Discovery of Potent Dipeptidyl Peptidase IV Inhibitors through Pharmacophore Hybridization and Hit-to-Lead Optimization Postprint

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Abstract

A novel dipeptidyl peptidase IV inhibitor hit (5, IC50 = 0.86 mu M) was structurally derived from our recently disclosed preclinical candidate 4 by replacing the cyanobenzyl with a butynyl based on pharmacophore hybridization. A hit-to-lead optimization effort was then initiated to improve its potency. Most N-substituted analogs exhibited good in vitro activity, and compound 180 (IC50 = 1.55 nM) was identified to be a potent dipeptidyl peptidase IV inhibitor with a significantly improved pharmacokinetic properties (bioavailablity: 41% vs 82.9%; T-1/2: 2 h vs 4.9 h).

Full Text

Discovery of Potent Dipeptidyl Peptidase IV Inhibitors Through Pharmacophore Hybridization and Hit-to-Lead Optimization

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Keywords: DPP-IV inhibitor, Type 2 diabetes, Linagliptin, Pharmacophore hybridization, Hit-to-lead optimization

1. Introduction

A novel dipeptidyl peptidase IV (DPP-IV) inhibitor hit (compound **5**, IC = 0.86 M) was structurally derived from our recently disclosed preclinical candidate **4** by replacing the cyanobenzyl group with a butynyl moiety based on pharmacophore hybridization principles. A subsequent hit-to-lead optimization campaign was initiated to improve its potency. Most N-substituted analogs exhibited good in vitro activity, and compound **18o** (IC = 1.55 nM) was identified as a potent, selective, and orally bioavailable DPP-IV inhibitor.

Type 2 diabetes (T2D), formerly referred to as non-insulin-dependent or adult-onset diabetes, results from the body' s ineffective use of insulin and comprises over 90% of diabetes cases. With more than 220 million people affected worldwide, diabetes has emerged as an epidemic, reflecting insufficient glycemic control and the urgent need for additional therapeutic options. Glucagon-like peptide-1 (GLP-1), an incretin hormone, contributes to increased insulin secretion and sensitivity, beta cell mass, and satiety, while also reducing glucagon secretion and gastric emptying, which is beneficial for glucose control in type 2 diabetics. However, under normal physiological conditions, GLP-1 is rapidly truncated by dipeptidyl peptidase IV (DPP-IV) and loses its function. Thus, inhibition of DPP-IV could effectively maintain GLP-1 activity and control glucose levels.

Compared to conventional anti-diabetic drugs, DPP-IV inhibitors offer good patient compliance, reduced risks of hypoglycemia, and fewer side effects. Consequently, the first DPP-IV inhibitor reached the market in 2006, and this class gradually became a major intervention for type 2 diabetes.

Traditional medicinal chemistry strategies for protease inhibitors mostly rely on direct tight binding to the target, which often leads to covalent compounds. However, the emergence of non-covalent compounds that achieve satisfactory binding affinity with lower risk of selectivity issues has been witnessed in recent years. Among DPP-IV inhibitors, the first non-covalent inhibitor with good selectivity against DPP-8 and DPP-9, Sitagliptin (**1**, Figure 1 [Figure 1: see original paper]), was marketed in 2006. Alogliptin (**2**, Figure 1) was the second non-covalent DPP-IV inhibitor approved by the EMA, exhibiting better efficacy than sitagliptin. The most potent and long-lasting drug is Linagliptin (**3**, Figure 1). Other DPP-IV inhibitors include Vildagliptin and Saxagliptin.

[Figure 1: see original paper] Figure 1. Marketed non-covalent DPP-IV inhibitors.

Our recently disclosed compound **4** was derived from Alogliptin by retaining its pharmacophore (the 3-aminopiperidinyl region, red box, Figure 2 [Figure 2: see original paper]) while modifying the scaffold. Compound **4** displayed

better in vivo efficacy than Alogliptin in lean mice and showed dose-dependent glucose reduction in the T2D ob/ob mouse model. Although **4** had a similar pharmacokinetic profile to Alogliptin in rats, its 40% bioavailability and 2-hour half-life still did not meet the requirements for an oral therapeutic candidate for a chronic disease. In our subsequent medicinal chemistry efforts on **4**, we conducted several optimizations, and herein we present our results on the adoption of a pharmacophore hybridization strategy in this process.

[Figure 2: see original paper] Figure 2. Generation of hit **5**.

