Discovery of novel pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors based on a pharmacokinetic property-driven optimization Postprint

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Abstract

Subsequent pharmacological evaluation revealed extensive hepatic first-pass effects in our recently disclosed DPP-IV inhibitors bearing a thienopyrimidine scaffold. Through scaffold replacement with pyrrolopyrimidine, compound 1a exhibited substantially improved metabolic stability (from 6.6% to 65.07%), yet displayed severely poor absorptive properties. Further modification via incorporation of varied substituents and structural conversion yielded compounds possessing both permeability and metabolic stability. The comprehensive pharmacokinetic property-based optimization successfully balanced overall properties, resulting in compound 1j, which demonstrates excellent efficacy as a potential anti-diabetic candidate.

Full Text

Preamble

Discovery of Novel Pyrrolopyrimidine Analogues as Potent Dipeptidyl Peptidase IV Inhibitors Based on Pharmacokinetic Property-Driven Optimization

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Subsequent pharmacological evaluation revealed extensive hepatic first-pass metabolism in our recently disclosed DPP-IV inhibitors bearing a thienopyrimidine scaffold. Through scaffold replacement with pyrrolopyrimidine, compound **1a** showed substantially improved metabolic stability (from 6.6% to 65.07% remaining), yet exhibited severely poor absorptive properties. Further modification through incorporation of various substituents and structural modifications yielded compounds with both improved permeability and metabolic stability. This comprehensive pharmacokinetic property-based optimization successfully balanced overall drug-like properties and resulted in compound **1j**, which demonstrates excellent efficacy as a potential anti-diabetic candidate.

Introduction

With over 220 million people affected, diabetes has emerged as an epidemic of this century and represents a major global health challenge. Type 2 diabetes (T2D), formerly called non-insulin-dependent or adult-onset diabetes, results from the body' s ineffective use of insulin and accounts for 90% of all diabetes cases. The growing number of deaths attributable to diabetes reflects insufficient glycemic control achieved by past and current treatments [**?**]. Thus, new therapeutic agents are needed to effectively combat this disease.

Glucagon-like peptide-1 (GLP-1) is an incretin secreted from the L cells of the small intestine in a glucose-dependent manner [**?**]. This hormone exerts several beneficial biological functions including stimulation of insulin secretion, inhibition of glucagon secretion, induction of satiety, retardation of gastric emptying, and stimulation of islet -cell regeneration and differentiation [**?**]. However, despite these attractive anti-diabetic functions, GLP-1 is rapidly degraded by dipeptidyl peptidase IV (DPP-IV) through cleavage of a dipeptide from the N-terminus, resulting in a half-life of only 0.5 minutes under normal physiological conditions [**?**].

DPP-IV inhibitors represent a new class of oral glucose-lowering agents that act by reducing GLP-1 turnover and prolonging its lifetime. With good patient compliance and lower risk of hypoglycemia or other side effects, DPP-IV inhibitors have demonstrated effective and safe blood glucose control [**?**]. To date, sitagliptin (**1**) [**?**], vildagliptin (**2**) [**?**], saxagliptin (**3**) [**?**], alogliptin (**4**) [**?**], and

linagliptin (**5**) are already marketed in many countries (Figure 1 [Figure 1: see original paper]).

For chronic disease treatment, drugs are preferred to have similar beneficial effects but with lower toxicity at reduced doses. Although several DPP-IV inhibitors are currently available, highly potent and selective compounds remain in great demand. Consequently, many novel DPP-IV inhibitors have been synthesized based on the structures of **1**, **2**, and **3** in recent years [**?**], leaving substantial chemical space unexplored since few alogliptin analogues have been disclosed. In a previous report from our laboratory [**?**], we discovered a novel series of thienopyrimidine scaffold-based DPP-IV inhibitors that were intrinsically potent against DPP-IV and selective over other dipeptidyl peptidase (DPP) family enzymes (see compound **6** in Figure 1 as an example). However, subsequent pharmacological evaluation revealed that compound **6** had only 23.3% bioavailability in rats [**?**]. Since DPP-IV inhibitors are oral anti-diabetic agents, this raised concerns about potential low in vivo efficacy. We suspected that insufficient oral bioavailability might explain why the highly potent compound **6** (IC = 0.33 nM) [**?**] exhibited similar in vivo efficacy as alogliptin.

In our continued medicinal chemistry efforts to develop anti-diabetic candidates, we conducted a pharmacokinetic (PK) property-driven optimization of this scaffold, leading to a new series of pyrrolopyrimidine analogues. By replacing the thienopyrimidine scaffold with its isostere pyrrolopyrimidine, we successfully resolved the severe metabolic biotransformation issues observed in thienopyrimidine analogues. We further introduced various substituents on the new scaffold to increase permeability and reduce transporter-mediated efflux, thereby avoiding low oral bioavailability caused by poor absorption. This PK-driven optimization yielded a new series of highly potent and selective DPP-IV inhibitors, exemplified by compound **1j**, which achieves a good balance of activity, selectivity, PK properties, and in vivo efficacy. Herein, we report the synthesis and optimization process of these novel pyrrolopyrimidine analogues, including their pharmacological activities, PK properties, and pharmacodynamic (PD) evaluations.

