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Amino acid anthranilamide derivatives as a new class of glycogen phosphorylase inhibitors

Karen A. Evans [a,*], Yue H. Li [a], Frank T. Coppo [a], Todd L. Graybill [a], Maria Cichy-Knight [a], Mehul Patel [a], Jennifer Gale [a], Hu Li [a], Sara H. Thrall [a], David Tew [a], Francis Tavares [b], Stephen A. Thomson [b], James E. Weiel [b], Joyce A. Boucheron [b], Daphne C. Clancy [b], Andrea H. Epperly [b], Pamela L. Golden [b]

[a] *Discovery Research, GlaxoSmithKline Pharmaceuticals, 1250 South Collegeville Road, PO Box 5089, Collegeville, PA 19426-0989, USA*
[b] *Metabolic and Viral Diseases Center of Excellence for Drug Discovery, GlaxoSmithKline Pharmaceuticals, Five Moore Drive, PO Box 13398, Research Triangle Park, NC 27709-3398, USA*

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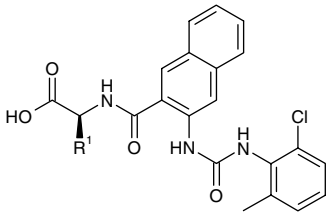
ABSTRACT

A series of amino acid anthranilamide derivatives identified from a high-throughput screening campaign as novel, potent, and glucose-sensitive inhibitors of human liver glycogen phosphorylase *a* are described. A solid-phase synthesis using Wang resin was also developed which provided efficient access to a variety of analogues, and resulted in the identification of key structure–activity relationships, and the discovery of a potent exemplar (IC_{50} = 80 nM). The SAR scope, synthetic strategy, and in vitro results for this series are presented herein.

According to the International Diabetes Federation (IDF), more than 230 million people, almost 6% of the world's adult population, now live with diabetes. The incidence of diabetes and associated complications, such as obesity and heart disease, is escalating rapidly in various societies around the world, placing a significant economic burden on healthcare resources. Despite the use of various hypoglycemic agents with or without co-therapy with insulin, current treatments often fail to achieve significant lowering of serum glucose to a level where the severity of diabetic complications is effectively reduced. Thus, there is a clear need for novel hypoglycemic agents targeting the underlying mechanism(s) of elevated serum glucose.

Studies have shown that excessive hepatic glucose production (HGP) is a significant factor contributing to hyperglycemia in patients with type 2 diabetes (non-insulin dependent diabetes mellitus).[1] Glucose is produced by both gluconeogenesis and glycogenolysis, the release of glucose-1-phosphate from glycogen. Since glycogenolysis is a major component of HGP and human liver glycogen phosphorylase *a* (GPa) catalyzes this reaction as the rate-limiting enzyme, it is thought that inhibiting GPa will limit glycogenolysis, reduce HGP, and thus lower blood glucose, thereby providing a potential new treatment for type 2 diabetes. There are several examples in the literature of small-molecule allosteric inhibitors of this enzyme.[2] Herein, the discovery, synthesis, and structure–activity relationships (SAR) of a series of amino acid anthranilamide derivatives as novel glucose-sensitive GPa inhibitors are presented.



1a, $R^1 = CH_2Cy$
IC_{50} (+glu) = 2.2 uM
IC_{50} (-glu) = 51 uM
IC_{50} (cell) = 7.4 uM

1b, $R^1 = CH_2CO_2H$
IC_{50} (+glu) = 0.39 uM
IC_{50} (-glu) = 1.6 uM
IC_{50} (cell) = 4.7 uM

Compounds **1a** (IC_{50} = 2.2 μM) and **1b** (IC_{50} = 0.39 μM) were identified as part of a high-throughput screen of the GSK proprietary compound collection. Both compounds, and compound **1b** in particular, were found to have good enzyme inhibition in the presence of glucose (+glu), but were observed to be less potent in the absence of glucose (−glu), suggesting glucose sensitivity for this

* Corresponding author. Tel.: +1 610 917 7764; fax: +1 610 917 7391.
E-mail address: karen.a.evans@gsk.com (K.A. Evans).

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series of compounds.[3] In addition, both compounds were active in cells, demonstrating micromolar potencies. Significant metabolic advantages should exist for glucose-sensitive GPa inhibitors which act primarily at the high blood glucose levels typically seen in diabetic patients, and then lose potency as glucose levels fall in order to avoid hypoglycemia.

