Identification of Alkylidene Hydrazides as Glucagon Receptor Antagonists

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High throughput screening of our small molecule combinatorial library identified a class of benzoylnaphthalenehydrazones with modest affinity for the human glucagon receptor. Optimization of this initial hit through a series of targeted libraries and traditional medicinal chemistry led to ligands with nanomolar affinities. Pharmacological evaluation demonstrated that these ligands were competitive glucagon receptor antagonists. Intravenous administration of a representative benzoylnaphthalenehydrazone into rats attenuated glucagon-stimulated glucose levels.

Introduction

Glucagon is a hormone synthesized and released from the pancreas in response to low blood glucose levels, such as during fasting or starvation. In diabetes, the bihormonal hypothesis implicates not only the lack of an insulin effect but also a paradoxically elevated relative level of circulating glucagon. Glucagon binds to specific receptors in the liver where it has a number of actions that oppose the effects of insulin. Glucagon stimulates glycogenolysis (breakdown of glycogen) and gluconeogenesis (glucose production), resulting in increased levels of glucose in the blood.[1,2] In vivo studies in various animal species suggest that neutralization of circulating glucagon alleviates hyperglycemia.[3−5] Researchers have focused on the modification of amino acids in the glucagon molecule in an attempt to turn the agonist into an antagonist.[6,7] Results with these peptide antagonists also support that glucagon receptor antagonists may be useful as agents for the treatment of diabetes.[8] Several small organic molecule glucagon antagonists have been disclosed in the primary literature and in patent applications. Among those reported are the hydroxybenzoyl-benzimidazoles,[9] triaryl-pyrroles,[10] triaryl imidazoles,[11] styryl quinoxalines,[12] pyrroloquinoxalines,[13] pyrimidinones,[14] quinolines,[15] phenylpyridines, and biphenyls.[16] Reviews of the latest glucagon antagonists have been summarized by Connell,[17] Livingston,[18] and Madsen.[19] In this paper, we report the synthesis and structure−activity relationships (SAR) of a series of benzoylnaphthylenehydrazones as competitive glucagon antagonists. The in vivo activity of a representative compound in rats is also reported. The structures described here are covered in two patent applications[20,21] and were previously presented at an American Chemical Society meeting.[22−24] The glucagon receptor is described in three U.S. patents[25−27] and in an international patent application.[28]

Chemistry

Compounds of the general structure **1** were synthesized from the condensation of acylhydrazides and aldehydes or ketones in the presence of DMF or DMSO containing acetic acid or trifluoroacetic acid in catalytic amounts, Scheme 1, procedure B. In general, the hydrazones were prepared in a library format, and screening hits were resynthesized. Desired acylhydrazides that were not commercially available were prepared by treating the corresponding methyl or ethyl ester with hydrazine in ethanol under reflux conditions, procedure A. The acylhydrazides obtained in this manner were usually solid or crystalline. The alkylidenehydrazide **42** was obtained from the reduction of hydrazone **41** in the presence of excess sodium borohydride.

Results and Discussion

Screening of our small molecule library led to the identification of benzoic acid arylidenehydrazides of the general formula **1** that bound to the human glucagon receptor. Resynthesis of the hits resulted in three 3-methoxy-4-hydroxybenzoic acid benzylidenehydrazides **2**−**4** that had IC_{50} values between 3 and 5 μM, Figure 1. The presence of the methoxy group at the C4 position of the naphthalene system did not contribute to the overall potency of the molecules since structures **3** and **4** were equipotent. Optimization of the hits was accomplished by exploring the right-portion, left-portion, and the acylhydrazide moeity of the structural series in parallel.

Optimization of the right-hand portion of **3** and **4** was carried out through the condensation of 4-hydroxy-3-

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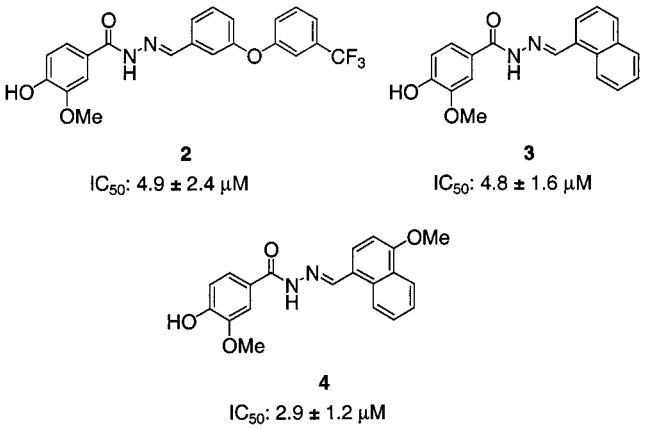
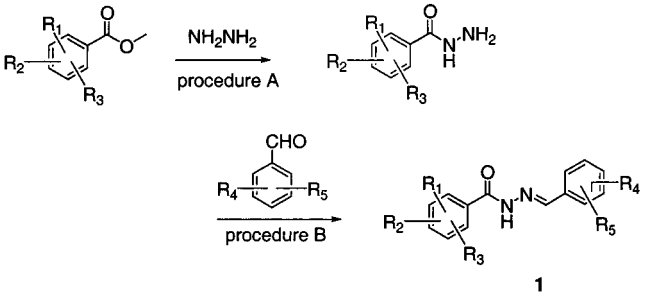


Figure 1. Hits from exploratory library. Standard deviations are shown (± SD). Number of determinations ≥ 3.

Scheme 1. General Synthetic Route for the Synthesis of Acyl Hydrazones

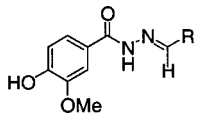


methoxybenzoylhydrazide with various aldehydes. Inspection of Table 1 showed that hydrazones derived from aldehydes containing polar features, such as compounds **5** and **6**, and benzaldehydes containing polar groups, such as methoxy and/or hydroxy groups (**7**−**9**), had IC_{50} values that were greater than 100 μM. Hydrazones derived from benzaldehydes containing hydrophobic groups such as isopropyl **12** or *tert*-butyl **13** and from aryl-aldehydes such as biphenyl **14**, furyl-phenyl **15**, and indole **16** had IC_{50} values between 10 and 50 μM. Hydrazones containing the bicyclic naphthyl-like system such as compounds **3**, **4**, **18**, **19**, and **20** bound to the receptor with the highest affinity (IC_{50} values of 10 μM or better). Replacement of the naphthalene unit in **3** for an indole or a quinoline moiety such as in **16** or **18**, respectively, led to compounds with binding affinities within an order of magnitude compared to the original hit. Replacement of the naphthyl C4 methoxy group in **4** for a dimethylamino group, compound **19**, decreased the affinity by only 3-fold. However, replacement of the methoxy with the hydroxy group in **20** led to a substantial increase in affinity.

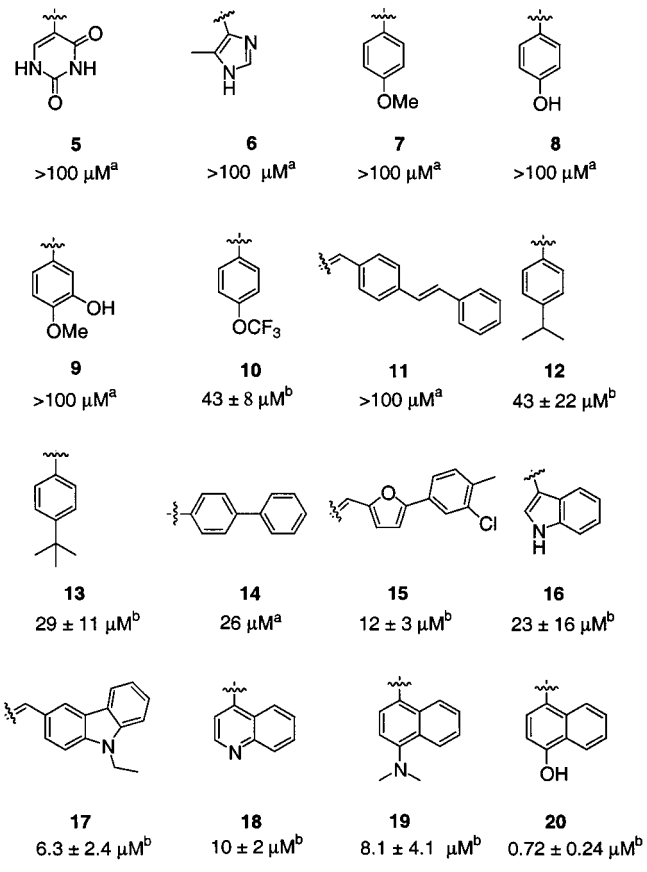
An investigation of the benzoic acid hydrazide region was carried out by keeping the 4-methoxynaphthaldehyde moiety constant. Table 2 shows the compounds that were prepared. Removal of the C3 methoxy group in the benzoyl ring from the initial hit **4** (2.9 μM) resulted in the equipotent compound **21** (4.9 μM). Removal of the C4 hydroxyl group in **4** led to **22**, which was found to be inactive. These results indicated that an essential hydrogen bond interaction between the C4 phenolic group and the receptor was identified.