Chemistry

The syntheses of compounds **1a** and **1b** are outlined in Scheme 1. The preparation of **1a** began with commercially available ethyl 2-cyano-4,4-diethoxybutanoate **2a**, which was heated with urea at 80 °C, then acidified with concentrated HCl to afford diol compound **4a**. Compound **1b** was synthesized starting from commercially available 6-aminopyrimidine-2,4(1H,3H)-dione **3b**, which was heated with 1-chloro-2-propanone to obtain compound **4b**. Chlorination of **4a** and **4b** with phosphoryl trichloride yielded **5a** and **5b**, which were subsequently hydrolyzed with aqueous sodium hydroxide to provide key intermediates **6a** and **6b**. Selective N-alkylation was performed using a previously published method [**?**] to produce compounds **8a** and **8b**. The final compounds **1a** and **1b** were obtained in high yields through amination of the

chloro precursors **8a** and **8b** with 3-(R)-aminopiperidine.

Scheme 1. Synthesis of compounds **1a** and **1b**. Reagents: (a) EtOH, Na, urea, 80°C; (b) conc. HCl; (c) 1N KOH; (d) POCl , DIEA, toluene; (e) (Boc) O, DMAP, Et N; (f) 2-CNPhCH Br, NaH, LiBr; (g) 3-(R)-aminopiperidine, NaHCO , 150°C; (h) 1-chloro-2-propanone, NaOAc.

The synthesis of compound **1c** is outlined in Scheme 2. Starting from compound **1a**, treatment with (Boc) O followed by bromination with NBS afforded the final compound **1c**.

Scheme 2. Synthesis of compound **1c**. Reagents: (i) (Boc) O, K CO ; (j) NBS, DCM; (k) TFA, DCM, then NaHCO (eq).

The syntheses of compounds **1d** and **1e** are outlined in Scheme 3. These syntheses began with compound **5a**, which was treated with CH I to obtain key intermediate **2d**. Subsequent reactions followed procedures similar to those for **1a** and **1b**.

Scheme 3. Synthesis of compounds **1d** and **1e**. Reagents: (l) NaH, MeI, THF.

Scheme 4. Synthesis of compounds **1f-1h**. Reagents: (m) Boronic acid, Pd(PPh) , t-butylamine, iso-propanol/H O.

The synthesis of compound **1i** is outlined in Scheme 4. Key intermediate **6i** was prepared from 6-methylpyrimidine-2,4(1H,3H)-dione **2i** according to a previously reported procedure [**?**]. Subsequent reactions followed procedures similar to those for **1a** and **1b**.

Scheme 5. Synthesis of compound **1i**. Reagents: (n) sulfuric acid, fuming nitric acid; (o) dimethylformamide-dimethyl acetal, DMF; (p) AcOH, Zn.

Scheme 6. Synthesis of compound **1j**. Reagents: (i) (Boc) O, K CO ; (j) NBS, DCM; (k) TFA, DCM, then NaHCO (eq).

Scheme 7. Synthesis of compounds **1k-1m**.

Results and Discussion

Key Scaffold Modification of Compound 6

In follow-up pharmacological evaluations of the highly potent compound **6** (IC = 0.33 nM) recently disclosed by our laboratory, we found it displayed similar anti-diabetic effects to alogliptin in an oral glucose tolerance test (OGTT) [**?**]. We hypothesized that this disconnect between in vitro activity and in vivo efficacy likely stemmed from poor pharmacokinetic (PK) properties, a assumption supported by its 23.3% oral bioavailability in Sprague Dawley rats [**?**]. To better understand the PK characteristics of this scaffold and determine the causes of low oral bioavailability, we investigated potential contributing factors.

As oral DPP-IV inhibitors, compounds are highly susceptible to hepatic first-pass metabolism. We assessed the in vitro metabolic stability of compound **6**

using pooled rat liver microsomes (RLM), which revealed 93% turnover after 30 minutes of incubation, consistent with our hepatic first-pass hypothesis (Table 1). We initially hypothesized that the unsubstituted thienyl ring might be a potential metabolic site and synthesized methyl-substituted compound **7**. Metabolite studies showed that methyl substitution slightly stabilized the thienyl ring, but alkyl substitution did not significantly stabilize the compounds overall, nor did electron-withdrawing substitution or structural rotation (compounds **7–10** in Table 1). These results prompted us to replace the thienyl ring with a more metabolically robust moiety such as a pyrryl ring.

We initially synthesized a simple pyrrolopyrimidine analogue, compound **1a**, to test the feasibility of this scaffold modification. Gratifyingly, **1a** demonstrated excellent stability in RLM with 65.07% remaining after 30 minutes incubation (Table 2). However, **1a** exhibited even worse oral bioavailability (5.7%) in rats than compound **6**. We reasoned that while the pyrrolopyrimidine scaffold could eliminate the hepatic first-pass effect, it might simultaneously induce other effects that compromise the PK profile. The cascade of events determining oral bioavailability is well established, with substantial evidence revealing that the human intestine serves as a major barrier for many oral drugs through biotransformation and active secretion by transporters such as P-glycoprotein (P-gp) [**?**]. Thus, the poor oral bioavailability of **1a** likely resulted from inadequate absorption rather than severe first-pass metabolism. We employed Caco-2 cell monolayers, derived from human intestine, as an in vitro model to evaluate the absorption of **1a** (Table 2). As expected, **1a** showed very low permeability from the apical (A) to basolateral (B) side and high transporter-mediated efflux from B to A, resulting in an extremely high efflux ratio.