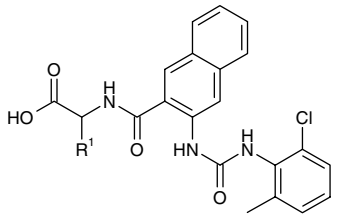
Initial objectives for this chemical series were thus to increase the potency, establish key structure–activity relationships, develop efficient synthetic methods, and evaluate the pharmacokinetic profile. Structural modifications of this template were primarily achieved through solid-phase synthesis using Irori® AccuTag-100™ (miniKan) technology with Wang resin-bound amino acids (Scheme 1).

Commercially available Fmoc-protected L- or D-amino acids (**2**) were loaded onto 4-hydroxymethylphenoxymethyl polystyrene (Wang) resin[4] using standard conditions.[5] Whenever possible, commercially available enantiopure Fmoc-protected amino acids preloaded onto Wang resin (**3**) were used.[6] Compound **3** was treated with piperidine in DMF to remove the Fmoc protecting group, and then reacted with substituted unprotected amino benzoic acids **4** to give resin-bound amides **5**. No undesired byproducts were observed in this step, presumably due to the reactivity difference between the less reactive aniline and the more reactive primary amine. Lastly, compounds **5** were treated with the corresponding isocyanates, and the final products were cleaved from the resin using a 1:1 solution of trifluoroacetic acid and dichloromethane. Resin-bound intermediates **5** could also be reacted with sulfonyl chlorides, chloroformates, acid chlorides, or carboxylic acids in a similar fashion.[7]

Thus, this solid-phase synthesis strategy proved to be a highly efficient and robust method for rapid SAR identification for this series, using one synthetic sequence to explore three points of diversity simultaneously. All compounds were purified by reverse-phase HPLC[8] to purities of >95% (LCMS, UV 214 nm detection),[9] and then evaluated in the enzyme inhibition assay against human liver glycogen phosphorylase. Compounds were then selected for further evaluation in the cellular assay.[10] The results are outlined below.

Initially, the SAR around the amino acid side chain (R^1) was investigated. The *S*-enantiomer (**1c**-(***S***),[11] IC_{50} = 80 nM) was established as the preferred isomer compared to the *R*-enantiomer (**1c**-(***R***), IC_{50} = 4.2 μM). A number of *S*-amino acid derivatives were prepared which showed that a variety of substituents were tolerated at the R^1 position with submicromolar enzyme potency and micromolar potency in the cellular assay. Compounds with a longer alkyl side chain were less potent, as in **1o** versus **1b**, **1p** versus **1g**, and **1a** versus **1c**-(***S***). The most dramatic result observed was in shortening the side chain of the cyclohexylmethyl analogue **1a**. By simply removing the methylene linker (**1c**-(***S***)), the enzyme

Table 1
In vitro GPa inhibition: amino acid side chain variations



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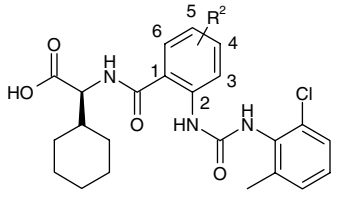
Compound	R^1	GPa IC_{50}, μM	GPa (cell) IC_{50}, μM
1a-(***S***)	CH_2Cyclohexyl	2.2 ± 2.9	7.4 ± 1.8
1c-(***S***)	Cyclohexyl (Cy)	0.08	0.60 ± 0.11
1c-(***R***)	Cy	4.2	
1d-(***S***)	Trp	0.27 ± 0.005	2.5
1e-(2***S***,3***S***)	$CH(CH_3)CH_2CH_3$	0.28	1.1
1f-(***S***)	2-Thienyl	0.36	1.7
1b-(***S***)	CH_2CO_2H	0.39 ± 0.37	4.7 ± 0.7
1g-(***S***)	CH_2CONH_2	0.42	2.1
1h-(2***S***,3***R***)	$CH(OH)CH_3$	0.43	1.9
1i-(***S***)	CH_2(4-OHPhenyl)	0.45	3.0
1j-(***S***)	CH_3	0.52	
1k-(***S***)	H	0.57	
1m-(***S***)	Pro	0.58	2.9
1n-(***S***)	$CH_2CH(CH_3)_2$	0.60	3.3
1o-(***S***)	$CH_2CH_2CO_2H$	0.70	>10
1p-(***S***)	$CH_2CH_2CONH_2$	1.3	
1q-(***S***)	$(CH_2)_4NH_2$	3.0 ± 0.31	>10