2. Chemistry

The synthesis of compounds **5**, **6**, and **18a–o** is outlined in Scheme 1. The conversion of compound **7** to **13** was described in our recent report. Alkylation of **13** with 1-bromo-2-butyne provided precursor **14**, which was deprotected to give key intermediate **15**. Bromination of **15** with N-bromosuccinimide (NBS) afforded compound **16**, which was converted to **5** by amination. Direct amination of **15** with 3-(R)-aminopiperidine afforded compound **6**. Compounds **18a–o** were obtained by N-alkylation of **15** followed by replacement of the chloro group with a 3-(R)-aminopiperidinyl group.

Scheme 1. Synthesis of compounds **5**, **6**, and **18a–o**. Reagents: (a) sulfuric acid, fuming nitric acid, rt; (b) dimethylformamide-dimethyl acetal, DMF, 80 °C then 140 °C; (c) AcOH, Zn, 80 °C; (d) POCl , DIEA, toluene, 70–80 °C; (e) 1 N NaOH/H O, 100 °C; (f) (Boc) O, DMAP, Et N, THF, rt; (g) 1-bromo-2-butyne, DIEA, DMF, rt; (h) HCl/H O, MeOH, rt; (i) NBS, DCM, rt; (j) RX, NaH, DMF, rt; (k) 3-(R)-aminopiperidine, NaHCO , 120°C, ethanol.

3. Results and Discussion

3.1. Hit Identification Through Pharmacophore Hybridization

Close comparison of the pharmacophores of Alogliptin, compound **4**, and Linagliptin revealed that the cyanobenzyl group of **4** could be freely exchanged with a butynyl group without sacrificing in vitro activity, provided the 3-(R)-aminopiperidinyl group remained present (Figure 2). Because Linagliptin is the most potent and longest-lasting DPP-IV inhibitor on the market, we decided to test our hypothesis using a pharmacophore hybridization approach. However, to our disappointment, compound **5** lost significant activity (IC = 0.86 M). Although this compound did not achieve the desired level of DPP-IV inhibition, it represented a promising hit worthy of further optimization.

3.2. Hit-to-Lead Optimization of Compound 5

With compound **5** in hand, we immediately initiated a hit-to-lead optimization effort. First, compound **6**, lacking any substituent on the pyrrolopyrimidine ring, was synthesized and found to have improved activity with an IC of 0.46 M. By simply adding a methyl group to the nitrogen (compound **18a**), the

activity was further increased. This trend suggested that N-substitution might be a powerful strategy to enhance inhibition. Consequently, we prepared a series of N-substituted analogs (**18b–18o**).

[Figure 3: see original paper] Figure 3. Hit-to-lead optimization strategy.

Although the first N-heterocyclic compound (**18b**) afforded further increased activity, it remained relatively inactive compared to the parent compound **4** (IC = 44.0 nM). Inspired by the discovery of Linagliptin, where it was found that the N-5 position could accommodate larger steric substituents, we synthesized a series of compounds with bicyclic rings at the N-position (**18c–18o**). Several of these compounds displayed activity in the nanomolar range, such as **18f**, **18l**, and **18m** (<10 nM). Surprisingly, the most potent compound (**18o**, IC = 1.55 nM) contained the same N-substituent as Linagliptin.

Table 1. Modifications at the N-5 position.

3.3. Biological Evaluation of Compound 18o

Compound **18o** was selected for preliminary evaluation due to its high DPP-IV inhibitory activity. It showed no inhibition against DPP-8 or DPP-9 up to 10 M. Additionally, **18o** exhibited no CYP3A inhibition up to 30 M, which is likely to decrease the risk of drug-drug interactions. The pharmacokinetic study of **18o** also displayed a favorable drug-like profile and successfully overcame the deficiencies of the starting compound **4** (Table 2 and Figure 4 [Figure 4: see original paper]).

[Figure 4: see original paper] Figure 4. Concentration-time curve of **18o** in rat.

Compared to its parent compound **4**, **18o** had a longer half-life in rats (approximately 5 hours) and better oral bioavailability (82.9%) that was comparable with marketed DPP-IV inhibitors (Alogliptin, 45%; Linagliptin, 50.7%). Combined with the published data and current research progress on compound **4**, we believe that **18o** has a high probability of demonstrating superior in vivo efficacy compared to compound **4** and Alogliptin, with doubled half-life and bioavailability.

Table 2. Pharmacokinetic parameters of **18o** in rat.

Dose (iv/po) mg/kg	AUC ∞ po (g · h · mL ^{1})	T / po (h)	Clz iv (L · h ^{1} · kg ^{1})	Vz iv (L · kg ^{1})	MRT ∞ po (h)
10/30	25.3 ± 2.6	4.9 ± 2.5	1.0 ± 0.1	4.0 ± 1.3	8.3 ± 1.6

po: oral administration; iv: intravenous injection.