Absorptive Property Optimization of Compound 1a

Currently, no direct structure-function relationships or pharmacophore models describe the structural features responsible for transporter substrate recognition, and the processes governing drug absorption and oral bioavailability are highly complex [**?**]. In our continued efforts to develop orally bioavailable DPP-IV inhibitors, we adopted several straightforward strategies based on literature precedents to improve absorption and reduce transporter-mediated efflux, leading to a series of pyrrolopyrimidine analogues [**?**]. Initially, we designed compounds **1b** and **1c** by incorporating alkyl and electron-withdrawing substituents to create steric hindrance that might reduce transporter interactions, while simultaneously increasing lipophilicity to improve permeability. The data are summarized in Table 2 . Compounds **1b** and **1c** showed improved A→B permeability and significantly lower efflux ratios. Notably, different substituents displayed varying B→A efflux capabilities, with electron-withdrawing groups increasing B→A transport (compound **1a** vs. **1c** and **1e**). To further block potential P-gp binding to the secondary amine functionality, we added a methyl group at the 1-position to remove the possible hydrogen bond donor, leading to compounds **1d** and **1e**. This modification would also reduce desolvation energy

costs and increase membrane penetration. The 1-position substitution improved permeability and decreased the efflux ratio to varying degrees. Based on these preliminary results and the promising profile of compound **1c**, which had the lowest efflux ratio of 2.05, we continued to explore other R groups (compounds **1f–1h**) and evaluated their absorption properties, among which compound **1h** showed an acceptable ratio.

Since interactions between compounds and transporters are configuration-dependent, we synthesized and evaluated rotated analogues (compounds **1i–m** in Table 3) to reduce potential P-gp interactions. Generally, the position of heteroatoms played a significant role in these interactions. Compound **1i** had a much lower efflux ratio than **1a**, similar to the relationship between compounds **6** and **8** (data not shown). Building on the results from compounds **1a–h**, we selected several flanking substituents to modify **1i**, yielding compounds **1j–m** (Table 3).

In Vitro Pharmacokinetic and Biological Evaluations

We evaluated the metabolic stability of all pyrrolopyrimidine analogues (Tables 2 and 3). This new scaffold successfully resolved the hepatic first-pass effect observed in thienopyrimidine analogues. However, N-substituted compounds (**1d–e**) and thienyl/para-pyridyl substituted compounds (**1l–m**) were not particularly stable, with the latter two groups potentially undergoing hepatic metabolism to some extent.

We selected analogues with acceptable in vitro PK properties (efflux ratio < 7; % remaining $> 60\%$) for further biological screening, using alogliptin as a reference compound [**?**, **?**]. As previously reported, selectivity of DPP-IV against DPP-8 and DPP-9 is critical because inhibition of these two enzymes may be associated with profound toxicities [**?**]. Fortunately, all compounds exhibited >1000-fold selectivity for DPP-IV over DPP-8 and DPP-9. Additionally, according to new FDA guidelines regarding cardiovascular risk of new T2D therapies [**?**], we conducted human Ether-à-go-go Related Gene (hERG) studies. The results are summarized in Table 4 . Under our experimental conditions, the IC value for alogliptin was 3.4 nM, consistent with literature values (IC $<$ 10 nM) [**?**]. Replacement of the thienopyrimidine scaffold with pyrrolopyrimidine resulted in a slight decrease in DPP-IV activity from subnanomolar to nanomolar potency. Nevertheless, all pyrrolopyrimidine analogues maintained comparable DPP-IV inhibitory activity with single-digit nanomolar IC values, except for compound **1b** (IC $= 13.78$ nM).

In Vivo Pharmacokinetic and Pharmacodynamic Evaluations

We conducted in vivo PK evaluation on compounds **1c**, **1h**, and **1j** based on their favorable potency, selectivity, and minimal hERG channel affinity. Unfortunately, the plasma concentrations of compound **1h** were too low for detection by LC-MS/MS. In vivo PK experiments and plasma DPP-IV inhibitory activity

were assessed, with results summarized in Table 5 and Figure 2 [Figure 2: see original paper]. Gratifyingly, compounds **1c** and **1j** exhibited good PK parameters in rats. Notably, compound **1j** inhibited plasma DPP-IV activity by >50% for over 10 hours, with approximately 30% inhibition persisting for 24 hours.

Table 5. Selected PK parameters for compounds **1c** and **1j** (hydrochloride) in male Sprague Dawley rats.

No.	Dose (mg/kg) iv/oral	iv T / (h)	oral T / (h)	CL (L · h ^{1} · kg ^{1})	V_d (L · kg ^{1})	poAUC (µg · h · mL ^{1})
1c	5/20	2.17 ± 0.36	3.48 ± 0.60	2.50 ± 0.61	7.04 ± 1.60	35.90 ± 12.65
1j	5/25	1.03 ± 0.30	1.25 ± 0.25	3.55 ± 1.06	7.44 ± 2.59	13.37 ± 5.08

i.v., intravenous injection; p.o., oral administration.