Having established the importance of the phenolic group, analogues capable of altering the acidity of the

Table 1. Exploration of Various Aldehydes/Shapes



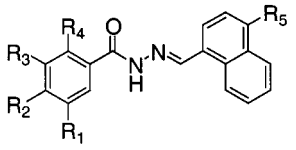
R: / IC_{50}:



[a] Number of determinations = 2. [b] Number of determinations ≥ 3. Standard deviations are shown as ± SD.

phenolic hydrogen were prepared from the condensation of various substituted 4-hydroxybenzoic acid hydrazides with 4-hydroxynaphthaldehyde. Incorporation of an appropriate small substituent at C3 of the phenyl ring proved to be desirable for activity compared to **23**, which was not substituted at this position, Table 2. Placement of an amino or a hydroxy substituent (compounds **24** or **25**) led to compounds with three to 4-fold decrease in affinity compared to the methoxy **20**. Relocation of the hydroxyl group from the C3 position, **25**, to the C2 position as in **26** decreased the potency by approximately 10 fold. Incorporation of a halogen substituent at the C3 position of the phenyl ring led to compounds **27**−**29** having IC_{50} values less than 1 μM. Placement of a chlorine atom in the C3 position resulted in compound **27** (0.20 μM), which was the most active antagonist in this series. It thus appeared that there was a preference for electron withdrawing groups at the C3 position. Addition of a second chlorine atom or methoxy group in the C5 position such as in **30** or **31** resulted in loss of affinity. This may be explained by the fact that either the addition of a second chlorine atom caused the phenol group to exist largely in the phenolate form and/or the possibility that the placement

Table 2. SAR: 4-Hydroxybenzoyl



No.	R_1	R_2	R_3	R_4	R_5	IC_{50} (± S.D., μM)
4	OMe	OH	H	H	OMe	2.9 ± 1.2[b]
21	H	OH	H	H	OMe	4.9 ± 1.2[b]
22	OMe	H	H	H	OMe	>100[a]
20	OMe	OH	H	H	OH	0.72 ± 0.24[b]
23	H	OH	H	H	OH	1.2 ± 0.1[b]
24	NH_2	OH	H	H	OH	2.0 ± 0.7[b]
25	OH	OH	H	H	OH	3.4 ± 0.7[b]
26	H	OH	H	OH	OH	28 ± 4[b]
27	Cl	OH	H	H	OH	0.20 ± 0.11[b]
28	F	OH	H	H	OH	0.39 ± 0.15[b]
29	Br	OH	H	H	OH	0.83 ± 0.22[b]
30	Cl	OH	Cl	H	OH	34 ± 6[b]
31	Cl	OH	OMe	H	OH	60 ± 32[b]
32		OH	H	H	OH	>100[a]

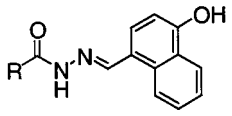
[a] Number of determinations = 2. [b] Number of determinations ≥ 3. Standard deviations are shown as ± SD.

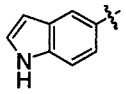
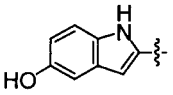
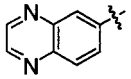
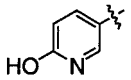
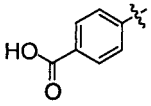
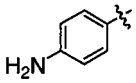
of an additional substituent increased the steric demands necessary for optimal ligand−receptor interaction, thus resulting in loss of activity. Incorporation of the morpholino group in **32** also led to substantial loss of activity.

Further exploration of the benzoic acid moiety was also investigated through replacement of the 4-hydroxybenzoyl region with other groups having similar properties. The phenolic replacements investigated were in general hydrophobic and aromatic and possessed the ability to participate in hydrogen bond donation and/or acceptor interactions. Hydrazones containing phenolic replacements, such as the indole **33**, the hydroxyindole **34**, the quinoxaline **35**, the 2-hydroxypyridinyl **36**, the carboxyphenyl **37**, and the amino phenyl **38**, were prepared and are shown in Table 3. These compounds were not active at the glucagon receptor.

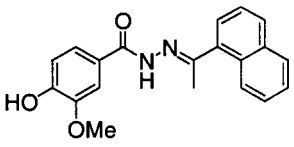
Early modifications to the acylhydrazone moiety, Figure 2, such as incorporation of a methyl group at the hydrazone carbon (**39**) or reversing the acylhydrazone connectivity between the phenol and the naphthyl ring (**40**) led to structures with IC_{50} values greater than 100 μM compared to the original hit **3** which was a 4.8 μM compound. Reduction of the hydrazone **41** (1.8 μM) to

Table 3. Exploration of 4-Hydroxyphenyl Replacements

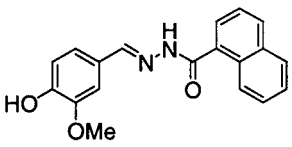


No.	R	IC_{50} (± S.D., μM)
33		>100[a]
34		>100[a]
35		>100[a]
36		>100[a]
37		>100 ± 80[b]
38		>100[a]

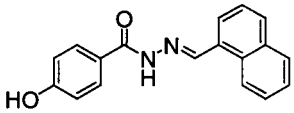
[a] Number of determinations = 2. [b] Number of determinations ≥ 3. Standard deviations are shown as ± SD.



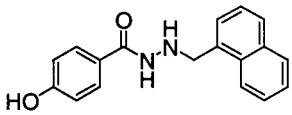
39
IC_{50}: >100 μM



40
IC_{50}: >100 μM



41
IC_{50}: 1.8 μM



42
IC_{50}: >100 μM

Figure 2. Hydrazone isostere replacements. Number of determinations = 2.

the hydrazide **42** (>100 μM) also led to substantial loss of activity. These early results indicated that the acylhydrazone moiety and the proper placement of the group was essential for activity. Thus our strategy was to delay the exploration of hydrazone isosteres until structures with much higher activities were found. This work is being prepared and will be published in due time.

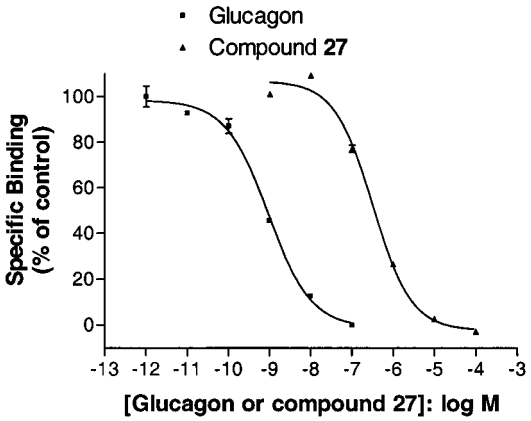


Figure 3. Displacement of [^{125}I]glucagon from membranes expressing human glucagon receptors by compound **27**. The results shown are representative of five binding experiments.

