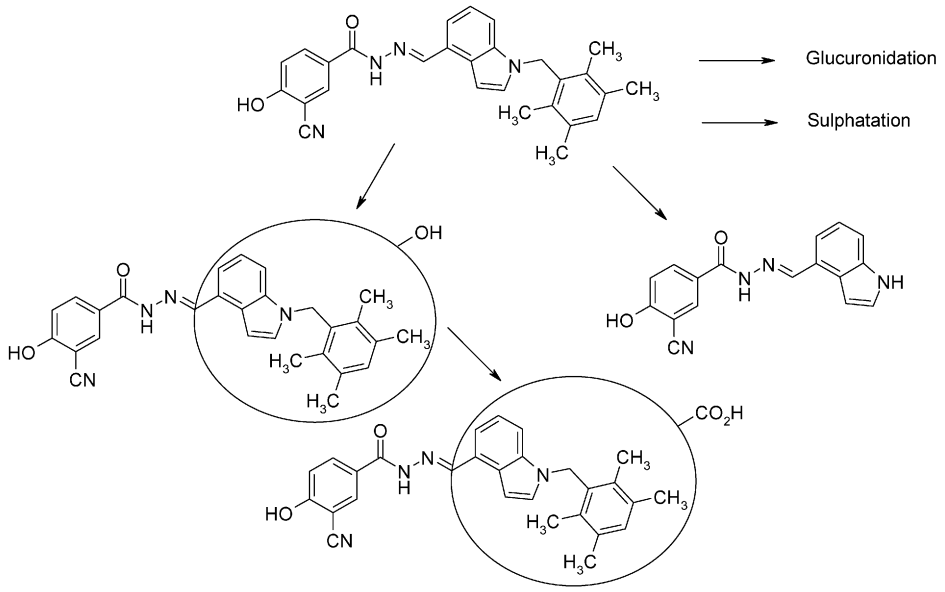

Figure 3. Metabolic profile of **74** in rat hepatocytes analyzed by LC−MS−MS.

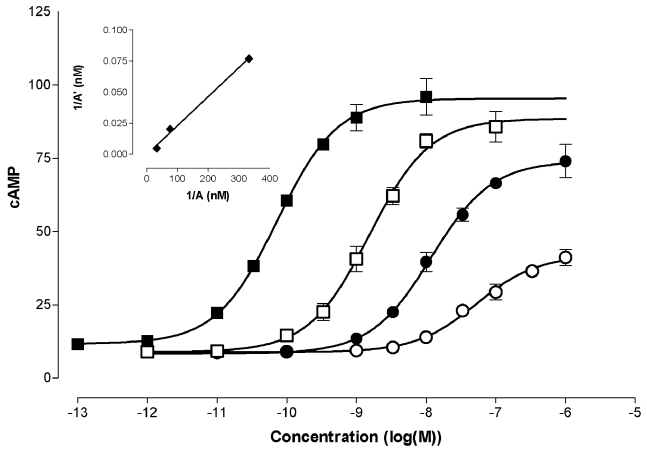

Figure 4. Inhibition of glucagon-stimulated cAMP by **74** using the recombinant human glucagon receptor in BHK cells. Shown is the observed shift in the glucagon dose−response curve by addition of **74**: glucagon (■), glucagon + 25 nM **74** (□), glucagon + 250 nM **74** (●), glucagon + 2500 nM **74** (○). Shown are data from one representative experiment out of three (mean ± SD of triplicate samples). Inserted is the K_B plot, where the 2500 nM **74** curve was used. K_B was determined to be 0.76 ± 0.18 nM.

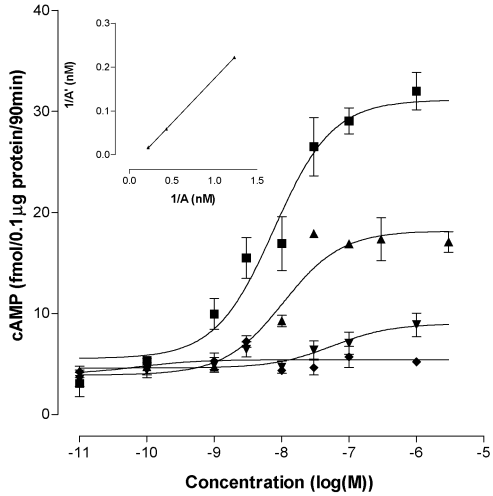

Figure 5. Inhibition of glucagon-stimulated cAMP by **74** using the isolated rat liver glucagon receptor. Shown is the observed shift in the glucagon dose−response curve by addition of **74**: glucagon (■), glucagon + 1 nM **74** (▲), glucagon + 10 nM **74** (▼), glucagon + 100 nM **74** (◆). Shown are data from one representative experiment out of three (mean ± SD of triplicate samples). Inserted is the K_B plot, where the 1 nM **74** curve was used. K_B was determined to be 0.38 ± 0.26 nM.

the presence of 0.1% human serum albumin in the incubation buffer, since, as previously mentioned,[23] this compound is able to bind to albumin. A further difference from isolated membranes is that primary hepatocytes are metabolically active. The discrepancy in the mode of inhibition, noncompetitive in isolated rat liver membranes versus competitive in the primary rat hepatocytes, is not obvious.

Pharmacokinetics. To assess the pharmacokinetic properties of **74**, the compound was dosed to purpose-bred male dogs. The PK profiles (Figure 7A) after intravenous administration of 0.5 mg/kg **74** gave uniform profiles with a harmonic mean elimination half-life of 1.11 h. After per oral administration by gavage (2.0 mg/kg) and use of the same dosing solution as used for intravenous administration (Figure 7B), three profiles were obtained with very fast absorption (t_{max} = 30−45 min) and one dog showed some delay with respect to absorption (t_{max} = 75 min). The elimination half-life after per oral administration was slightly longer than after intravenous administration with a harmonic mean half-life of 1.4 h. The bioavailability was 15%. The PK analysis was performed using noncompartmental methods.

In Vivo Pharmacology. Results from the in vivo test of **74** in the glucagon-challenged rat model are shown in Figure 8. At 3 mg/kg, **74** was inactive. However, at 10 mg/kg, the hyperglycaemic effect of the exogenously administered glucagon was completely abolished. In fact, the change (Δ) in blood glucose value after administration of 10 mg/kg was negative (below basal level), which suggests that the compound may also, at least partly, have inhibited the action of the endogenous glucagon responsible for maintenance of euglycemia.

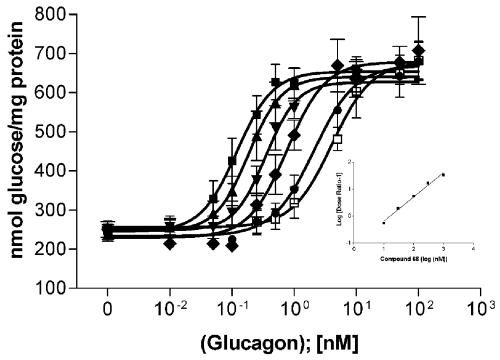

Figure 6. Inhibition of glucagon-stimulated glucose production in primary rat hepatocytes by **74**. The hepatocytes were incubated with glucagon and increasing concentrations of **74**: 0 nM (■), 10 nM (▲), 30 nM (▼), 100 nM (◆), 300 nM (●), and 1000 nM (□). Results are presented as the mean ± SEM of four independent experiments (*n* is defined as the number of separate hepatocytes preparations). Inserted is the Schild plot.

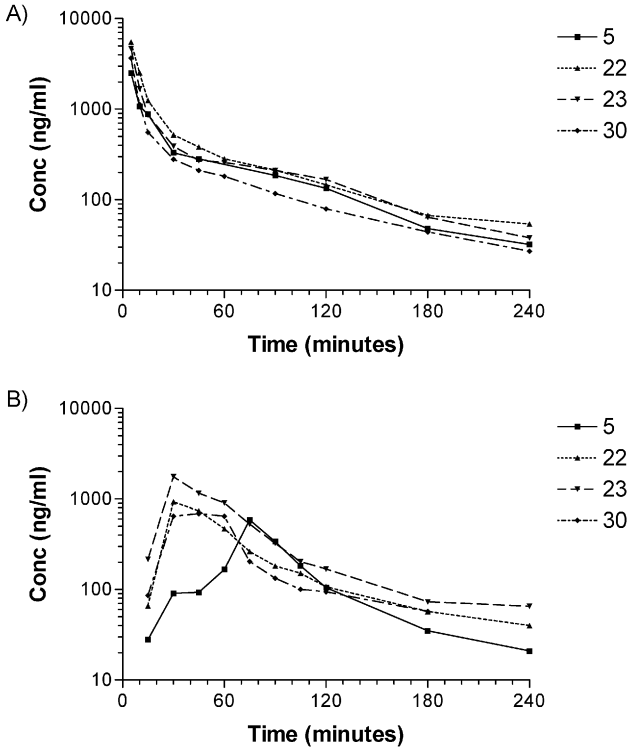

Figure 7. Plasma concentration vs time plots of **74** after (A) intravenous dosing of 0.5 mg/kg to dogs and (B) peroral administration of 2.0 mg/kg by gavage to dogs. Legends identify dogs. Mean half-lives were 1.11 and 1.40 h after iv and po administration, respectively. The mean oral bioavailability was 15%.

Conclusions

Optimization of the lead structures **1** and **2** led to series of high-affinity human glucagon receptor antagonists, addressing pharmacokinetic issues with in vitro metabolism screening and metabolite profiling while optimizing hGluR affinity. The SAR of these series revealed that it was not necessarily a requirement to have a distal aromatic ring to obtain high-affinity glucagon receptor antagonists, since, for example, the diethyl acetamide (**29**) and the 2-ethylpiperidyl acetamide (**34**) had IC_{50} values of 17 and 7.4 nM, respectively. The presence of a distal aromatic ring, however, often increased the affinity further.

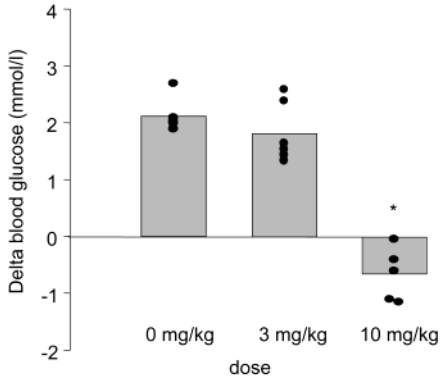

Figure 8. Test of **74** in glucagon-challenged Sprague−Dawley rats. Dots represents individual changes (Δ) in the values, and bars represent the corresponding mean values (n = 5−6 per dose). The asterisk (∗) represents $p < 0.0005$ vs vehicle (0 mg/kg).