Figure 2. Plasma concentrations and DPP-IV inhibition in rats for compounds **1c** and **1j** (hydrochloride, 20 mg/kg of **1c** and 25 mg/kg of **1j**, p.o.).

Preliminary oral glucose tolerance tests (OGTT) in ICR and KM mice demonstrated that compound **1j** showed better efficacy than **1c** and alogliptin at 1 and 3 mg/kg, attributable to its lower IC value and stronger plasma DPP-IV inhibition. We further evaluated the in vivo efficacy of **1j** using ob/ob model mice. Compound **1j** was orally administered at indicated doses 60 minutes before glucose administration (2 g/kg), and blood samples were collected and analyzed for glucose concentration at various time intervals from 0–120 minutes. The glucose AUC was calculated from 0–120 minutes, with OGTT data summarized in Figure 3 [Figure 3: see original paper]. The results demonstrated that **1j** reduced blood glucose excursion in a dose-dependent manner at 1 mg/kg, 3 mg/kg, and 9 mg/kg.

Through PK-driven optimization of our previously disclosed highly potent DPP-IV inhibitors [**?**], we have synthesized a new series of potent and selective DPP-IV inhibitors bearing a pyrrolopyrimidine scaffold and conducted systematic pharmacological evaluation. We identified compound **1j** as a promising anti-diabetic candidate for further scale-up.

Conclusions

In summary, further pharmacological evaluation of our recently reported DPP-IV inhibitors [**?**] revealed a disconnect between in vitro activity and in vivo efficacy. In subsequent optimization, compound **1a** successfully reduced hepatic first-pass metabolism by replacing the thienyl ring with a pyrryl ring, decreasing 30-minute turnover from 93% to 35%. However, **1a** exhibited very poor

intestinal absorptive properties (efflux ratio = 19 in Caco-2 cell monolayers). Further efforts to increase permeability and reduce transporter-mediated efflux led to compounds **1b–m**. Among these, compounds **1c**, **1h**, and **1j** demonstrated favorable in vitro properties in terms of inhibitory activity, selectivity, and pharmacokinetics. Additional PK and PD evaluation identified compound **1j**, which showed an acceptable PK profile in rats and excellent in vivo efficacy in T2D model mice. This paper reports a successful PK-driven optimization that yielded several analogues with drug-like properties, exemplified by compound **1j**. Further preclinical studies will be conducted, and new analogues with improved activity and efficacy may be developed in the future.

Experimental Section

General Methods

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer. Chemical shifts are expressed in parts per million (ppm) and coupling constants in Hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Low-resolution mass spectra (MS) and compound purity data were acquired on a Waters ZQ LC/MS single quadrupole system equipped with an electrospray ionization (ESI) source, UV detector (220 nm and 254 nm), and evaporative light scattering detector (ELSD). Preparative HPLC was conducted on the same system using mixtures of TFA (0.05%) buffered water and acetonitrile. Thin-layer chromatography was performed on 0.25 mm Merck silica gel plates (60F-254) and visualized with UV light, 5% ethanolic phosphomolybdic acid, ninhydrin, or p-anisaldehyde solution. Flash column chromatography was performed on silica gel (230–400 mesh, Merck).

Synthetic Procedures

7H-Pyrrolo[2,3-d]pyrimidine-2,4-diol (4a). Urea (30 g) was added to a solution of sodium ethoxide prepared from sodium (22 g) and absolute ethanol (300 mL) and cooled to 5–10°C. The mixture was stirred for 5 minutes, then treated with ethyl 2-cyano-4,4-diethoxybutanoate **1a** (110 g) and allowed to warm with stirring. The sodium salt dissolved with evolution of heat, and the temperature was maintained at 80°C overnight. The reaction mixture was cooled, poured into 300 mL of water, and concentrated in vacuo. The residue was acidified with concentrated HCl to give a white precipitate, which was collected by filtration, washed with water, and dried to afford the title compound **4a** (66.8 g, 92.1% yield). ^{1}H NMR (400 MHz, DMSO-d): 11.44 (1H, s), 11.09 (1H, s), 10.47 (1H, s), 6.56 (1H, t, J = 2.4 Hz), 6.22 (1H, t, J = 2.4 Hz). MS: 152.1 [M+H] .

2,4-Dichloro-7H-pyrrolo[2,3-d]pyrimidine (5a). The title compound was prepared according to a previously reported procedure (WO2007/012953). A total of 51.20 g of **5a** was obtained in 81.3% yield. ^{1}H NMR (400 MHz, DMSO-

d): 12.77 (1H, s), 7.72 (1H, t, J = 2.8 Hz), 6.65 (1H, dd, J = 2.0 Hz, 1.6 Hz). MS: 190.0 [M+H] .

