Discovery of Alogliptin: A Potent, Selective, Bioavailable, and Efficacious Inhibitor of Dipeptidyl Peptidase IV†

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Abstract: Alogliptin is a potent, selective inhibitor of the serine protease dipeptidyl peptidase IV (DPP-4). Herein, we describe the structure-based design and optimization of alogliptin and related quinazolinone-based DPP-4 inhibitors. Following an oral dose, these noncovalent inhibitors provide sustained reduction of plasma DPP-4 activity and a lowering of blood glucose in animal models of diabetes. Alogliptin is currently undergoing phase III trials in patients with type 2 diabetes.

The World Health Organization estimates the number of people with diabetes to be approximately 180 million. This number is projected to double by 2030. Type 2 diabetes (T2D) is a progressive disease characterized by high levels of glucose resulting from insulin resistance and impairment of insulin secretion. If left untreated, hyperglycemia may cause nephropathy, neuropathy, retinopathy, and atherosclerosis. T2D causes significant morbidity and mortality and results in considerable expense to patients, their families, and society.[1]

Glucagon-like peptide-1 (GLP-1 (7−36 amide or 7−37)), a 30-amino acid peptide hormone, is secreted by intestinal L-cells in response to meal ingestion and stimulates insulin secretion from β-cells while inhibiting hepatic glucose production.[2] Furthermore, GLP-1 has been shown in mammals to stimulate insulin biosynthesis, inhibit glucagon secretion, slow gastric emptying, reduce appetite, and stimulate the regeneration and differentiation of islet β-cells.[3] Continuous infusion of GLP-1 to patients with T2D results in significant reduction of blood glucose and hemoglobin A_{1c} levels.[4] However, active GLP-1 is rapidly converted to inactive GLP-1 (9−36 amide or 9−37) by the serine protease dipeptidyl peptidase IV (DPP-4[a]), thus limiting its therapeutic practicality. Inhibition of DPP-4 increases the levels of endogenous intact GLP-1. Consequently, inhibition of DPP-4 is rapidly emerging as a novel therapeutic approach for the treatment of type 2 diabetes.[5] Clinical proof of concept has already been established with DPP-4 inhibitors such as 1-[[(3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine (LAF-237)[6] and (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (MK-0431).[7]

The active site of DPP-4 is shown in Figure 1 with important residues labeled. Figure 2 shows the surface of the site. Using

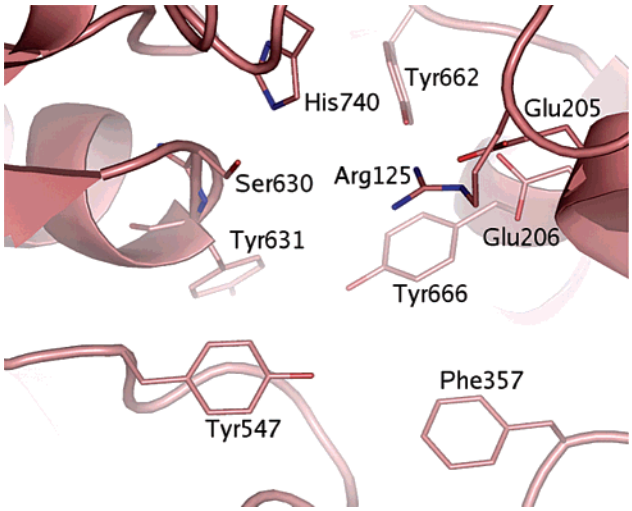

Figure 1. Active site of DPP-4.

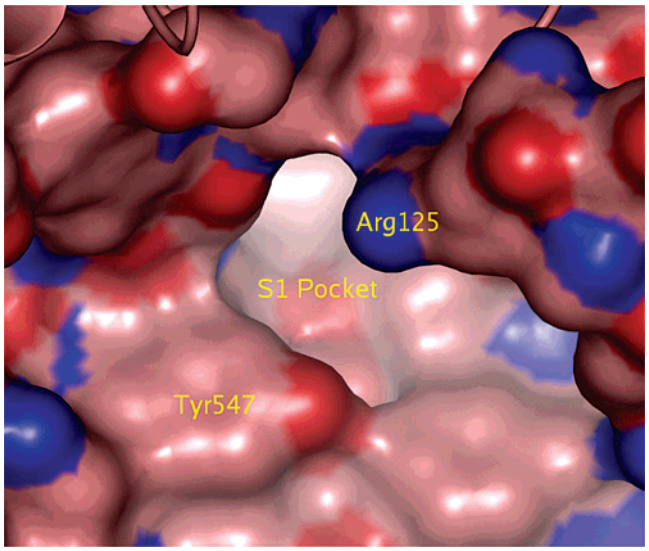

Figure 2. DPP-4 active site surface.