The SAR was quite insensitive toward structural changes in the linker region. High-affinity compounds could be obtained with both long and short linkers as well as with polar and apolar linkers. One noteworthy exception was the activity of the enantiomers **14** and **15** with (*R*) and (*S*) configurations, respectively, where the (*R*)-isomer was 10-fold more potent than the (*S*)-isomer.

One promising compound (**74**) with low in vitro metabolic turnover was a highly potent noncompetitive antagonist of the human glucagon receptor (K_B = 760 pM) and of the isolated rat receptor (K_B = 380 pM). Glucagon-stimulated glucose production from isolated primary rat hepatocytes was inhibited competitively by **74** (K_i = 14 nM). This compound was orally available in dogs (F_{po} = 15%) and was active in a glucagon-challenged rat model of hyperglucagonemia and hyperglycemia.

Experimental Section

General. ^{1}H NMR spectra were recorded in deuterated solvents at 200, 300, and 400 MHz (DRX 200, DRX 300, and AMX2 400 from Bruker Instruments, respectively). Chemical shifts are reported as δ values (ppm) relative to internal tetramethylsilane (δ = 0 ppm). Elemental analyses were performed by the microanalytical laboratories at Novo Nordisk A/S, Denmark, and at Numega and Gailbraith. Column chromatography[24] was performed on silica gel 60 (40−63 μm). Melting points were determined in open capillary tubes on a Büchi 535 apparatus and are uncorrected. Chemicals and solvents used were commercially available and were used without further purification. Yields refer to pure materials and are not optimized. The preparation methods are illustrated by single representative experimental procedures. Further experimental details (including NMR data) have been published in the patent applications.[25,26]

3-Chloro-4-hydroxybenzoic Acid Hydrazide (I). To methyl 3-chloro-4-hydroxybenzoate (2 g, 11 mmol) dissolved in EtOH (50 mL) was added hydrazine (1.8 mL). The reaction mixture was refluxed overnight under nitrogen. When the mixture was cooled, the desired product crystallized. The white solid was isolated by filtration. Recrystallization from hot EtOH afforded 1.2 g (60%) of 3-chloro-4-hydroxybenzoic acid hydrazide (**I**). ^{1}H NMR (DMSO-d_6): δ 4.49 (bs, 2H), 7.05 (dd, 1H), 7.71 (dd, 1H), 7.89 (d, 1H), 9.69 (s, 1H), 10.72 (bs, 1H).

3-Fluoro-4-hydroxybenzoic Acid Hydrazide (II). ^{1}H NMR (DMSO-d_6): δ 9.45 (bs, 1H), 7.5 (d, 1H), 7.43 (d, 1H), 6.85 (t, 1H), 5.55 (bs, 3H).

2,3-Dichloro-4-hydroxybenzoic Acid Hydrazide (III). 4-Amino-2-chlorobenzoic acid (5 g, 29 mmol) was dissolved in

H_2SO_4 (12 N, 120 mL) with heating. After the solution was cooled in an ice bath, aqueous $NaNO_2$ (2.5 M, 25 mL) was added dropwise such that the internal temperature remained at 5 °C. Urea was added to the mixture after stirring for 15 min to destroy excess $NaNO_2$ (monitored by starch iodine test). $CuSO_4$ (100−200 mg) was added, and the mixture was heated to 90 °C until evolution of gas stopped. After cooling, the mixture was extracted with ethyl ether (3×). The combined organic fractions were extracted with 3 N NaOH (3×). The combined aqueous layer was acidified with concentrated HCl, and the product was extracted with ethyl ether (3×). The organic fractions were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue 2-chloro-4-hydroxybenzoic acid was dissolved in anhydrous MeOH and added thionyl chloride (1.5 equiv). After the solution was stirred at room temperature for 16 h, the solvent was evaporated. The residue was taken up in EtOAc and washed with saturated aqueous $NaHCO_3$, water, and brine, dried over $MgSO_4$, and concentrated in vacuo to afford 3.5 g (65%) of methyl 2-chloro-4-hydroxybenzoate. ^{1}H NMR ($CDCl_3$): δ 3.91 (s, 3H), 6.67 (bs, 1H), 6.80 (dd, 1H), 6.97 (d, 1H), 7.83 (d, 1H).

To a solution of methyl 2-chloro-4-hydroxybenzoate (13.6 g, 73.1 mmol) in acetic acid (300 mL) was added *N*-chlorosuccinimide (9.8 g, 73.7 mmol). The solution was refluxed for 24 h, and the solvent was evaporated in vacuo. The residue was taken up in chloroform, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. Methyl 2,3-dichloro-4-hydroxybenzoate precipitated out of EtOAc. Chromatography of the residue using EtOAc/hexane (1:9 to 3:7) afforded methyl 2,5-dichloro-4-hydroxybenzoate (1.4 g, 9%) as well as an additional crop of methyl 2,3-dichloro-4-hydroxybenzoate (total yield 8.4 g, 52%). ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 3H), 7.02 (d, 1H), 7.70 (d 1H), 11.52 (s, 1H).

2,3-Dichloro-4-hydroxybenzoic acid hydrazide was prepared from the methyl 2,3-dichloro-4-hydroxybenzoate as described above with the exception that pentanol was used as the solvent. The product was purified via silica gel column chromatography using DCM/MeOH (95:5 to 80:20) to afford 2,3-dichloro-4-hydroxybenzoic acid hydrazide (**III**) (50%). ^{1}H NMR (DMSO-d_6): δ 4.41 (bs, 2H), 6.99 (1, 1H), 7.37 (s, 1H), 9.46 (s, 1H), 11.04 (s, 1H).

3-Cyano-4-hydroxybenzoic Acid Hydrazide (IV). Methyl 4-hydroxybenzoate (35.5 g, 0.233 mol) was dissolved in warm (65 °C) HOAc (200 mL). A solution of iodine monochloride (37.8 g, 0.233 mol) in HOAc (50 mL) was added over 40 min while maintaining a temperature of 65 °C and vigorous stirring. The product crystallized from solution upon cooling to room temperature and standing overnight. The crystals were filtered, washed with water, and dried in vacuo to afford 28.6 g (44%) of methyl 4-hydroxy-3-iodobenzoate. ^{1}H NMR (DMSO-d_6): δ 3.79 (s, 3H), 6.95 (d, 1H), 7.81 (dd, 1H), 8.22 (d, 1H).

Methyl 4-hydroxy-3-iodobenzoate (2.00 g, 7.2 mmol) was dissolved in dry DMF (5 mL). CuCN (0.72 g, 8.0 mmol) and a small crystal of NaCN were added. The mixture was flushed with nitrogen and stirred at 100−110 °C for 16 h. The mixture was cooled and filtered. The solids were extracted with DMF (3 mL). The combined filtrate and washings were dissolved in EtOAc (100 mL) and washed with brine (3×). The solids and aqueous washings were combined and extracted with a mixture of EtOAc (50 mL) and an $FeCl_3$ solution (4 g of hydrated $FeCl_3$ in 7 mL of concentrated HCl). The EtOAc phases were combined, washed with brine containing sodium metabisulfite, dried (Na_2SO_4), filtered, and evaporated in vacuo. The residue was purified by flash chromatography on silica gel (20% EtOAc/ hexane) to afford 0.93 g (73%) of methyl 3-cyano-4-hydroxybenzoate. ^{1}H NMR (DMSO-d_6): δ 3.79 (s, 3H), 7.07 (d, 1H), 8.02 (dd, J = 8.7, 1.9, 1H), 8.10 (d, 1H).

Methyl 3-cyano-4-hydroxybenzoate (2.71 g, 15.3 mmol) was dissolved in THF (50 mL). The solution was cooled in an ice bath, and 2.0 M KOH (17 mL, 34 mmol) was added dropwise. The resulting mixture was stirred at room temperature overnight. The organic solvent was removed by rotary evaporation in vacuo, and the aqueous residue was acidified with 1 N HCl. The product was extracted with EtOAc (3×), and the combined organic extracts were washed with water (3×) and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was recrystallized from MeOH/H_2O to afford 2.1 g (84%) of 3-cyano-4-hydroxybenzoic acid. ^{1}H NMR (DMSO-d_6): δ 7.09 (d, 1H), 8.00 (dd, 1H), 8.07 (d, 1H), 12.50 (bs, 2H).

3-Cyano-4-hydroxybenzoic acid (1.88 g, 11.5 mmol) was dissolved in a 1:1 mixture of DCM/DMF (20 mL) and cooled in an ice bath. Diisopropylethylamine (12 mL, 69 mmol), *tert*-butyl carbazate (1.76 g, 13.3 mmol), and PyBroP (bromotrispyrrolidinophosphonium hexafluorophosphate, 6 g, 12.9 mmol) were added, and the mixture was stirred to form a clear solution. The solution was left at 5 °C for 16 h. Additional diisopropylethylamine (22 mL, 127 mmol), *tert*-butyl carbazate (0.85 g, 6.4 mmol), and PyBroP (3.0 g, 6.4 mmol) were added. After 8 h at 0 °C, the mixture was concentrated in vacuo. The residue was diluted with EtOAc (100 mL) and washed with several portions of 0.1 M HCl (until the wash remained acidic to litmus paper). The EtOAc layer was further washed with brine (three portions), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (6:4 hexane/EtOAc) to afford 1.8 g (56%) of *tert*-butoxycarbonyl (3-cyano-4-hydroxy)benzoic acid hydrazide as a white solid. ^{1}H NMR (DMSO-d_6): δ 1.42 (s, 9H), 7.09 (d, 1H), 7.98 (m, 1H), 8.11 (bs, 1H), 8.92 (s, 1H), 10.15 (s, 1H), 11.73 (bs, 1H).

tert-Butoxycarbonyl(3-cyano-4-hydroxy)benzoic acid hydrazide (0.554 g, 2 mmol) was suspended in cold water (10 mL) and cooled in an ice bath. Concentrated HCl (10 mL) was added, and the mixture was stirred at room temperature. Upon being stirred, the suspension became a solution and then turned back into a suspension. The reaction vessel was warmed until a solution was obtained. Upon sitting overnight, the product crystallized as white needles. This afforded 0.24 g (68%) of 3-cyano-4-hydroxybenzoic acid hydrazide (**IV**). ^{1}H NMR (DMSO-d_6): δ 7.16 (d, 1H), 8.00 (dd, 1H), 8.14 (d, 1H), 10.47 (bs, 5H).

