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5-Chloroindoloyl glycine amide inhibitors of glycogen phosphorylase: synthesis, in vitro, in vivo, and X-ray crystallographic characterization

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Abstract—The synthesis, in vitro, and in vivo biological characterization of a series of achiral 5-chloroindoloyl glycine amide inhibitors of human liver glycogen phosphorylase A are described. Improved potency over previously reported compounds in cellular and in vivo assays was observed. The allosteric binding site of these compounds was shown by X-ray crystallography to be the same as that reported previously for 5-chloroindoloyl norstatine amides.

Type 2 diabetes (formerly non-insulin-dependent diabetes mellitus) is a severe and increasingly prevalent disease.[1] Diabetics may suffer debilitating cardiovascular, eye, kidney, and nerve damage are at risk of premature handicap and death due to these and other diabetic complications, which are the result of glucose toxicity caused by their hyperglycemia. A progressive reduction in insulin sensitivity and insulin secretion are hallmarks of the disease, which eventually result in failure of the pancreatic islet cells and dependence on exogenous insulin. Clinical studies have been carried out to define the primary defect that causes the elevated fasting blood glucose levels observed in Type 2 diabetics. The results have suggested that excessive hepatic glucose output is a principal cause.[2] Hepatic glucose output, in turn, derives from the breakdown of hepatic glycogen (glycogenolysis) and synthesis of glucose from 3-carbon fragments such as pyruvate (gluconeogenesis). Gluconeogenic flux has been shown to be excessive in Type 2 diabetics.[3] Because phosphorylated glycogen phosphorylase a (GPa, EC 2.4.1.1) is responsible for the release of glucose-1-phosphate from glycogen, the rate determining step in glycogenolytic glucose production, and at least a portion of the gluconeogenic flux has been shown to cycle through the glycogenolytic pathway,[4] inhibition of glycogen phosphorylase may represent a useful therapy for the treatment of Type 2 diabetes. Glucose lowering by inhibition of GPa with small molecule inhibitors has been reported for several structural classes.[5] Interest in novel inhibitors of this enzyme remains high as evidenced by the continued appearance of patents and publications describing new inhibitors that bind at both the AMP allosteric site[6] and a novel allosteric site[7] identified previously by these laboratories.[8]

We sought to identify novel allosteric chloroindole inhibitors of GPa that were structurally distinct from the norstatine series of inhibitors previously reported by us.[9] In particular, we wanted to explore an achiral 5-chloroindole glycine template, to learn if exocyclic amides were active as inhibitors of GPa within this series. Our rationale for this approach stemmed from a desire to reduce molecular weight and hydrophobic surface area in order to improve aqueous solubility and increase

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plasma free fraction over our previous series. Molecular modeling suggested that the 5-chloroindole glycine template was not an unreasonable starting point for compound design, and the lack of stereocenters was an added benefit. In this paper we report the results of this investigation.

Recombinant human liver GPa (50 ng) activity was measured in the direction of glycogen synthesis by the release of phosphate from glucose-1-phosphate as described previously,[11] using malachite green to detect phosphate.[12] To evaluate the activity of inhibitors in cells, an assay was established in a human liver-derived tissue culture cell line as previously described.[9,13] Our first investigation centered upon the preparation of a set of tertiary amides with various alkyl and aryl groups pendant to the amide nitrogen. For simplicity, the second amide nitrogen substituent was held constant as a methyl group (Table 1). We were gratified to find inhibitors within this set of analogs whose enzyme inhibitory potency compared favorably with those identified earlier (e.g., CP-91149, Fig. 1).[9] In addition, we were pleased to find that certain analogs (**1b**, **1c**, **1d**, **1e**, **1g**) had good potency in the cellular assay. Increased enzyme potency, however, did not always translate into improved cell potency (**1f**, **1j**, **1h**). Saturated nitrogen heterocycles in particular were poorly tolerated (**1a** in the cell; **1v** and **1w** in the enzyme assay).

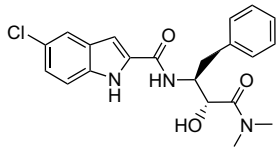
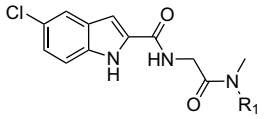


Figure 1. Structure of CP-91149.