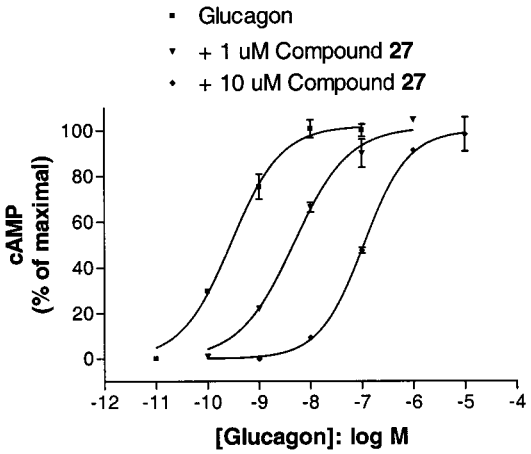


Figure 4. Inhibition of glucagon-stimulated cAMP in cells expressing human glucagon receptors by compound **27**. The results shown are representative of two experiments.

Pharmacology Results and Discussion

Compound **27** bound to the human glucagon receptor with an IC_{50} value of 0.20 μM as determined in a competition-binding assay (Figure 3). To determine the functional activity, we examined the effect of compound **27** on glucagon-stimulated cAMP accumulation in BHK cells transfected with the human glucagon receptor. Increasing concentrations of compound **27** shifted the glucagon concentration−response curves rightward, while the maximal response to glucagon was not reduced (Figure 4). Schild analysis of these data indicated a pA_2 of 6.99 for compound **27**, correlating closely with the IC_{50} value determined in the binding assay. The addition of compound **27** alone to these cells over a wide concentration range did not increase cAMP levels. These data suggested that compound **27** was a competitive antagonist at the human glucagon receptor.

Compound **27** was also tested in a rat receptor binding assay. The IC_{50} value was found to be 0.027 ± 0.0042 μM. Thus, compound **27** bound to the rat receptor with greater affinity than to the human receptor. However, the assays were not directly comparable as they were carried out under different conditions and a cloned receptor was used for the human assay and isolated livers for the rat assay.

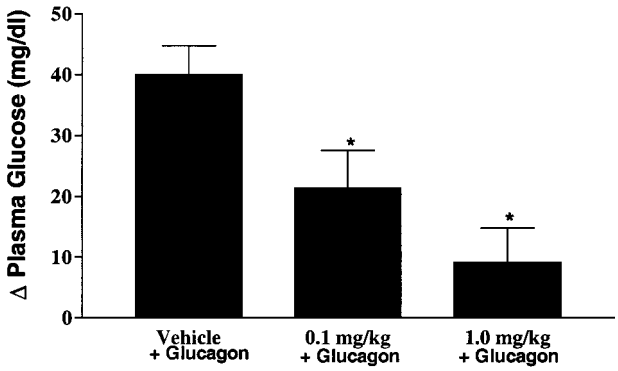


Figure 5. Attenuation of glucagon-stimulated blood glucose in rats by compound **27** is dose-dependent. Figure shows means ± SEM. *p < 0.05 vs vehicle + glucagon.

In Vivo Results and Discussion

Intravenous administration of glucagon to rats elicits a transient hyperglycemic response.[5] A time course for the dose of glucagon (10 μg) administered in these studies showed the maximum increase in plasma glucose occured 20 min post dose. The glucagon antagonist **27** was administered intravenously at 5, 10, 20, 30, or 60 min prior to glucagon challenge. Compound **27** inhibited the hyperglycemic response at 10 and 20 min. At 5 min, partial inhibition was observed, while at 30 and 60 min compound **27** did not inhibit the glucose response to exogenous glucagon. Compound **27** alone did not effect resting plasma glucose. Figure 5 represents compound **27** administered 20 min prior to glucagon challenge. These data suggested that inhibition of the hyperglycemic effect of glucagon by **27** was dose-dependent and was mediated through the glucagon receptor.

Summary

In summary, a series of 4-hydroxybenzoic acid naphthylidene hydrazides were discovered and found to have high affinity for the human glucagon receptor. These hydrazide antagonists required the presence of a hydroxy functionality at the C-4 position of the benzoic acid moiety of the hydrazide. The most potent compound described in this paper was 4-hydroxy-3-methoxybenzoic acid (4-hydroxy-1-naphthylmethylene)hydrazide (**27**) with an IC_{50} value of 0.20 μM. Intravenous administration of this antagonist into rats suppressed glucagon-stimulated increase of plasma glucose.

Experimental Section

Chemistry. All reactions described were carried out under a nitrogen atmosphere. Column chromatography was performed using silica gel (220−400 mesh) as the stationary phase and nitrogen pressure. Thin-layer chromatography was performed on silica gel 60 F_{254} glass-backed plates and visualized with UV light, 3,5-dinitrophenylhydrazine, and/or ninhydrin. Chromatographic elution solvent systems are reported as volume/volume ratios. NMR spectra were obtained in a Bruker 300 MHz at room temperature employing deuterated solvents. The δ scale is reported in ppm in reference to the deuterated solvent. Coupling constants (J) are reported in hertz (Hz). Mass spectra (FAB) were obtained at the mass spectrometry laboratory at Scripps Research Institute in La Jolla, and data obtained via APCI were obtained in house in an Agilant 1100 LC-MSD. Elemental combustion experiments were performed at Atlantic Microlab, Numega, or Galbraith.

General Procedure for the Preparation of Substituted Benzoic Acid Hydrazides (Procedure A). The methyl or ethyl ester of the corresponding benzoic acid was refluxed with excess hydrazine in methanol or ethanol solution under a nitrogen atmosphere overnight. Upon cooling and concentration of the solution in vacuo, the hydrazide precipitated out of solution. The benzoylhydrazides were recrystallized from ethanol. Excess hydrazine was also removed by washing the products with water.

3-Chloro-4-hydroxybenzoic Acid Hydrazide. To a sample of methyl 3-chloro-4-hydroxybenzoate (2 g, 10.7 mmol) dissolved in ethanol (50 mL) was added hydrazine (1.8 mL, 54 mmol). The reaction was refluxed overnight under nitrogen. Upon cooling the reaction vessel, the desired product crystallized. The white solid was isolated by filtration and recrystallized from hot ethanol, affording 3-chloro-4-hydroxybenzoic acid hydrazide in 60% yield. ^{1}H NMR (DMSO-d_6) δ 4.49 (br s, 2H), 7.05 (dd, 1H), 7.71 (dd, 1H), 7.89 (d, 1H), 9.67 (s, 1H), 10.72 (br s, 1H).

5-Hydroxyindole-2-carboxylic Acid Hydrazide. To a sample of methyl 5-hydroxyindole-2-carboxylate (5 g, 24 mmol) dissolved in ethanol (250 mL) was added hydrazine (4 mL, 121 mmol). The reaction was refluxed overnight under nitrogen. Upon cooling the reaction vessel, the desired product crystallized. Recrystallization from hot ethanol gave the 5-hydroxyindole-3-carboxylic acid hydrazide in 85% yield. ^{1}H NMR (DMSO-d_6) δ 4.38 (s, 2H), 6.62 (dd, 1H), 6.76 (dd, 2H), 7.13 (d, 1H), 8.70 (s, 1H), 9.57 (s, 1H), 11.21 (s, 1H); MS (FAB): *m*/*z* 192 (M+H)$^+$.

3-Fluoro-4-hydroxybenzoic Acid Hydrazide. Same procedure as described above. ^{1}H NMR (DMSO-d_6) δ 5.55 (br s, 3H), 6.85 (t, 1H), 7.43 (d, 1H), 7.50 (d, 1H), 9.45 (br s, 1H).

3-Bromo-4-hydroxybenzoic Acid Hydrazide. Same procedure as described above. ^{1}H NMR (DMSO-d_6) δ 4.40 (br s, 2H), 6.95 (d, 1H), 9.61 (br s, 1H), 9.65 (d, 1H), 9.95 (s, 1H); MS (FAB): *m*/*z* 233.1 (M+H)$^+$.

