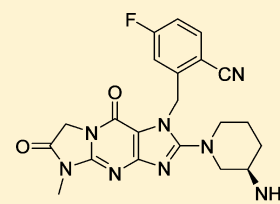


Discovery of Novel Tricyclic Heterocycles as Potent and Selective DPP-4 Inhibitors for the Treatment of Type 2 Diabetes

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Supporting Information

ABSTRACT: In our efforts to develop second generation DPP-4 inhibitors, we endeavored to identify distinct structures with long-acting (once weekly) potential. Taking advantage of X-ray cocrystal structures of sitagliptin and other DPP-4 inhibitors, such as alogliptin and linagliptin bound to DPP-4, and aided by molecular modeling, we designed several series of heterocyclic compounds as initial targets. During their synthesis, an unexpected chemical transformation provided a novel tricyclic scaffold that was beyond our original design. Capitalizing on this serendipitous discovery, we have elaborated this scaffold into a very potent and selective DPP-4 inhibitor lead series, as highlighted by compound 17c.



17c

h DPP-4 IC₅₀ = 1.7 nM
FAP IC₅₀ = 16.3 μM
DPP-8, DPP-9, QPP
IC₅₀ > 100 μM

KEYWORDS: Diabetes, dipeptidyl peptidase IV (DPP-4), inhibitor, tricyclic heterocycles, crystal structure, OGTT

Diabetes mellitus remains a challenging health problem that affects 415 million adults worldwide as of 2015, with an alarming growth predicted over the next quarter of century.¹ Type 2 diabetes (T2DM), a chronic disease characterized by elevated blood sugar levels and insulin resistance, is the most prevalent form. Dipeptidyl peptidase IV (DPP-4), a serine protease in blood, regulates glucose metabolism through degradation of the incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) that are secreted following a meal. Inhibition of DPP-4 thus increases the levels of GLP-1 and GIP, which inhibits glucagon release and in turn stimulates insulin secretion thereby reducing blood glucose levels.² Indeed, the usefulness of DPP-4 inhibitors for treatment of T2DM has been demonstrated by the successful launch of JANUVIA (sitagliptin, **1**, Figure 1), the first DPP-4 inhibitor approved by the U.S. Food and Drug Administration.³ Systematic structure–activity relationship (SAR) studies of conformationally restricted analogues of sitagliptin culminated in discovery, development, and launch of MARIZEV (omarigliptin, **2**) as a once-weekly treatment for T2DM.⁴ Over the past decade, several other DPP-4 inhibitors with distinct structures have also been developed, such as **3** (alogliptin)⁵ and **4** (linagliptin).⁶

In our continued efforts to develop second generation DPP-4 inhibitors, we endeavored to identify distinct structures with long-acting (once weekly, QW) potential. Taking advantage of available X-ray cocrystal structures of sitagliptin and other DPP-4 inhibitors under late-stage development, such as alogliptin

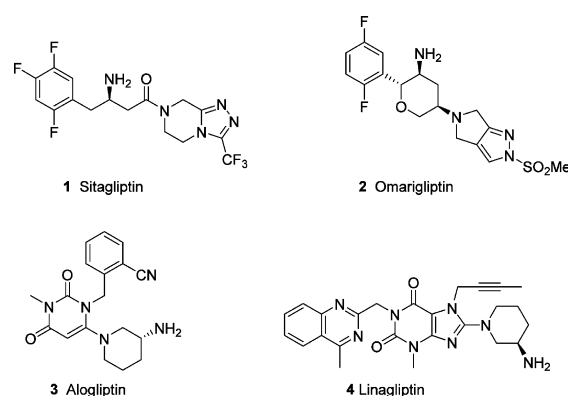


Figure 1. Representative DPP-4 inhibitors.

and linagliptin bound to DPP-4, and assisted by molecular modeling, we designed several series of heterocyclic compounds as initial synthetic targets (Figure 2).⁷ Over the course of the synthesis, an unexpected chemical transformation provided a novel tricyclic scaffold. Capitalizing on this serendipitous discovery, we elaborated the scaffold into a very potent and selective DPP-4 inhibitor lead series. In this

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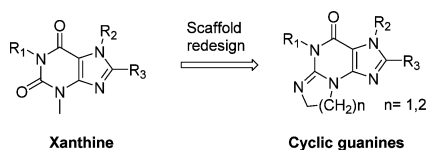
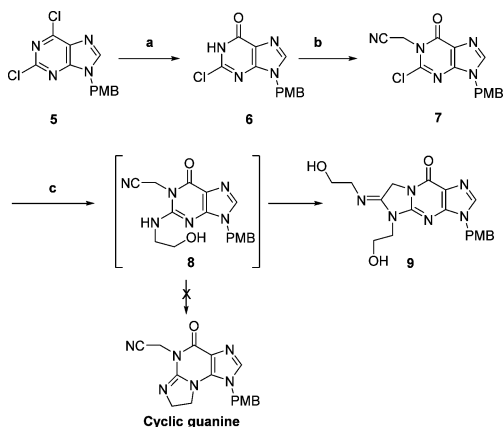


Figure 2. Design of cyclic guanine scaffold.

communication, we report our preliminary findings and results of initial SAR exploration.

