# Novel Dipeptidyl Peptidase-IV Inhibitors Identified through Scaffold Hopping and Molecular Hybridization (Postprint)

**Authors:** Wang Shanchun, Zeng Lili, Ding Yuyang, Zeng Shaogao, Song Hongrui, Hu Wenhui, Xie Hui, Hu Wenhui, Xie Hui

**Date:** 2017-11-17T00:00:00+00:00

## Abstract

To date, seven dipeptidyl peptidase IV (DPP-IV) inhibitors have been approved as anti-diabetic drugs, and their structural differences and intrinsic correlations provide new insights for further structural modification. In this study, focusing on the structural features of alogliptin and linagliptin, and employing the principles of scaffold hopping and drug hybridization, we rapidly obtained a novel DPP-IV inhibitor 8g (IC50 = 4.9 nM), whose activity and selectivity are both comparable to those of marketed drugs. Therefore, applying classical medicinal chemistry strategies to perform molecular manipulation on marketed drugs targeting the same target can effectively generate novel active molecules.

## Full Text

### Discovery of Novel Dipeptididyl Peptidase IV Inhibitors through Scaffold Hopping and Drug Splicing Strategy

**Shan-chun Wang[1], Li-li Zeng[2], Yu-yang Ding[3], Shao-gao Zeng[2], Hong-rui Song[1], Wen-hui Hu[2], *Hui Xie***

[1]Jiangsu Chia-Tai Tianqing Pharmaceutical Co. Ltd, Jiangsu, 222006, China
[2]Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, 510530, China
[3]School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, 110016, China
First Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510120, China

**Abstract**

To date, seven dipeptidyl peptidase IV (DPP-IV) inhibitors have been approved as new anti-diabetic drugs, and their structural differences and inherent correlations provide new insights for further structural modification. In this study, focusing on the structural features of alogliptin and linagliptin, we rapidly obtained a novel DPP-IV inhibitor **8g** (IC = 4.9 nM) using scaffold hopping and drug splicing principles. This compound exhibits activity and selectivity comparable to marketed drugs. Therefore, applying classical medicinal chemistry strategies to perform molecular operations on marketed drugs targeting the same target can effectively generate novel bioactive molecules.

**Keywords:** type 2 diabetes; DPP-IV inhibitor; scaffold hopping; drug splicing strategy
**Funding:** Guangzhou Science and Information Technology Bureau (XXXXXX, please add number, belongs to DDP project)
**Corresponding Authors:** Tel: 86-20-32015211; 86-20-83062528, E-mail: hu_wenhui@gibh.ac.cn; brennan_huixie@126.com

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex endocrine and metabolic disorder that has become the third most prevalent non-communicable disease threatening human health, following cardiovascular diseases and malignant tumors. According to the International Diabetes Federation (IDF), the global diabetic population reached 371 million in 2012, with over 90% being type 2 diabetes [**?**]. Conventional hypoglycemic agents primarily include three categories: insulin sensitizers, insulin secretagogues, and -glucosidase inhibitors [**?**]. However, these drugs often cause adverse effects such as weight gain, drug resistance, and hypoglycemia [**?**], making the development of novel anti-diabetic agents with new mechanisms of action and low toxicity a persistent focus in both academia and industry [**?**].

In recent years, DPP-IV inhibitors have emerged as first-line drugs for treating type 2 diabetes in developed countries. DPP-IV inhibitors exert their therapeutic effects by attenuating the degradation of incretins, thereby enhancing the activity of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). This leads to increased insulin sensitivity, promoted insulin secretion, and stimulated pancreatic -cell growth [**?**]. Compared with traditional anti-diabetic medications, DPP-IV inhibitors demonstrate definitive efficacy, fewer adverse reactions, and favorable oral bioavailability, making them suitable for chronic disease management.

Currently marketed DPP-IV inhibitors include sitagliptin [**?**] (1), vildagliptin [**?**] (2), saxagliptin [**?**] (3), alogliptin [**?**] (4), linagliptin [**?**] (5), gemigliptin [**?**] (6), and teneligliptin [**?**] (7). As shown in [Figure 1: see original paper], although

these drugs bind to the same target, they exhibit substantial structural differences, particularly among sitagliptin, alogliptin, and linagliptin. Our previous studies revealed that alogliptin and linagliptin share certain pharmacophoric similarities and significant structural correlations, making them suitable candidates for molecular operations such as scaffold hopping and fragment splicing [**?**].

[Figure 1: see original paper]

Therefore, we employed a scaffold hopping strategy to replace the pyrimidinedione scaffold of alogliptin with a xanthine scaffold, rapidly obtaining a novel class of DPP-IV inhibitors. Through structural modification of the xanthine scaffold, particularly by splicing the quinazoline fragment from linagliptin at the N-1 position, we discovered the highly active and selective DPP-IV inhibitor **8g** (IC = 4.9 nM) ([Figure 2: see original paper]). The discovery of compound **8g** further confirms that for marketed drugs targeting the same protein, exploring their similarities and applying classical medicinal chemistry molecular operations can rapidly establish novel bioactive molecules.

