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Anthranilimide-based glycogen phosphorylase inhibitors for the treatment of type 2 diabetes: 1. Identification of 1-amino-1-cycloalkyl carboxylic acid headgroups

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ABSTRACT

Optimization of the amino acid residue within a series of anthranilimide-based glycogen phosphorylase inhibitors is described. These studies culminated in the identification of anthranilimides **16** and **22** which displayed potent in vitro inhibition of GP*a* in addition to reduced inhibition of CYP2C9 and excellent pharmacokinetic properties.

Type 2 diabetes is a major worldwide public health problem with devastating chronic complications such as cardiovascular disease, retinopathy, neuropathy, and nephropathy. Type 2 diabetes affects between 6% and 20% of the population of Western industrialized societies and the growth rate is predicted to increase at 6% per year.[1] The disease is polygenic in nature and characterized by hyperglycemia, defects in pancreatic insulin secretion, and insulin resistance in skeletal muscle, adipose tissue, and liver. In addition to these derangements, the rate of endogenous glucose production is significantly elevated in type 2 diabetics relative to healthy subjects.[2,3] The liver accounts for approximately 90% of the body's endogenous glucose production (the kidney produces the remaining 10%). In normal individuals, hepatic glucose production (HGP) is tightly regulated by insulin and insulin's counter-regulatory hormone glucagon. However, in the type 2 diabetic state hepatic insulin resistance coupled with elevated levels of glucagon leads to excessive HGP which contributes to the observed hyperglycemia.

HGP is the sum of two metabolic processes: glycogenolysis, which is the release of monomeric glucose from its polymeric storage form called glycogen, and gluconeogenesis, which is the de novo synthesis of glucose from lactate, amino acids, and glycerol. Estimates vary on the relative contribution of gluconeogenesis and glycogenolysis to HGP in humans; however, studies performed in type 2 diabetics estimate the glycogenolytic contribution anywhere from 12% to 75% of total HGP.[3] Despite the discordance regarding the relative contribution of glycogenolysis to HGP, inhibition of glycogenolysis represents a novel approach to the development of new antihyperglycemic agents.

Among potential biochemical targets which could lead to the amelioration of the hyperglycemic state, glycogen phosphorylase *a* (GP*a*), which catalyzes the phosphorolytic cleavage of the glucose polymer glycogen at the δ-1,4-glycosidic linkage to produce glucose 1-phosphate (G-1-P), has been investigated.[4] Herein we report the optimization of the amino acid fragment of a series of anthranilimide-based glycogen phosphorylase inhibitors, leading to potent, glucose-sensitive, orally bioavailable GP*a* inhibitors.

Compound **1** was identified as a starting point for optimization following a high throughput synthesis campaign.[5] Compound **1** is a potent inhibitor of human liver GP*a* (IC_{50} = 73 nM) and was found to be 6 times less potent in the absence of glucose, thus reducing the risk of hypoglycemia during periods of low blood glucose with this class of inhibitors. While the potency of anthranilimide **1** was acceptable, the compound was rapidly metabolized both in vitro and in vivo. In vitro metabolite ID studies identified the cyclohexyl residue as the major site of metabolism. In addition, analogs closely related to **1** showed submicromolar inhibition of CYP2C9. Thus, the primary goals of the optimization were to increase potency, improve metabolic stability, and reduce P450 inhibition (see Fig. 1).

The general synthesis of the anthranilimides is shown in Scheme 1.[6] Amide bond formation between the appropriate amino

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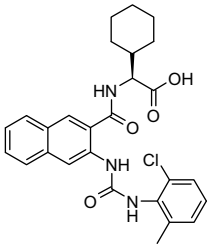
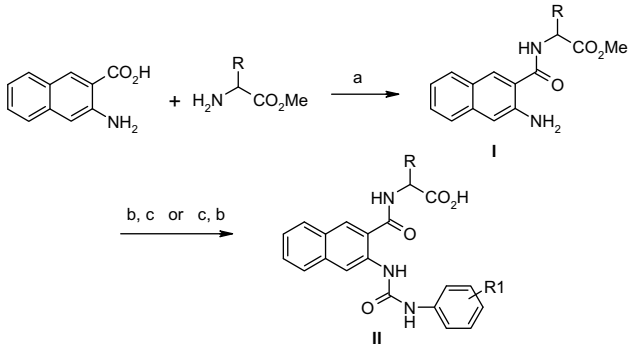


1
IC_{50} (+glu) = 73 nM
IC_{50} (-glu) = 460 nM
IC_{50} (cell) =1290 nM
CYP2C9 IC_{50} = 1000 nM

Figure 1. In vitro profile of anthranilimide **1**.