Preparation Method A. 3-Chloro-4-hydroxybenzoic Acid (4-Hydroxymethylnaphthalen-1-ylmethylene)hydrazide (3). 1,4-Naphthalenedicarboxylic acid (25 g, 116 mmol) was added in portions to a mixture of $LiAlH_4$ (15 g, 395 mmol) in anhydrous THF (600 mL) and refluxed for 2 days. The mixture was cooled in an ice bath, and excess $LiAlH_4$ was decomposed by the slow addition of MeOH followed by ice chips. THF was removed in vacuo, and the residue was acidified with 1 N HCl. The aqueous phase was extracted with EtOAc (3×), and the combined organic phases were washed with aqueous $NaHCO_3$ (3×), water, and brine, dried ($MgSO_4$), and evaporated in vacuo to afford 15.3 g (70%) of 1,4-bishydroxymethylnaphthalene as a solid. This product can be used in the subsequent oxidation step without further purification. ^{1}H NMR (DMSO-d_6): δ 5.19 (s, 4H), 7.77 (m, 4H), 8.32 (m, 2H).

To a solution of 1,4-bishydroxymethylnaphthalene (12 g, 65 mmol) in EtOAc (300 mL) was added MnO_2 (28 g, 325 mmol). After being stirred for 45 min, the mixture was passed through a bed of Celite and eluted with additional volumes of EtOAc. The solvent was evaporated in vacuo, and the residue was purified by column chromatography using hexane/EtOAc (80:20 to 75:25) to afford 6.0 g (50%) of 4-hydroxymethyl-1-naphthaldehyde (**V**). ^{1}H NMR (DMSO-d_6): δ 5.19 (s, 2H), 5.71 (brd s, 1H), 7.73 (t, 1H), 7.78 (t, 1H), 7.95 (d, 1H), 8.26 (m, 2H), 9.34 (d, 1H), 10.46 (s, 1H).

3-Chloro-4-hydroxybenzoic acid hydrazide (**I**) (186 mg, 1 mmol) was dissolved in DMSO (1 mL) and added to a solution of 4-hydroxymethyl-1-naphthaldehyde (**V**) (186 mg, 1 mmol) in DMSO (0.5 mL) followed by HOAc (50 μL). The mixture was stirred at 25 °C for 16 h. The mixture was diluted with EtOAc (10 mL) and washed with brine (10 mL) and water (5 mL) and dried ($MgSO_4$). As the solvent was evaporated, the product (276 mg, 78%) 3-chloro-4-hydroxybenzoic acid (4-hydroxymethylnaphthalen-1-ylmethylene)hydrazide (**3**) precipitated out of solution. Mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 5.02 (s, 2H), 5.43 (t, 1H), 7.10 (d, 1H), 7.66 (m, 3H), 7.80 (d, 1H), 7.90 (d, 1H), 8.02 (s, 1H), 8.15 (d, 1H), 8.87 (d, 1H), 9.08

(s, 1H), 10.98 (s, 1H), 11. 79 (s, 1H). Anal. ($C_{19}H_{15}ClN_2O_3$): C, H. N: calcd, 7.90; found, 7.21.

5-Hydroxymethyl-1-naphthaldehyde (VI). To a suspension of 5-bromo-1-naphthoic acid (5 g, 20 mmol) in anhydrous MeOH (200 mL) was added concentrated H_2SO_4 (5 mL), and the resulting mixture was refluxed overnight. The mixture was cooled and concentrated in vacuo to one-third the volume. The residue was diluted with water and extracted with Et_2O. The organic phase was washed with water (2×), dried ($MgSO_4$), and concentrated in vacuo. Silica gel column chromatography using hexane/EtOAc (2:1) afforded 4.91 g (92%) of methyl 5-bromo-1-naphthylcarboxylate. ^{1}H NMR ($CDCl_3$): δ 4.01 (s, 3H), 7.44 (dd, 1H), 7.61 (dd, 1H), 7.85 (dd, 1H), 8.22 (dd, 1H), 8.51 (d, 1H), 8.90 (d, 1H).

A mixture of methyl 5-bromo-1-naphthylcarboxylate (5.2 g, 19 mmol) and CuCN (3.4 g, 38 mmol) in anhydrous DMF (100 mL) was refluxed overnight. After the mixture was cooled to 70 °C, a solution of NaCN (2 g) in water (50 mL) was added. The mixture was extracted with EtOAc. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Column chromatography on silica gel using hexane/EtOAc (5:1) afforded 3.8 g (95%) of methyl 5-cyano-1-naphthylcarboxylate. ^{1}H NMR ($CDCl_3$): δ 4.01 (s, 3H), 7.60−7.80 (m, 3H), 7.98 (d, 1H), 8.30 (d, 1H), 8.45 (d, 1H), 9.21 (d, 1H).

To a cooled (0 °C) solution of methyl 5-cyano-1-naphthylcarboxylate (1 g, 5 mmol) in anhydrous THF (20 mL) was added DIBAL (1 M in hexane, 20 mL, 20 mmol) via syringe. The mixture was stirred at 50−60 °C overnight. The mixture was then cooled to room temperature and poured into cold (0 °C) 2 N HCl (100 mL). The mixture was extracted with Et_2O (2×), and the organic phase was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Silica gel column chromatography using hexane/EtOAc (2:1) afforded 0.85 g (91%) of 5-hydroxymethyl-1-naphthaldehyde (**VI**). ^{1}H NMR ($CDCl_3$): δ 4.95 (s, 2H), 7.45−7.58 (m, 3H), 7.82 (dd, 1H), 8.24 (d, 1H), 9.03 (dd, 1H), 10.21 (s, 1H).

3-Chloro-4-hydroxybenzoic Acid (4-Methoxymethylnaphthalen-1-ylmethylene)hydrazide (8). 4-Hydroxymethyl-1-naphthaldehyde (**V**) (7 g, 37 mmol) was suspended in aqueous HBr (50 mL, 48%) and cooled in an ice bath. Concentrated H_2SO_4 (50 mL) was added, and the mixture was stirred for 4 h, diluted with EtOAc, and washed with brine (3×) and aqueous $NaHCO_3$ (3×), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified though a bed of silica gel using ethyl acetate/hexane (5−50%). Two recrystallizations from EtOAc afforded 7 g (75%) of 4-bromomethyl-1-naphthaldehyde (**VIII**). ^{1}H NMR (DMSO-d_6): δ 5.27 (s, 2H), 7.76 (m, 2H), 7.92 (d, 1H), 8.13 (d, 1H), 8.15 (m, 1H), 9.20 (, 1H), 10.39 (s, 1H).

To a solution of 4-bromomethyl-1-naphthaldehyde (1 g, 4 mmol) in toluene (70 mL) was added ethylene glycol (2.2 mL, 40 mmol) followed by a catalytic amount of *p*-TsOH. The mixture was refluxed overnight and diluted with EtOAc. The mixture was washed with brine (3×), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified via silica gel column chromatography using ethyl acetate/hexane (5:95) to afford 0.51 g (44%) of 2-(4-bromomethylnaphthalen-1-yl)-[1,3]-dioxolane. ^{1}H NMR (DMSO-d_6): δ 4.10 (m, 4H), 5.22 (s, 2H), 6.38 (s, 1H), 7.59−7.71 (m, 4H), 8.24 (t, 2H).

To a solution of 2-(4-bromomethylnaphthalen-1-yl)-[1,3]-dioxolane (0.2 g, 0.68 mmol) in anhydrous DMF (20 mL) was added sodium methoxide (0.75 mmol). After the mixture was stirred for 1 h, the mixture was diluted with EtOAc and washed with brine (3×), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was dissolved in acetone (7 mL), concentrated HCl (2 mL) was added, and the mixture was stirred for 16 h. The mixture was diluted with EtOAc, washed with brine (3×), dried ($MgSO_4$), and concentrated in vacuo to afford 0.15 g (100%) of 4-methoxymethyl-1-naphthaldehyde (**VII**). ^{1}H NMR (DMSO-d_6): δ 3.52 (s, 3H), 4.97 (s, 2H), 7.62−7.72 (m, 3H), 7.96 (d, 1H), 8.10 (d, 1H), 9.32 (d, 1H), 10.37 (s, 1H).

To a solution of 4-hydroxy-3-chlorobenzoic acid hydrazide (**I**) (0.15 g, 0.8 mmol) in DMSO (10 mL) was added 4-methoxymethyl-1-naphthaldehyde (0.15 g, 0.70 mmol) followed by a catalytic amount of acetic acid. The reaction was stirred at room temperature for 16 h. The mixture was diluted with EtOAc, washed with aqueous $NaHCO_3$ (3×) and brine (3×), dried ($MgSO_4$), and filtered. Upon concentration of the solvent in vacuo, 69 mg (23%) of 3-chloro-4-hydroxybenzoic acid (4-methoxymethylnaphthalen-1-ylmethylene)hydrazide (**8**) precipitated out of solution and was isolated by filtration, mp 204−206.5 °C. ^{1}H NMR (DMSO-d_6): δ 3.43 (s, 3H), 4.91 (s, 2H), 7.10 (d, 1H), 7.62−7.71 (m, 3H), 7.82 (d, 1H), 7.91 (d, 1H), 8.01 (s, 1H), 8.13 (d. 1H), 8.85 (d, 1H), 9.09 (s, 1H), 11.0 (brd s, 1H), 11.82 (s, 1H). Anal. ($C_{20}H_{17}ClN_2O_3 \cdot 0.25H_2O$) C, H, N.

Preparation Method B. 3-Chloro-4-hydroxybenzoic Acid [5-(4-Trifluoromethoxybenzyloxymethyl)naphthalene-1-ylmethylene]hydrazide (10). To a solution of 5-hydroxymethyl-1-naphthaldehyde (**VI**) (0.5 g, 2.1 mmol), 4-trifluoromethoxybenzyl bromide (0.64 g, 2.5 mmol), and 100 mg of *n*-Bu_4NCl (100 mg) in DCM (20 mL) was added aqueous 5% KOH (20 mL). The reaction mixture was refluxed for 16 h, and the two phases were separated. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography using hexane/EtOAc to afford 0.32 g (89%) of 5-(4-trifluoromethoxybenzyloxy)methyl-1-naphthaldehyde. ^{1}H NMR ($CDCl_3$): δ 4.54 (s, 2H), 5.05 (s, 2H), 7.21 (d, 2H), 7.39 (d, 2H), 7.59−7.74 (m, 3H), 8.01 (d, 1H), 8.45 (d, 1H), 9.30 (d, 1H), 10.43 (s, 1H).