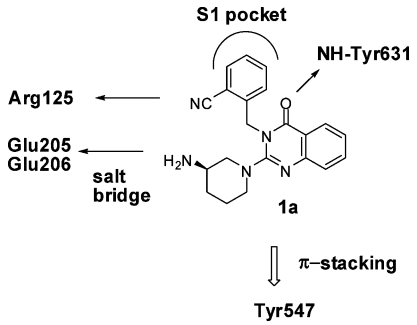

Figure 3. Structure-based design of compound **1a**.

structure-based design, we hypothesized that a quinazolinone scaffold could effectively display groups known to interact with the active site residues of DPP-4.[8] As shown in Figure 3, placing the aminopiperidine motif at C-2 was predicted to provide a salt bridge to E205/E206 while a cyanobenzyl group at N-3 was expected to effectively fill the S1 pocket (formed by V656, Y631, Y662, W659, Y666, and V711) and interact with Arg125. The carbonyl at C-4 was anticipated to provide an important hydrogen bond to the backbone NH of Tyr631, and the bicyclic

† Compound **1a** has been deposited into the Protein Data Bank: PDB code 2ONC.

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[a] Abbreviations: DPP-4, dipeptidyl peptidase IV.

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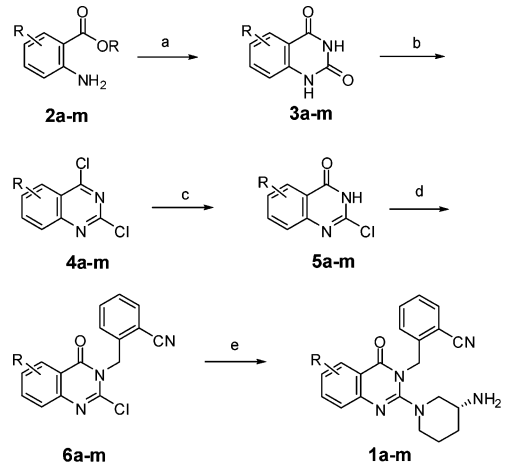
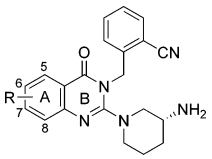
Scheme 1. Synthesis of Compounds **1a**−**m**[a]



[a] Reagents: (a) urea, 200 °C; (b) $POCl_3$, Me_2NPh, reflux; (c) NaOH; (d) NaH, LiBr, 2-CNPhCH$_2$Br; (e) 3-(*R*)-aminopiperidine, $NaHCO_3$, 150 °C.

Table 1. Selected Data for Quinazolinones **1a**−**m**



compd	R	DPP-4 IC_{50} (μM)	DPP-8 IC_{50} (μM)	RLM[b] $t_{1/2}$ (min)	HLM[c] $t_{1/2}$ (min)	3A4[d] IC_{50} (μM)
1a[a]	H	0.013 ± 0.006	>100	2.5	>200	2.0
1b	5-F	0.005 ± 0.0002	>100	1.8	125	0.50
1c	6-F	0.004 ± 0.0008	>100	31	>200	2.5
1d	6-Cl	0.005 ± 0.0008	>50	40	>200	0.25
1e	7-Cl	0.015 ± 0.002	>100	NT[e]	NT[e]	0.25
1f[a]	8-Cl	0.029 ± 0.011	>100	3.7	80	0.13
1g	6,8-diCl	0.013 ± 0.003	>100	17	64	0.08
1h	6-Br	0.005 ± 0.001	>100	34	113	0.25
1i	6-OMe	0.008 ± 0.0003	>50	10	82	0.20
1j	6-OMe, 7-F	0.004 ± 0.0008	>100	35	136	0.25
1k	6,7-di-OMe	0.019 ± 0.004	>100	107	146	0.50
1l[a]	8-OMe	0.030 ± 0.005	>100	7.2	61	0.63
1m	6-F, 7-morpholinyl	0.018 ± 0.003	>50	6.1	33	0.79

[a] Racemic. [b] RLM = incubation with rat liver microsomes. [c] HLM = incubation with human liver microsomes. [d] 3A4 = cytochrome P450 3A4. [e] NT = not tested.

heterocycle was predicted to π-stack with Tyr547. In addition, since the quinazolinone scaffold is well represented in bioactive natural products and drugs, we surmised that it would impart favorable physical properties to our inhibitors.[9]

The syntheses of **1a**−**m** (Scheme 1) began with commercially available 2-aminobenzoic acids or esters **2a**−**m**, which were heated with urea at 200 °C to generate quinazolinediones **3a**−**m**. Chlorination with $POCl_3$ followed by selective hydrolysis gave **4a**−**m**. Selective N-alkylation was performed using conditions reported by Curran and co-workers.[10] Displacement of the chloride in **6** with 3-(*R*)-aminopiperidine was performed in a sealed tube or in a microwave reactor.