Encouraged by the results obtained with **1b**, we prepared a series of tertiary amides with a cyclopentyl group pendant to the amide nitrogen. The second amide nitrogen substituent was varied, with particular focus on substituents containing polar functionalities in order to enhance aqueous solubility[14] (Table 2).

Within this series of compounds some new SAR trends were readily apparent. First, a clear preference for OH and CN groups in the side chain was evident (**2a**–**e**,

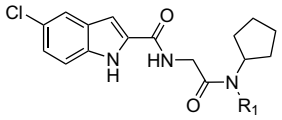
Table 1. Variation of amide substituents in *N*-methyl amides **1**



Compound	R_1	Gpa IC_{50}, μM (sd)[a]	SK-Hep IC_{50}, μM (sd)
1a	3-(*N*CHO)pyrollidinyl[b]	0.25 (0.1)	19 ($n = 1$)
1b	Cyclopentyl	0.055 (0.01)	1.7 ($n = 1$)
1c	*n*-Butyl	0.15 (0.1)	1.4 (0.07)
1d	Cyclohexyl	0.15 (0.02)	1.4 ($n = 1$)
1e	Cyclobutyl	0.17 (0.01)	1.0 ($n = 1$)
1f	Phenyl	0.21 (0.02)	7.1 (1)
1g	Isobutyl	0.23 (0.02)	0.62 ($n = 1$)
1h	Cycloheptyl	0.32 (0.06)	>10 ($n = 1$)
1i	3-Pyridyl	0.33 (0.04)	1.7 ($n = 1$)
1j	2-Pyridyl	0.34 (0.08)	9.9 ($n = 1$)
1k	3-Sulfolanyl[c]	0.41 (0.01)	2.0 (0.8)
1l	(Cyclopropyl)methyl	0.47 (0.4)	1.2 ($n = 1$)
1m	4-Tetrahydropyranyl	0.51 (0.4)	3.4 ($n = 1$)
1n	Benzyl	0.55 (0.5)	>10 ($n = 1$)
1o	Cyclopropyl	0.67 (0.2)	nd[d]
1p	*t*-Bu	0.67 (0.3)	nd
1q	(2-Pyridyl)methyl	1.5 (0.07)	nd
1r	(4-Pyridyl)methyl	1.6 (0.2)	nd
1s	(3-Pyridyl)methyl	1.9 (0.4)	nd
1t	2,2,2-Trifluoroethyl	1.9 (0.4)	nd
1u	(4-Hydroxy)cyclohexyl	2.1 (0.07)	nd
1v	4-(*N*CHO)piperidinyl[e]	8.4 (4)	nd
1w	3-(*N*Me)pyrollidinyl[f]	9.6 (0.1)	nd
1x	2-Phenylethyl	>10	nd
CP-91149[g]		0.11 (0.07)	1.5

[a] Standard deviation.
[b] 3-(*N*-Formyl)pyrollidinyl.
[c] 3-Tetrahydrothiophenyl-*S*,*S*-dioxide.
[d] Not determined.
[e] 4-(*N*-Formyl)piperidinyl.
[f] 3-(*N*-Methyl)pyrollidinyl.
[g] Ref. 9.