Hydrazone formation was done as described in preparation method A to afford 3-chloro-4-hydroxybenzoic acid [5-(4-trifluoromethoxybenzyloxymethyl)naphthalene-1-ylmethylene]-hydrazide (**10**), mp 183−185 °C. ^{1}H NMR (DMSO-d_6): δ 4.67 (s, 2H), 5.05 (s, 2H), 7.11 (d, 1H), 7.35 (d, 2H), 7.50 (d, 2H), 7.57−7.75 (m, 3H), 7.82 (d, 1H), 7.95−8.08 (m, 2H), 8.22 (d, 1H), 8.78 (s, 1H), 9.14 (s, 1H), 11.01 (s, 1H), 11.85 (s, 1H). Anal. ($C_{27}H_{20}ClF_3N_2O_4$) C, H, N.

(*S*)-2-({4-[(3-Chloro-4-hydroxybenzoyl)hydrazonomethyl]naphth-1-ylmethyl}amino)-3-phenylpropionamide (15). 4-Bromomethyl-1-naphthaldehyde (**VIII**) (5.7 g, 23 mmol) was suspended in Et_2O (125 mL). To this suspension was added neopentylglycol (3.6 g, 34.58 mmol), trimethyl orthoformate (3.66 g, 34.53 mmol), and *p*-TsOH (20 mg). The reaction mixture was refluxed for 12 h. The solvent was evaporated in vacuo, the residue was dissolved in EtOAc, and the solution was washed with H_2O (3×) and brine (2×), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography using 10% EtOAc/hexane. Recrystallization from MeCN afforded 5 g (65%) of 2-[4-(bromomethyl)-1-naphthyl]-5,5-dimethyl-1,3-dioxane. ^{1}H NMR (DMSO-d_6): δ 0.81 (s, 3H), 1.27 (s, 3H), 3.79 (m, 4H), 5.22 (s, 2H), 6.03 (s, 1H), 7.67 (m, 4H), 8.22 (d, 1H), 8.30 (d, 1H).

(*S*)-Phenylalanine amide hydrochloride (0.45 g, 2.25 mmol) was suspended in CH_3CN (3 mL), and saturated aqueous $NaHCO_3$ (2 mL) was added. To this mixture, 2-[4-(bromomethyl)-1-naphthyl]-5,5-dimethyl-1,3-dioxane (0.25 g, 0.75 mmol) dissolved in MeCN (3 mL) was added. The reaction mixture was refluxed for 48 h and cooled to room temperature. The solid formed was collected by filtration and washed with H_2O. Drying in vacuo afforded 0.25 g (80%) of *N*-{[4-(5,5-dimethyl-1,3-dioxan-2-yl)-1-naphthyl]methyl}-(*S*)-phenylalaninamide. ^{1}H NMR (DMSO-d_6): δ 0.8 (s, 3H), 1.25 (s, 3H), 2.27 (m, 1H), 2.75 (dd, 1H), 2.87 (dd, 1H), 3.77 (brd s, 4H), 3.92 (dd, 1H), 4.12 (dd, 1H), 5.97 (s, 1H), 7.12 (s, 1H), 7.23 (m, 5H), 7.34 (d, 1H), 7.51 (m, 4H), 8.03 (d, 1H), 8.23 (d, 1H).

N-{[4-(5,5-Dimethyl-1,3-dioxan-2-yl)-1-naphthyl]methyl}-*S*-phenylalaninamide (0.25 g, 0.6 mmol) was dissolved in acetone (25 mL) by gentle warming. Water (5 mL) and pyridinium *p*-toluenesulfonic acid (0.05 g) were added, and the reaction mixture was refluxed for 4 days. The mixture was concentrated in vacuo and dissolved in EtOAc. The organic solution was washed with H_2O (3×) and brine (2×), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography using 35% EtOAc/hexane to afforded 66 mg (33%) of *N*-[(4-formyl-1-naphthyl)methyl]-(*S*)-phenylalaninamide. ^{1}H NMR (DMSO-d_6): δ 3.26 (m, 2H), 4.16 (brd s, 1H),

4.59 (m, 2H), 7.12 (d, 1H), 7.31 (m, 5H), 7.49 (d, 1H), 7.80 (m, 4H), 8.24 (m, 2H), 9.24 (d, 1H), 10.44 (s, 1H).

3-Chloro-4-hydroxybenzoic acid hydrazide (**I**) (41 mg, 0.22 mmol) and *N*-[(4-formyl-1-naphthyl)methyl]-*S*-phenylalaninamide (66 mg, 0.2 mmol) were combined and dissolved in DMSO (1.5 mL). AcOH (0.2 mL) was added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc (30 mL) and washed with H_2O (2×) and brine (2×), and upon concentration in vacuo to about 10 mL a white solid precipitated. The solid was filtered, washed with EtOAc, and dried in vacuo to afford 10 mg (10%) of (*S*)-2-({4-[(3-chloro-4-hydroxybenzoyl)hydrazonomethyl]naphth-1-ylmethyl}amino)-3-phenylpropionamide (**15**). ^{1}H NMR (DMSO-d_6): δ 2.76 (m, 1H), 2.91 (m, 1H), 3.97 (d, 1H), 3.36 (brd s, 1H), 4.17 (d, 1H), 7.12 (m, 2H), 7.25 (m, 5H), 7.49 (m, 3H), 7.65 (t, 1H), 7.80 (m, 2H), 8.02 (d, 1H), 8.1 (d, 1H), 8.82 (d, 1H), 9.07 (brd s, 1H), 11.04 (brd s, 1H), 11.8 (s, 1H). Anal. ($C_{28}H_{25}ClN_4O_3 \cdot 1^1/_2H_2O$) C, H, N.

3-Chloro-4-hydroxybenzoic Acid (4-Cyclopentanesulfonylmethylnaphthalen-1-ylmethylene)hydrazide (19). A mixture of 4-bromomethyl-1-naphthaldehyde (**VIII**) (120 mg, 0.48 mmol), cyclopentylthiol (0.06 mL, 0.48 mmol), and K_2CO_3 (130 mg) was stirred at room temperature for 12 h. After filtration, the mixture was concentrated in vacuo and the residue was purified by column chromatography using EtOAc/hexane (1:5) to afford 96 mg (74%) of 4-cyclopentylthiomethyl-1-naphthaldehyde as an oil. ^{1}H NMR ($CDCl_3$): δ 13.1 (s, 1H), 9.3 (d, 1H), 8.2 (d, 1H), 7.9 (d, 1H), 7.7 (m, 3H), 7.6 (d, 1H), 4.2 (s, 2H), 3.1 (m, 1H), 1.9 (m, 2H), 1.7 (m, 2H), 1.5 (m, 4H).

To a solution of 4-cyclopentylthiomethyl-1-naphthaldehyde (96 mg) in DCM (3 mL) at room temperature was added *m*CPBA (226 mg, 48.7%). The reaction mixture was stirred at room temperature for 1 h and was washed with 0.5 M of $NaHSO_3$ followed by 0.5 M of K_2CO_3. The solution was dried ($MgSO_4$) and concentrated in vacuo to give 4-cyclopentylsulfonylmethyl-1-naphthaldehyde as an oil that was used without characterization in the next step.

Hydrazone formation was done as described in preparation method A to afford 3-chloro-4-hydroxybenzoic acid (4-cyclopentanesulfonylmethylnaphthalen-1-ylmethylene)hydrazide (**19**), mp 245−247 °C. ^{1}H NMR (DMSO-d_6): δ 1.60 (4H, m), 1.95 (4H, m), 3.71 (1H, m), 5.04 (2H, s), 8.31 (1H, d), 7.67 (3H, m), 7.79 (1H, d), 7.94 (1H, d), 8.01 (1H, s), 8.31 (1H, d), 8.78 (1H, d), 9.12 (1H, s), 11.0 (1H, s). Anal. ($C_{24}H_{23}ClN_2O_4S \cdot {}^1/_4H_2O$) C, H, N.

3-Chloro-4-hydroxybenzoic Acid [4-(4-Trifluoromethoxyphenylmethanesulfonylmethyl)naphthalen-1-ylmethylene]hydrazide (11). To a mixture of 4-bromomethyl-1-naphthaldehyde (**VIII**) (113 mg, 0.45 mmol) and 4-trifluoromethoxybenzylmercaptan (99 mg, 0.47 mmol) in DMF (2 mL) was added K_2CO_3 (200 mg), and the resulting mixture was stirred at room temperature for 2 h. Then *m*CPBA (281 mg, 55% pure, 0.9 mmol) was added to the mixture. The reaction mixture was stirred at room temperature for another 2 h and poured into a cold 10% K_2CO_3 solution. The aqueous phase was extracted with EtOAc (3 × 20 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue (54% crude yield) was reacted with 3-chloro-4-hydroxybenzoic acid hydrazide (**I**) as described in preparation method A to afford 61 mg (23%) of 3-chloro-4-hydroxybenzoic acid [4-(4-trifluoromethoxyphenylmethanesulfonylmethyl)-naphthalen-1-ylmethylene]hydrazide (**11**), mp 240−241 °C. ^{1}H NMR (DMSO-d_6): δ 11.7 (b, 1H), 10.4 (b, 1H), 9.2 (s, 1H), 8.6 (d, 1H), 8.1 (m, 2H), 8.0 (s, 1H), 7.9 (d, 1H), 7.6 (m, 2H), 7.5 (d, 2H), 7.2 (d, 2H), 7.0 (d, 1H), 4.9 (s, 2H), 4.5 (s, 2H). HR-MS: calcd for $C_{27}H_{20}ClF_3N_2O_5S$, 577.08063; found, 577.0800.

Preparation Method C. 3-Chloro-4-hydroxybenzoic Acid {4-[2-(4-Trifluoromethoxybenzylamino)ethoxy]naphthalen-1-methylene}hydrazide (20). A solution of 4-hydroxy-1-naphthaldehyde (0.37 g, 2.15 mmol) and 1-chloro-2-bromoethane (0.3 g, 2.15 mmol) in DMF (3 mL) containing K_2CO_3 (0.3 g, 2.15 mmol) was stirred at 80 °C for 12 h. The solvent was evaporated in vacuo, and the residue was diluted with ethyl acetate. The organic phase was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Silica gel column chromatography using ethyl acetate/hexane (1:9 to 2:8) afforded 0.21 g (41%) of 4-(2-chloroethoxy)-1-naphthaldehyde. ^{1}H NMR (DMSO-d_6): δ 3.99 (t, 2H), 4.51 (t, 2H), 6.90 (d, 1H), 7.26 (s, 1H), 7.63 (t, 1H), 7.72 (t, 1H), 7.91 (d, 1H), 8.39 (d, 1H), 9.30 (d, 1H), 10.22 (s, 1H).