4. Conclusion

In our continued medicinal chemistry efforts to develop potent DPP-IV inhibitors based on our previously published compound **4**, we explored several optimization strategies. Although compound **4** demonstrated better in vivo efficacy than Alogliptin, its 40% bioavailability and 2-hour half-life still did not meet the requirements for oral chronic disease intervention. During this process, inspired by the pharmacophore similarities between Alogliptin, compound **4**, and Linagliptin, we identified a novel DPP-IV inhibitor (**5**) through a pharmacophore hybridization strategy. While its in vitro activity remained modest, compound **5** (IC = 0.86 M) served as a logical starting point for optimization and yielded a series of DPP-IV inhibitors with significantly improved activity. Compound **18o** was ultimately obtained with the desired in vitro activity (IC = 1.55 nM) and a substantially improved pharmacokinetic profile (bioavailability: 82.9%; half-life: 4.9 hours), suggesting a high probability of competent in vivo efficacy. Reviewing this work, we noted similarities with peer research, yet **18o** demonstrates a successful optimization pathway through classical pharmacophore hybridization. As part of our broader optimization efforts on compound **4**, further evaluations of **18o** will be conducted and additional work will be reported in due course.

5. Experimental Section

5.1. Chemistry

All commercially available compounds and solvents were of reagent grade and used without further purification unless otherwise noted. Reactions were monitored by TLC using Qingdao Haiyang GF254 silica gel plates (5 × 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) and by spraying with an ethanol solution of 2,4-DNP or ninhydrin, or by exposure to iodine vapor. Silica gel column chromatography was performed on silica gel (200–300 mesh) from Qingdao Haiyang. NMR spectra were recorded on a Bruker AVANCE 400 (400 MHz) or Bruker AVANCE 500 (500 MHz) spectrometer. Chemical shifts ( ) are reported in ppm and coupling constants in Hz. Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). MS data were measured on an Agilent MSD-1200 ESI-MS system.

Synthesis of Compound 5: A mixture of **16** (301 mg, 1.0 mmol), 3-(R)-aminopiperidine dihydrochloride (207 mg, 1.2 mmol), and NaHCO (336 mg, 4.0 mmol) in a sealed tube containing 15 mL of ethanol was heated at 120°C and stirred overnight. The reaction mixture was subsequently cooled to room temperature and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to yield compound **5**. Yield: 81.8%. ^{1}H NMR (400 MHz, MeOD) ppm: 7.37 (1H, s), 4.84–4.82 (2H, m), 3.53–3.50 (1H, m), 3.47–3.43 (1H, m), 3.06 (1H, s), 2.92–2.98 (1H, m), 2.03–2.00 (1H, m), 1.98–1.96 (1H, m), 1.89–1.87 (1H, m), 1.81 (3H, s), 1.79–1.73 (1H, m), 1.33–1.27 (1H, m). ^{13}C

NMR (500 MHz, MeOD) ppm: 24.92 (1C), 28.68 (1C), 34.07 (1C), 35.63 (1C), 52.68 (1C), 58.99 (1C), 75.29 (1C), 80.54 (1C), 81.04 (1C), 91.44 (1C), 116.26 (1C), 129.02 (1C), 142.13 (1C), 155.80 (1C), 156.49 (1C). ESI-MS calculated for C H N O [M+H] 363.07, 365.07, found 364.0, 366.0.

Compounds **6** and **18a–o** were prepared using procedures identical to that described for **5**.

Compound 6: Yield: 67.8%. ^{1}H NMR (400 MHz, CDCl) ppm: 10.87 (1H, s), 7.23 (1H, d, J = 2.8 Hz), 6.36 (1H, d, J = 2.8 Hz), 4.85 (2H, s), 3.49–3.46 (1H, m), 3.36–3.33 (1H, m), 3.11 (1H, m), 2.92 (1H, m), 2.80–2.78 (1H, m), 2.27 (2H, s), 1.99–1.97 (1H, m), 1.88–1.86 (1H, m), 1.79 (3H, s), 1.73–1.70 (1H, m), 1.37–1.32 (1H, m). ESI-MS calculated for C H N O [M+H] 286.16, found 286.1.

