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Anthranilimide-based glycogen phosphorylase inhibitors for the treatment of Type 2 diabetes: 2. Optimization of serine and threonine ether amino acid residues

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ABSTRACT

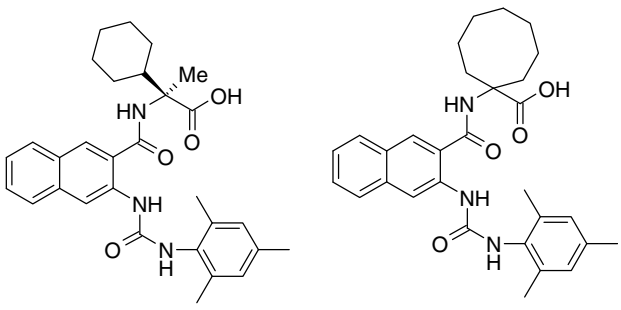
Optimization of the amino acid residue of a series of anthranilimide-based glycogen phosphorylase inhibitors is described leading to the identification of serine and threonine ether analogs. *t*-Butylthreonine analog **20** displayed potent in vitro inhibition of GP*a*, low potential for P450 inhibition, and excellent pharmacokinetic properties.

Therapies targeting the reduction of hepatic glucose production (HGP) as a method to control the hyperglycemia associated with Type 2 diabetes have recently attracted considerable attention.[1] Gluconeogenesis, the de novo synthesis of glucose from lactate, amino acids, and glycerol, and glycogenolysis, the release of monomeric glucose from its polymeric storage form glycogen, are the two processes which contribute to HGP. Pharmacological interventions aimed at inhibiting both processes have been investigated with the goal of reducing endogenous glucose production.[2]

Among biological targets which directly inhibit both gluconeogenesis and glycogenolysis, antagonists of the glucagon receptor[3] and inhibitors of glucose-6-phosphatase translocase[4] have been examined. The selective inhibition of gluconeogenesis has been studied with the development of inhibitors for fructose 1,6-bisphosphatase[5] and phosphoenolpyruvate carboxykinase,[2b] while inhibition of glycogen phosphorylase[6] (GP) has been studied for the selective inhibition of glycogenolysis. A variety of targets have also been studied for the indirect reduction of HGP. These include inhibitors of 11-β-hydroxysteroid dehydrogenase-1,[1b] dipeptidyl peptidase-IV,[7] and pyruvate dehydrogenase kinase,[8] in addition to activators of glucokinase[9] and antagonists of the glucocorticoid receptor.[10]

In the preceding communication we described the optimization of the amino acid fragment of a series of anthranilimide based glycogen phosphorylase (GP) inhibitors leading to the identification of orally bioavailable compounds **1** and **2** with diminished activity at CYP2C9 (Fig. 1).[11] In this communication we report further optimization of the amino acid residue.

Early lead exploration efforts resulting from a high throughput synthesis campaign revealed that anthranilimides with acyclic amino acid residues provided submicromolar enzyme potency



1
IC_{50} (+glu) = 10 nM
IC_{50} (cell) = 350 nM
CYP2C9 IC_{50} = 9,200 nM
37 %F (rat)

2
IC_{50} (+glu) = 5 nM
IC_{50} (cell) = 498 nM
CYP2C9 IC_{50} > 33,000 nM
28 %F (rat)

Figure 1. In vitro profile of anthranilimides **1** and **2**.

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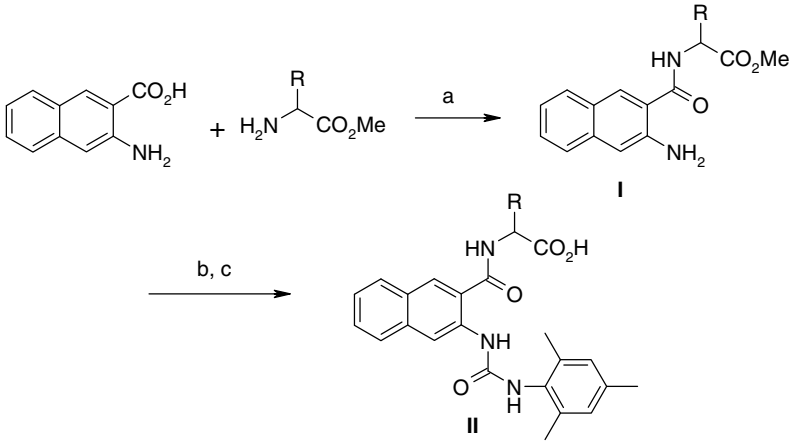
and micromolar cellular potency.[12] Thus, the primary goals of these efforts were to identify acyclic amino acid residues which provided increased potency at the enzyme and cellular levels while maintaining the pharmacokinetic profile of anthranilimides **1** and **2**.