A solution of 4-(2-chloroethoxy)-1-naphthaldehyde (2.35 g, 10 mmol), 3-chloro-4-hydroxybenzoic acid hydrazide (**I**) (1.87 g, 10 mmol), HOAc (0.2 mL), and DMSO (15 mL) were stirred at room temperature overnight. Ethyl acetate (100 mL) was added, and the solution was treated with water and brine, which induced precipitation. Filtration afforded 3.1 g (77%) of 3-chloro-4-hydroxybenzoic acid [4-(2-chloroethoxy)-1-naphthylmethylene]hydrazide. ^{1}H NMR (DMSO-d_6): δ 11.5 (s, 1H), 10.7 (s, 1H), 8.7 (bs, 2H), 8.1 (m, 1H), 7.8 (s, 1H), 7.6−7.3 (m, 2H), 7.0 (m, 2H), 4.3 (t, 2H), 3.7 (t, 2H).

To 4-trifluoromethoxybenzylamine (0.29 g, 1 mmol) in DMF (5 mL) was added 3-chloro-4-hydroxybenzoic acid [4-(2-chloroethoxy)-1-naphthylmethylene]hydrazide (0.403 g, 1 mmol) and triethylamine (0.1 g, 1 mmol). The reaction mixture was heated at 80 °C for 16 h. Removal of most of the solvent in vacuo followed by flash chromatography (10:1 $CHCl_3$/MeOH) on silica gel afforded 0.15 g (27%) of 3-chloro-4-hydroxybenzoic acid {4-[2-(4-trifluoromethoxy)benzylaminoethoxy]-1-naphthylmethylene}hydrazide (**20**) as a solid, mp 125−127 °C. ^{1}H NMR (DMSO-d_6): δ 11.6 (s, 1H), 9.0 (m, 2H), 8.3 (m, 1H), 8.0 (m, 1H), 7.8 (s, 2H), 7.7 (m, 1H), 7.6 (m, 1H), 7.5 (m, 3H), 7.3 (m, 2H), 7.1 (m, 2H), 4.3 (t, 2H), 3.9 (s, 2H), 3.0 (t, 2H). Anal. ($C_{28}H_{23}ClF_3N_3O_4 \cdot 1.75H_2O$): C, N. H: calcd, 4.53; found, 3.97.

Preparation Method D. 3-Chloro-4-hydroxybenzoic Acid {4-[2-(1,2,3,4-Tetrahydroisoquinolin-2-yl)ethoxy]-2-methoxybenzylidene}hydrazide (24). Polystyrene resin loaded with the Wang linker (15 g, 0.92 mmol/g) was successively washed with DMF (3 × 40 mL) and DCM (3 × 40 mL). The resin was suspended in DCM (80 mL), and diisopropylethylamine (60 mL) was added. The mixture was cooled to 0 °C, and methanesulfonyl chloride (5.8 mL) dissolved in DCM (30 mL) was added dropwise while maintaining the temperature below 5 °C. When addition was complete, the mixture was stirred at 0 °C for 30 min and at room temperature for 30 min. The resin was successively washed with DCM (3 × 80 mL) and *N*-methylpyrrollidone (NMP) (3 × 80 mL). This resin and Cs_2CO_3 (12.3 g) were added to ethyl 3-chloro-4-hydroxybenzoate (15 g) dissolved in NMP (200 mL), and the mixture was stirred at 80 °C for 4 h. After cooling, the resin was successively washed with NMP (3 × 80 mL) and MeOH (3 × 80 mL). The resin was washed with THF (3 × 300 mL) and treated with $NaOSiMe_3$ in THF (1 M, 300 mL) for 4 h at 25 °C. After filtration, the resin was washed with THF (2 × 300 mL), and added THF (300 mL) and HOAc (75 mL) were added. The mixture was stirred overnight at 25 °C. The resulting resin-bound carboxylic acid was washed with THF (2 × 300 mL) and DCM (300 mL). To the resin was added a solution of PyBoP (46.8 g, 90 mmol) in DCM (300 mL) followed by *N*-methylmorpholine (30 mL). The mixture was stirred for 45 min at 25 °C, and hydrazine hydrate (30 mL) was added. After the mixture was shaken for 4 h at 25 °C, the resin was filtered and washed with DCM (2 × 300 mL) and DMF (300 mL) to afford the resin-bound 3-chloro-4-hydroxybenzoic acid hydrazide (**I**).

1,2-Dibromoethane (57 mL, 0.66 mol) was added to a mixture of 4-hydroxy-2-methoxybenzaldehyde (10 g, 66 mmol) and K_2CO_3 (45 g, 0.33 mol) in DMF (130 mL), and the resulting mixture was stirred vigorously at room temperature for 16 h. The mixture was poured into water (0.8 L) and extracted with EtOAc (3 × 300 mL). The combined organic phases were washed with brine (400 mL), dried ($MgSO_4$), and evaporated in vacuo to afford 17.4 g (99%) of 4-(2-bromoethoxy)-2-methoxybenzaldehyde, mp 78−79 °C.

The resin-bound 3-chloro-4-hydroxybenzoic acid hydrazide (**I**) (3 g, ∼3 mmol) was swelled in DMF (35 mL) for 30 min. Then 4-(2-bromoethoxy)-2-methoxybenzaldehyde (2.33 g, 9 mmol) and triethyl orthoformate (18 mL) were added and the mixture was shaken at room temperature for 16 h. The resin was repeatedly swelled in DMF (4 × 35 mL), DCM (6 × 35

mL), and *N*-methyl-2-pyrrolidinone (NMP) (2 × 35 mL) and filtered. The resin was swelled in NMP (40 mL), and 1,2,3,4-tetrahydroisoquinoline (3.75 mL, 30 mmol) and KI (1.0 g, 6 mmol) were added. The resin was shaken at room temperature for 16 h and filtered. The resin was repeatedly washed with DMF (5 × 40 mL) and DCM (10 × 40 mL) and filtered. The compound was cleaved off the resin by shaking for 1 h at room temperature with a 50% solution of TFA in DCM (40 mL). The mixture was filtered, and the resin was extracted with DCM (2 × 40 mL). The combined DCM extracts were concentrated in vacuo. The residue was dissolved in DCM (40 mL) and concentrated in vacuo. The residue was dissolved in MeOH (40 mL) and concentrated in vacuo. The residue was partitioned between EtOAc (50 mL) and saturated $NaHCO_3$ (50 mL). The aqueous phase was extracted with EtOAc (50 mL), and the combined organic extracts were dried over ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography over silica gel (200 mL), eluting with a mixture of DCM and MeOH (9:1). This afforded 280 mg of 3-chloro-4-hydroxybenzoic acid {4-[2-(1,2,3,4-tetrahydroisoquinolin-2-yl)ethoxy]-2-methoxybenzylidene}hydrazide (**24**), mp 170−171.9 °C. ^{1}H NMR (DMSO-d_6): δ 2.80 (4H, m), 2.90 (2H, t), 3.69 (2H, s), 3.86 (3H, s), 4.25 (2H, t), 6.68 (2H, m), 7.04 (1H, d), 7.07−7.14 (5H, m), 7.75 (1H, dd), 7.80 (1H, bs), 7.96 (1H, d), 8.58 (1H, s), 11.6 (1H, s). HR-MS: calcd for $C_{26}H_{26}ClN_3O_4$, 479.1611; found, 479.1604.

Preparation Method E. Library Method. 4-[(3-Chloro-4-hydroxybenzoyl)hydrazonomethyl]naphthoic Acid (IX). A mixture of 4-methyl-1-naphthoic acid (10 g, 54 mmol), NBS (10 g, 56 mmol), and AIBN (100 mg) in CCl_4 (250 mL) was refluxed for 3 h. The reaction mixture was concentrated in vacuo and dissolved in EtOAc. The organic solution was washed with water and brine and dried ($MgSO_4$). Evaporation of the solvent in vacuo afforded 16 g, (80%) of 4-bromomethyl-1-naphthoic acid. ^{1}H NMR (DMSO-d_6): δ 5.24 (s, 2H), 7.73 (m, 3H), 8.03 (d, 1H), 8.28 (d, 1H), 8.86 (d, 1H), 13.29 (bs, 1H).

4-Bromomethyl-1-naphthoic acid (16 g, 60 mmol) in an aqueous solution of K_2CO_3 (10%, 100 mL) was stirred at 70 °C for 30 min. The reaction mixture was cooled and made acidic with concentrated HCl. The resulting precipitate was filtered off and dried to afford 12 g (100%) of 4-hydroxymethyl-1-naphthoic acid. ^{1}H NMR (DMSO-d_6): δ 5.01 (s, 2H), 5.96 (s, 1H), 7.70 (m, 3H), 8.10 (m, 2H), 8.90 (d, 1H).

A mixture of 4-hydroxymethyl-1-naphthoic acid (10 g, 50 mmol), MeOH (300 mL), and concentrated H_2SO_4 (2 mL) was refluxed overnight. Insoluble material was filtered off, and the filtrate was concentrated. The residue was dissolved in EtOAc and washed with aqueous $NaHCO_3$ (2×) and brine, dried ($MgSO_4$), and concentrated in vacuo. Column chromatography on silica gel using EtOAc/hexane (1:3) afforded 3.3 g (35%) of methyl 4-hydroxymethyl-1-naphthoate as an oil. ^{1}H NMR ($CDCl_3$): δ 2.05 (t, 1H), 4.01 (s, 3H), 5.22 (s, 2H), 7.66 (m, 3H), 8.09 (d, 1H), 8.16 (d, 1H), 8.96 (d, 1H).

To a solution of methyl 4-hydroxymethyl-1-naphthoate (3.3 g, 15.3 mmol) in DCM (20 mL) was added MnO_2 (6.6 g, 76 mmol). After the mixture was stirred for 16 h, the mixture was filtered through a bed of Celite. Evaporation of the solvent in vacuo afforded 3.3 g (quantitative) of methyl 4-formyl-1-naphthoate as a white solid. ^{1}H NMR ($CDCl_3$): δ 4.06 (S, 3H), 7.75 (m, 2H), 8.03 (d, 1H), 8.20 (d, 1H), 8.80 (d, 1H), 9.27 (d, 1H), 10.50 (s, 1H).

A mixture of methyl 4-formyl-1-naphthoate (2.3 g, 1 mmol) and Na_2CO_3 (1.25 g, 12 mmol) in water (30 mL) was heated at 100 °C until a clear solution was obtained (approximately 2 h). The solution was cooled and filtered, and the filtrate was acidified with concentrated HCl. The solid was isolated by filtration and dried for 16 h to afford 1.86 g (87%) of 4-formyl-1-naphthoic acid. ^{1}H NMR (DMSO-d_6): δ 7.76 (m, 2H), 8.22 (m, 2H), 8.71 (d, 1H), 9.20 (d, 1H), 10.49 (s, 1H).