The compounds shown in Table 1 are potent DPP-4 inhibitors and demonstrate excellent selectivity over the related protease, DPP-8. Remarkably, the first compound synthesized in the quinazolinone series, **1a**, is a 10 nM inhibitor of DPP-4. We obtained a cocrystal structure of this compound in the active site of DPP-4 (Figure 4).[11] The interactions observed in this cocrystal structure were consistent with our design (compare Figures 5 and 3).

Compound **1a** suffers from a short metabolic half-life in the rat, making in vivo assessments difficult. Metabolite studies

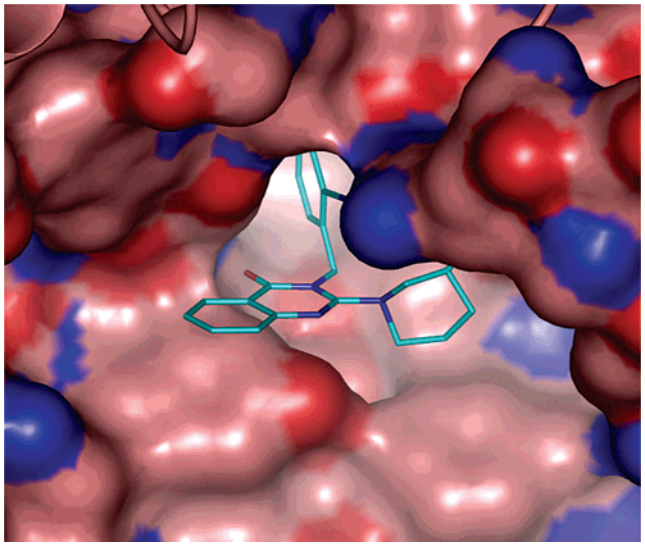


Figure 4. Compound **1a** in the active site of DPP-4.

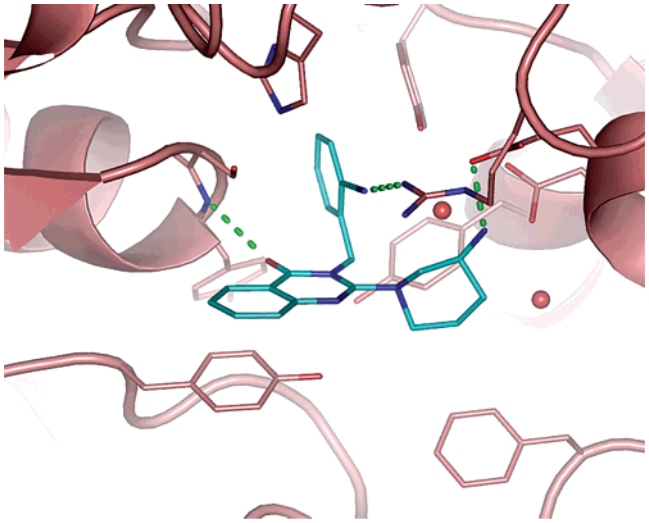


Figure 5. Compound **1a** in the active site of DPP-4 with key interactions shown.

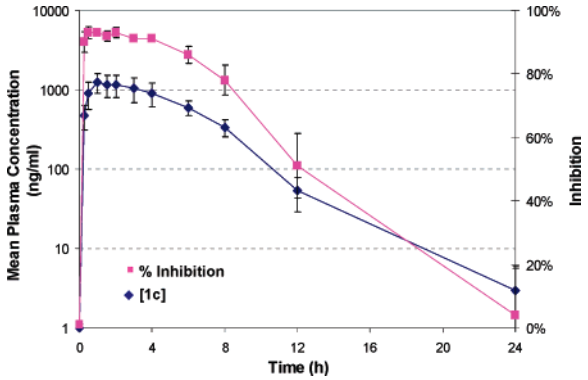


Figure 6. Plasma concentrations and DPP-4 inhibition in rats for **1c** (TFA salt, 10 mpk po).

using rat liver microsome preparations revealed that the short metabolic half-life in rat was due to oxidation of the A-ring phenyl group at position 6 or 7. To address this problem, fluorinated derivative **1c** was synthesized. This compound showed a 10-fold improvement in metabolic stability in the rat. Figure 6 shows the pharmacokinetic (PK) and pharmacodynamic (PD) profile of **1c** in the rat.[12] Selected PK parameters are listed in Table 2.