Table 2
In vitro GPa inhibition: phenyl spacer variations



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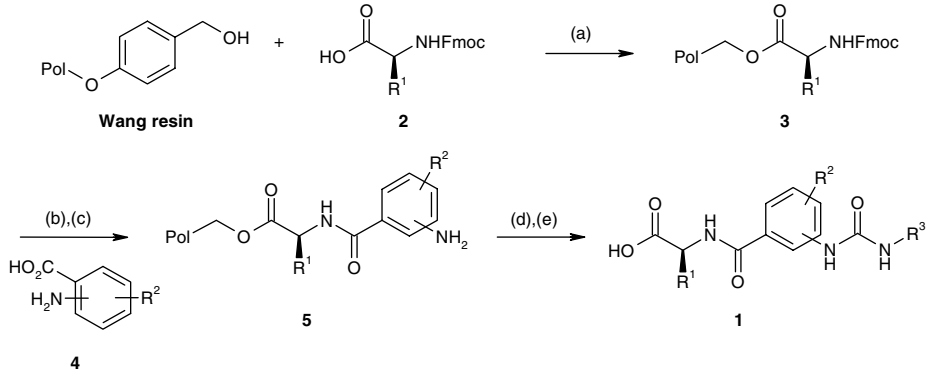
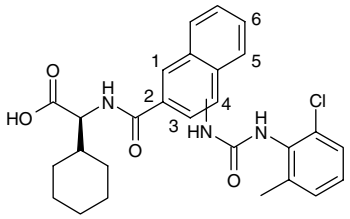
Compound	R^2	GPa IC_{50}, μM	GPa (cell) IC_{50}, μM
1c	4,5-Phenyl (fused)	0.08	0.60 ± 0.11
6a	H	100	>7.5 ± 2.5
6b	4,5-Dimethoxy	7.0	
6c	4,5-Difluoro	0.86	6.8 ± 3.2
6d	3,5-Dimethyl	>25	
6e	3,6-Dichloro	>25	



Scheme 1. Reagents and conditions: (a) 0.3 M 2,6-dimethylbenzoyl chloride; 0.5 M pyridine; 0.3 M $R^1CHNHFmocCO_2H$; DMF, rt, 20 h, (b) 20% piperidine/DMF, rt, 20 h, (c) 0.3 M EDC; 0.3 M HOAT; 0.3 M DIEA; 0.3 M R^2CO_2H; NMP, rt, 24 h, (d) 10 equiv R^3NCO, pyridine, rt, 24 h, (e) 50% TFA/DCM, rt, 30 min.

Table 3
In vitro GPa inhibition: naphthyl spacer variations



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Compound	Carbonyl/urea relationship	GPa IC_{50}, μM
1c	2,3-	0.08
7a	1,4-	100
7b	2,6-	100

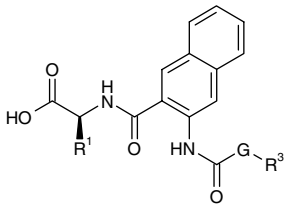
potency improved by 30-fold and the cellular potency improved to submicromolar levels.

With compound **1c**-(***S***) now in hand as a potent lead, variations on the central aryl ring R^2 were investigated to better understand the role of the naphthyl ring, and therefore a small number of disubstituted phenyl analogues were prepared (Table 2). Replacement of the naphthyl ring with an unsubstituted phenyl ring (**6a**) was unfavorable, as were 3,5- and 3,6-disubstitutions (compounds **6d** and **6e**, respectively). While 4,5-disubstitutions (**6b**, **6c)** were tolerated, the naphthyl moiety (**1c)** remained the most potent.

The relationship of the urea and carbonyl group position on the naphthyl ring was then studied (Table 3). It was quickly established by the preparation of compounds **7a** and **7b** that the urea and the carbonyl must be ortho to each other on the aryl ring.