2-Chloro-7H-pyrrolo[2,3-d]pyrimidin-4-ol (6a). A mixture of 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine **5a** (51.3 g) and 1.36 L of 1 N KOH was stirred at 80°C overnight. The solution was then chilled and adjusted to pH 6 with AcOH. The resulting precipitate was collected, washed with water, and dried to afford **6a** as a solid (37.3 g, 80.7% yield). ^{1}H NMR (400 MHz, DMSO-d): 12.75 (1H, br s), 12.02 (1H, s), 7.06 (1H, t, J = 2.8 Hz), 6.45 (1H, t, J = 2.8 Hz). MS: 168.0 [M+H] .

Tert-Butyl 2-chloro-4-hydroxy-7H-pyrrolo[2,3-d]pyrimidine-7-carboxylate (7a). 2-Chloro-7H-pyrrolo[2,3-d]pyrimidin-4-ol **6a** (20 g) was dissolved in dry THF (400 mL), then treated with dry Et N (16.4 mL) and DMAP (1 g). The mixture was stirred for 30 minutes at 0 °C, then a solution of (Boc) O (27 g) in dry THF (100 mL) was slowly added. The solution was stirred at room temperature overnight, concentrated in vacuo, and extracted with ethyl acetate. The organic layer was dried (Na SO) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH = 20:1) to give **7a** (30 g, 94.3% yield). ^{1}H NMR (400 MHz, CDCl): 12.76 (1H, br s), 7.37 (1H, d, J = 4.0 Hz), 6.73 (1H, d, J = 3.6 Hz), 1.68 (9H, t, J = 7.6 Hz). MS: 292.0 [M+Na] .

2-((2-Chloro-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (8a). NaH (2.1 g, 51.6 mmol) was added to a stirred solution of **7a** (8.4 g, 44.9 mmol) in DME (120 mL) and DMF (30 mL) at 0°C. After 20 minutes, LiBr (7.9 g, 89.7 mmol) was added, and the mixture was warmed to room temperature. After 15 minutes, -bromo-o-tolunitrile (10.15 g, 51.6 mmol) was added, and the mixture was heated at 65°C overnight. After cooling, the mixture was poured into water (1,000 mL), extracted with ethyl acetate, and the organic layer was dried (Na SO) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH = 20:1) to give **8a**. ^{1}H NMR (400 MHz, DMSO-d): 12.20 (1H, s), 7.90 (1H, d, J = 7.6 Hz), 7.65 (1H, t), 7.50 (1H, t), 7.15 (2H, m), 6.54 (1H, t), 5.58 (2H, s). MS: 285.0 [M+H] , 307.0 [M+Na] .

(R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (1a). A mixture of **8a** (13.1 g, 43.4 mmol), 3-(R)-aminopiperidine dihydrochloride (11.5 g, 66.0 mmol), and NaHCO (17.4 g, 173.6 mmol) in 300 mL of ethanol in a sealed tube was heated at 150°C for 6 hours. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to give **1a** (10.2 g, 63% yield). ^{1}H NMR (400 MHz, CDCl): 10.90 (1H, br s), 7.59 (1H, d, J = 7.6 Hz), 7.36 (1H, t, J = 7.6 Hz), 7.25 (1H, t, J = 7.6 Hz), 6.97 (1H, d, J = 7.6 Hz), 6.76 (1H, d, J = 7.6 Hz), 6.59 (1H, d, J = 7.6 Hz), 5.53 (2H, d, J = 5.2 Hz), 3.13 (1H, t, J = 2.4 Hz), 2.96 (2H, m), 2.71 (2H, t), 1.86 (2H, m), 1.69 (1H, m), 1.56 (1H, m). MS: 349.1 [M+H] , 371.1 [M+Na] .

6-Methyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diol (4b). A solution of 6-aminopyrimidine-2,4(1H,3H)-dione **3b** (5.0 g) was treated at room temperature with sodium acetate (5.0 g) dissolved in H O (200 mL). The mixture was stirred for 30 minutes, then 1-chloro-2-propanone (5 mL) was added, and the solution was heated to boiling for 72 hours. The reaction was cooled and filtered to give **4b** (5.2 g, 80% yield). MS: 166.0 [M+H] .

4-Dichloro-6-methyl-7H-pyrrolo[2,3-d]pyrimidine (5b). Following the procedure described for **5a**, **5b** was prepared (400 mg, 20% yield). MS: 202.0 [M+H] .

2-Chloro-6-methyl-4,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (6b). Following the procedure described for **6a**, **6b** was prepared (260 mg, 71.6% yield). MS: 184.0 [M+H] .

Tert-Butyl 2-chloro-6-methyl-4-oxo-4H-pyrrolo[2,3-d]pyrimidine-7(4aH)-carboxylate (7b). Following the procedure described for **7a**, **7b** was prepared.

2-((2-Chloro-6-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (8b). Following the procedure described for **8a**, **8b** was prepared. MS: 299.1 [M+H] .

(R)-2-((2-(3-Aminopiperidin-1-yl)-6-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (1b). Following the procedure described for **1a**, **1b** was prepared. ^{1}H NMR (400 MHz, CDCl): 7.63 (1H, m), 7.42 (1H, m), 7.29 (1H, m), 7.01 (1H, m), 6.28 (1H, s), 5.55 (2H, s), 3.14 (1H, m), 2.97 (2H, m), 2.72 (2H, m), 2.30 (3H, s), 1.90 (1H, m), 1.70 (1H, m), 1.58 (1H, m), 1.34 (1H, m). MS: 363.2 [M+H] .