6-Hydroxynicotinic Acid Hydrazide. This compound was prepared by a modification of a reported procedure.[29] A mixture of 2-hydroxypyridine-5-carboxylic acid (13.91 g, 0.1 mol), sulfuric acid (10 mL), and methanol (100 mL) was refluxed overnight. The methanol was removed in vacuo, and the resulting residue was basified with aqueous sodium carbonate and extracted with ethyl acetate. The extract was dried over sodium sulfate, filtered, and concentrated under reduced pressure, affording a slurry. The solid was filtered, and the cake was washed with ethyl acetate to give 8.44 g (55%) of the methyl ester. This material was treated with anhydrous hydrazine (20 mL, 0.637 mol, 11.6 equiv) and anhydrous ethanol (20 mL), and the mixture was refluxed for 1 h, resulting in the formation of a solid mass. This was triturated with water (20 mL), filtered, then washed with water and ethanol, and dried, affording the hydrazide as white powder: 6.94 g (82% from ester). ^{1}H NMR (DMSO-d_6 + 20% D_2O) δ 7.96 (d, J = 2.6 Hz, 1 H), 7.87 (dd, J = 9.6, 2.6 Hz, 1 H), 6.43 (d, J = 9.6 Hz, 1 H).

3,5-Dichloro-4-hydroxybenzoic Acid Hydrazide. This compound was prepared by a modification of a reported procedure.[30] A mixture of methyl 3,5-dichloro-4-hydroxybenzoate hydrate (11.95 g, 0.050 mol), anhydrous hydrazine (16 mL), and water (2 mL) was heated at 80 °C for 2 h. The excess hydrazine was removed in vacuo, and the residue was triturated with water and filtered. The filter cake was washed with water, methanol, and ether and then dried, affording 9.26 g (84%) of desired product. ^{1}H NMR (MeOH-d_4) δ 7.75 (s).

3-(4-Morpholinomethyl)-4-hydroxybenzoic Acid Hydrazide. This was prepared from the carboxylic acid monohydrate as described above for 6-hydroxynicotinic acid hydrazide with the following modifications: In the workup of the methyl ester intermediate, sodium bicarbonate was used instead of sodium carbonate. The crude ester was refluxed with 30 equiv of hydrazine monohydrate in ethanol for 10 h. The volatiles were completely removed in vacuo (80 °C bath temperature, 10 Torr), and the solid was triturated with 1:4 ethanol/ether, filtered, and washed with ether, affording the title product in 73% yield overall from the acid. An analytically pure sample was prepared by recrystallization from ethanol: mp 168−170 °C; ^{1}H NMR (DMSO-d_6) δ 2.45 (br s, 4H), 3.6 (br s, 6H), 4.4 (br s, 2H), 7.62 (dd, J = 8.2, 1.8 Hz, 1H), 7.65 (d, J = 1.8 Hz, 1H), 7.66 (d, J = 8.2, Hz, 1H), 9.49 (br s, 1H); MS (APCI) *m*/*z* 252.1 (M+H$^+$). Anal. ($C_{12}H_{17}N_3O_3$) C, H, N.

General Procedure for the Preparation of Alkylidene Hydrazides (Procedure B). The acylhydrazides were treated with the corresponding aldehyde, and a catalytic amount of acetic acid or trifluoroacetic acid in ethanol, DMF, or DMSO. After the solution was stirred at 80 °C overnight under a blanket of nitrogen, the desired products were crystallized out of solution upon cooling. In many instances, the reaction proceeds at room temperature. The products were usually either recrystallized from ethanol or ethyl acetate/hexane or chromatographed over silica gel.

3-Chloro-4-hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]benzohydrazide (27). To a solution of 3-chloro-4-hydroxybenzoic acid hydrazide (220 mg, 1.1 mmol) in DMSO (2 mL) were added 4-hydroxynaphthaldehyde (180 mg, 1.1 mmol) and a catalytic amount of glacial acetic acid (5 drops). The reaction was stirred overnight under nitrogen and diluted with ethyl acetate. The solution was washed with saturated sodium bicarbonate, water, and brine and dried over $MgSO_4$. The organic volume was concentrated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel using CH_2Cl_2/MeOH as the mobile phase. Yield 26%.;mp 252−254 °C; ^{1}H NMR (DMSO-d_6) δ 6.90 (d, J = 7.98 Hz, 1H), 7.02 (d, J = 8.49 Hz, 1H), 7.47 (t, J = 7.50, J = 14.97 Hz, 1H), 7.58 (t, J = 7.11, 14.82 Hz, 1H), 7.66 (d, J = 8.04 Hz, 1H), 7.73 (dd, J = 1.65, 8.49 Hz, 1H), 7.93 (s, 1H), 8.17 (d, J = 8.19 Hz, 1H), 8.84 (s, 1H), 8.88 (d, J = 8.46 Hz, 1H), 10.75 (br s, 1H), 10.88 (br s, 1H), 11.54 (s, 1H); MS (APCI) *m*/*z* 341.2 (M+H$^+$). Anal. ($C_{18}H_{13}ClN_2O_3$·0.25H_2O), C, H, N.

3,5-Dichloro-4-hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl-)methylidene]benzohydrazide (30). A mixture of the 3,5-dichloro-4-hydroxybenzoylhydrazide (442 mg, 2.0 mmol) and 4-hydroxynaphthaldehyde (344 mg, 2.0 mmol) was dissolved in hot (~100 °C) DMSO (3 mL). The mixture was allowed to cool and allowed to stand at room temperature for 2 days. The reaction mixture was diluted with water, filtered, and washed with a minimum amount of methanol, then ether, affording 325 mg of solid. This was recrystallized from methanol, affording 238 mg (32%) of the product as a yellow solid. mp 266−269 °C; ^{1}H NMR (DMSO-d_6) δ 6.96 (d, J = 8.29, 1H), 7.54 (dd, J = 7.5 Hz, J = 7.2 Hz, 1H), 7.65 (dd, J = 7.9 Hz, J = 6.2 Hz, 1H), 7.73 (d, J = 8.3 Hz, 1H), 7.98 (s, 2H), 8.23 (d, J = 7.9 Hz, 1H), 8.29 (s, 1H), 8.94 (d, J = 8.3, 1H), 10.82 (s, 1H), 10.96 (br s, 1H), 11.69 (s, 1H); MS (APCI) *m*/*z* 376.0 (M+H$^+$). Anal. ($C_{18}H_{12}Cl_2N_2O_3$) C, H, N.

6-Hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]-nicotinohydrazide (36). A mixture of 6-hydroxynicotinoyl hydrazide (306 mg, 2.0 mmol) and 4-hydroxynaphthalene-1-carboxaldehyde (344 mg, 2.0 mmol) was dissolved in hot DMSO (2 mL), allowed to cool to room temperature, and allowed to stand at room temperature overnight. The solution was diluted with water (50 mL) and filtered, and the cake was washed with methanol and dried with heating to constant weight, affording 543 mg (88%) of the acylhydrazone as a tan solid. mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 6.43 (d, J = 9.4 Hz, 1H), 6.96 (d, J = 7.9 Hz, 1H), 7.53 (dd, J = 7.9 Hz, J = 7.2 Hz, 1H), 7.63 (dd, J = 7.9 Hz, J = 7.2 Hz, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.95 (d, J = 9.4 Hz, 1H), 8.15 (s, 1H), 8.23 (d, J = 8.3 Hz, 1H), 8.81 (s, 1H), 8.94 (d, J = 7.5 Hz, 1H), 10.80 (s, 1H), 11.51 (s, 1H), 12.11 (s, 1H); MS (APCI) *m*/*z* 308.07 (M+H$^+$). Anal. ($C_{17}H_{13}N_3O_3$·1.75H_2O) C, H, N.

4-Hydroxy-3-methoxy-*N*′-((*E*)-{3-[4-(trifluoromethyl)-phenoxy]phenyl}methylidene)-benzohydrazide (2). Yield 49%; mp 150.5−152.5 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H), 6.85 (d, 1H, J = 8.1 Hz), 7.16 (dd, 1H, J = 8.1, 2.1 Hz), 7.36 (m, 5H), 7.44 (m, 3H), 7.61 (t, 1H, J = 8.0 Hz), 8.43 (s, 1H), 9.75 (s, 1H), 11.69 (s, 1H); MS (APCI) *m*/*z* 431.14 (M+H$^+$). Anal. ($C_{22}H_{17}F_3N_2O_4$) C, H, N.