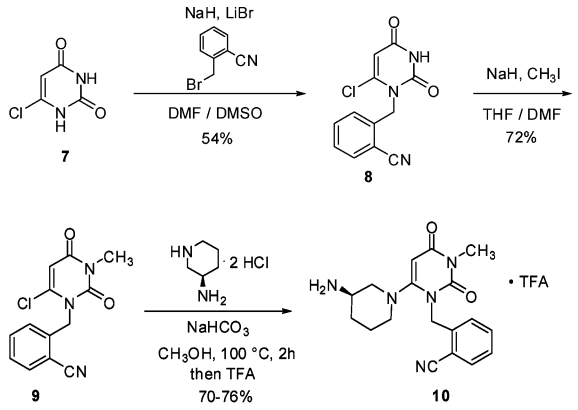
Table 2. Selected Rat PK Parameters for Compound **1c** (TFA Salt)

species	dose, iv/oral (mg/kg)	iv $t_{1/2}$ (h)	oral $t_{1/2}$ (h)	AUC_{po} (μg h mL^{-1})	CL (mL kg^{-1} min^{-1})	V_{dss} (mL kg^{-1})	F (%)
rat	1/10	2.60 ± 1.85	2.43 ± 0.31	7.56 ± 1.80	20.61 ± 8.31	2865 ± 632	85 ± 20

Table 3. Selected PK Parameters for Compound **10**

species	salt	doses, iv/oral (mg/kg)	iv $t_{1/2}$ (h)	oral $t_{1/2}$ (h)	AUC_{po} (μg h mL^{-1})	CL (mL kg^{-1} min^{-1})	V_{dss} (mL kg^{-1})	F (%)
dog	HCl	1/3	2.93 ± 0.84	3.04 ± 0.75	1.61 ± 0.51	22.96 ± 7.81	3508 ± 255	68 ± 22
monkey	benzoate	1.1/10	5.74 ± 1.70	5.66 ± 0.23	16.63 ± 2.52	8.81 ± 1.07	2602 ± 561	87 ± 13

Scheme 2. Synthesis of **10**



As shown in Figure 6, there is a strong correlation between plasma levels of compound **1c** and the level of DPP-4 inhibition, with a 10 mpk oral dose providing 50% inhibition of DPP-4 activity after 12 h. Consistent with this effective in vivo inhibition, compound **1c** (also known as Syrrx106124) reduced glucose excursion following an oral glucose tolerance test (OGTT) in mice.[13]

Preliminary safety assessments of compound **1c** included an Ames test, a safety pharmacology screen, and a 4-day rat toxicology study. The results of these assessments were favorable; however, compound **1c** was found to inhibit CYP450 3A4

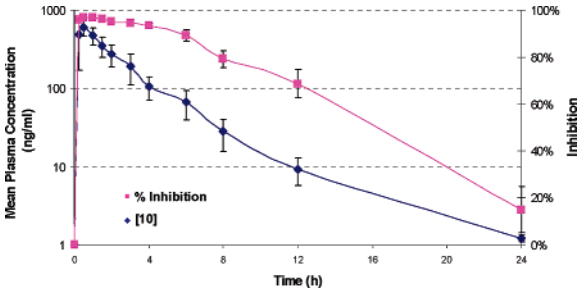


Figure 7. Plasma concentrations and DPP-4 inhibition in dogs for **10** (HCl salt, 3 mpk po).

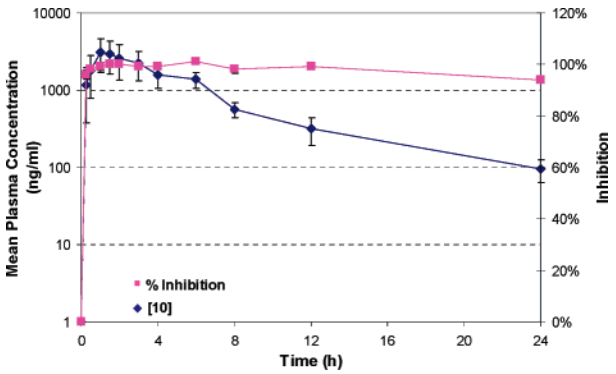


Figure 8. Plasma concentrations and DPP-4 inhibition in monkeys for **10** (benzoate salt, 10 mpk po).