(R)-Tert-Butyl 2-(3-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3-(2-cyanobenzyl)-4-oxo-3H-pyrrolo[2,3-d]pyrimidine-7(4H)-carboxylate (2c). Compound **1a** (348.4 mg) was dissolved in DCM (20 mL) and treated with K CO (346 mg). The mixture was stirred for 30 minutes at 0 °C, then a solution of (Boc) O (873 mg) in dry DCM (10 mL) was slowly added. The solution was stirred at room temperature overnight, then filtered. The residue was purified by silica gel chromatography (PE/EA = 2:1) to give **2c**. MS: 549.3 [M+H] , 571.3 [M+Na] .

(R)-2-((2-(3-Aminopiperidin-1-yl)-6-bromo-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (2c) and (R)-2-((2-(3-aminopiperidin-1-yl)-5,6-dibromo-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (1c). Compound **2c** (100 mg) was dissolved in DCM (20 mL) and treated with NBS (31 mg). The mixture was stirred for 4 hours at room temperature, then concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (DCM) to give (R)-tert-butyl(1-(6-bromo-3-(2-cyanobenzyl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-2-yl)piperidin-3-yl)carbamate **(3c)**. To a solution of **3c** in DCM was added TFA at 0 °C. After stirring at room temperature

for 3 hours, the solution was treated with saturated NaHCO to pH 6–7, then concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (DCM/MeOH) to give **1c**. ^{1}H NMR (400 MHz, CDCl): 7.64 (1H, dd, J = 7.6 Hz), 7.36 (1H, t, J = 7.6 Hz), 7.30 (1H, t, J = 7.6 Hz), 6.96 (1H, d, J = 7.2 Hz), 6.58 (1H, s), 5.53 (2H, m), 3.19 (1H, m), 3.06 (1H, m), 2.97 (1H, m), 2.80 (1H, mt), 1.93 (2H, m), 1.76 (2H, m), 1.64 (2H, m). MS: 426.9 [M+H] .

2,4-Dichloro-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (2d). To a solution of 60% NaH (750 mg) in dry THF (20 mL) was slowly added a solution of **5a** (1.7 g) in dry THF (50 mL). The solution was stirred at 0 °C for 30 minutes, then MeI (0.79 mL) was added at 0 °C. The mixture was stirred at room temperature overnight. Water (20 mL) was added, and the solution was extracted with ether (3 × 10 mL) and washed with saturated brine. The organic phase was dried with anhydrous sodium sulfate and filtered. Ether was evaporated in vacuo, and the crude residue was purified by silica gel chromatography (PE/EA = 10:1) to give **2d** (1.53 g, 84.1% yield). MS: 202.1 [M+H] .

2-Chloro-7-methyl-4a,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (3d). Following the procedure described for **6a**, **3d** was prepared. MS: 184.0 [M+H] .

2-((2-Chloro-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (4d). Following the procedure described for **8a**, **4d** was prepared. MS: 299.0 [M+H] .

(R)-2-((2-(3-Aminopiperidin-1-yl)-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (1d). Following the procedure described for **1a**, **1d** was prepared. ^{1}H NMR (400 MHz, CDCl): 7.64 (1H, d, J = 7.6 Hz), 7.45 (1H, d, J = 7.6 Hz), 7.31 (1H, t, J = 7.6 Hz), 7.00 (1H, d, J = 7.6 Hz), 6.73 (1H, d, J = 3.2 Hz), 6.60 (1H, d, J = 3.2 Hz), 5.56 (2H, s), 3.71 (3H, s), 3.15 (1H, m), 3.01 (1H, m), 2.92 (1H, m), 2.74 (1H, m), 2.58 (1H, m), 1.92 (1H, m), 1.72 (1H, m), 1.64 (1H, m), 1.44 (2H, s), 1.20 (1H, m). MS: 363.2 [M+H] .

(R)-Tert-Butyl(1-(3-(2-cyanobenzyl)-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-2-yl)piperidin-3-yl)carbamate (2e). Following the procedure described for **2c**, **2e** was prepared.

(R)-2-((2-(3-Aminopiperidin-1-yl)-5,6-dibromo-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (1e). Following the procedure described for **1c**, **1e** was prepared. ^{1}H NMR (400 MHz, CDCl): 7.65 (1H, dd, J = 7.6 Hz), 7.46 (1H, t, J = 7.6 Hz), 7.32 (1H, t, J = 7.6 Hz), 7.04 (1H, d, J = 7.6 Hz), 6.66 (1H, s), 5.51 (2H, s), 3.80 (1H, s), 3.68 (3H, m), 3.29 (1H, m), 3.17 (1H, s), 3.01 (3H, m), 1.78 (2H, m), 1.65 (2H, m), 1.58 (1H, m). MS: 441.0 [M+H] .