Table 2. Variation of amide substituents in *N*-cyclopentyl amides **2**

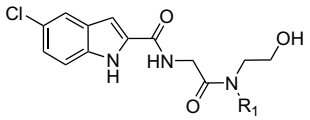


Compound	R_1	GPa IC_{50}, μM (sd)[a]	SK-Hep IC_{50}, μM (sd)
1b	Methyl	0.055 (0.01)	1.7 ($n = 1$)
2a	$CH_2CH(OH)CH_3$	0.016 (0.005)	1.3 ($n = 1$)
2b	CH_2CN	0.030 (0.004)	0.68 ($n = 1$)
2c	$(CH_2)_3OH$	0.042 (0.01)	0.76 (0.4)
2d	$CH_2CH(OH)CH_2OH$	0.054 (0.02)	1.3 (0.1)
2e	$(CH_2)_2OH$	0.057 (0.002)	0.14 ($n = 1$)
2f	*n*-Butyl	0.082 (0.04)	9.4 ($n = 1$)
2g	$CH_2CMe(OH)CH_2OH$	0.083 (0.05)	2.4 (1)
2h	Allyl	0.096 (0.05)	0.90 ($n = 1$)
2i	$CH_2CH(OH)CH_2OMe$	0.10 (0.04)	0.87 ($n = 1$)
2j	CH_2CH_2CN	0.14 (0.1)	0.75 ($n = 1$)
2k	CH_2CMe_2OH	0.15 (0.05)	1.4 ($n = 1$)
2l	$(CH_2)_2OMe$	0.20 (0.04)	1.5 (0.8)
2m	Ethyl	0.21 (0.02)	0.41 ($n = 1$)
2n	$CHMe(CH_2)_2OH$	0.21 (0.02)	1.4 ($n = 1$)
2o	$(CH_2)_2O(CH_2)_2OH$	0.23 (0.08)	3.8 ($n = 1$)
2p	CH_2CO_2H	0.26 (0.05)	>10 ($n = 1$)
2q	*n*-Propyl	0.34 (0.15)	nd[b]
2r	$CH(CH_2OH)_2$	0.35 (0.25)	9.4 (5)
2s	$(CH_2)_3OMe$	0.37 (0.01)	nd
2t	CH_2CONMe_2	0.41 (0.09)	13 (5)
2u	$CH_2CH(NH_2)CH_2OH$	0.51 (0.1)	8.3 ($n = 1$)
2v	CH_2CO_2Et	0.53 (0.4)	5.2 ($n = 1$)
2w	Cyclopentyl	0.66 (0.2)	nd
2x	$(CH_2)_3O$-*i*-Pr[c]	0.67 (0.01)	nd
2y	$CH_2CH(OH)CH_2O$-*i*-Pr	0.93 (0.1)	nd
2z	$(CH_2)_2O$-*i*-Pr	1.1 (0.8)	nd
2aa	$CH_2CH(NH_2)CH_2OMe$	1.2 (0.07)	nd
2bb	$(CH_2)_2OPh$	1.4 (0.05)	nd
2cc	$(CH_2)_2N[(CH_2)_2]_2O$[d]	1.5 (0.1)	nd
2dd	$(CH_2)_2NH_2$	1.6 (0.6)	nd
2ee	$(CH_2)_2OCH_2Ph$	1.6 (0.03)	nd
2ff	$(CH_2)_3NH_2$	3.0 (0.9)	nd
2gg	$(CH_2)_4OH$	3.9 (0.3)	nd
2hh	CH_2CONH_2	4.6 (0.5)	nd
2ii	$(CH_2)_3NMe_2$	5.0 (0.7)	nd
2jj	$(CH_2)_2NMe_2$	9.7 (0.9)	nd

[a] Standard deviation.
[b] Not determined.
[c] *i*-Pr = isopropyl.
[d] (4-Ethyl)morpholinyl.

2g). In every case examined, the conversion of an OH group to the corresponding methyl ether resulted in a loss of potency (e.g., **2e** vs **2l**, **2c** vs **2s**, **2d** vs **2i**). Conversion of an OH group to the corresponding isopropyl ether resulted in a greater loss of potency than the methyl ether (e.g., **2s** vs **2x**, **2i** vs **2y**). Sterically larger, more hydrophobic ethers were even worse (**2bb**, **2ee**). The incorporation of a strongly basic amine group resulted in a significant loss of potency (e.g., **2e** vs **2dd** and **2jj**, **2c** vs **2ff** and **2ii**). Less basic amines were also less potent but to a lesser extent (e.g., **2cc** and **2d** vs **2u**). By contrast, a glycine substituent had acceptable enzyme potency (**2p**) but failed in the cellular assay, presumably due to poor cellular penetration or excessive nonspecific protein binding. Attempts to convert the glycine residue into nonionized carboxylic acid derivatives decreased potency and did little to enhance cellular potency (**2t**, **2v**, **2h**). The SAR of unfunctionalized alkyl side chains in both the enzyme and cellular assays was considerably less predictive (**2f**, **2h**, **2m**, **2q**, **2w**). The poor aqueous solubility and high microsomal lability of these compounds indicated that they were poor targets for further compound design. From the enzyme and cellular potency data, the ethanol (**2e**), acetonitrile (**2b**), and propanol (**2c**) side chains emerged as the most desirable. The acetonitrile side chain was viewed with some concern as it was thought that this group could eventually lead to toxicity problems by undergoing loss of cyanide ion in what can be considered as similar to a reversed Strecker reaction. In as much as the ethanol derivative **2e** exhibited the least loss of potency (only about 2-fold) between the enzyme and cellular assays,