To a solution of 3-chloro-4-hydroxybenzoic acid hydrazide (**I**) (1.53 g, 8.23 mmol) in DMSO (20 mL) was added a solution of 4-formyl-1-naphthoic acid (1.65 g, 8.23 mmol) in DMSO (2 mL). After the solution was stirred for 16 h, the reaction mixture was diluted with EtOAc (30 mL) and water (30 mL). The precipitate was filtered, washed with hexane, and dried to afford 3.0 g (quantitative) of 4-[(3-chloro-4-hydroxybenzoyl)-hydrazonomethyl]naphthoic acid (**IX**) as a white solid. ^{1}H NMR (DMSO-d_6): δ 4.70 (d, 1H), 7.70 (m, 2H), 7.83 (d, 1H), 8.03 (m, 2H), 8.18 (d, 1H), 8.72 (s, 1H), 8.90 (d, 1H), 9.17 (s, 1H), 11.0 (bs, 1H), 11.94 (s, 1H), 13.4 (bs, 1H).

{4-[(3-Chloro-4-hydroxybenzoyl)hydrazonomethyl]-naphth-1-yl}acetic Acid (X). 4-Formylnaphthalene-1-ylacetic acid methyl ester was prepared from the reduction of 4-cyano-1-naphthylacetic acid in the presence of 85% formic acid and Raney alloy as described in the literature.[27] By condensation of 4-formylnaphthalene-1-ylacetic acid and 3-chloro-4-hydroxybenzoic acid hydrazide (**I**) as described above, {4-[(3-chloro-4-hydroxybenzoyl)hydrazonomethyl]naphth-1-yl}-acetic acid (**X**) was prepared. ^{1}H NMR (DMSO-d_6): δ 4.1 (s, 2H), 7.1 (d, 1H), 7.5 (d, 1H), 7.7 (qt, 2H), 7.8 (d, 1H), 7.9 (d, 1H), 8.0 (s, 1H), 8.1 (d, 1H), 8.8 (d, 1H), 9.1 (s, 1H), 11.0 (bs, 1H), 11.8 (s, 1H), 12.2 (bs, 1H).

{4-[(3-Chloro-4-hydroxybenzoyl)hydrazonomethyl]-naphth-1-yloxy}acetic Acid (XI). 4-Hydroxy-1-naphthaldehyde (10 g, 58 mmol), K_2CO_3 (16 g, 110 mmol), and methyl bromoacetate (16 g, 100 mmol) were refluxed in acetone (120 mL) for 16 h. The reaction mixture was poured into ice (500 mL). Filtration and drying in vacuo afforded 13 g (86%) of 4-formylnaphthalene-1-yloxyacetic acid methyl ester. ^{1}H NMR ($CDCl_3$): δ 3.86 (s, 3H), 4.93 (s, 2H), 6.80 (d, 1H), 7.61 (t, 1H), 7.72 (t, 1H), 7.90 (d, 1H), 8.42 (d, 1H), 9.29 (d, 1H), 10.22 (s, 1H).

4-Formylnaphthalene-1-yloxyacetic acid methyl ester (13 g, 50 mmol) was dissolved in MeOH (100 mL), and 2 M NaOH (40 mL) was added. The reaction mixture was stirred for 16 h and concentrated in vacuo to approximately 100 mL. The residue was poured into ice (500 mL), and the mixture was acidified with the addition of 3 N HCl. Filtration, washing with water, and drying afforded 9 g (78%) of 4-formylnaphthalene-1-yloxyacetic acid, which was used in the next step without characterization. To a solution of 3-chloro-4-hydroxybenzoic acid hydrazide (**I**) (2 g, 10.7 mmol) in DMSO (20 mL) was added 4-formylnaphthalene-1-yloxyacetic acid (3 g, 13 mmol) and a catalytic amount of acetic acid (10 drops). The solution was stirred for 16 h and diluted with EtOAc. The solution was washed with water (3×) and brine and dried ($MgSO_4$). The volume was reduced to approximately 100 mL by evaporation in vacuo and cooled to 0 °C. The resulting solid was filtered and washed with cold EtOAc to afford 4 g (99%) of {4-[(3-chloro-4-hydroxybenzoyl)hydrazonomethyl]naphth-1-yloxy}-acetic acid (**XI**). ^{1}H NMR (DMSO-d_6): δ 4.91 (s, 2H), 6.95 (d, 1H), 7.02 (d, 1H), 7.55 (t, 1H), 7.64 (t, 1H), 7.74 (d, 1H), 7.92 (d, 1H), 8.27 (d, 1H), 8.90 (m, 2H), 10.92 (bs, 1H), 11.63 (s, 1H), 13.14 (bs, 1H).

{4-[(3-Chloro-4-hydroxybenzoyl)hydrazonomethyl]-indol-1-yl}acetic Acid (XII). Under a nitrogen atmosphere, methyl indole-4-carboxylate (2 g, 11.4 mmol) was dissolved in anhydrous THF (20 mL). The solution was cooled in an ice bath, and $LiAlH_4$ (12 mL, 1 M/THF, 12 mmol) was added dropwise. The reaction mixture was stirred for 2 h at room temperature, diluted with Et_2O, and washed with water (3×) and brine, dried ($MgSO_4$), and filtered. Evaporation of the solvent gave the crude product, which was purified by column chromatography using 25% ethyl acetate/hexanes. This afforded 1.6 g (97%) of 4-(hydroxymethyl)indole. The reaction was repeated with 6 g of the ester, and 5.6 g (78%) of the product was isolated. ^{1}H NMR (DMSO-d_6): δ 4.98 (s, 2H), 6.66 (m, 1H), 7.13 (d, 1H), 7.18 (m, 1H), 7.21 (d, 1H), 7.33 (d, 1H,), 8.50 (s, 1H).

4-Hydroxymethylindole (5.6 g, 38.6 mmol) was dissolved in a minimum volume of EtOAc (125 mL). To this, MnO_2 (10 g, 116 mmol) was added, and the reaction mixture was refluxed for 10 h. After cooling, the reaction mixture was filtered and concentrated in vacuo to afford 4.9 g (89%) 4-formylindole. ^{1}H NMR (DMSO-d_6): δ 7.10 (s, 1H), 7.30 (t, 1H), 7.64 (m, 2H), 7.76 (d, 1H), 10.18 (s, 1H), 11.61 (s, 1H).

To a solution of 4-formylindole (1 g, 7 mmol) and ethyl bromoacetate (1.75 g, 10.5 mmol) in DMSO (20 mL) was added

K_2CO_3 (2 g, 14.4 mmol). The reaction mixture was stirred at room temperature for 12 h, and water (100 mL) was added. The mixture was extracted with EtOAc (3 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography using hexane/ethyl acetate (2:1) as eluent to afford 1.55 g (96%) of ethyl (4-formylindol-1-yl)-acetate. ^{1}H NMR ($CDCl_3$): δ 1.13 (t, 3H), 4.15 (q, 2H), 4.86 (s, 2H), 7.22−7.35 (m, 3H), 7.49 (d, 1H), 7.60 (d, 1H), 10.20 (s, 1H).

Ethyl (4-formylindol-1-yl)acetate (1 g, 4.9 mmol) was dissolved in MeOH (50 mL), 5% aqueous KOH (10 mL) was added, and the resulting mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated in vacuo to one-third of the original volume, diluted with aqueous KOH (20 mL, 10%), and washed with Et_2O (2 × 30 mL). The aqueous layer was acidified by the addition of concentrated HCl. This afforded, after filtration and drying, 0.9 g (90%) of (4-formylindol-1-yl)acetic acid. ^{1}H NMR (DMSO-d_6): δ 5.15 (s, 2H), 7.12 (d, 1H), 7.36 (d, 1H), 7.61 (d, 1H), 7.71 (d, 1H), 7.82 (d, 1H), 10.20 (s, 1H), 12.94 (brd s, 1H).

Condensation of (4-formylindol-1-yl)acetic acid with 3-chloro-4-hydroxybenzoic acid hydrazide (**I**) according to the general procedure for the synthesis of alkylidene hydrazides afforded 4-[(3-chloro-4-hydroxybenzoyl)hydrazonomethyl]indol-1-yl acetic acid (**XII**). ^{1}H NMR (DMSO-d_6): δ 5.09 (s, 2H), 7.09 (d, 1H), 7.16−7.25 (m, 2H), 7.32 (d, 1H), 7.45−7.55 (m, 2H), 7.81 (d, 1H), 8.01 (d, 1H), 8.68 (s, 1H), 10.96 (s, 1H), 11.71 (s, 1H), 12.90 (b, 1H).

Library Preparation Procedure. To a solution of a derivative of alkylidene hydrazide carboxylic acid (**IX**−**XII**) in DMSO was added carbonyldiimidazole (1.2 equiv). The solution was agitated for 5 min and diluted with DMSO to a concentration of 50 mM. The solution was then dispensed into 88 deep-well plates containing solutions of amines in DMSO (50 mM). The plates were covered and agitated for 16 h. The products were purified by HPLC.

Scale-Up Procedure. 2-{4-[(3-Cyano-4-hydroxybenzoyl)-hydrazonomethyl]naphthalen-1-yl}-*N*,*N*-diethylacetamide (29). To a solution of 4-formylnaphthalene-1-ylacetic acid (2.5 g, 11.6 mmol) in DMF (10 mL) was added CDI (2.5 g, 15.4 mmol). The mixture was stirred at room temperature for 1 h, and freshly distilled diethylamine (1.3 mL, 12.5 mmol) was added. After the mixture was stirred at room temperature for 16 h, EtOAc (100 mL) was added and the mixture was washed with H_2O (2 × 50 mL), 0.5 N HCl (50 mL) and brine (2 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (hexane/EtOAc 1:1) afforded 1.6 g (50%) of *N*,*N*-diethyl-4-formylnaphthalene-1-ylacetamide. ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 6H), 3.37 (q, 2H), 3.47 (q, 2H), 4.19 (s, 2H), 7.53 (d, 1H), 7.71 (m, 2H), 7.94 (d, 1H), 8.01 (d, 1 H), 9.33 (d, 1H), 10.36 (s, 1H).

N,*N*-Diethyl-4-formylnaphthalene-1-ylacetamide (1.6 g, 5.92 mmol) was dissolved in DMSO (10 mL), and 3-cyano-4-hydroxybenzoylhydrazide (1.05 g, 5.92 mmol) in DMSO (5 mL) and a catalytic amount of HOAc were added. After the mixture was stirred at room temperature for 16 h, water was added and the precipitate was filtered and washed with water (3 × 10 mL) and DCM (3 × 10 mL) to afford 2.33 g (92%) of 2-{4-[(3-cyano-4-hydroxybenzoyl)hydrazonomethyl]naphthalen-1-yl}-*N*,*N*-diethylacetamide (**29**), mp 265−268 °C. ^{1}H NMR (DMSO-d_6): δ 1.08 (3H, t), 1.18 (3H, t), 3.35 (2H, q), 3.49 (2H, q), 4.20 (2H, s), 7.16 (1H, d), 7.45 (1H, d), 7.65 (3H, m), 7.89 (1H, d), 8.01 (1H, d), 8.12 (1H, d), 8.30 (1H, s), 8.97 (1H, d), 9.10 (1H, s), 11.9 (1H, bs). Anal. ($C_{25}H_{24}N_4O_3 \cdot 0.5H_2O$) C, H, N.