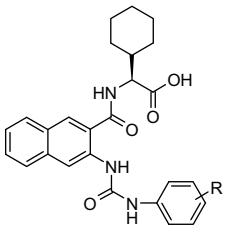


Scheme 1. General syntheses of the anthranilimide core. Reagents and conditions: (a) HATU, *i*-PrNEt$_2$, DMF, rt; (b) R1PhNCO, pyridine, rt; (c) 2 M LiOH, MeOH, THF.

acid ester and 2-aminonaphthoic acid provided the amide derivatives **I**, which were subsequently condensed with substituted phenylisocyanates and saponified to provide the desired anthranilimide analogs **II**. Alternatively, the compounds could be assembled by reversing the order of steps with saponification of the amide intermediates **I** followed by urea formation to deliver the desired compounds **II**.

Prior to optimizing the amino acid residue, an initial study of the SAR around the phenyl urea moiety was undertaken utilizing commercially available phenylisocyanates. Previous work revealed that 2,6-disubstitution around the phenyl urea was minimally required to deliver potent GP*a* inhibitors and therefore a series of

Table 1
In vitro GP*a* inhibition: phenyl urea substitution



Compound	R	GP*a* IC_{50} (nM)	GP*a* (cell)[a] IC_{50} (nM)
1	2-Cl-6-Me	73	1290 (177)
2	2,6-diCl	21 (10)	320
3	2,6-diCl-4-OCF_3	5 (2)	273 (76)
4	2,4,6-triCl	65 (8)	224 (85)
5	2,6-diMe	120 (21)	1060 (250)
6	2,4,6-triMe	6 (1)	373 (110)

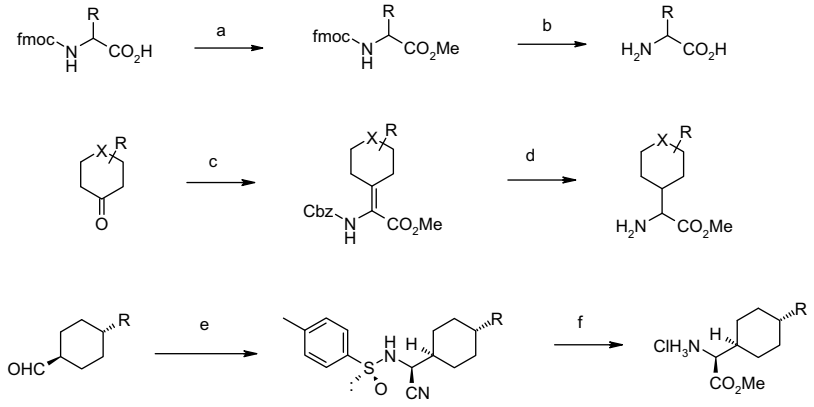
[a] Values are means of three experiments, standard error is given in parentheses.

2,6-disubstituted and 2,4,6 trisubstituted phenyl ureas was prepared (Table 1).[5] The compounds were evaluated in an enzyme assay utilizing human liver glycogen phosphorylase and subsequently progressed to a cellular assay measuring inhibition of forskolin induced glycogenolysis.[7,8] SAR around the phenyl group of the urea revealed that 2,4,6-trisubstitution provided the most potent analogs with the 2,4,6-trimethylphenyl urea derivative **6** providing the optimal balance of enzyme inhibition (GP*a* IC_{50} = 6 nM) and cellular activity (IC_{50} = 373 nM).