Table 3. Variation of amide substituents in ethanolamine amides **3**



Compound	R_1	GPa IC_{50}, μM (sd)[a]	SK-Hep IC_{50}, μM (sd)
2e	Cyclopentyl	0.057 (0.002)	0.14 ($n = 1$)
3a	$CH[(CH_2)_2]_2SO_2$[b]	0.012 (0.004)	>30 ($n = 1$)
3b	Cyclononyl	0.082 (0.06)	28 ($n = 1$)
3c	Cycloheptyl	0.030 (0.008)	2.3 ($n = 1$)
3d	Cyclooctyl	0.032 (0.01)	12 ($n = 1$)
3e	1-(2,3-Dihydro)indanyl	0.049 (0.03)	1.8 ($n = 1$)
3f	3-Tetrahydrofuryl	0.055 (0.02)	1.8 ($n = 1$)
3g	Cyclohexyl	0.099 (0.005)	0.71 ($n = 1$)
3h	Cyclodecyl	0.21 (0.1)	>30 ($n = 1$)
3i	Phenyl	0.12 (0.02)	0.27 ($n = 1$)
3j	*n*-Butyl	0.12 (0.006)	1.0 ($n = 1$)
3k	Cyclobutyl	0.13 (0.04)	2.0 ($n = 1$)
3l	*n*-Propyl	0.15 (0.02)	0.49 ($n = 1$)
3m	Cyclopropylmethyl	0.16 (0.01)	1.5 ($n = 1$)
3n	*i*-Bu	0.18 (0.01)	2.5 ($n = 1$)
3o	Cyclopropyl	0.20 (0.07)	4.6 ($n = 1$)
3p	*i*-Pr	0.21 (0.05)	3.4 ($n = 1$)
3q	$CH[(CH_2)_2]_2O$[c]	0.25 (0.07)	1.4 ($n = 1$)
3r	(2-Furyl)methyl	0.26 (0.1)	1.5 ($n = 1$)
3s	*t*-Bu	0.29 (0.06)	6.7 ($n = 1$)
3t	Benzyl	0.32 (0.02)	2.5 ($n = 1$)
3u	3-Tetrahydrothiophenyl	0.34 (0.1)	5.0 ($n = 1$)
3v	(2-Imidazolyl)methyl	0.43 (0.08)	14 ($n = 1$)
3w	(2-THF)methyl[d]	0.55 (0.05)	5.7 ($n = 1$)
3x	Methyl	0.57 (0.08)	4.0 ($n = 1$)
3y	Cyclododecyl	0.95 (0.4)	>30 ($n = 1$)
3z	(3-Pyridyl)methyl	1.1 (0.5)	nd[e]
3aa	3-Sulfolanyl[f]	1.3 (0.06)	nd

[a] Standard deviation.
[b] 4-Tetrahydrothiopyranyl-*S*,*S*-dioxide.
[c] 4-Tetrahydropyranyl.
[d] (2-Tetra-hydrofuranyl)methyl.
[e] Not determined.
[f] 3-Tetrahydrothiophenyl-*S*,*S*-dioxide.

the ethanol side chain was chosen for further analog production (Table 3).

Of immediate note within this series was the good enzyme inhibitory potency observed with a broad variety of both saturated and aromatic carbocycles and heterocycles and alkyl groups (**3a**–**u**) encompassing a somewhat surprising range of ring sizes. Only upon increasing the cycloalkyl ring size from cyclooctyl (**3d**) to cyclononyl (**3b**) was potency diminished. Even a methyl group (**3x**) afforded an active analog. By contrast, the diminished potency observed with the sulfolane analog **3aa** was surprising and not easy to rationalize, particularly in contrast to the *N*-methyl sulfolane analog **1k**.

Results in the cellular assay revealed a much stricter preference for relatively compact substituents with few rotatable bonds (**3i**, **3l**, **3g**, **3q**, **3m**, **3r**, **3e**, **3f**, **3k**). The larger C_7–C_{10} rings that were so well accommodated in the enzyme assay led, in a linear fashion, to increasing loss of potency in the cellular assay. Predictive SAR was difficult to discern in many cases, such as the good cellular potency observed with the *n*-propyl analog **3l** but the lower potency observed with closely related compounds (e.g., **3p**, **3s**).