Preparation Method F. Cyclopentanecarboxylic Acid {4-[(3-Chloro-4-hydroxybenzoyl)hydrazonomethyl]-naphthalen-1-yl}amide (54). To a cold (0 °C) suspension of 1-methylnaphthalene (5 g, 35 mmol) in HNO_3 was added H_2SO_4 (5 mL) dropwise. After the reaction mixture was stirred for 1 h at room temperature, the solution was diluted with EtOAc and washed with water (3×), aqueous saturated $NaHCO_3$ (2×), and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography using EtOAc/hexane (5:95) and recrystallized from MeOH to afford 1.75 g (27%) of 4-nitro-1-methylnaphthalene as yellow needles. ^{1}H NMR ($CDCl_3$): δ 2.79 (s, 3H), 7.38 (d, 1H), 7.65−7.73 (m, 2H), 8.10 (d, 1H), 8.14 (d, 1H), 8.61 (d, 1H).

To a stirred refluxing solution of sulfur (3.7 g) in 12% aqueous NaOH (50 mL) was added a solution of 4-nitro-1-methylnaphthalene (8 g, 43 mmol) in EtOH (50 mL). After the mixture was refluxed for 1 h, the mixture was diluted with EtOAc (500 mL) and washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified via silica gel column chromatography using EtOAc/hexane (5:95 to 10:90) to afford 2.54 g (34%) of 4-amino-1-naphthaldehyde (**XIII**). The product was stored at −78 °C. ^{1}H NMR (DMSO-d_6): δ 6.55 (d, 1H), 6.95 (bs, 2H), 7.25 (t, 1H), 7.45 (t, 1H), 7.60 (d, 1H), 8.05 (d, 1H), 9.10 (d, 1H), 9.68 (s, 1H).

To a solution of 4-amino-1-naphthaldehyde (210 mg, 1.12 mmol) and 4-(dimethylamino)pyridine (410 mg, 3.36 mmol) in EtOAc (3 mL) was added cyclopentanecarbonyl chloride (2 mL, 15 mmol). After the mixture was stirred for 12 h at room temperature, the mixture was diluted with EtOAc and washed with 1 N HCl (3×) and brine (3×), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using EtOAc/hexane (1:9) to afford 147 mg (45%) of *N*-(4-formyl-1-naphthyl)cyclopentanecarboxamide. ^{1}H NMR ($CDCl_3$): δ 1.71−1.74 (m, 2H), 1.84−1.89 (m 2H), 2.01−2.10 (m, 4H), 2.91 (quintet, 1H), 7.64−7.73 (m, 2H), 7.89 (d, 1H), 8.00 (d, 1H), 8.46 (d, 1H), 9.44 (d, 1H), 10.29 (s, 1H).

To a solution of 4-hydroxy-3-chlorobenzoic acid hydrazide (**I**) (100 mg, 0.55 mmol) in DMSO (3 mL) was added *N*-(4-formyl-1-naphthyl)cyclopentanecarboxamide (147 mg, 0.55 mmol) followed by a catalytic amount of acetic acid. After the mixture was stirred for 16 h, the mixture was diluted with ethyl acetate and washed with water (2×), dried ($MgSO_4$), and concentrated in vacuo to about 10 mL. The mixture was filtered and dried to afford 190 mg (36%) of cyclopentanecarboxylic acid {4-[(3-chloro-4-hydroxybenzoyl)hydrazonomethyl]-naphthalen-1-yl}amide (**54**), mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 1.61 (m, 2H), 1.71 (m 2H), 1.82 (m, 2H), 1.90 (m, 2H), 3.06 (quintet, 1H), 7.10 (d, 1H), 7.65 (quintet, 2H), 7.83 (qt, 2H), 7.90 (d, 1H), 8.02 (s, 1H), 8.18 (d, 1H), 8.90 (d, 1H), 9.04 (s, 1H), 10.01 (s, 1H), 10.99 (s, 1H), 11.78 (s, 1H). Anal. ($C_{24}H_{22}ClN_3O_3$) C, H, N.

Preparation Method G. *N*-{4-[(3-Chloro-4-hydroxybenzoyl)hydrazonomethyl]-3-methoxyphenyl}-2-(4-trifluoromethylphenyl)propionamide (60). Methyl 4-amino-2-methoxybenzoate (14.7 g, 81 mmol) and Fmoc-OSu (26.1 g, 77.3 mmol) were stirred in a mixture of MeCN and water (1:1, 320 mL) at reflux for 16 h. The reaction mixture was concentrated in vacuo to half the volume, and the precipitate was isolated by filtration. The isolated solid was dissolved in EtOAc (300 mL) and washed with 0.4 N HCl (200 mL), 0.2 N HCl (200 mL), water (200 mL), and a 20% saturated solution of sodium chloride (200 mL). After the mixture was dried ($MgSO_4$), the organic phase was evaporated in vacuo. The residue was washed with MeOH and dried. The crude product (12 g) was dissolved in DCM (1 L) under nitrogen, and a solution of diisobutylaluminum hydride (90 mL, 1.2 M in toluene) was added dropwise at 0−5 °C. The reaction mixture was stirred at 20 °C for 16 h and quenched by dropwise addition of water (58 mL) at 0−5 °C. The reaction mixture was stirred at 20 °C for 3 h and filtered. The filtrate was concentrated in vacuo. The crude product (6.8 g) was suspended in DCM (400 mL), and MnO_2 (15.6 g, 180 mmol) was added. The mixture was stirred for 16 h at 20 °C and filtered. The filtrate was concentrated in vacuo to afford 5.1 g (17%) of (4-formyl-3-methoxyphenyl)carbamic acid 9*H*-fluoren-9-ylmethyl ester, mp 187−188 °C. Anal. ($C_{23}H_{19}NO_4$) C, H, N.

A sample of 0.75 g of Wang resin loaded with 3-chloro-4-hydroxybenzoic acid hydrazide (**I**) was swelled in DMF (6 mL) for 30 min and filtered. (4-Formyl-3-methoxyphenyl)carbamic acid 9*H*-fluoren-9-ylmethyl ester (0.5 g, 1.36 mmol) dissolved in DMF (3 mL) was added followed by addition of triethyl orthoformate (1.5 mL). The mixture was shaken for 16 h at

20 °C and drained. The resin was successively washed with DMF (5 × 4 mL), DCM (5 × 4 mL), and DMF (5 × 4 mL). The coupling of the aldehyde was repeated once. The resin was swelled in DMF (5 mL), and piperidine (1.25 mL) was added. After being shaken for 30 min, the resin was drained and successively washed with DMF (5 × 4 mL), NMP (5 × 4 mL), and DMF (5 × 4 mL). The resin-bound 3-chloro-4-hydroxybenzoic acid (4-amino-2-methoxybenzylidene)hydrazide (0.5 g) was swelled in DMF (2.5 mL) and filtered. 3-(4-Trifluoromethylphenyl)propionic acid (0.5 g, 2.3 mmol) was dissolved in DMF (2 mL) together with diisopropylcarbodiimide (DIC) (1.13 mmol), and after 10 min, this mixture was added to the drained resin. After 30 min of shaking, a 1 M solution of DMAP in DMF (0.32 mL) was added and the mixture was shaken for 16 h and filtered. The resin was successively washed with DMF (5 × 4 mL) and DCM (5 × 4 mL). Coupling with the acid was repeated once. The resin was swelled in DCM (2 mL), and TFA (2 mL) was added. After being shaken for 30 min, the resin was filtered. The filtrate was collected and concentrated in vacuo. The residue was crystallized from MeOH to afford 64 mg (27%) of *N*-{4-[(3-chloro-4-hydroxybenzoyl)hydrazonomethyl]-3-methoxyphenyl}-2-(4-trifluoromethylphenyl)propionamide (**60**), mp 233−235 °C. ^{1}H NMR (DMSO-d_6): δ 2.72 (2H, t), 3.03 (2H, t), 3.84 (3H, s), 7.07 (1H, d), 7.18 (1H, d), 7.5 (3H, m), 7.69 (2H, d), 7.78 (2H, m), 7.99 (1H, s), 8.70 (1H, s), 10.2 (1H, s), 10.9 (1H, s), 11.7 (1H, s). Anal. ($C_{25}H_{21}ClF_3N_3O_4 \cdot {}^1/_4H_2O$) C, H, N.

Preparation Method H. *N*-{4-[(3-Cyano-4-hydroxybenzoyl)hydrazonomethyl]-3-methoxyphenyl}-2-(3-trifluoromethylphenyl)acetamide (59). DIC (8.1 g, 42 mmol) was added to a solution of 3-(trifluoromethyl)phenylacetic acid (17 g, 83 mmol) in DCM (50 mL). After 10 min, methyl 4-amino-2-methoxybenzoate (5.0 g, 28 mmol) was added and the mixture was stirred at reflux for 4 h and at 20 °C for 16 h. The mixture was diluted with DCM (100 mL) and extracted with a saturated solution of sodium hydrogen carbonate (3 × 50 mL) and water (3 × 50 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography on silica gel using heptane/EtOAc (3:2) as eluent to afford 8 g (78%) of 2-methoxy-4-[2-(3-trifluoromethylphenyl)acetetylamino]benzoic acid methyl ester. ^{1}H NMR (DMSO-d_6): δ 10.5 (s, 1H), 7.7−7.5 (m, 6H), 7.2 (d, 1H), 3.85 (s, 2H), 3.75 (s, 3H), 3.7 (s, 3H). Anal. ($C_{18}H_{16}NO_4$) C, H, N.