General Procedures

Coupling Reaction Affording Compounds 2f-2h and 2k-2m. (R)-Tert-butyl(1-(6-bromo-3-(2-cyanobenzyl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-2-yl)piperidin-3-yl)carbamate (**3c**) or (R)-2-((2-(3-aminopiperidin-1-yl)-7-bromo-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (**3j**) (500 mg, 0.95 mmol) was mixed with arylboronic acid (1.42 mmol), Pd(PPh) (77 mg, 0.0665 mmol), and t-butylamine (4 mL) in iso-propanol/H O (2:1). The mixture was flushed with argon for 5 minutes, then stirred and refluxed (oil bath, 100 °C) in a sealed tube until starting material was consumed (TLC monitoring). After cooling, the solution was concentrated to dryness under reduced pressure, and the residue was purified by column chromatography on silica gel.

General Procedure for Tert-Butyloxycarbonyl (Boc) Deprotection. To a solution of **2f-2h** or **2k-2m** in DCM was slowly added TFA, and the solution was stirred at 0 °C. After stirring at room temperature for 2 hours, saturated NaHCO was added to adjust pH to 7 with stirring in an ice bath. The solution was extracted with ethyl acetate, the organic layer was dried (Na SO) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH = 15:1) to afford compounds **1f-1h** or **1k-1m**.

Compound 1h. ^{1}H NMR (400 MHz, MeOD): 9.00 (1H, m), 8.69 (1H, m), 8.22 (1H, m), 8.10 (1H, m), 7.72 (1H, d, J = 7.6 Hz), 7.61 (1H, t, J = 7.2 Hz), 7.43 (1H, t, J = 7.2 Hz), 7.28 (2H, m), 5.59 (1H, d, J = 15.2 Hz), 5.50 (1H, d, J = 15.2 Hz), 3.68 (1H, m), 3.54 (1H, m), 3.22 (2H, m), 3.00 (1H, m), 2.16 (1H, m), 1.83 (1H, m), 1.67 (2H, m). MS: 426.1 [M+H] .

Compound 1k. ^{1}H NMR (400 MHz, DMSO-d): 12.66 (1H, s), 9.55 (1H, s), 9.10-9.12 (1H, d, J = 8 Hz), 8.69-8.70 (1H, d, J = 4 Hz), 8.34 (3H, s), 8.27-8.28 (1H, d, J = 4 Hz), 7.99-8.03 (1H, t, J = 8 Hz), 7.83-7.85 (1H, d, J = 8 Hz), 7.59-7.63 (1H, t, J = 8 Hz), 7.43-7.47 (1H, t, J = 8 Hz), 7.11-7.13 (1H, d, J = 8 Hz), 5.42-5.53 (2H, dd, J = 24 Hz, J = 16 Hz), 3.57 (1H, s), 3.60 (2H, s), 3.10-3.13 (1H, d, J = 8 Hz), 2.95 (1H, s), 1.99 (1H, s), 1.85 (1H, s), 1.58-1.60 (1H, d, J = 8 Hz). MS: 426.2, 427.2, 428.2 [M+1] .

5,7-Dichloro-1H-pyrrolo[3,2-b]pyridine (6i). The title compound was prepared from 6-methylpyrimidine-2,4(1H,3H)-dione according to a previously reported procedure (WO2009/062258). MS: 187.9 [M+H] .

2-Chloro-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (7i). The title compound was prepared from 5,7-dichloro-1H-pyrrolo[3,2-b]pyridine **6i** in 94% yield using a method analogous to that for **6a**. MS: 170.0 [M+H] .

Tert-Butyl 2-chloro-4-oxo-3H-pyrrolo[3,2-d]pyrimidine-5(4H)-carboxylate (8i). The title compound was prepared from 2-chloro-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one **7i** in 57% yield using a method analogous to that for **7a**. MS: 292.0 [M+Na] .

2-((2-Chloro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (9i). The title compound was prepared from tert-butyl 2-chloro-4-oxo-3H-pyrrolo[3,2-d]pyrimidine-5(4H)-carboxylate **8i** in 90% yield using a method analogous to that for **8a**. ^{1}H NMR (400 MHz, CDCl): 7.73 (1H, m), 7.53 (1H, m), 7.39 (2H, m), 7.12 (1H, d), 6.53 (1H, m), 5.79 (2H, s).

(R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (1i). The title compound was prepared from **9i** in 75% yield using a method analogous to that for **1a**. ^{1}H NMR (400 MHz, CDCl): 7.66 (1H, m), 7.43 (1H, m), 7.31 (1H, m), 7.24 (1H, d), 6.93 (1H, d), 6.41 (1H, d), 5.62 (2H, s), 3.15 (1H, m), 2.94 (2H, m), 2.79 (1H, t), 2.66 (1H, m), 1.91 (1H, m), 1.74 (1H, m), 1.59 (1H, m), 1.23 (1H, m). MS: 349.1 [M+H] .

(R)-2-((2-(3-Aminopiperidin-1-yl)-7-bromo-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (1j). The title compound was prepared from (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile **1i** using a method analogous to that for **1c**. ^{1}H NMR (400 MHz, MeOD+DMSO-d) : 8.04–8.06 (1H, d, J = 8 Hz), 7.86–7.90 (1H, t, J = 8 Hz), 7.70–7.74 (2H, m), 7.40–7.42 (1H, d, J = 8 Hz), 5.73–5.91 (2H, dd, J = 15 Hz, J = 15 Hz), 3.75–3.78 (1H, m), 3.65–3.71 (1H, m), 3.38–3.46 (2H, m), 3.15–3.20 (1H, m), 2.35–2.37 (1H, m), 2.11–2.15 (1H, m), 1.92–1.98 (2H, m). MS: 427.0, 428.0, 429.0 [M+1] .