Based on their potency in the enzyme and cellular assays, selected compounds were evaluated for their hypoglycemic effect in diabetic (*ob*/*ob*) mice in the fed state at 3 h post-dosing as described previously.[15] Compounds were administered orally at 10 mg/kg initially and subsequently at lower doses. The assay was carried out at successively lower doses to determine the minimum effective dose (MED) for in vivo reduction of plasma glucose (Table 4). The chloroindole norstatine inhibitor CP-91149 was used as a positive control in these experiments. In this assay, the two ethanolamine derivatives **3q** and **2e** distinguished themselves by their relatively low MED. The derivatives **1b, 2k**, and **2b** were also active in vivo, as might have been anticipated from their performance in the enzyme and cellular assays. Surprisingly, closely related compounds with similar or greater potency in the enzyme and cellular assays failed to exhibit in vivo glucose lowering in this model (e.g., **3m**, **3i**, **3l**, **3g**, **2c**). Among the active compounds, cellular potency

Table 4. In vivo MED determinations for inhibitors of GPa

Compound	GPa IC_{50}, μM	SK-Hep IC_{50}, μM	*ob/ob* mouse MED, mg/kg at 3 h	% glucose lowering at 3 h at MED[a] (*P* value)[b]
3q	0.25	1.4	5	27 (0.01)
2e	0.057	0.14	5	25 (0.02)
1b	0.055	1.7	10	36 (0.01)
2k	0.15	1.4	10	30 (0.01)
2b	0.030	0.68	10	40 (0.001)
3m	0.16	1.5	>10	
2d	0.054	1.3	10	34 (0.01)
3k	0.13	2.0	25	30 (0.002)
3i	0.12	0.27	>10	
3l	0.15	0.49	>25	
3g	0.099	0.71	>10	
2c	0.042	0.76	>10	
1d	0.15	1.4	>10	
2l	0.20	1.5	>10	
2g	0.083	2.4	>10	
3n	0.18	2.5	25	25 (0.002)
3p	0.21	3.4	>10	
3j	0.12	1.0	>10	
2o	0.23	3.8	>10	
CP-91149	0.11	1.5	25	32 (0.05)

[a] Percent decrease in drug treated plasma glucose concentration, relative to vehicle-treated control at 3 h within the experiment.
[b] Significance.

could not be used to predict or rank—order the in vivo MED. However, no compounds with modest potency in the cellular assay ($IC_{50} > 2.0\,\mu M$) were found to afford in vivo activity.

In order to better understand the binding mode of our compounds, we co-crystallized recombinant HLGPa with **2e** as previously described.[16] The data for the GPa complex with **2e** was measured using a Brandeis B4 CCD detector[17] at beamline X12C, Brookhaven National Light Source and diffracted to 2.2 Å resolution. The structure was solved by molecular replacement using the published structure of the catalytically inactive HLGPb (Protein Data Bank ID code 1EXV) as a starting model.[18] Subsequent refinement was carried out as described previously.[19] Our structure revealed that **2e** bound at the same allosteric inhibitor site as previously described (Fig. 2),[8,16] and achieves inhibition of GPa by the same mechanism. Two molecules of inhibitor **2e** are bound to each phosphorylase dimer. Similarly to previous chloroindole inhibitors of GPa at this site, the chloroindole residue is buried in a hydrophobic pocket and is involved in a pi-stacking interaction with Arg60. The indole NH and the amide carbonyls make hydrogen bonds to backbone carbonyl of Glu190 and to the side chain of Lys191. Unlike the chloroindole norstatine compounds, however, the cyclopentyl ring is bound at right angles to the plane normally occupied by the benzene ring in the norstatine. The hydroxyethyl amide substituent is directed towards the solvent-exposed subunit interface and may be involved in a hydrogen bond to backbone carbonyl of Tyr185 (Fig. 3).