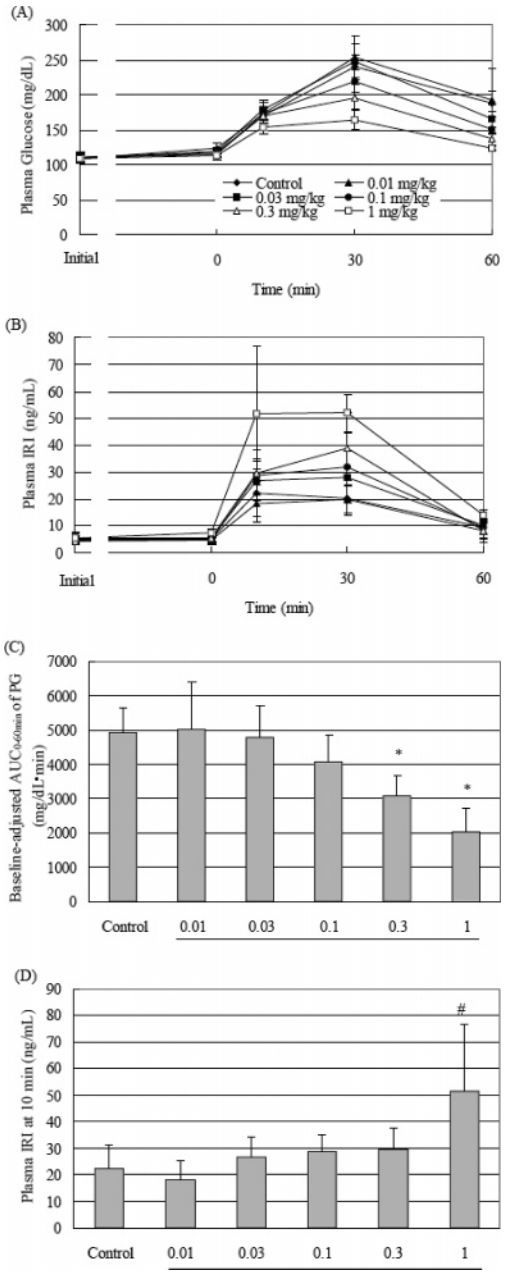


Figure 9. Effects of **10** on plasma glucose (A), plasma immunoreactive insulin (IRI) (B), baseline (0 min)-adjusted $AUC_{0-60min}$ of plasma glucose levels (C), and plasma IRI levels at 10 min after the oral glucose load (D) in glucose tolerance test of female Wistar fatty rats. These studies were conducted using alogliptin benzoate. Doses are normalized to reflect the amount of free base administered. Values are the mean and SD (n = 6): (*) $p \leq 0.025$ vs control by one-tailed Williams' test; (#) $p \leq 0.025$ vs control by one-tailed Shirley−Williams' test.

with an IC_{50} of 2.5 μM and to block the hERG channel at micromolar concentrations.

In an effort to improve upon **1c**, we adopted two strategies. The first relied on modifications to the quinazolinone substit-

uents. The second relied on replacing the quinazolinone with other heterocycles. It was the second strategy that yielded compounds that were chosen for further development. Interestingly, we found that the phenyl ring of the quinazolinone could be eliminated without loss of DPP-4 inhibition.

Replacing the quinazolinone with a pyrimidinedione resulted in **10** (alogliptin, SYR-322), whose synthesis is shown in Scheme 2. Selective alkylation,[10] methylation and displacement of the chloride with 3-(*R*)-aminopiperidine gave **10**.

Compound **10** is a potent (IC_{50} < 10 nM) inhibitor of DPP-4 and exhibits greater than 10,000 fold selectivity over the closely related serine proteases DPP-8 and DPP-9.[14] In addition, in rat (data not shown), dog, and monkey treated with **10** (Figures 7 and 8, respectively, and Table 3), the plasma concentration of the compound and the level of DPP-4 inhibition displayed a good correlation. Compound **10** also produced dose-dependent improvements in glucose tolerance and increased plasma insulin levels in female Wistar fatty rats as shown in Figure 9.

Compound **10** is not an inhibitor of CYP-450 enzymes and does not block the hERG channel at concentrations up to 30 μM. Further, **10** was profiled in a safety pharmacology screen with very favorable results. Based on the data presented above, **10** was selected for preclinical evaluation. Following scale-up, GLP toxicology studies in rat and dog demonstrated the compound to be well tolerated. In phase I human trials, **10** demonstrated human PK−PD suitable for once daily dosing. **10** has now progressed to phase III testing for the treatment of type 2 diabetes.[15]

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Supporting Information Available: X-ray diffraction data, DPP-4 assay procedure, microsomal stability procedure, general chemistry procedures, experimental details for synthesis of the target compounds, and purity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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