4-Hydroxy-3-methoxy-*N*′-[(*E*)-1-naphthylmethylidene]-benzohydrazide (3). Yield 64%; mp 165−167 °C; ^{1}H NMR (DMSO-d_6) δ 3.94 (s, 3H), 6.74 (d, J = 8.2 Hz, 1H), 7.37−7.52 (m, 6H), 7.77 (d, J = 7.1 Hz, 1H), 7.85 (m, 2H), 8.67 (d, J = 8.1 Hz, 1H), 9.13 (s, 1H), 10.90 (br s, 1H); MS (APCI) m/z 321.07 (M+H$^+$). Anal. ($C_{19}H_{16}N_2O_3$·0.5H_2O) C, H, N.

4-Hydroxy-3-methoxy-*N*′-[(*E*)-(4-methoxy-1-naphthyl)-methylidene]benzohydrazide (4). mp 184.5−186 °C; ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H), 4.80 (s, 3H), 6.00 (s, 1H), 6.59 (d, 1H), 6.83 (d, 1H), 7.39 (m, 3H), 7.52 (s, 1H), 7.73 (s, 1H), 8.18 (d, 1H), 8.58 (d, 1H), 8.88 (s, 1H), 9.95 (s, 1H); MS (APCI) m/z 351 (M+H$^+$). Anal. ($C_{20}H_{18}N_2O_4$) C, H, N.

***N*′-[(*E*)-(2,4-Dioxo-1,2,3,4-tetrahydro-5-pyridinyl)methylidene]-4-hydroxy-3-methoxybenzohydrazide (5).** Yield 66%; mp 259−261 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (s, 3H), 6.78 (d, J = 8.2 Hz, 1H), 7.36 (d, J = 8.3 Hz, 1H), 7.40 (s, 1H), 7.82 (s, 1H), 8.27 (s, 1H), 9.66 (br s, 1H), 11.20 (br s, 1H), 11.37 (s, 1H), 11.45 (s, 1H); MS (APCI) m/z 304.97 (M+H$^+$). Anal. ($C_{13}H_{12}N_4O_5$·0.9H_2O) C, H, N.

4-Hydroxy-3-methoxy-*N*′-[(*E*)-(5-methyl-1*H*-imidazol-4-yl)methylidene]benzohydrazide (6). Yield 99%; mp 264 °C, dec; ^{1}H NMR (DMSO-d_6) δ 2.60 (s, 3H), 3.99 (s, 3H), 8.72 (s, 1H), 14.52 (br s, 1H); MS (APCI) m/z 274.99 (M+H$^+$). Anal. ($C_{13}H_{14}N_4O_3$·0.75H_2O) C, H, N.

4-Hydroxy-3-methoxy-*N*′-[(*E*)-(4-methoxyphenyl)methylidene]benzohydrazide (7). Yield 73%; mp 202−203 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H), 3.84 (s, 3H), 6.86 (d, J = 8.29 Hz, 1H), 7.01 (d, J = 8.67 Hz, 2H), 7.43 (d, J = 9.42 Hz, 1H), 7.47 (s, 1H), 7.64 (d, J = 8.67 Hz, 2H), 8.38 (s, 1H), 9.61 (s, 1H), 11.34 (s, 1H); MS (APCI) m/z 301.05 (M+H$^+$). Anal. ($C_{16}H_{16}N_2O_7$) C, H, N.

4-Hydroxy-*N*′-[(*E*)-(4-hydroxyphenyl)methylidene]-3-methoxybenzohydrazide (**8**). Yield 48%; mp 250−252 °C; ^{1}H NMR (DMSO-d_6) δ 3.84 (s, 3H), 6.84 (m, 3H), 7.42 (dd, J = 8.29 Hz, 1.88 Hz, 1H), 7.47 (d, J = 1.88 Hz, 1H), 7.53 (d, J = 8.29 Hz, 2H), 8.32 (s, 1H), 9.59 (s, 1H), 9.82 (s, 1H), 11.35 (s, 1H); MS (APCI) m/z 287.05 (M+H$^+$). Anal. ($C_{15}H_{14}N_2O_7$) C, H, N.

4-Hydroxy-*N*′-[(*E*)-(3-hydroxy-4-methoxyphenyl)methylidene]-3-methoxybenzohydrazide (9). Yield 22%; mp 188−189 °C; ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3H), 3.84 (s, 3H), 6.85 (d, J = 8.29 Hz, 1H), 6.92 (d, J = 8.29, 1H), 7.02 (d, J = 8.29 Hz, 1H), 7.25 (s, 1H), 7.42 (dd, J = 8.29, 1.88 Hz, 1H), 7.47 (d, J = 1.51 Hz, 1H), 8.26 (s, 1H), 9.21 (s, 1H), 9.60 (s, 1H), 11.37 (s, 1H); MS (APCI) m/z 317.07 (M+H$^+$). Anal. ($C_{16}H_{16}N_2O_5$·0.1H_2O) C, H, N.

4-Hydroxy-3-methoxy-*N*′-{(*E*)-[4-(trifluoromethoxy)-phenyl]methylidene}benzohydrazide (**10**). Yield 53%; mp 202−203 °C; ^{1}H NMR (DMSO-d_6) δ 4.01 (s, 3H), 7.06 (d, J = 8.11 Hz, 1H), 7.64 (m, 4H), 8.03 (d, J = 8.81 Hz, 2H), 8.64 (s, 1H), 9.93 (s, 1H), 11.89 (s, 1H); MS (APCI) m/z 355.00 (M+H$^+$). Anal. ($C_{16}H_{13}F_3N_2O_4$) C, H, N.

4-Hydroxy-3-methoxy-*N*′-(*E*)-{4-[(*E*)-2-phenylethenyl]-phenyl}methylidene]benzohydrazide (**11**). Yield 75%; mp 238−239 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H), 6.84 (d, 1H, J = 7.91 Hz), 7.27 (dd, J = 7.54, 6.78 Hz, 1H), 7.33 (d, J = 4.52 Hz, 2H), 7.38 (t, J = 7.54 Hz, 2H), 7.44 (dd, J = 8.29, 1.88 Hz, 1H), 7.47 (s, 2H), 7.62 (d, J = 7.54 Hz, 2H), 7.70 (m, 4H), 8.42 (s, 1H), 11.64 (s, 1H); MS (APCI) m/z 373.07 (M+H$^+$). Anal. ($C_{23}H_{20}N_2O_3$·2H_2O) C, H, N.

4-Hydroxy-*N*′-[(*E*)-(4-isopropylphenyl)methylidene]-3-methoxybenzohydrazide (12). Yield 45%; mp 192−193.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (d, J = 7.2 Hz, 6H), 2.91 (septet, 1H), 3.93 (s, 3H), 6.00 (br s, 1H), 6.95 (d, J = 7.9 Hz, 1H), 7.22 (m, 3H), 7.52 (m, 1H), 7.63 (m, 2H), 8.24 (br s, 1H), 9.37 (br s, 1H); MS (APCI) m/z 313.07 (M+H$^+$). Anal. ($C_{18}H_{20}N_2O_3$· 0.33H_2O) C, H, N.

4-Hydroxy-*N*′-[(*E*)-(4-*tert*-butylphenyl)methylidene]-3-methoxybenzohydrazide (13). Yield 49%; mp 135−139 °C; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 9H), 3.92 (s, 3H), 6.06 (br s, 1H), 6.92 (d, J = 7.54, 1H), 7.38 (m, 3H), 7.54 (m, 1H), 7.63 (m, 2H), 8.28 (br s, 1H), 9.59 (br s, 1H); MS (APCI) m/z 327.08 (M+H$^+$). Anal. ($C_{19}H_{22}N_2O_3$·0.7H_2O) C, H, N.