The general synthesis of the anthranilimide inhibitors is shown in Scheme 1. Amide bond formation between the appropriate amino acid ester and 2-aminonaphthoic acid provided the amide derivatives **I**, which were then condensed with substituted 2,4,6-trimethylphenyl-isocyanate and saponified to provide the desired anthranilimide analogs **II**.

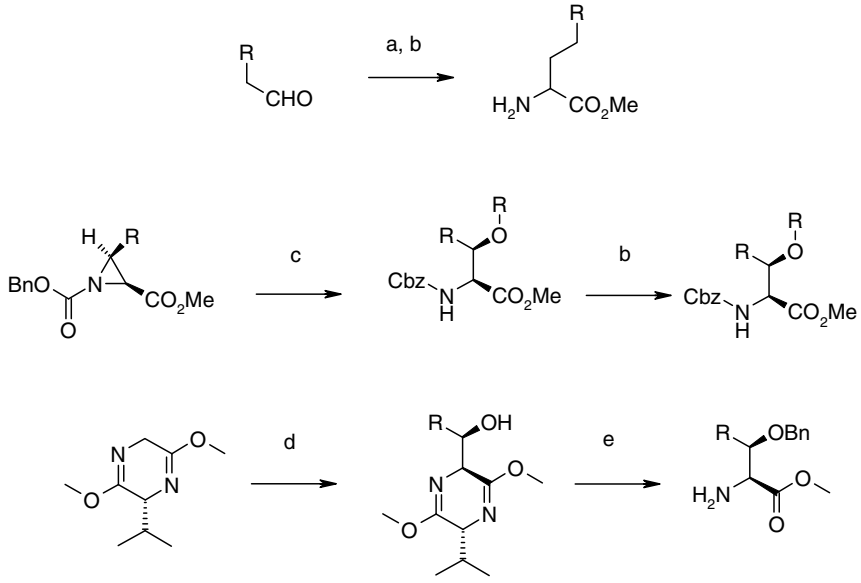
Custom amino acid esters were prepared via several methods detailed in Scheme 2. Dehydroamino esters were prepared by the condensation of aldehydes with phosphorylglycine esters and subsequently reduced to provide the racemic amino acid esters.[13] In select cases, the racemic anthranilimides derived from this method were separated by chiral SFC to deliver enantiomerically pure final products. Serine and threonine ether derivatives were prepared by the Lewis acid assisted ring opening of N-acylated aziridines with alcohols[14] and C-4 substituted threonine analogs were prepared utilizing the methods of Schöllkopf.[15]

Our initial studies revealed that the enzyme preferred anthranilimide inhibitors with nonpolar lipophilic amino acid residues.[11] Based on this understanding, we surveyed several commercially available amino acids with acyclic lipophilic residues (Table 1). 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.[11,16]

Analogs derived from valine, *tert*-leucine, and norvaline (**3–5**) revealed that increasing chain length provided a 10-fold increase in enzyme potency while maintaining cellular potency near 1 μM (Table 1). Examination of methyl substitution (**6–8**) revealed that both β-substitution (isoleucine **6**) and chain elongation (norleucine **8**) provided inhibitors with submicromolar potency in the cellular assay. Further modifications to the terminal atom of the residue including elongation (**9** and **10**) produced a 3- to 4-fold reduction in cellular potency. Having determined that a 4-atom residue was optimal for both enzyme and cellular potency, δ-substitution (**11** and **12**) was evaluated which provided excellent enzyme and cellular potency, but also increased CYP2C9 inhibition (**8** vs **11** and **12**).

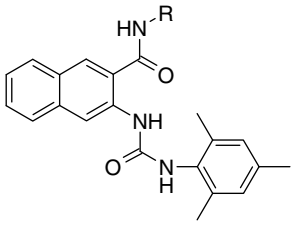


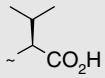
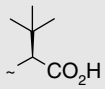
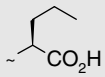
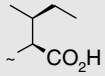
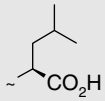
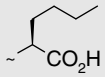
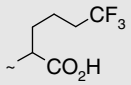
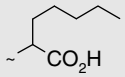
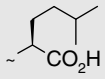
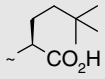
Scheme 1. General Syntheses of the anthranilimide core. Reagents and conditions: (a) HATU, *i*PrNEt$_2$, DMF, rt (b) 2,4,6-trimethylphenyl isocyanate, pyridine, rt (c) 2 M LiOH, MeOH, THF.



Scheme 2. General syntheses of the amino acid esters. Reagents and conditions: (a) N-Cbz-2-(diethoxyphosphoryl)glycine methyl ester, DBU, DCM (b) H_2, 10% Pd/C (c) ROH, $BF_3{\cdot}EtO_2$, $CHCl_3$ (d) *n*-BuLi, THF, −78 °C; RCHO (e) NaH, BnBr, DMF; aq HCl, CH_3CN.