Compound 18a: Yield: 52.6%. ^{1}H NMR (400 MHz, CDCl) ppm: 6.87 (1H, d, J = 2.8 Hz), 6.17 (1H, d, J = 2.8 Hz), 5.19 (2H, s), 4.77–4.63 (2H, AB q, J = 34.8 Hz, 16.4 Hz), 3.96 (1H, s), 3.50–3.47 (1H, m), 3.32 (2H, m), 3.03–2.98 (1H, m), 2.90 (1H, m), 2.05 (1H, m), 1.84 (1H, m), 1.75 (3H, s), 1.65–1.63 (2H, m). ^{13}C NMR (500 MHz, CDCl) ppm: 22.64 (1C), 28.34 (1C), 30.38 (1C), 34.41 (1C), 35.57 (1C), 47.69 (1C), 51.55 (1C), 55.40 (1C), 74.46 (1C), 79.35 (1C), 101.61 (1C), 115.10 (1C), 131.69 (1C), 142.73 (1C), 153.49 (1C), 155.42 (1C). ESI-MS calculated for C H N O [M+H] 300.17, found 300.1.

Compound 18b: Yield: 54.2%. ^{1}H NMR (400 MHz, CDCl) ppm: 7.14 (1H, d, J = 2.8 Hz), 6.39 (1H, d, J = 2.8 Hz), 5.46 (2H, s), 4.76 (2H, s), 3.49–3.47 (1H, m), 3.38–3.35 (1H, m), 3.15 (1H, m), 2.96–2.91 (1H, m), 2.80 (2H, s), 2.00–1.96 (1H, m), 1.88–1.84 (1H, m), 1.80 (3H, s), 1.75–1.65 (2H, m), 1.42 (1H, m). ^{13}C NMR (500 MHz, CDCl) ppm: 22.95 (1C), 28.35 (1C), 32.54 (1C), 34.80 (1C), 35.47 (1C), 47.58 (1C), 50.39 (1C), 51.26 (1C), 58.04 (1C), 73.97 (1C), 79.86 (1C), 104.73 (1C), 114.60 (1C), 130.28 (1C), 143.98 (1C), 154.66 (1C), 155.57 (1C). ESI-MS calculated for C H N O [M+H] 325.17, found 325.1.

Compound 18c: Yield: 54.5%. ^{1}H NMR (400 MHz, MeOD) ppm: 8.65–8.63 (2H, d, J = 4.8 Hz), 7.32 (1H, d, J = 2.8 Hz), 7.31–7.28 (1H, t, J = 4.8 Hz), 6.31 (1H, d, J = 2.8 Hz), 5.81 (2H, s), 4.76–4.67 (2H, q, J = 18.8 Hz), 3.46–3.43 (1H, m), 3.36–3.29 (1H, m), 3.12–3.08 (1H, m), 2.90 (1H, m), 2.81 (1H, m), 2.78–2.76 (1H, m), 2.01–1.98 (1H, m), 1.88–1.86 (1H, m), 1.85–1.75 (1H, m), 1.72 (3H, s), 1.43–1.39 (1H, m). ^{13}C NMR (500 MHz, MeOD) ppm: 22.34 (1C), 28.87 (1C), 32.60 (1C), 35.16 (1C), 49.51 (1C), 50.51 (1C), 52.77 (1C), 54.39 (1C), 58.06 (1C), 75.36 (1C), 80.41 (1C), 103.23 (1C), 116.32 (1C), 121.15 (1C), 134.41 (1C), 145.10 (1C), 155.54 (1C), 156.79 (1C), 158.70 (1C), 167.68 (1C). ESI-MS calculated for C H N O [M+H] 378.20, found 378.1.

Compound 18d: Yield: 51.7%. ^{1}H NMR (400 MHz, MeOD) ppm: 7.46–7.44 (1H, d, J = 8.0 Hz), 7.30 (1H, s), 7.28 (1H, s), 7.28 (1H, d, J = 2.8 Hz), 7.04–7.00 (1H, m), 6.92–6.88 (1H, m), 6.13 (1H, d, J = 2.8 Hz), 5.73 (2H, s), 4.81 (2H, s), 3.57 (1H, m), 3.47–3.44 (1H, m), 3.26–3.25 (1H, m), 3.07–3.01 (1H, m), 2.05 (1H, m), 1.89–1.87 (1H, m), 1.78 (1H, m), 1.77 (3H, s), 1.63–1.61 (1H, m), 1.22 (1H, m). ^{13}C NMR (500 MHz, MeOD) ppm: 22.47 (1C), 28.92

(1C), 35.44 (1C), 44.25 (1C), 48.49 (1C), 49.51 (1C), 52.92 (1C), 53.85 (1C), 58.06 (1C), 61.44 (1C), 75.36 (1C), 80.41 (1C), 102.73 (1C), 112.44 (1C), 115.73 (1C), 119.23 (1C), 120.29 (1C), 122.77 (1C), 125.81 (1C), 127.80 (1C), 133.07 (1C), 138.14 (1C), 144.66 (1C), 154.56 (1C), 156.82 (1C). ESI-MS calculated for C H N O [M+H] 415.22, found 415.1.