***N*′-[(*E*)-(1,1′-Biphenyl]-4-ylmethylidene]-4-hydroxy-3-methoxybenzohydrazide (14).** Yield 23%; mp 205−206 °C; ^{1}H NMR (DMSO-d_6) δ 4.02 (s, 3H), 7.04 (d, J = 8.2 Hz, 1H), 7.63−7.68 (m, 5H), 7.88−7.96 (m, 6H), 8.65 (s, 1H), 9.90 (br s, 1H), 11.83 (s, 1H); MS (APCI) m/z 347.09 (M+H$^+$). Anal. ($C_{21}H_{18}N_2O_3$) C, H, N.

***N*′-{(*E*)-[5-(3-Chloro-4-methylphenyl)-2-furyl]methylidene}-4-hydroxy-3-methoxybenzohydrazide (15).** Yield 76.7%; mp 210−212 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 6.89 (d, J = 9.0 Hz, 1H), 7.05 (d, J = 3.0 Hz, 1H), 7.28 (s, 1H), 7.48 (m, 4H), 7.74 (d, J = 3.0 Hz, 1H), 7.84 (s, 1H), 8.39 (s, 1H), 9.77 (s, 1H), 11.67 (s, 1H). Anal. ($C_{19}H_{15}ClN_2O_4$·0.4H_2O) C, H, N.

4-Hydroxy-*N*′-[(*E*)-1*H*-indol-3-ylmethylidene]-3-methoxybenzohydrazide (16). Yield 45%; mp 224−226 °C; ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3H), 6.80 (d, J = 8.2 Hz, 1H), 7.11 (m, 2H), 7.39 (m, 3H), 7.74 (s, 1H), 8.23 (d, J = 7.5 Hz, 1H), 8.54 (s, 1H), 9.58 (s, 1H), 11.23 (s, 1H), 11.50 (s, 1H); MS (APCI) m/z 310.06 (M+H$^+$). Anal. ($C_{17}H_{15}N_3O_3$) C, H, N.

***N*′-[(*E*)-(9-Ethyl-9*H*-carbazol-3-yl)methylidene]-4-hydroxy-3-methoxybenzohydrazide (17)**. This was recrystallized from ethanol in 26% yield; mp 126−128 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.33 (t, J = 6.80 Hz, 3H), 3.86 (s, 3H), 4.49 (q, J = 7.18 Hz, 2H), 6.89 (d, J = 7.93 Hz, 1H), 7.25 (t, J = 7.37, 1H), 7.49 (m, 3H), 7.68 (m, 2H), 7.89 (d, J = 8.31 Hz, 1H), 8.24 (d, J = 7.55 Hz, 1H), 8.46 (s, 1H), 8.61 (s, 1H), 9.71 (s, 1H), 11.56 (s, 1H). Anal. ($C_{23}H_{21}N_3O_3$·1EtOH) C, H, N.

4-Hydroxy-3-methoxy-*N*′-[(*E*)-4-quinolinylmethylidene]-benzohydrazide (18). Yield 76%; ^{1}H NMR (DMSO-d_6) δ 3.58 (s, 3H), 6.52 (d, J = 8.1 Hz, 1H), 7.28 (m, 2H), 7.48 (t, J = 7.1 Hz, 1H), 7.61 (m, 2H), 7.86 (d, J = 8.4 Hz, 1H), 8.49 (d, J = 8.4 Hz, 1H), 8.73 (d, J = 4.5 Hz, 1H), 8.94 (s, 1H); MS (APCI) m/z 322.08 (M+H$^+$). Anal. ($C_{18}H_{15}N_3O_3$·0.25H_2O) C, H, N.

***N*′-{(*E*)-[4-(Dimethylamino)-1-naphthyl]methylidene}-4-hydroxy-3-methoxybenzohydrazide (19).** Yield 39%; mp 221−222 °C; ^{1}H NMR (DMSO-d_6) δ 3.05 (s, 6H), 3.97 (s, 3H), 7.06 (d, J = 8.2 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.66 (m, 2H), 7.77 (m, 2H), 7.97 (d, J = 7.9 Hz, 1H), 8.38 (d, J = 7.9 Hz, 1H), 9.10 (d, J = 8.2 Hz, 1H), 9.14 (s, 1H), 9.90 (s, 1H), 11.74 (s, 1H); MS (APCI) m/z 364.08 (M+H$^+$). Anal. ($C_{21}H_{21}N_3O_3$· 0.5H_2O) C, H, N.

4-Hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]-3-methoxybenzohydrazide (20). Yield 75%; mp 230.5−231.5; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 6.89 (d, J = 7.9 Hz, 1H), 6.96 (d, J = 7.9 Hz, 1H), 7.48 (m, 3H), 7.63 (dd, J = 7.9, 7.2 Hz, 1H), 7.72 (d, J = 7.9 Hz, 1H), 8.23 (d, J = 8.7 Hz, 1H), 8.92 (m, 2H), 9.62 (br s, 1H), 10.69 (s, 1H), 11.44 (s, 1H); MS (APCI) m/z 337 (M+H$^+$). Anal. ($C_{19}H_{16}N_2O_4$·1.45H_2O) C, H, N.

4-Hydroxy-*N*′-[(*E*)-(4-methoxy-1-naphthyl)methylidene]-benzohydrazide (21). Yield 68.1%; mp 230.5−233 °C; ^{1}H NMR (DMSO-d_6) δ 3.95 (s, 3H), 6.81 (d, J = 8.58 Hz, 2H), 7.03 (d, J = 8.2 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 7.62 (t, J = 6.8 Hz, 1H), 7.77 (m, 3H), 8.19 (d, J = 8.2 Hz, 1H), 8.89 (m, 2H), 10.07 (s, 1H); MS (APCI) m/z 321.06 (M+H$^+$). Anal. ($C_{19}H_{16}N_2O_3$· 0.6H_2O) C, H, N.

3-Methoxy-*N*′-[(*E*)-(4-methoxy-1-naphthyl)methylidene]-benzohydrazide (22). Yield 39%; ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 3H), 4.10 (s, 3H), 7.50−6.61 (m, 3H), 7.65 (t, J = 9 Hz, 1H), 7.76 (t, J = 9 Hz, 1H), 7.92 (d, J = 9 Hz, 1H), 8.32 (d, J = 9 Hz, 1H), 9.03 (s, 1H), 9.06 (s, 1H), 11.79 (s, 1H); MS (APCI) m/z 335.09 (M+H$^+$). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

4-Hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]-benzohydrazide (23). Yield 40%; mp 258−260 °C; ^{1}H NMR (DMSO-d_6) δ 6.85 (d, 2H, J = 8.7 Hz), 6.95 (d, J = 7.9 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.63 (t, J = 7.5 Hz, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 8.7 Hz, 2H), 8.23 (d, J = 7.9 Hz, 1H),8.94 (m, 2H), 10.11 (s, 1H), 10.76 (s, 1H), 11.50 (s, 1H); MS (APCI) m/z 307.06 (M+H$^+$). Anal. ($C_{18}H_{14}N_2O_3$·0.25H_2O) C, H, N.

3-Amino-4-hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]benzohydrazide (24). Yield 92%; mp 254−260 °C dec; ^{1}H NMR (DMSO-d_6) δ 6.66 (dd, J = 8.2, 1.2 Hz, 1H), 6.90 (d, J = 7.3 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H), 7.15 (s, 1H), 7.46 (t, J = 7.1 Hz, 1H), 7.56 (t, J = 7.9 Hz, 1H), 7.63 (d, J =

7.8 Hz, 1H), 8.16 (d, J = 8.3 Hz, 1H), 8.83 (d, J = 7.6 Hz, 1H), 8.86 (s, 1H), 9.68 (br s, 1H), 10.71 (br s, 1H), 11.34 (s, 1H); MS (APCI) m/z 321.94 ($M+H^+$). Anal. ($C_{18}H_{15}N_3O_3 \cdot 0.75H_2O$) C, H, N.