Table 1
In vitro GP*a* inhibition: acyclic amino acids **3**–**11**



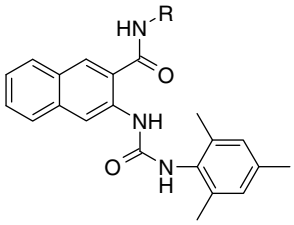
Compound	R	GP*a* IC_{50}, nM[a]	GP*a* (cell) IC_{50}, nM[a]	CYP2C9 IC_{50}, nM
3		387 (118)	1170 (89)	12,000
4		311 (14)	1138 (194)	2300
5		39 (8)	1019 (177)	8400
6		72 (16)	688 (70)	1940
7		101 (21)	1427 (131)	nt
8		23 (4)	501 (84)	3000
9		123 (46)	2003 (450)	nt
10		114 (14)	1533 (288)	nt
11		9	452 (67)	740
12		25 (2)	311 (21)	450

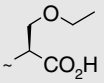
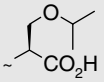
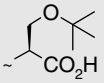
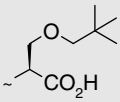
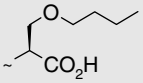
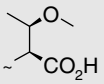
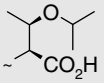
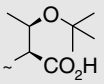
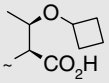
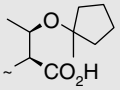
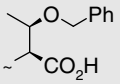
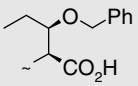
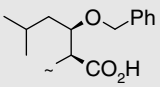
[a] Values are means of three experiments, standard error is given in parentheses.

To further explore the SAR around the acyclic amino acids, incorporation of oxygen into the residues examined (Table 2). Serine and threonine ethers were targeted with the goal of further improving potency and incorporating polar functionality to decrease CYP2C9 inhibition.[17] Serine ethers (**13–17**) were initially prepared and confirmed that δ-substitution provided excellent cellular potency (**14** and **15**) and revealed that incorporation of oxygen into the residue provided a greater than 10-fold reduction in CYP2C9 activity (**12** vs **15**). Incorporation of a β-methyl group in the form of threonine ethers (**18–23**) provided further improvements in enzyme and cellular potency along with additional reductions in CYP2C9 inhibition. *t*-Bu-Thr analog **20** was identified as having the optimal combination of enzyme (5 nM) and cellular (139 nM) activity along with reduced potential for CYP2C9 inhibition. Substitution off of the threonine γ-carbon was explored with analogs **24** and **25**, which afforded a decrease in both enzyme and cellular activity and a dramatic increase in CYP2C9 inhibition.

With potent GP inhibitors incorporating acyclic amino acid residues in hand, pharmacokinetic (PK) studies were undertaken (Table 3). Analogs **8, 15**, and **20** were evaluated in rat PK experiments. The PK profiles were characterized by moderate oral exposures with low to moderate clearances and low steady-state volumes of distribution. Half-lives ranged from 2 to 4 h. Of the analogs examined, *t*-butylthreonine analog **20** showed superior bioavailability (40%) and improved oral exposure. These parameters coupled with the compound's superior cellular activity (IC_{50} = 139 nM) and reduced CYP2C9 inhibition (IC_{50} = 10 μM) dictated selection of the compound for in vivo efficacy studies.

Table 2
In vitro GP*a* inhibition: serine and threonine analogs **12–24**



Compound	R	GP*a* IC_{50} (nM)[a]	GP*a* (cell) IC_{50} (nM)[a]	CYP2C9 IC_{50} (nM)
13		56	4467 (396)	3300
14		148 (94)	451 (95)	6920
15		44 (18)	387 (70)	6000
16		79 (31)	949 (103)	4700
17		95 (28)	1139 (141)	2800
18		298 (54)	2457 (711)	32,000
19		17 (2)	156 (22)	14,000
20		7 (2)	139 (41)	10,000
21		17 (5)	434 (64)	2100
22		6	159 (17)	7230
23		16 (3)	420 (15)	1900
24		24 (4)	309 (19)	680
25		73 (18)	613 (111)	50

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

In summary, systematic modifications to the amino acid residue of a series of anthranilimide based glycogen phosphorylase inhibitors led to the identification of a series of serine and threonine ethers with excellent potency and attenuated CYP2C9 inhibition. Anthranilimide **20**, containing the *t*-butylthreonine residue, was identified for further progression into in vivo efficacy models, the results of which will be reported in the following communication.

Table 3
Rat pharmacokinetic data for anthranilimide GPIs

Compound	$AUC_{0\rightarrow\infty,\mathbf{po}}$(ng.h/mL)[a]	Cl[b] (mL/min/kg)	Vss (L/kg)	$T_{1/2,\ po}$[b](h)	*F*(%)
8	320	4	0.6	2.8	8
15	256	26	1.9	4.0	17
20	514	13	0.5	3.0	40

[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.

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