A significant effort was also spent probing changes and requirements at the urea position R^3 (Table 4). Replacement of the urea with aryl sulfonamides, substituted phenyl amides (i.e., removal of G in compound **8**) (not shown, GPa IC_{50} = 100 μM), and urethanes (**8n**) resulted in loss of potency. In fact, removal of any of the three hydrogen bond positions in this template by replacement of NH with NMe resulted in reduction in potency (GPa IC_{50} > 1 μM,

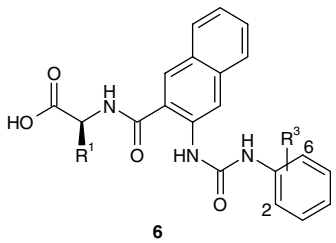
Table 4
In vitro GPa inhibition: urea variations



8

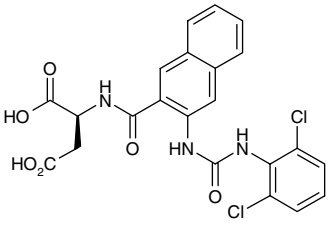
Compound	R^1	R^3	G	GPa IC_{50}, μM	GPa (cell) IC_{50}, μM
1c	Cy	2-Cl-6-MePhenyl	N	0.08	0.60 ± 0.11
8a	Cy	2,6-diClPhenyl	N	0.10	0.32
8b	Cy	2,6-diClPhenyl	C	6.8	1.6
8c	Cy	2,6-diMePhenyl	N	0.12 ± 0.02	1.1 ± 0.25
8d	Cy	2,6-diEtPhenyl	N	0.59 ± 0.28	4.5 ± 1.9
8e	Cy	2,6-diFPhenyl	N	4.9	
8f	Cy	2-MePhenyl	N	30 ± 41	>5
8g	Cy	2-ClPhenyl	N	100	
1b	CH_2CO_2H	2-Cl-6-MePhenyl	N	0.39	4.7
8h	CH_2CO_2H	2,6-diClPhenyl	N	0.23	0.64 ± 0.43
8i	CH_2CO_2H	2,6-diMePhenyl	N	0.12 ± 0.08	1.1
8j	CH_2CO_2H	2,6-diFPhenyl	N	5.0	
8k	CH_2CO_2H	2-MePhenyl	N	1.2	
8m	CH_2CO_2H	2-ClPhenyl	N	1.6 ± 1.5	
8n	CH_2CO_2H	2-ClPhenyl	O	100	

Table 5
In vitro metabolic stability in liver microsomes



6

Compound	R^1	R3	$T_{1/2}$ (min) human	$T_{1/2}$ (min) rat	Clint (ml/min/kg) human	Clint (ml/min/kg) rat
1c	Cy	2-Cl,6-Me	16	<15	118	1317
8a	Cy	2,6-diCl	16	<15	120	857
8h	CH_2CO_2H	2,6-diCl	>120	>120	<15	<23
8i	CH_2CO_2H	2,6-diMe	>120	>120	<15	<23
1q	$(CH_2)_4NH_2$	2-Cl,6-Me	>120	19	<15	151



8h

$T_{1/2}$ = 4.0 h
AUC (iv) = 45247 ng-hr/mL
AUC (po) = 1671 ng-hr/mL
Cl_b = 0.92 mL/min/kg
Vss = 0.25 L/kg
%F = 1

Figure 1. In vivo rat DMPK of **8h** (10 mg/kg po, 2.5 mg/kg iv, vehicle (2:98) DMSO:30% solutol in saline).

not shown). However, modification of the urea to a phenylacetic amide (G═C) was weakly tolerated (**8b** vs **8a**). The key finding was that appropriate 2,6-disubstitution on the aryl ring was minimally required for good potency with methyl or chloro substituents being the most preferred (**1c**, **8a**, **8c**, **1b**, **8h–i**). Simple 2-substitution showed a decrease in potency (**8f–g**, **k–m**), most pronounced with the cyclohexyl side chain (**8f–g**).

In ADME studies, compound **1c**-(***S***) was rapidly metabolized in rat liver microsomes, with a half-life of <15 min and displayed very high intrinsic clearance (CLint) of 1317 mL/min/kg (Table 5). This observation was consistent with results from in vivo rat pharmacokinetic studies, which showed very rapid clearance following intravenous administration (2.5 mg/kg), and very low C_{max} of 7.3 ng/mL when administered orally (10 mg/kg). Compound **8h** had a much better metabolic stability profile, and comparable cellular potency to **1c**-(***S***), and was therefore also tested in vivo. While compound **8h** showed reasonable half-life (4 h) and lower plasma clearance compared to **1c**-(***S***), it unfortunately had poor oral exposure (Fig. 1). While it is not surprising that the diacid **8h** has poor bioavailability, this result demonstrates that by proper modifications of the amino acid side chain, the clearance and half-life can be dramatically improved.