Compound 18e: Yield: 77.1%. ^{1}H NMR (400 MHz, MeOD) ppm: 7.47 (2H, m), 7.39 (1H, d, J = 2.8 Hz), 7.18–7.15 (2H, m), 6.32 (1H, d, J = 2.8 Hz), 5.84 (1H, s), 4.78 (2H, s), 3.46–3.44 (1H, m), 3.43–3.42 (1H, m), 3.41–3.40 (1H, m), 3.03–2.98 (1H, m), 2.08–2.05 (1H, m), 1.91–1.88 (1H, m), 1.82–1.79 (1H, m), 1.77–1.75 (1H, m), 1.73 (3H, s), 1.60–1.57 (1H, m). ESI-MS calculated for C H N O [M+H] 416.21, found 416.2.

Compound 18f: Yield: 51.6%. ^{1}H NMR (400 MHz, MeOD) ppm: 8.67 (1H, d, J = 8.0 Hz), 8.25–8.23 (1H, d, J = 8.0 Hz), 8.08–8.06 (1H, d, J = 8.0 Hz), 7.85–7.81 (1H, m), 7.73–7.69 (1H, m), 7.40 (1H, d, J = 2.8 Hz), 6.62 (1H, d, J = 2.8 Hz), 6.43–6.42 (1H, m), 6.24 (2H, s), 4.79–4.70 (2H, t, J = 19.2 Hz), 3.48–3.45 (1H, m), 3.41–3.38 (1H, m), 3.03–2.98 (1H, m), 2.93–2.87 (1H, m), 2.76–2.70 (1H, m), 2.01–1.98 (1H, m), 1.90–1.87 (1H, m), 1.79–1.76 (1H, m), 1.74 (3H, s), 1.40–1.30 (1H, m). ^{13}C NMR (500 MHz, MeOD) ppm: 24.52 (1C), 33.74 (1C), 35.42 (1C), 48.53 (1C), 49.55 (1C), 49.58 (1C), 52.76 (1C), 59.35 (1C), 75.28 (1C), 80.52 (1C), 103.96 (1C), 116.09 (1C), 119.10 (1C), 124.29 (1C), 127.06 (1C), 128.60 (1C), 129.89 (1C), 131.22 (1C), 134.10 (1C), 145.54 (1C), 147.43 (1C), 148.48 (1C), 151.30 (1C), 156.14 (1C), 156.94 (1C). ESI-MS calculated for C H N O [M+H] 427.22, found 427.1.

Compound 18g: Yield: 54.8%. ^{1}H NMR (400 MHz, CDCl) ppm: 8.20–8.18 (1H, d, J = 8.0 Hz), 7.98–7.96 (1H, d, J = 8.8 Hz), 7.83–7.81 (1H, d, J = 8.0 Hz), 7.85–7.81 (1H, m), 7.73–7.70 (1H, m), 7.54–7.51 (1H, m), 7.40 (1H, d, J = 2.8 Hz), 6.35 (1H, d, J = 2.8 Hz), 5.87 (2H, s), 4.79–4.69 (2H, t, J = 21.6 Hz), 3.44–3.41 (1H, m), 3.36–3.29 (1H, m), 2.99–2.94 (1H, m), 2.88–2.85 (1H, m), 2.71–2.63 (1H, m), 1.99–1.95 (1H, m), 1.86–1.83 (1H, m), 1.73 (1H, m), 1.73 (3H, s), 1.38–1.26 (1H, m). ^{13}C NMR (500 MHz, MeOD) ppm: 24.48 (1C), 33.69 (1C), 35.32 (1C), 48.48 (1C), 49.51 (1C), 52.69 (1C), 54.44 (1C), 59.33 (1C), 75.36 (1C), 80.43 (1C), 103.79 (1C), 115.92 (1C), 120.19 (1C), 127.82 (1C), 128.85 (1C), 128.94 (1C), 129.19 (1C), 131.24 (1C), 133.92 (1C), 139.13 (1C), 145.41 (1C), 148.50 (1C), 155.96 (1C), 156.92 (1C), 159.53 (1C). ESI-MS calculated for C H N O [M+H] 427.22, found 427.1.