2-Methoxy-4-[2-(3-trifluoromethylphenyl)acetylamino]benzoic acid methyl ester (2.0 g, 5.4 mmol) was dissolved in dry DCM (100 mL) under nitrogen and cooled to −20 °C. Diisobutylaluminum hydride (1.2 M in toluene, 16 mL, 18.9 mmol) was added dropwise over 40 min. The reaction mixture was warmed to 20 °C and stirred at this temperature for 2 h. After dilution with DCM (100 mL), the reaction mixture was quenched by dropwise addition of water (10 mL) at 20−25 °C. After 16 h, the mixture was filtered and the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using heptane/EtOAc (3:2) as eluent to afford 0.7 g (37%) of *N*-(4-hydroxymethyl-3-methoxyphenyl)-2-(3-trifluoromethylphenyl)acetamide. ^{1}H NMR (DMSO-d_6): δ 10.2 (s, 1H), 7.7−7.5 (m, 4H), 7.35 (s, 1H), 7.25 (d, 1H), 7.1 (d, 1H), 4.9 (t, 1H), 4.4 (d, 2H), 3.8 (s, 2H), 3.7 (s, 3H).

N-(4-Hydroxymethyl-3-methoxyphenyl)-2-(3-trifluoromethylphenyl)acetamide (0.7 g, 2 mmol) was dissolved in EtOAc (40 mL), and MnO_2 (3 g, 34 mmol) was added. The reaction mixture was stirred at 20 °C for 3 h and filtered. The organic phase was concentrated in vacuo to give crude 0.67 g (99%) of *N*-(4-formyl-3-methoxyphenyl)-2-(3-trifluoromethylphenyl)acetamide, which was used for the next step without further purification. ^{1}H NMR (DMSO-d_6): δ 10.65 (s, 1H), 10.2 (s, 1H), 7.7−7.5 (m, 6H), 7.2 (d, 1H), 3.85 (s, 5H).

N-(4-Formyl-3-methoxyphenyl)-2-(3-trifluoromethylphenyl)-acetamide (0.67 g, 2 mmol) was dissolved in DMSO (10 mL). 3-Cyano-4-hydroxybenzoic acid hydrazide (**IV**) (0.35 g, 2 mmol) was added followed by addition of glacial acetic acid (0.3 mL). The reaction mixture was stirred for 16 h at 20 °C, diluted with EtOAc (125 mL), and washed with water (100 mL). The aqueous phase was extracted with EtOAc (100 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated in vacuo. The crude product was crystallized from MeOH/DCM (1:9) to afford 0.5 g (50%) of *N*-{4-[(3-cyano-4-hydroxybenzoyl)hydrazonomethyl]-3-methoxyphenyl}-2-(3-trifluoromethylphenyl)acetamide (**59**), mp 257−260 °C. ^{1}H NMR (DMSO-d_6): δ 11.8 (s, 1H), 11.7 (s, 1H), 10.5 (s, 1H), 8.7 (s, 1H), 8.2 (s, 1H), 8.05 (dd, 1H), 7.8 (d, 1H), 7.7 (s, 1H), 7.65−7.5 (m, 4H), 7.15 (d, 1H), 7.1 (d, 1H), 3.8 (s, 5H).

Preparation Method I. Library Procedure. 4-Formylindole (1.45 g, 10 mmol) was dissolved in dry DMF (8.6 mL). With an atmosphere of nitrogen or argon over the solution, NaH (0.27 g of 95% dry reagent, 1.1 equiv) was transferred to the indole solution. The mixture was stirred for 15 min. Amber glass vials (for preparing stock solutions) were dried for at least 4 h at 110 °C, then were allowed to cool under an argon atmosphere in a desiccator. Halide solutions (1.0 M) were prepared in anhydrous DMF in the dried vials. Each halide solution (100 μL) was added to its corresponding well of a deep-well plate. An amount of 100 μL of the 1.0 M indole salt solution was quickly delivered to each halide in the deep-well plates. The plates were vortexed briefly to mix, then allowed to stand for 2 h. 3-Substituted 4-hydroxybenzoic acid hydrazides (10 mmol) were dissolved in 5 mL of dry DMSO, followed by TFA (0.77 mL). The resulting solutions were diluted to final volumes of 10.0 mL. The hydrazide solutions (100 μL) were added to each well of the deep-well plate. The plates were vortexed for 1 min to mix and then allowed to stand for 30 min. The products were purified by chromatography on silica gel with EtOAc/hexane eluent.

Scale-Up Procedure. 3-Cyano-4-hydroxybenzoic Acid [1-(2,3,5,6-Tetramethylbenzyl)-1*H*-indol-4-ylmethylene]-hydrazide (74). To a solution of 4-formylindole (4 g, 28 mmol) in DMF at 0 °C was added sodium hydride (1.2 g, 60%, 30 mmol). The mixture was stirred for 30 min, and 2,3,5,6-tetramethylbenzyl chloride (6.13 g, 33.6 mmol) was added. The mixture was stirred for 2 h, diluted with EtOAc, washed with H_2O (3×) and brine (3×), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified via column chromatography using ethyl acetate/hexane (1:9) to afford 5.9 g (71%) of 4-formyl-1-(2,3,5,6-tetramethylbenzyl)-1*H*-indole. ^{1}H NMR ($CDCl_3$): δ 2.15 (s, 6H), 2.30 (s, 6H), 5.35 (s, 2H), 6.84 (d, 1H), 7.08 (s, 1H), 7.20 (s, 1H), 7.42 (t, 1H), 7.69 (d, 1H), 7.81 (d, 1H), 10.27 (s, 1H).

To a solution of 3-cyano-4-hydroxybenzoic acid hydrazide (**IV**) (0.84 g, 4.7 mmol) in a minimum volume of DMSO was added 4-formyl-1-(2,3,5,6-tetramethylbenzyl)-1*H*-indole (1.2 g, 4.2 mmol) and a catalytic amount of HOAc (five drops). The mixture was stirred overnight under nitrogen and diluted with EtOAc. The organic phase was washed with saturated $NaHCO_3$, water, and brine and dried ($MgSO_4$). Upon concentration of the solvent in vacuo, 1.7 g (83%) of 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6-tetramethylbenzyl)-1*H*-indol-4-ylmethylene]hydrazide (**74**) crystallized out of solution, mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 2.08 (s, 6H), 2.21 (s, 6H), 5.37 (s, 2H), 6.78 (s, 1H), 7.05 (m, 3H), 7.26 (t, 1H), 7.33 (d, 1H), 7.76 (d, 1H), 8.01 (d, 1H), 8.19 (s, 1H), 8.64 (s, 1H), 11.68 (s, 1H). Anal. ($C_{28}H_{26}N_4O_2 \cdot {}^1/_4H_2O$) C, H, N.

Biological Assays. Binding and Functional Assays. Receptor binding was carried out using membranes from BHK cells expressing the cloned human glucagon receptor. The assay was carried out as earlier described[20] with some modifications. The buffer was 50 mM HEPES, 5 mM EGTA, 5 mM $MgCl_2$, 0.005% Tween 20, pH 7.4, and compounds were diluted in 100% DMSO. A total of 140 (for standard curve) or 165 μL of buffer was added to wells. A total of 10 μL samples in DMSO or DMSO (for standard curve) were added, and 25 μL of different glucagon concentrations were added. The 50.000 CPM tracer was added in 25 μL, and 4 μg of plasma membrane was added in 25 μL. Incubation was for 2 h at 30 °C. The functional assay was carried out as previously described.[20]

Primary Hepatocytes Assays. Hepatocytes were isolated from male Sprague−Dawly rats fed ad libitum using a two-

step perfusion technique and cultured essentially as previously described.[21] To study glycogenolysis, hepatocytes were incubated with 15 mM glucose and 10 nM insulin for 24 h to induce glycogen synthesis. The hepatocytes were washed twice in prewarmed buffer A (117.6 mM NaCl, 5.4 mM KCl, 0.82 mM Mg_2SO_4, 1.5 mM KH_2PO_4, 20 mM HEPES, 9 mM $NaHCO_3$, 0.1% w/v HSA, and 2.25 mM $CaCl_2$, pH 7.4 at 37 °C) and incubated with increasing concentrations of **74** (0−1000 nM) in the presence of 0.5 nM glucagon or with increasing concentration of glucagon (0−100 nM) in buffer A for 70 min. The glucose released into the medium and the glycogen levels were measured as described previously.[21]

In Vitro Metabolism. Metabolic Rate of Disappearance. Test compounds were screened in rat liver microsomes in order to determine the metabolic rate of disappearance. Incubation times were 0, 5, 10, 10, 30 min ($n = 3$), total incubation volume was 150 μL, protein content was 0.331 mg/mL, the incubation mixture contained 1 mM UDP-GA, 1 mM NADPH, KH_2PO_4 (pH 7.4) buffer up to 150 μL, and the compound concentration was 10 μM. Incubation temperature was 37 °C. All incubations were performed in a 96-well plate format, and a Packard liquid handler was applied for incubations and liquid handling in general. Microsomal incubations were terminated by addition of 150 μL of MeCN. The metabolic rates were determined via LC−MS analysis (disappearance of parent).

Metabolic Profiling. Incubation times were 0 and 60 min ($n = 3$), total incubation volume was 1000 μL, protein content was 1 mg/mL, compound concentration was 25 μM, and incubation temperature was 37 °C. The incubation mixture contained 1 mM UDP-GA, 1 mM NADPH, and KH_2PO_4 (pH 7.4) buffer up to 1000 μL. Microsomal incubations were terminated by solid-phase extraction (SPE).

SPE Method. SPE column: SPEC (C_8) SPE cartridge. Activation: 1000 μL of MeOH + 1000 μL of $NaHPO_4$ (pH 7.4). Sample volume: 1000 μL. Washing: 2 × 1000 μL of $NaHPO_4$ (pH 7.4). Eluate: 1000 μL of MeOH. The three samples (LC−MS) at each time point were pooled, and the solvent was evaporated under N_2 gas at 40 °C. A total of 250 μL of mobile phase was then added to the evaporated sample and analyzed by LC−MS.

Chromatography. A reversed-phase chromatographic column (4.6 mm × 30 mm i.d., 5 μm particles, Luna C18) (Phenomenex, Torrance, CA) was used with a flow rate of 1 mL/min. Eluent A consisted of 5% MeCN in ammonium acetate (20 mM). Eluent B consisted of 21% MeCN in ammonium acetate (20 mM). The linear gradient system applied was the following: initially the mobile phase contained 10% B and 90% A, which was increased to 100% B in 5 min.

LC−MS Analysis. A Perkin-Elmer HPLC system (Norwalk, CT) consisting of an autosampler 200AS An intelligent pump 200LC and a UV detector APB 785A (operating at 254 nm) was used, and the Sciex API 150 mass spectrometer running with a turbo Ionspray interphase (Thornhill, Canada) was also used.

Acknowledgment. The skillful technical assistance of Annette Nielsen, Paw Bloch, Dorthe E. Jensen, Sanne Kold, Claus B. Jensen, Ingrid Sveistrup, Ina Dresner, Karin H. Albrechtsen, Jørgen S. Jensen, Lone Risegaard, and Brian Hansen is gratefully acknowledged.

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JM0208572