Having secured that 2,4,6-trisubstition on the phenyl group of the urea was advantageous for potency, optimization of the amino acid residue was investigated. Custom and commercially available amino acid esters were employed directly in the sequence shown in Scheme 1 to provide the desired anthranilimide targets. The custom amino esters were prepared via several routes detailed in Scheme 2.[9]

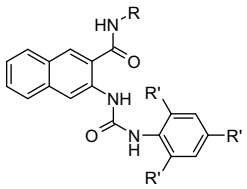
Initial investigation of the SAR around the amino acid residue of the anthranilimides revealed the (*S*)-cyclohexylglycine residue provided potent enzyme inhibition and submicromolar cellular activity but suffered from rapid in vitro and in vivo metabolism. This observation was reaffirmed with the trimethylphenyl urea derivative **6** which has excellent enzyme and cellular potency (Table 1) but had submicromolar inhibition of CYP2C9 and was rapidly metabolized in rat liver microsomes ($t_{1/2}$ < 15 min).

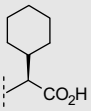
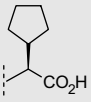
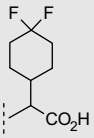
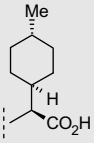
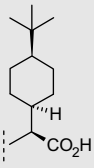
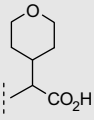
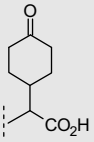
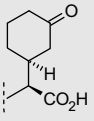
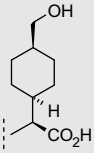
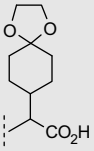
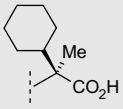
To further explore the SAR around the cyclohexane ring, an examination of substitution was undertaken with the goal of



Scheme 2. General syntheses of the amino acid esters. Reagents and conditions: (a) $TMSCH_2N_2$, DCM; (b) PS-*N*-methylpiperazine, DCM; (c) *N*-Cbz-2-(diethoxyphosphoryl)glycine methyl ester, DBU, DCM; (d) H_2, 10% Pd/C; (f) (*S*)-+-*p*-tolyl-sulfoximine, $Ti(OEt)_4$, DCM; Et_2AlCN, *i*-PrOH, THF; (g) HCl, MeOH.

Table 2
In vitro GP*a* inhibition: cyclohexylglycine analogs



Compound	R	R′	GP*a* IC_{50} (nM)	GP*a* (cell) IC_{50}[a] (nM)	CYP2C9 IC_{50} (nM)
6		Me	6 (1)	373 (110)	610
7		Me	14 (2)	321 (85)	1200
8		Me	13 (8)	1005 (223)	650
9		Cl	56	845 (235)	180
10		Cl	556	2400	210
11		Me	97 (39)	4873 (2170)	2400
12		Me	199 (37)	>10,000	9400
13		Me	132 (38)	>10,000	nt
14		Me	17,000	nt	nt
15		Me	106 (87)	2,053 (623)	6700
16		Me	10 (3)	350 (60)	9200

[a] Values are means of three experiments, standard error is given in parentheses (nt, not tested).

increasing the metabolic stability by blocking metabolism at the cyclohexane ring and decreasing inhibition of CYP2C9 (Table 2). The effect of the ring size of the amino acid residue was explored with cyclopentylglycine derivative **7** which maintained enzyme and cellular potency with a modest decrease in CYP2C9 activity. Substitution at the 4-position of the cyclohexylglycine residue was evaluated with difluoro analog **8** and 1,4-disubstituted cyclohexane derivatives **9** and **10**, which provided a 2- to 100-fold loss of enzyme activity with an accompanying increase in 2C9 inhibition. A greater than 10-fold loss of enzyme activity was seen with the incorporation of polar functionality exemplified by the pyran derivative **11**, ketone derivatives **12** and **13**, and the *cis*-4-hydroxymethylcyclohexylglycine analog **14**, indicating that nonpolar lipophilic residues are preferred. Armed with this knowledge, α-methyl-(*S*)-cyclohexylglycine derivative **16** was prepared which maintained activity at the enzyme and cellular level comparable to cyclohexylglycine derivative **6** but also afforded a >10-fold decrease in CYP2C9 inhibition.