As depicted in Scheme 1, we planned to construct the tricyclic guanine scaffold starting from the readily available 2,6-

Scheme 1. Synthesis of Cyclic Guanines^a

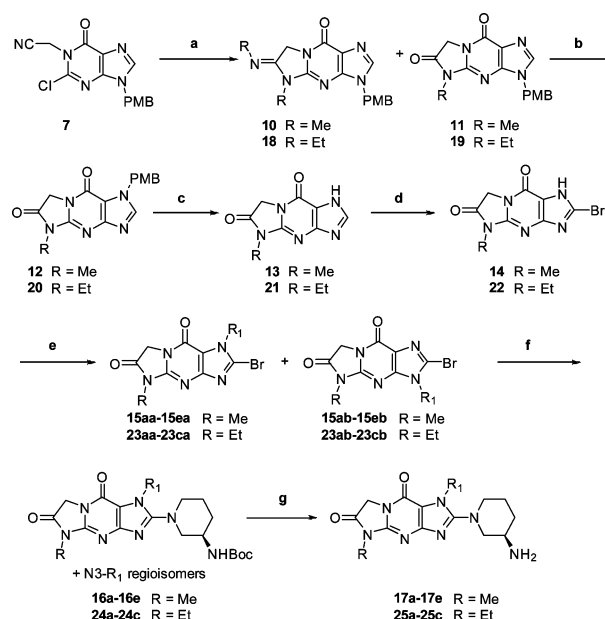


^aReagents and conditions: (a) NaOH, 90%; (b) BrCH₂CN, DIEA, DMF, 55%; (c) HOCH₂CH₂NH₂, DMF, 120 °C, 65%.

dichloropurine derivative **5**.⁸ Compound **5** was hydrolyzed selectively to give **6**, which in turn was alkylated under basic conditions to afford intermediate **7**. At this stage, we reasoned that substitution of a β -aminoalcohol for the chlorine should give intermediate **8**, and activation of the hydroxy of **8** would result in ring closure to provide the tricyclic guanine scaffold. However, treatment of **7** with 2-aminoethanol in DMF under microwave heating conditions did not give any expected product **8** as judged by LC–MS, although the starting material was consumed. Instead, an amidine compound **9** was isolated and its structure has been verified rigorously.⁹

Although our plan did not work out, we were curious to explore the reaction at lower temperatures using simple methylamine, as outlined in Scheme 2. Mixing compound **7** with an excess amount of methylamine as a solution in 1,4-dioxane at room temperature, or even at 0 °C, brought about a fast reaction (in less than 10 min) to give amidine **10** as the major product along with some lactam **11**. This result was quite surprising because amidine formation from a nitrile usually occurs under harsh conditions or in the presence of catalysts.¹⁰ What was more striking was identification of lactam **11** as a minor product, presumably through hydrolysis of **10**, hinting that the amidine moiety might be easily converted to the more stable lactam. To our great pleasure, a mixture of compounds **10** and **11** was refluxed in 6 N HCl to give compound **12**, with migration of the N-PMB group from N3 to N1.¹¹ Lactams **11** and **12** can be differentiated by LC–MS and TLC, but their difference in protection regiochemistry is inconsequential because the PMB group was removed by TFA in the next step to give tricyclic core **13**.

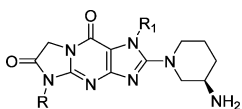
Scheme 2. Identification of a Novel Scaffold and Elaboration to Tricyclic DPP-4 Inhibitors^a











^aReagents and conditions: (a) MeNH₂ or EtNH₂, 1,4-dioxane, room temperature; (b) 6 N HCl, reflux, ~100% over 2 steps; (c) TFA, ~90%; (d) Br₂, 50–60%; (e) R₁Br, NaH, 40–60%; (f) (R)-3-(Boc-amino)piperidine; (g) TFA, DCM, ~20–40% over two steps.