3,4-Dihydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]benzohydrazide (25). Yield 41%; mp 255−256 °C; ^{1}H NMR (DMSO-d_6) δ 6.83 (d, J = 8.3 Hz, 1H), 6.95 (d, J = 7.9 Hz, 1H), 7.32 (dt, J = 8.3 Hz, 1.9 Hz, 1H), 7.38 (d, J = 1.9 Hz, 1H), 7.53 (dd, J = 7.5 Hz, 7.2 Hz, 1H), 7.63 (dd, J = 7.9 Hz, 7.2 Hz, 1H), 7.70 (d, J = 7.9 Hz, 1H), 8.23 (d, J = 8.3 Hz, 1H), 8.92 (m, 2H), 9.25 (br s, 1H), 9.63 (br s, 1H), 10.76 (br s, 1H), 11.45 (s, 1H); MS (APCI) m/z 323.11 ($M+H^+$). Anal. ($C_{18}H_{14}N_2O_4$) C, H, N.

2,4-Dihydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]benzohydrazide (26). Yield 46%; mp 268−269 °C dec; ^{1}H NMR (DMSO-d_6) δ 6.32 (d, J = 2.6 Hz, 1H), 6.37 (dt, J = 8.7, 2.3 Hz, 1H), 6.96 (d, J = 7.9 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.65 (dd, J = 7.5, 7.2 Hz, 1H), 7.74 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 8.7 Hz, 1H), 8.23 (d, J = 8.3 Hz, 1H), 8.89 (s, 1H), 8.99 (d, J = 8.7 Hz, 1H), 10.32 (br s, 2H), 11.63 (br s, 1H), 12.52 (br s, 1H); MS (APCI) m/z 323.04 ($M+H^+$). Anal. ($C_{18}H_{14}N_2O_4 \cdot 0.2H_2O$) C, H, N.

3-Fluoro-4-hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]benzohydrazide (28). Yield 45%; mp > 265 °C; ^{1}H NMR (DMSO-d_6) δ 6.90 (d, J = 8.0 Hz, 1H), 7.00 (t, J = 8.6 Hz, 1H), 7.47 (t, J = 7.4 Hz, 1H), 7.65 (m, 5H), 8.17 (d, J = 8.6 Hz, 1H), 8.84 (s, 1H), 8.89 (d, J = 8.5 Hz, 1H), 10.50 (br s, 1H), 11.50 (s, 1H); MS (APCI) m/z 325.1 ($M+H^+$). Anal. ($C_{18}H_{13}FN_2O_3 \cdot 0.75H_2O$) C, H, N.

3-Bromo-4-hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]benzohydrazide (29). Yield 57%; mp 218−221 °C; ^{1}H NMR (DMSO-d_6) δ 6.90 (d, J = 8.0 Hz, 1H), 7.00 (d, J = 8.5 Hz, 1H), 7.45 (t, 1H), 7.60 (t, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 8.08 (d, J = 2.0 Hz, 1H), 8.17 (d, J = 7.4 Hz, 1H), 8.80 (s, 1H), 8.90 (d, 1H), 10.50 (br s, 1H), 11.54 (s, 1H); MS (APCI) m/z 384.9, 386.9 ($M+H^+$). Anal. ($C_{18}H_{13}BrN_2O_3 \cdot 0.25H_2O$) C, H, N.

3-Chloro-4-hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]-5-methoxybenzohydrazide (31). Yield 20.5%; mp 243−244 °C dec; ^{1}HNMR (DMSO-d_6) δ 3.93 (s, 3H), 6.97 (d, J = 7.93 Hz, 1H), 7.53 (m, 2H), 7.65 (m, 2H), 7.75 (d, J = 7.93 Hz, 1H), 8.24 (d, J = 8.31 Hz, 1H), 8.94 (m, 2H), 10.18 (s, 1H), 10.80 (s, 1H), 11.60 (s, 1H); MS (APCI) m/z 371.0, 373.0 ($M+H^+$). Anal. ($C_{19}H_{15}ClN_2O_4 \cdot 1H_2O$) C, H, N.

4-Hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]-3-(4-morpholinylmethyl)benzohydrazide (32). Yield 37%; mp 221.5−222 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.48 (br s, 4H, overlaps DMSO-d_5), 3.65 (s, br s overlap, 6 H), 6.88 (d, J = 8.4 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.64 (t, J = 7.3 Hz, 1H), 7.70−7.86 (s, m overlap, 3H), 8.24 (d, J = 8.4 Hz, 1H), 8.9 (br s, 2H), 10.7 (br s, 1H), 11.52 (s, 1H); MS (APCI) m/z 406.2 ($M+H^+$). Anal. ($C_{23}H_{23}N_3O_4$) H, N; C: calcd 68.13; found, 67.70

***N*′-[(*E*)-(4-Hydroxy-1-naphthyl)methylidene]-1*H*-indole-5-carbohydrazide (33).** Yield 14%; mp > 260 °C; ^{1}H NMR (DMSO-d_6) δ 6.59 (d, 1H) 6.85 (d, 1H), 7.4−7.70 (m, 6H), 8.15 (m, 1H), 8.29 (br s, 1H), 8.90 (br s, 1H), 10.68 (br s, 1H), 11.12 (br s, 1H), 11.59 (br s, 1H); MS (APCI) m/z 330.0 ($M+H^+$). Anal. ($C_{20}H_{15}N_3O_2 \cdot 2H_2O$) C, H, N.

5-Hydroxy-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]-1*H*-indole-2-carbohydrazide (34). Yield 29%; mp 231−232 °C; ^{1}H NMR (DMSO-d_6) δ 6.71 (d, J = 8.8 Hz, 1H), 6.88 (d, J = 2.1 Hz, 1H), 7.09 (s, 1H), 7.22 (d, J = 8.8 Hz, 1H), 7.58 (m, 3H), 7.92 (m, 3H), 8.74 (m, 2H), 9.05 (s, 1H), 11.48 (s, 1H), 11.82 (s, 1H); MS (APCI) m/z 341.11 ($M+H^+$). Anal. ($C_{20}H_{15}N_2O_3 \cdot 0.4H_2O$) C, H; N: calcd, 11.92; found, 11.34.

***N*′-[(*E*)-(4-Hydroxy-1-naphthyl)methylidene]-6-quinoxalinecarbohydrazide (35).** Yield 17%; mp 254−255 °C dec; ^{1}H NMR (DMSO-d_6) δ 6.92 (d, J = 7.6 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.62 (t, J = 7.0 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 8.21 (m, 2H), 8.30 (d, J = 8.8 Hz, 1H), 8.69 (s, 1H), 8.95 (m, 2H), 9.01 (m, 2H), 10.79 (s, 1H), 12.03 (s, 1H); MS (APCI) m/z 343.06 ($M+H^+$). Anal. ($C_{20}H_{14}N_4O_2 \cdot 0.5H_2O$) C, H, N.

4-({2-[(*E*)-(4-Hydroxy-1-naphthyl)methylidene]hydrazino}carbonyl)benzoic acid (37). Yield 53.1%; mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 7.01 (d, J = 7.9 Hz, 1H), 7.54 (dd, J = 9.9, 7.2 Hz, 1H), 7.65 (dd, J = 8.3, 7.2 Hz, 1H), 7.74 (d, J = 7.9 Hz, 1H), 7.82 (d, J = 8.3 Hz, 1H), 7.97 (d, J = 7.9, 2H), 8.03 (d, J = 8.3 Hz, 1H), 8.24 (d, J = 8.3, 1H), 8.96 (m, 2H), 11.82 (s, 1H); MS (APCI) m/z 335.03 ($M+H^+$). Anal. ($C_{19}H_{14}N_2O_4 \cdot 0.5H_2O$) C, H, N.

4-Amino-*N*′-[(*E*)-(4-hydroxy-1-naphthyl)methylidene]benzohydrazide (38). Yield 52%; mp 255 °C dec; ^{1}H NMR (DMSO-d_6) δ 5.71 (s, 1H), 6.54 (d, J = 8.6 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 7.56 (m, 5H), 8.16 (d, J = 7.8 Hz, 1H), 8.85 (m, 2H), 10.66 (s, 1H), 11.25 (s, 1H); MS (APCI) m/z 306.08 ($M+H^+$). Anal. ($C_{18}H_{15}N_3O_2 \cdot 0.2H_2O$) C, H, N.