To understand the structural requirements for the binding of the anthranilimide inhibitors to glycogen phosphorylase, X-ray crystallographic analysis was undertaken. Crystallization studies utilizing hLGP*a* with α-methyl-(*S*)-cyclohexylglycine derivative **16** revealed the anthranilimide inhibitors bind to the AMP site of the enzyme (Fig. 2).[10] Interestingly, the amino acid moiety of compound **16** was present in two conformations, **A** and **B**, suggesting that cyclic α,α-disubstitued amino acids would be accommodated in the binding site. With this information in hand, a series of amino-cycloalkyl-carboxylic acid (AC_xC) derivatives were prepared (Table 3).

α,α-Dipropyl derivative **17** was initially prepared and although less potent at GP*a*, exhibited dramatically decreased CYP2C9 inhibition. Constraint of the α,α-disubstitution into a ring was examined with AC_xC derivatives **18**–**23** which revealed that large lipophilic rings were preferred (**21–23**), consistent with results from the cyclohexylglycine analogs. Cyclooctyl analog **22** afforded excellent enzyme inhibition and cellular activity, as well as a greater than 50-fold reduction in CYP2C9 inhibitory activity. Incorpora-

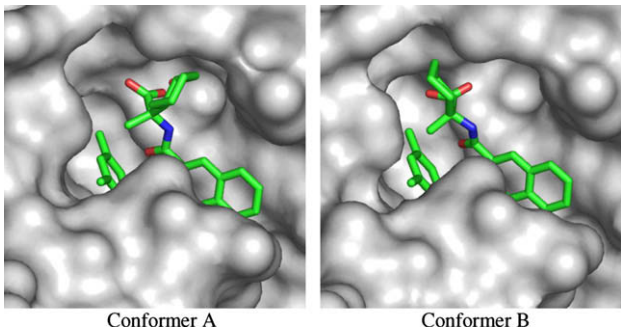
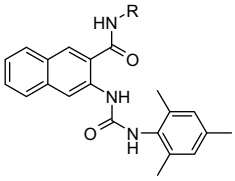
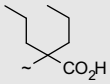
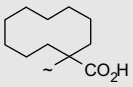
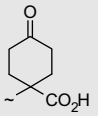
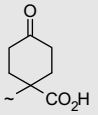
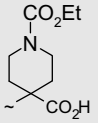
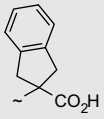
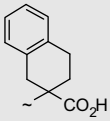


Figure 2. α-Methyl cyclohexylglycine analog **16** exists in two conformations in each of the two hLGP*a* AMP binding-sites seen in the crystal structure. Each site contains a different major conformer within the site as shown above. Minor conformers in each site were not built.

Table 3
In vitro GP*a* inhibition: amino-1-cycloalkyl carboxylic acid analogs



Compound	R	GP*a* IC_{50} (nM)	GP*a* (cell) IC_{50}[a] (nM)	CYP2C9 inhibition IC_{50} (nM)
17		732 (59)	2517 (863)	>33,000
18		720 (117)	9023 (972)	>33,000
19		279	1773 (485)	>33,000
20		339 (93)	1390 (63)	>33,000
21		36 (5)	585 (76)	4,100
22		5 (1)	498 (86)	>33,000
23		14 (5)	443 (84)	nt
24		1147	nt	nt
25		1391 (139)	nt	nt
26		970 (308)	>10,000	nt
27		91 (16)	4788 (653)	4877
28		27 (5)	1070 (302)	nt

[a] Values are means of three experiments, standard deviation is given in parentheses (nt, not tested).

tion of heteroatoms into AC_6C derivatives **24, 25**, and **26** resulted in diminished inhibitory activity of GP*a*, consistent with SAR gained from the cyclohexylglycine series. Indene and tetrahydronaphthalene derivatives **27** and **28** were also prepared and showed good enzyme inhibition but diminished cellular activity (>1 μM).