Biological Assays

Caco-2 Permeability Bidirectional Study. Test compounds (10 µM) were added to either the apical or basolateral side of a confluent Caco-2 cell monolayer. Permeability was measured by monitoring compound appearance on the opposite side of the cell membrane by LC-MS/MS after 60 minutes incubation.

Inhibition of DPP-IV, DPP-8, and DPP-9 in Vitro. Test compound solutions at varying concentrations (10 mM final concentration) were prepared in dimethyl sulfoxide (DMSO) and diluted into assay buffer comprising 20 mM Tris (pH 7.4), 20 mM KCl, and 0.1 mg/mL BSA. Human DPP-IV (0.1 nM final concentration) was added to the dilutions and pre-incubated for 10 minutes at ambient temperature before initiating the reaction with Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; Sigma-Aldrich; 10 µM final concentration). The total reaction volume was 100 µL. The reaction was monitored kinetically (excitation at 400 nm; emission at 505 nm) for 5–10 minutes or measured as an endpoint after 10 minutes. Inhibition constants (IC) were calculated from enzyme progress curves using standard mathematical models.

Microsomal Stability. Test compounds (1 mM final concentration) were incubated with pooled rat liver microsomes (3 mg/mL final concentration) for 5 minutes. Samples were removed at five time points (0, 2, 4, 12, 20, 30 minutes) and parent compound concentrations were determined by LC-MS/MS.

In Vivo Pharmacokinetic Study. Adult male SD rats (n = 4/group) were administered test compounds dissolved in distilled water at a single oral dose of 20 mg/kg or 25 mg/kg (5 mg/mL for intravenous administration). Blood samples (100–200 µL) were collected from the orbital sinus at time points indicated in Figure 2 [Figure 2: see original paper]. Test compound concentrations in blood were determined by LC-MS/MS. PK parameters were obtained using DAS 2.0 pharmacokinetic software.

DPP-IV Inhibition in SD Rats. Adult male SD rats (n = 4/group) were orally gavaged with test compounds dissolved in distilled water at a single dose of 20 mg/kg or 25 mg/kg. Blood samples (20–25 µL) were collected from the orbital sinus at time points indicated in Figure 2 [Figure 2: see original paper], and plasma fractions were kept frozen until DPP-IV activity measurement. Plasma DPP-IV activity was determined by the cleavage rate of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; Sigma-Aldrich). Plasma (10 µL) was mixed with 140 µL of 150 µM Gly-Pro-AMC in assay buffer composed of 25 mM tris(hydroxymethyl)aminomethane HCl (pH 7.4), 140 mM NaCl, 10 mM KCl, and 0.1% bovine serum albumin. Fluorescence was measured using a Thermo Scientific Fluoroskan Ascent FL (excitation at 400 nm, emission at 505 nm). DPP-IV inhibition in plasma was calculated as (vehicle control activity - remaining activity)/vehicle control activity. One unit of activity is defined as the amount of enzyme producing 1 µM product per minute.

OGTT in ob/ob Mice. Male 13–14 week-old ob/ob mice (Jackson Laboratories) were maintained under constant temperature and humidity conditions with a 12 h/12 h light-dark cycle and had free access to a 10% fat rodent diet (D1245B Research Diets) and tap water. After an overnight fast, animals were orally dosed with vehicle (water) or DPP-4 inhibitor (1, 3, 9 mg/kg) at -60 minutes. Two blood samples were collected at -60 and 0 minutes by tail bleed for glucose determination. Glucose (2 g/kg) was then administered orally at 0 minutes. Additional blood samples were collected at 15, 30, 60, and 120 minutes for glucose determination. Plasma glucose was measured with an Accu-Chek Advantage (Roche) glucometer. Data represent the mean of at least 6 mice/group. Statistical analysis was performed using one-way ANOVA followed by Dunnett' s test. All procedures were performed according to GIBH-IACUC guidelines.

Cytochrome P450 Inhibition Assay. Test compounds (0.1–100 µM) were incubated with human pooled liver microsomes and NADPH in the presence of specific CYP P450 probe substrates. Each reaction was performed under linear conditions with respect to time and microsomal protein concentration. Metabolites were monitored by LC-MS/MS. A decrease in metabolite formation compared to vehicle control was used to calculate IC values using Grafit 5 software.

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Supporting Information Available

Supporting Information may include: (1) large tables, (2) extensive figures, (3) lengthy experimental procedures, (4) mathematical derivations, (5) analytical and spectral characterization data, (6) molecular modeling coordinates, (7) modeling programs, (8) crystallographic information files (CIF), (9) instrument and circuit diagrams, and (10) expanded discussions of peripheral findings. For complete instructions on preparing this material for publication, consult the Guide, Notes, Notice, or Instructions for Authors that appear in each publication' s first issue of the year and at http://pubs.acs.org.

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Note: Figure translations are in progress. See original paper for figures.