Compound 18h: Yield: 43.8%. ^{1}H NMR (400 MHz, MeOD) ppm: 8.14–8.11 (1H, d, J = 8.8 Hz), 8.00–7.99 (1H, d, J = 2.0 Hz), 7.90–7.87 (1H, m), 7.80–7.77 (1H, m), 7.44 (1H, d, J = 2.8 Hz), 7.23–7.21 (1H, d, J = 8.8 Hz), 6.42 (1H, d, J = 2.8 Hz), 5.91 (2H, s), 4.83–4.81 (2H, m), 3.53–3.50 (1H, m), 3.45–3.42 (1H, m), 3.10–3.04 (1H, m), 2.96–2.91 (1H, m), 2.80–2.75 (1H, m), 2.07–2.03 (1H, m), 1.95–1.90 (1H, m), 1.84 (3H, s), 1.83–1.75 (1H, m), 1.45–1.33 (1H, m). ^{13}C NMR (500 MHz, CDCl +MeOD) ppm: 24.27 (1C), 33.45 (1C), 35.29 (1C), 48.48 (1C), 49.50 (1C), 49.98 (1C), 52.50 (1C), 54.29 (1C), 59.07 (1C), 75.14 (1C), 80.40 (1C), 103.70 (1C), 115.68 (1C), 121.17 (1C), 129.63 (1C), 130.72

(1C), 131.04 (1C), 133.61 (1C), 134.19 (1C), 137.85 (1C), 145.06 (1C), 146.83 (1C), 155.63 (1C), 156.67 (1C), 159.63 (1C). ESI-MS calculated for C H BrN O [M+H] 505.13, found 505.1.

Compound 18i: Yield: 67.8%. ^{1}H NMR (400 MHz, CDCl) ppm: 7.96–7.93 (1H, m), 7.71 (1H, d, J = 2.4 Hz), 7.60–7.57 (1H, dd, J = 2.4 Hz, J = 9.2 Hz), 7.30–7.26 (1H, m), 7.20 (1H, d, J = 2.8 Hz), 6.34 (1H, d, J = 2.8 Hz), 5.88 (2H, s), 4.79 (2H, d, J = 2.0 Hz), 3.48–3.43 (1H, m), 3.06–3.00 (1H, m), 2.90–2.85 (1H, m), 2.72–2.67 (1H, m), 1.98–1.93 (1H, m), 1.89 (2H, s), 1.85–1.82 (1H, m), 1.80 (3H, s), 1.74–1.65 (1H, m), 1.32–1.24 (1H, m). ^{13}C NMR (500 MHz, CDCl) ppm: 23.41 (1C), 33.45 (1C), 34.49 (1C), 47.83 (1C), 51.36 (1C), 53.67 (1C), 59.26 (1C), 74.47 (1C), 77.00 (1C), 79.34 (1C), 103.12 (1C), 114.75 (1C), 120.82 (1C), 126.14 (1C), 127.93 (1C), 130.52 (1C), 130.74 (1C), 131.49 (1C), 132.14 (1C), 136.21 (1C), 143.72 (1C), 145.95 (1C), 154.24 (1C). ESI-MS calculated for C H ClN O [M+H] 461.18, found 461.1.

Compound 18j: Yield: 62.5%. ^{1}H NMR (400 MHz, CDCl) ppm: 8.02–7.97 (2H, m), 7.46–7.41 (1H, m), 7.36–7.33 (1H, m), 7.31–7.29 (1H, d, J = 8.4 Hz), 7.20 (1H, d, J = 2.8 Hz), 6.33 (1H, d, J = 2.8 Hz), 5.89 (2H, s), 4.79 (2H, d, J = 2.0 Hz), 3.48–3.43 (1H, m), 3.37–3.34 (1H, m), 3.06–3.00 (1H, m), 2.90–2.85 (1H, m), 1.98–1.93 (1H, m), 1.87–1.84 (1H, m), 1.83 (2H, s), 1.79 (3H, s), 1.74–1.66 (1H, m), 1.33–1.31 (1H, m). ^{13}C NMR (500 MHz, CDCl) ppm: 23.40 (1C), 33.45 (1C), 34.45 (1C), 47.81 (1C), 51.34 (1C), 53.63 (1C), 59.26 (1C), 74.48 (1C), 77.00 (1C), 79.31 (1C), 103.06 (1C), 110.37 (1C), 114.73 (1C), 119.68 (1C), 120.72 (1C), 127.90 (1C), 131.53 (1C), 136.45 (1C), 143.70 (1C), 144.63 (1C), 154.21 (1C), 155.70 (1C), 157.00 (1C), 159.34 (1C), 161.31 (1C). ESI-MS calculated for C H FN O [M+H] 445.21, found 445.1.