Table 4
Rat and dog pharmacokinetic data for anthranilimide GPIs

Compound (species)	$AUC_{0\rightarrow\infty,po}$ (ng h/mL)[a]	Cl (mL/min/kg)	Vss (L/kg)	$t_{1/2,\ po}$ (h)	*F* (%)
16 (rat[b])	1720	7.5	0.30	4.1	37
22 (rat[b])	4380	1.1	0.28	5.4	28
22 (dog[c])	620	13	0.50	4.0	45

[a] Dose adjusted oral AUC normalized to 1 mg/kg.
[b] Sprague–Dawley rats ($n = 2$), oral dose = 10 mg/kg, IV dose = 2.5 mg/kg.
[c] Beagles ($n = 2$), oral dose = 5 mg/kg, IV dose = 1 mg/kg.

With potent GP*a* inhibitors with reduced CYP2C9 inhibitory activity in hand, pharmacokinetic (PK) evaluation was undertaken (Table 4). Cyclohexylglycine analog **16** was evaluated in rat PK experiments and AC_8C analog **22** was evaluated in both rat and dog studies. The rat PK profiles of both compounds demonstrated low clearance and volumes of distribution and showed reasonable exposure upon oral dosing (>25% bioavailability) with oral half-lives of greater than 4 h. The PK of compound **22** in dog also exhibited good bioavailability and half-life with slightly increased clearance and lower oral exposure relative to the results in the rat.

In summary, the amino acid residue of a series of anthranilimide-based glycogen phosphorylase inhibitors was optimized to improve potency and stability while reducing the potential for CYP2C9 inhibition. Among the analogs described herein, anthranilimides **16** and **22** were chosen for evaluation in pharmacokinetic studies which supported progression into in vivo efficacy models. These results, along with additional reports on the SAR and optimization of this class of glycogen phosphorylase inhibitors, will reported in due course.

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6. All novel compounds were characterized by NMR and LC–MS and gave results consistent with the proposed structures.
7. Inhibitors were tested for human liver glycogen phosphorylase enzymatic activity using a fluorescence intensity endpoint assay. The change in fluorescence due to product formation was measured on a fluorescence plate reader (Viewlux, Perkin-Elmer) using a 525-nm excitation filter and 595 emission filter. The hGP*a* enzyme IC_{50} values given in Tables 1–4 are average values of at least 2 replicates where standard deviations are noted, and were measured in the presence of glucose (10 mM). Due to the specific activity of the enzyme, a concentration of 10–15 nM glycogen phosphorylase is used in the assay. Therefore, inhibitors with IC_{50} determined to be < approximately 5 nM (Kd < enzyme concentration) cannot be accurately evaluated in this assay format. Inhibitors falling into this category may have IC_{50} significantly lower than the estimate. See Ref. 11 for additional details.
8. This full curve assay was designed to detect the inhibition of glycogenolysis (glycogen breakdown) by test compounds. On the day before the assay the glycogen in HepG2 cells is prelabeled by overnight inclusion of ^{14}C-glucose in the culture medium. To begin the assay, the cells are treated with test compounds, and glycogenolysis is stimulated by forskolin treatment for 60 min. The cells are then lysed and the radiolabeled glycogen in the cells is quantified. If a test compound inhibits glycogenolysis, the radiolabeled glycogen content of the cells will be greater than control (forskolin treated). The hGP*a* (cell) IC_{50} values given in Tables 1–4 are average values of at least 2 replicates where standard errors are noted. See Ref. [11] for additional details.
9. Dehydroamino acid synthesis: (a) Schmidt, U.; Lieberknecht, A.; Schanbacher, U.; Beuttler, T.; Wild, J. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 776; Asymmetric Strecker chemistry: (b) Davis, F. A.; Portonovo, P. S.; Reddy, R. E.; Chiu, Y. *J. Org. Chem.* **1996**, *61*, 440; The amino acid for compound **13** was prepared by the

method of Corey: (c) Corey, E. J.; Noe, M. C.; Xu, F. *Tetrahedron Lett.* **1998**, *39*, 5347.

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