With identification of the compelling novel tricyclic scaffold of compound **13**, we endeavored to scale up this structure and elaborate it to generate tricyclic targets as DPP-4 inhibitors. The stability of the tricyclic core under the above harsh acidic hydrolysis conditions further augmented our confidence in its usefulness. After bromination at the C2 position of intermediate **13**, N-alkylation of the imidazole moiety of **14** gave a mixture of substituted regioisomers **15aa–15ea** and **15ab–15eb**. Finally, displacement of the C2-bromide of the isolated N1-alkylated compound **15ca** with the (R)-3-amino-piperidine moiety, an optimized structural feature in both alogliptin and linagliptin, gave intermediate **16c**, and removal of N-Boc group furnished target **17c**. Analogues **17a**, **17b**, **17d**, and **17e** were prepared from the corresponding mixtures of regioisomers **15** using the above two-step procedures. Ethyl substituted analogues **25a–25c** were prepared accordingly using ethylamine instead of methylamine in the first step of Scheme 2.

Analogues were evaluated for their *in vitro* inhibition of human DPP-4, and the results are summarized in Table 1. Analogue **17a** (IC₅₀ = 123 nM) bearing a but-2-ynyl group on N1 is much less potent than linagliptin (**4**, IC₅₀ = 0.10 nM), which has an extra quinazoline substituent at N1. Replacement of the buty-2-nyl group with a 2-cyanobenzyl moiety improved potency significantly (**17b**, IC₅₀ = 10.6 nM), comparable to that of alogliptin (**3**, IC₅₀ = 7.6 nM). Further improvement was achieved with an additional 5-fluoro substituent on the benzyl ring (**17c**, IC₅₀ = 1.7 nM). Electron withdrawing replacements of the cyano group (**17d** and **17e**) decreased potency. N5-Ethyl substituted analogues are similarly potent (**25b** vs **17d**) or less potent (**25a** vs **17c** and **25c** vs **17e**) than the corresponding N-methyl substituted counterparts. The most active analogues **17b**, **17c**, **17d**, **25a**, and **25b** (IC₅₀ < 100 nM) were also counterscreened against other related enzymes¹² such as

Table 1. In Vitro Biological Data^a


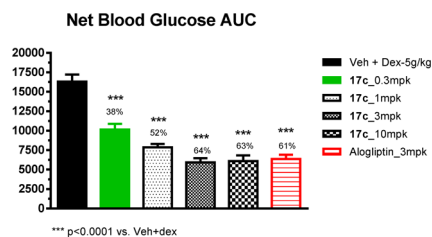
Compd	R	R1	IC ₅₀ (nM)	IC ₅₀ (nM)
			DPP-4	FAP
3			7.6	>100,000
4			0.1	70.8
17a	Me		123.0	ND
17b	Me		10.6	53,600
17c	Me		1.7	16,300
17d	Me		27.4	>100,000
17e	Me		156.1	ND
25a	Et		13.5	7,300
25b	Et		11.2	>100,000
25c	Et		625.0	ND

^aTwo internal standard compounds were included in all the assay runs to track the robustness of the assays ($n = 3$), and data were within 3-fold of the reported values. Standard deviation was within 10%. Literature compounds **3** and **4** were also tested for comparison.

QPP,¹³ DPP8,¹⁴ DPP9,¹⁵ and FAP.¹⁶ All analogues except **25a** showed no activity against QPP, DPP-8, and DPP-9 (>100 μM , data not reported in Table 1) and great selectivity against FAP. Compound **25a** showed no activity against QPP but modest activity against DPP-8 and DPP-9 (IC₅₀ = 21163 and 15828 nM, respectively).

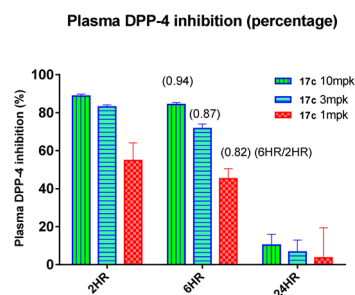
The most potent analogue **17c** in this new series of DPP-4 inhibitors was further characterized. It showed no significant CYP450 inhibition (>50 μM for CYP3A4, 2C8, 2C9, and 2D6) or time-dependent CYP3A4 inhibition. The compound displayed weak activity in the MK-499 displacement binding assay for hERG activity with an IC₅₀ of 40 μM . It had no PXR inhibition (IC₅₀ > 30 μM). Compound **17c** also exhibited low hepatocyte clearance across species (human, rat, cyno monkey, dog = 0.1, 0.3, <1, <1 $\mu\text{L min}^{-1} 10^6 \text{ cell}^{-1}$, respectively).¹⁷