Compound 18k: Yield: 69.4%. ^{1}H NMR (400 MHz, MeOD) ppm: 8.06–8.04 (1H, d, J = 8.4 Hz), 7.86–7.84 (1H, d, J = 8.4 Hz), 7.62 (1H, s), 7.61–7.59 (1H, dd, J = 8.8 Hz, J = 1.6 Hz), 7.39 (1H, d, J = 2.8 Hz), 7.30–7.28 (1H, d, J = 8.4 Hz), 6.34 (1H, d, J = 2.8 Hz), 5.73 (2H, s), 4.80–4.74 (2H, m), 3.45–3.43 (1H, m), 3.35–3.34 (1H, m), 3.01–2.95 (1H, m), 2.87–2.67 (1H, m), 2.65 (3H, s), 2.00–1.96 (1H, m), 1.88–1.84 (1H, m), 1.77 (3H, s), 1.74–1.66 (1H, m), 1.38–1.35 (1H, m). ^{13}C NMR (500 MHz, MeOD) ppm: 24.63 (1C), 33.73 (1C), 35.33 (1C), 48.49 (1C), 49.51 (1C), 52.19 (1C), 52.26 (1C), 55.50 (1C), 59.40 (1C), 75.49 (1C), 80.41 (1C), 103.56 (1C), 115.64 (1C), 123.60 (1C), 127.07 (1C), 127.79 (1C), 128.74 (1C), 130.34 (1C), 133.41 (1C), 137.70 (1C), 138.21 (1C), 145.28 (1C), 147.72 (1C), 155.81 (1C), 156.77 (1C), 160.52 (1C). ESI-MS calculated for C H N O [M+H] 441.23, found 441.1.

Compound 18l: Yield: 43.4%. ^{1}H NMR (400 MHz, CDCl) ppm: 8.03 (1H, d, J = 1.6 Hz), 8.01 (1H, s), 7.69–7.67 (1H, d, J = 8.8 Hz), 7.45–7.43 (1H, dd, J = 8.8 Hz, J = 2.0 Hz), 7.30–7.28 (1H, d, J = 8.8 Hz), 7.22 (1H, d, J = 2.8 Hz), 6.35 (1H, d, J = 2.8 Hz), 5.90 (2H, s), 4.84–4.75 (2H, m), 3.50–3.47 (1H, m), 3.37–3.34 (1H, m), 3.16–3.12 (1H, m), 2.97–2.92 (1H, m), 2.84–2.80 (1H, m), 2.43 (2H, s), 1.99–1.96 (1H, m), 1.89–1.85 (1H, m), 1.80 (3H, t, J = 2.4 Hz), 1.76–1.66 (1H, m), 1.42–1.40 (1H, m). ^{13}C NMR (500 MHz, MeOD) ppm:

23.07 (1C), 29.65 (1C), 32.65 (1C), 34.53 (1C), 47.70 (1C), 51.40 (1C), 53.67 (1C), 58.27 (1C), 74.45 (1C), 79.46 (1C), 103.11 (1C), 114.78 (1C), 120.14 (1C), 125.74 (1C), 127.53 (1C), 128.21 (1C), 128.71 (1C), 131.64 (1C), 135.49 (1C), 136.98 (1C), 143.59 (1C), 147.96 (1C), 154.12 (1C), 155.67 (1C), 158.74 (1C). ESI-MS calculated for C H ClN O [M+H] 461.18, found 461.1.

Compound 18m: Yield: 45.9%. ^{1}H NMR (400 MHz, CDCl) ppm: 8.04-8.02 (1H, d, J = 8.4 Hz), 7.75-7.64 (1H, m), 7.67-7.64 (1H, dd, J = 5.2 Hz, J = 2.0 Hz), 7.30-7.28 (1H, m), 7.24 (1H, s), 7.22 (1H, d, J = 2.8 Hz), 6.35 (1H, d, J = 2.8 Hz), 5.90 (2H, s), 4.80 (2H, d, J = 2.0 Hz), 3.49-3.47 (1H, m), 3.38-3.35 (1H, m), 3.07 (1H, m), 2.93-2.88 (1H, m), 2.76-2.71 (1H, m), 1.98-1.95 (1H, m), 1.88 (1H, m), 1.85 (2H, s), 1.80 (3H, s), 1.76-1.66 (1H, m), 1.33-1.31 (1H, m). ^{13}C NMR (500 MHz, CDCl) ppm: 23.17 (1C), 33.51 (1C), 34.49 (1C), 47.03 (1C), 51.37 (1C), 53.71 (1C), 74.51 (1C), 79.34 (1C), 103.12 (1C), 112.76 (1C), 114.80 (1C), 116.87 (1C), 119.24 (1C), 124.38 (1C), 129.45 (1C), 131.53 (1C), 137.02 (1C), 143.70 (1C), 148.55 (1C), 154.22 (1C), 155.73 (1C), 158.81 (1C), 162.13 (1C), 164.12 (1C). ESI-MS calculated for C H FN O [M+H] 445.21, found 445.1.