4-Hydroxy-3-methoxy-*N*′-[(*E*)-1-(1-naphthyl)ethylidene]benzohydrazide (39). A mixture of the 4-hydroxy-3-methoxybenzoylhydrazide (100 mg, 0.5 mmol) and 1-acetonaphthone (90 mg, 0.5 mmol) was stirred in hot (∼100 °C) DMSO (3 mL) for 10 h. The reaction mixture was diluted with ethyl acetate and washed with water (3×), brine (3×), dried over $MgSO_4$, filtered, and concentrated to give the product. This was recrystallized from dichloromethane (80 mg, 46%): mp 185−186 °C; ^{1}H NMR (DMSO-d_6) δ 3.27 (s, 3H), 3.83 (s, 3H), 6.86 (d, J = 7.91 Hz, 1H), 7.46 (dd, J = 8.29, 1.88 Hz, 1H), 7.50 (d, J = 1.51 Hz, 1H), 7.56 (m, 4H), 7.96 (ddd, J = 3.77, 3.39, 3.01 Hz, 2H), 8.26 (dd, J = 6.03 Hz, 1H), 9.59 (s, 1H), 10.60 (s, 1H); MS (APCI) m/z 334.87 ($M+H^+$). Anal. ($C_{20}H_{18}N_2O_3 \cdot 0.4H_2O$) C, H, N.

***N*′-[(*E*)-(4-hydroxy-3-methoxyphenyl)methylidene]-1-naphthohydrazide (40)**. Yield 94%; mp 264.5−265.5 °C; ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 3H), 6.62 (d, J = 8.08 Hz, 1H), 6.85 (dd, J = 8.15, 1.48 Hz, 1H), 7.11 (d, J = 1.51 Hz, 1H), 7.29−7.39 (m, 3H), 7.49 (d, J = 6.83 Hz, 1H), 7.77 (m, 2H), 7.83 (d, J = 8.15 Hz, 1H), 7.97 (m, 2H), 9.36 (s, 1H), 11.61 (s, 1H); MS (APCI) m/z 321.07 ($M+H^+$). Anal. ($C_{19}H_{16}N_2O_3 \cdot 0.25H_2O$) C, H, N.

3-Hydroxy-*N*′-[(*E*)-1-naphthylmethylidene]benzohydrazide (41). Yield 69%; mp 234−235 °C; ^{1}H NMR (DMSO-d_6) δ 6.89 (d, J = 8.67 Hz, 2H), 7.60 (t, J = 7.54 Hz, 2H), 7.66 (t, J = 6.78 Hz, 1H), 7.85 (d, J = 8.67 Hz, 2H), 7.92 (d, J = 7.16 Hz, 1H), 8.01 (d, J = 7.91 Hz, 2H), 8.84 (d, J = 8.29 Hz, 1H), 9.08 (s, 1H), 10.15 (s, 1H), 11.74 (s, 1H); MS (APCI) m/z 291.02 ($M+H^+$). Anal. ($C_{18}H_{14}N_2O_2 \cdot 0.2H_2O$) C, H, N.

4-Hydroxy-*N*′-(1-naphthylmethyl)benzohydrazide (42). To a solution of **41** in methanol (20 mL) was added $NaBH_4$ (0.143 g, 3.78 mmol) followed by catalytic amount of trifluoroacetic acid. After the solution was stirred for 10 h, the solvent was evaporated and replaced with ethyl acetate. The solution was washed with aqueous sodium bicarbonate (3×) and brine (2×), dried over $MgSO_4$, filtered, and concentrated. The crude product was dissolved in a minimum volume of hot ethyl acetate and precipitated with the addition of hexane to afford the desired product (0.105 g, 48%): mp 184.5−186.5 °C; ^{1}H NMR (DMSO-d_6) δ 4.38 (d, J = 3.91 Hz, 2H), 5.36 (d, J = 5.11 Hz, 1H), 6.78 (d, J = 8.66 Hz, 2H), 7.40−7.56 (m, 4H), 7.70 (d, J = 8.67 Hz, 2H), 7.84 (d, J = 7.71 Hz, 1H), 7.91 (dd, J = 7.29, 1.47 Hz, 1H), 8.36 (d, J = 8.12 Hz, 1H), 9.91 (d, J = 4.41 Hz, 1H), 9.99 (s, 1H); MS (APCI) m/z 293.10 ($M+H^+$). Anal. ($C_{18}H_{16}N_2O_2 \cdot 0.5H_2O$) C, H; N: Calcd, 9.30; found, 10.02.

In Vitro Pharmacology Methods. Receptor Binding Assay: Binding assays were carried out in duplicate in polypropylene tubes. The buffer consisted of 25 mM HEPES (pH 7.4) and 0.1% BSA. A total of 100 μL of test compound and 100 μL of [^{125}I]glucagon (∼25 000 cpm) was added to the tubes. Next, 100 μL (∼0.5 μg) of plasma membrane from BHK cells transfected with the cloned human glucagon receptor was added to the tubes to initiate the assay, and the binding proceeded for 1 h at 37 °C. Bound and unbound radioligand were then separated by vacuum filtration on a Brandel harvester, and the GF/C filters were counted in a scintillation counter.

Rat Receptor Binding Assay: Rat livers were isolated from male Wistar rats, and plasma membranes were prepared. The buffer used was 50 mM HEPES, 5 mM EGTA, 5 mM

$MgCl_2$, and 0.005% tween-20, pH 7.4. Samples were diluted in 100% DMSO and added in 10 μL to 96-well plates (Millipore PVDF filter plates 0.65 μm) together with approximately 50 000 CPM ^{125}I-glucagon in 25 μL, 2 μg protein in 25 μL, and 165 μL buffer. Nonspecific binding was determined with excess of unlabeled glucagon. Incubation proceeded for 2 h at 30 °C. Bound and unbound radioligand were then separated by vacuum filtration on a Millipore harvester, and the filters were counted in a γ-scintillation counter. Binding curves were fitted in Graph Pad Prism.

cAMP Accumulation Assay: The cAMP assay was carried out in borosilicate glass tubes. The buffer consisted of 10 mM HEPES (pH 7.4), 1 mM EGTA, 1.4 mM $MgCl_2$, 0.1 mM IBMX, 30 mM NaCl, 4.7 mM KCl, 2.5 mM NaH_2PO_4, 3 mM glucose, and 0.2% BSA. BHK cells transfected with the cloned human glucagon receptor (0.5 mL, 10^6/mL) were pretreated with various concentrations of compounds for 10 min at 37 °C, then challenged with increasing concentrations of glucagon for 20 min. Alternatively, the cells were treated with various concentrations of the compounds alone to determine if any of the compounds behaved as agonists. The reactions were terminated by centrifugation, followed by cell lysis by the addition of 500 μL of 0.1% HCl. Cellular debris was pelleted and the supernatant evaporated to dryness. cAMP was measured by using an RIA kit (NEN).

In Vivo Methods. Adult male Sprague−Dawley rats were purchased from Harlan Sprague Dawley (San Diego). Animals were housed two per cage and maintained in a temperature-controlled room (22 ± 2 °C) with a photoperiod of 12 h light/12 h dark cycle (lights on at 0600h). Rat chow (Teklad LM-485 rat diet, Madison, WI) and tap water was provided ad libitum.

Rat Glucagon Challenge Model. Animals were instrumented with indwelling femoral artery and vein cannula 48 h prior to study. Cannula were exteriorized behind the neck. On study day, extension tubing was attached to the cannula to facilitate remote blood sampling and injections. Glucagon was initially dissolved in Nanopure water and diluted in 0.9% saline. The antagonist **27** was formulated in 10% DMSO, 20% β-cyclodextrin, and 80% saline solution for iv administration. Animals were allowed to acclimate to the procedure room for 1 h prior to drawing baseline blood samples. Animals received an intravenous (iv) injection of vehicle or compound **27** (0.1 or 1.0 mg/kg) 5, 10, 20, 30, or 60 min prior to glucagon challenge (10 μg; iv). Twenty minutes after the glucagon challenge, blood samples were collected in cold glass tubes containing EDTA 10 μL (60 μg/μL). Samples were centrifuged, and plasma was stored at −20 °C until assayed. Plasma glucose was measured using the Sigma Glucose Procedure 115 (hexokinase method).

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