In summary, a series of amino acid anthranilamide derivatives were successfully identified from high-throughput screening as novel, potent, glucose-sensitive inhibitors of human liver glycogen phosphorylase *a*. A solid-phase synthesis strategy was employed to provide efficient access to a variety of analogues for rapid SAR generation. A potent exemplar of this series was quickly identified (**1c**-(***S***), GPa IC_{50} = 80 nM) which appeared to be rapidly metabolized both in vitro and in vivo, and a related analogue was identified with comparable enzyme potency (**8h**), GPa IC_{50} = 230 nM) and an improved metabolic stability profile. The potency of compound **1c**-(***S***) and the improved half-life of compound **8h** rendered this series worthy of continued exploration and development with additional modifications to the amino acid side chain, the phenylurea, and the central aryl ring. Details regarding the further optimization of the potency and pharmacokinetic profile of this novel series will be the subject of a future communication.

Acknowledgments

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References and notes

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3. Inhibitors were tested for human liver glycogen phosphorylase enzymatic activity using a fluorescence intensity endpoint assay. To aid in the identification of glucose-sensitive inhibitors of glycogen phosphorylase, the assay was performed with and without 10 mM glucose. 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. A concentration of 25 nM GPa enzyme was used. Therefore, inhibitors can be accurately evaluated to an IC_{50} = 12.5 nM in this assay format. The hGPa 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. See Ref. 7 for additional details.
4. The Wang resin was obtained from Polymer Laboratories, 1.7 mmol/g (PL-Wang, Product # 1463, 150–300 μm).
5. Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 6147.
6. Commercially available resins preloaded with L-amino acids were obtained from Polymer Labs (www.polymerlabs.com), typically 0.86 mmol/g, >99% optical purity.
7. For further experimental details, see Evans, K.A.; Cichy-Knight, M.; Coppo, F.T.; Dwornik, K.A.; Gale, J.P.; Garrido, D.M.; Li, Y.H.; Patel, M.; Tavares, F.X.; Thomson, S.A.; Dickerson, S.H.; Peat, A.J.; Sparks, S.M.; Banker, P.; Cooper, J.P. WO 2006/052722 A1.
8. Preparative HPLC purifications (reverse-phase) were performed using a Gilson 215 Liquid Handler with Unipoint software, typically with a YMC 20 × 50 combiprep column (or 30 × 75) ODS-A, 5u. A 5-min run (25 mL/min, 10% ACN/H_2O, 0.1%TFA to 90% ACN/H_2O, 0.1% TFA) with UV detection at 254 nm was typically used.
9. All novel compounds were characterized by LCMS, and gave satisfactory results in agreement with the proposed structure. Purity data were determined by a C18 reverse phase HPLC column [Keystone Aquasil (1 × 40 mm)] in 10–90% ACN/H_2O containing 0.02% TFA (3.6 min gradient) and monitored by a UV detector operating at 214 nm and by a SEDEX 75 evaporative light scattering detector (ELSD) operating at 42 °C. LCMS M+H signals were consistent with expected molecular weight for all reported products.
10. 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 hGPa (cell) IC_{50} values given in Tables 1–4 are average values of at least 2 replicates where standard deviations are noted.
11. Spectral data for compound **1c**-(***S***): ^{1}H NMR (400 MHz, MeOH-d_4) δ: ppm 1.21–1.36 (m, 5 H), 1.70 (d, *J* = 11.7 Hz, 1 H), 1.82 (d, *J* = 10.8 Hz, 1 H), 1.96 (br s, 1 H), 2.36 (s, 3 H), 4.52 (br s, 1 H), 7.21–7.26 (m, 2 H), 7.34 (d, *J* = 6.6 Hz, 1 H), 7.41 (dt, *J* = 7.0, 0.9 Hz, 1 H) 7.51 (dt, *J* = 8.2, 1.0 Hz, 1 H), 7.76 (d, *J* = 8.2 Hz, 1 H), 7.88 (d, *J* = 8.1 Hz, 1 H), 8.18 (s, 1 H), 8.57 (s, 1 H), 8.75 (br s, 1H). ESMS *m/z* $[M+H]^+$ = 494.4.