On the basis of its favorable in vitro profile, compound **17c** was evaluated in lean mice for its ability to improve glucose tolerance (Figure 3). In an oral glucose tolerance test (OGTT), a single dose of compound **17c** (0.3, 1, 3, and 10 mg/kg) was given orally to C57BL/6 mice 1 h prior to dextrose challenge. Compound **17c** significantly reduced blood glucose excursion in a dose-dependent manner from 0.3 to 3 mg/kg with a plateau between 3 and 10 mpk. The observed reduction of glucose in AUC was consistent with the measured plasma inhibitor concentration (0.3 mg/kg, 38% reduction in glucose AUC, plasma concentration 2 nM; 1 mg/kg, 52% reduction, 10

Figure 3. OGTT data of compound **17c**.

nM; 3 mg/kg, 64% reduction, 70 nM; 10 mg/kg, 63% reduction, 235 nM). The maximum efficacy of glucose lowering in this model was similar to that achieved with alogliptin at oral dose of 3 mg/kg.

In the corresponding ex vivo pharmacodynamic differentiating assay, compound **17c** was dosed orally at 1, 3, and 10 mg/kg to C57BL/6N mice, and a time course of plasma DPP-4 inhibition was measured at 2, 6, and 24 h. As shown in Figure 4, at doses of 3 and 10 mg/kg, plasma DPP-4 activity

Figure 4. Ex vivo pharmacodynamic data of compound **17c**.

was inhibited by >80% (uncorrected for assay dilution) at 2 h and maintained at 6 h. The effect had returned to near baseline at 24 h. The relative high ratio of inhibition at 6 h to that at 2 h (≥ 0.8) for all the three doses suggested long-acting potential for **17c** (ratios >0.7 are typically a good predictor of extended duration of action), which is consistent with what the low in vitro clearance would indicate.

As revealed in Figure 5, compound **17c** (yellow) binds in the DPP-4 binding site in a very similar way to that observed for alogliptin (**3**, blue). The amino group binds to the conserved glutamic acids (E205 and E206), and the CN group is within hydrogen bonding distance of Arg125. The tricyclic core is stacked against Y547, forming a more extensive interaction than that observed with alogliptin. Elements of the tricyclic core also engage in hydrogen bonds to the backbone NH of Tyr631, to the backbone NH of Trp629, to the side-chain of Lys544 (through a water molecule), and to two other ordered water molecules.

In summary, we have identified a series of potent and selective DPP-4 inhibitors with a novel tricyclic core. Among them, compound **17c** demonstrated robust glucose reduction efficacy after oral administration in mice, as well as potential for a long duration of action. The successful identification of this novel class of DPP-4 inhibitors was made possible by serendipity as well as careful analytical work and critical medicinal chemistry follow-up of the observed biology.

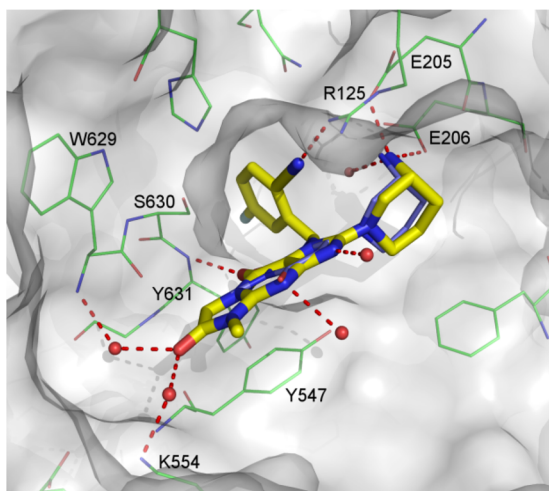


Figure 5. Superposition of compound 17c (yellow) and alogliptin (3, blue) in the DPP-4 active site using their cocrystal structures of DPP-4 (PDB codes 5I7U and 3G0B). The image was generated using PyMol.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmchemlett.6b00027](https://doi.org/10.1021/acsmchemlett.6b00027).

Experimental details for synthetic procedures and compound characterization for compounds 6–25 as well as biological assay conditions (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

T2DM, type 2 diabetes mellitus; DPP-4, dipeptidyl peptidase 4; DPP-8, dipeptidyl peptidase 8; DPP-9, dipeptidyl peptidase 9; FAP, seprase or fibroblast activation protein; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon like peptide-1; QPP, quiescent cell proline dipeptidase; OGTT, oral glucose tolerance test; QW, once-weekly; SAR, structure–activity relationship; PMB, *para*-methoxybenzyl; TFA, trifluoroacetic acid

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(17) See [Supporting Information](#) for more details and references on CYP, PXR, hERG, and clearance assays.