Compound 18n: Yield: 61.5%. ^{1}H NMR (400 MHz, MeOD) ppm: 8.67 (1H, s), 7.97-7.90 (2H, m), 7.74-7.69 (2H, m), 7.45 (1H, d, J = 2.8 Hz), 6.35 (1H, d, J = 2.8 Hz), 5.90 (2H, s), 4.78-4.71 (2H, m), 3.44-3.41 (1H, m), 3.36-3.32 (1H, m), 2.99-2.95 (1H, m), 2.86-2.84 (1H, m), 2.71-2.66 (1H, m), 1.99-1.94 (1H, m), 1.85-1.82 (1H, m), 1.73 (3H, s), 1.69-1.68 (1H, m), 1.37-1.26 (1H, m). ^{13}C NMR (500 MHz, MeOD) ppm: 24.46 (1C), 33.68 (1C), 35.33 (1C), 48.48 (1C), 49.98 (1C), 52.64 (1C), 54.40 (1C), 59.33 (1C), 75.35 (1C), 80.43 (1C), 103.71 (1C), 115.75 (1C), 129.67 (1C), 129.98 (1C), 131.16 (1C), 131.58 (1C), 134.10 (1C), 142.64 (1C), 142.93 (1C), 145.16 (1C), 145.45 (1C), 154.23 (1C), 155.90 (1C), 156.81 (1C). ESI-MS calculated for C H N O [M+H] 428.21, found 428.1.

Compound 18o: Yield: 53.4%. ^{1}H NMR (400 MHz, CDCl) ppm: 7.98-7.92 (2H, m), 7.69-7.61 (2H, m), 7.07 (1H, d, J = 2.8 Hz), 6.34 (1H, d, J = 2.8 Hz), 6.04 (2H, s), 4.78 (2H, d, J = 2.0 Hz), 3.49-3.46 (1H, m), 3.38-3.35 (1H, m), 3.06-3.01 (1H, m), 2.91-2.86 (1H, m), 2.72 (3H, s), 2.72 (1H, m), 1.98-1.94 (1H, m), 1.87-1.81 (1H, m), 1.77 (2H, s), 1.77 (3H, s), 1.72-1.68 (1H, m), 1.29-1.27 (1H, m). ^{13}C NMR (500 MHz, CDCl) ppm: 22.13 (1C), 23.44 (1C), 29.63 (1C), 33.46 (1C), 34.48 (1C), 47.84 (1C), 50.93 (1C), 51.37 (1C), 59.32 (1C), 74.46 (1C), 76.78 (1C), 79.35 (1C), 103.08 (1C), 115.00 (1C), 128.29 (1C), 128.98 (1C), 129.81 (1C), 131.41 (1C), 140.66 (1C), 141.61 (1C), 143.54 (1C), 150.83 (1C), 152.80 (1C), 154.17 (1C), 155.84 (1C). ESI-MS calculated for C H N O [M+H] 442.23, found 442.2.

5.2. In Vitro Inhibition of DPP-IV, DPP-8, and DPP-9

Solutions of test compounds at varying concentrations (10 mM final concentration) were prepared in dimethyl sulfoxide (DMSO) and diluted into assay buffer containing 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 the reaction was initiated by addition of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin, Sigma-Aldrich, 10 M final concentration). The total reaction volume was 100 L. Reaction kinetics were monitored (excitation at 400 nm, emission at 505 nm) for 5–10 minutes, or an endpoint was measured after 10 minutes. Inhibition constants (IC) were calculated from enzyme progress curves using standard mathematical models.

5.3. In Vivo Pharmacokinetic Study

Adult male SD rats (n = 4/group) were administered test compounds dissolved in distilled water at a single dose of 20 mg/kg or 25 mg/kg for oral administration and 5 mg/mL for intravenous injection. Blood samples of 100–200 L were collected from the orbital sinus at 11 time points within 24 hours. Blood concentrations of test compounds were determined by LC-MS/MS. PK parameters were obtained using the pharmacokinetic software DAS 2.0.

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