The compounds were prepared by the procedure outlined in Scheme 1, in which the preparation of **2e** is illustrated for example. The desired secondary amine was prepared by reductive amination using sodium

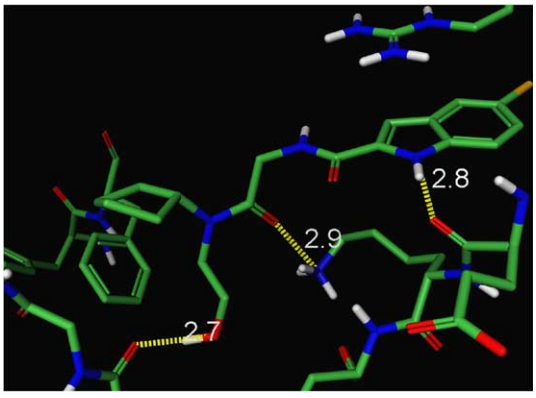


Figure 2. X-Ray structure of **2e** co-crystallized with GPa showing hydrogen bonds made to the backbone and side chains of GPa.

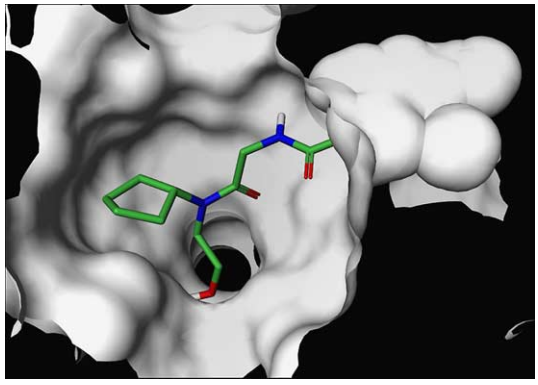
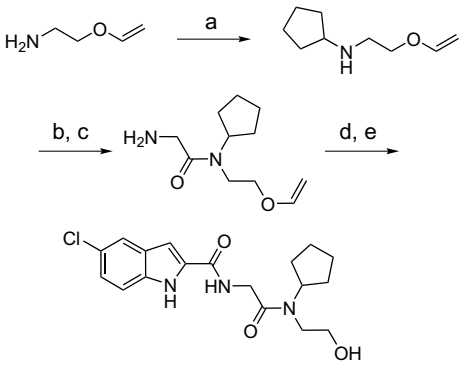


Figure 3. X-Ray structure of **2e** co-crystallized with GPa showing solvent accessible surface area of the enzyme. The chloroindole fragment is encapsulated at right in a hydrophobic pocket under Arg60.



Scheme 1. Reagents and conditions: (a) cyclopentanone, $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$, 25 °C; (b) $ClCH_2COCl$, Et_3N, THF, 0 °C; (c) NH_4OH, 2-PrOH, 25 °C; (d) 5-chloroindole-2-carbonyl chloride, Et_3N, THF, H_2O, 25 °C; (e) HCl, MeOH, H_2O, 25 °C.

triacetoxborohydride.[10] If necessary, potentially reactive functional groups were protected with acid—labile protecting groups prior to the reductive amination step. Typically alcohols were protected as the vinyl ethers, while 1,2-diols and 1,3-diols were protected as the acetonides. The resulting secondary amines were then converted to the glycine derivative by either (a) reaction of the secondary amine with chloroacetyl chloride followed by aminolysis using excess NH_4OH, (b) coupling to Boc-Gly-OH using 1,1′-carbonyldiimidazole followed by removal of the Boc group with aqueous HCl, or (c) coupling to CBZ-Gly-Cl followed by hydrogenolysis of the CBZ group. In all cases, the resulting glycine derivative was then acylated with 5-chloroindole-2-carbonyl chloride under Schotten–Baumann conditions to afford the target compound. Protecting groups, if present, were removed last by hydrolysis with aqueous HCl.

In summary, we have identified a structurally distinct series of potent chloroindole inhibitors of human liver glycogen phosphorylase, which lack the pendant phenyl rings and chiral centers present in the previous series. The simpler chloroindole glycine scaffold, coupled with the exocyclic amide, has allowed facile analog production and modification of physical chemical properties. We expect these newer compounds to exhibit lower overall hydrophobicity and an altered pharmacokinetic/pharmacodynamic profile as compared to previous chloroindole compounds because of the introduction of polar, hydrophilic side chains. While the X-ray crystallographic structure revealed many binding similarities to the earlier chloroindole norstatine analogs, the in vivo performance of the chloroindole glycines is enhanced over the previously reported compounds. In particular, the *N*-cyclopentyl ethanolamine amide **2e** and the *N*-(4-tetrahydropyranyl) ethanolamine amide **3q** have distinguished themselves by their ability to lower plasma glucose in the *ob*/*ob* mouse model at low doses.

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