[Figure 2: see original paper]

---

## 2. Chemical Synthesis

The synthetic route to compounds **8a-g** is illustrated in [Figure 3: see original paper]. Commercially available 2-cyanobenzyl bromide underwent nucleophilic substitution with 8-bromo-3-methylxanthine (compound **9**) to afford key intermediate **10**. N-alkylation with 2-bromomethyl-4-methylquinazoline yielded compound **11**, which was then reacted with phthalimide-protected (R)-3-aminopiperidine to give compound **13g**. Alternatively, compound **10** was first reacted with protected 3-aminopiperidine to obtain compound **12**, followed by N-alkylation to produce compounds **13a-f**. Finally, deprotection of compounds **13a-g** in ethanolamine/toluene at 90 °C furnished target compounds **8a-g**.

**Reagents:** i. DIEA, DMF, rt; ii. Na CO or K CO , NMP, rt-150 °C; iii. DIEA, NMP, 140 °C; iv. Ethanolamine, toluene, 90 °C.

---

## 3. Results and Discussion

### 3.1 Scaffold Hopping of Alogliptin to Obtain Novel DPP-IV Inhibitors

The concept of scaffold hopping originated from computational techniques and was extended to drug discovery to identify novel structures with different topological scaffolds that maintain biological activity from known databases [**?**]. Its advantage lies in the rapid and convenient generation of drug candidates with properties similar to marketed drugs when applied to approved therapeutics.

Through scaffold hopping strategies based on alogliptin, we previously reported several highly potent inhibitors, such as compound **6** (IC = 0.33 nM) [**?**] and compound **7** (IC = 1.4 nM) [**?**] ([Figure 4: see original paper]).

[Figure 4: see original paper]

Although these molecules demonstrated superior potency, they failed to advance to development due to deficiencies in pharmacokinetic properties and safety profiles [**?**, **?**], prompting us to focus on analyzing and performing molecular operations on marketed drugs. We noted that despite significant structural differences between alogliptin and linagliptin, their binding modes to the DPP-IV enzyme share considerable similarities [**?**, **?**]. Both compounds feature (R)-3-aminopiperidine groups that form hydrogen bonds with Glu205 and Glu206; their 2-cyanobenzyl and butynyl groups occupy the hydrophobic S1 pocket; and their molecular scaffolds engage in - stacking interactions with Tyr547. Notably, linagliptin, with its xanthine scaffold, demonstrates superior properties among marketed DPP-IV inhibitors in terms of in vitro biological activity, pharmacokinetics, and pharmacodynamics [**?**]. Therefore, in this new round of structural optimization, we retained the 2-cyanobenzyl and (R)-3-aminopiperidine pharmacophores of alogliptin while introducing the advantageous xanthine scaffold of linagliptin to synthesize a series of novel DPP-IV inhibitors.

### 3.2 Modification of Substituents at the N-1 Position of the Xanthine Scaffold

Through modification at the N-1 position, we synthesized compounds **8a-g** as shown in , all of which exhibited varying degrees of DPP-IV inhibitory activity and selectivity. The N-1 substituent significantly impacted activity, with aromatic groups particularly favorable for potency.

The results in demonstrate that the N-1 position is indeed a sensitive site for activity, with aromatic fused rings producing better inhibitory activity (compounds **8e-g**). Screening against DPP-VIII/IX revealed that compounds **8a-g** showed no inhibition against these DPP-IV isozymes, indicating excellent selectivity. Notably, compound **8g** (IC = 4.9 nM), obtained through drug splicing strategy, incorporates the 2-methyl-4-methylquinazolinyl fragment from linagliptin and maintains high activity and selectivity. Under identical experimental conditions, alogliptin showed a DPP-IV inhibitory IC value of 3.4 nM. The **8g** series retains the excellent DPP selectivity characteristic of marketed DPP-IV inhibitors, while its DPP-IV inhibitory activity approaches the level of commercial drugs.

---

## 4. Conclusion

In me-too drug development, scaffold hopping can rapidly generate novel active molecules, though gaps remain in overall drug-like properties. Building on our

previous work, we directly selected marketed DPP-IV inhibitors alogliptin and linagliptin for scaffold hopping and drug splicing operations, rapidly establishing a novel and highly potent DPP-IV inhibitor. This study, through the identification of compound **8g** (IC = 4.9 nM), demonstrates that integrating marketed drugs targeting the same target and applying medicinal chemistry strategies and molecular operations [**?**] can efficiently generate novel bioactive molecules.

---

## 5. Experimental Section

### 5.1 Instruments and Reagents

Instruments used included a ZF-20D dark-box UV analyzer (Gongyi Yuhua Instrument Co., Ltd.), R-1001-VN rotary evaporator (Zhengzhou Great Wall Scientific Industry and Trade Co., Ltd.), AVANCE AV 400 MHz and 500 MHz NMR spectrometers (Bruker, Switzerland), and an API 3000 LC-MS/MS system (mass spectrometer: AB Sciex API3000, autosampler: Gerstel UPS3C, HPLC: Shimadzu LC-10ADVP, Applied Biosystems, USA). Chromatography silica gel G and H (Qingdao Marine Chemical Factory) and silica gel plates GF254 (Qingdao Puke Separation Materials Co., Ltd.) were used for purification.

Major reagents were purchased from Guangzhou Jinhua Chemical Reagent Co., Ltd. (analytical grade). Key starting materials were obtained from Aladdin Reagent (purity >95%).

### 5.2 Synthetic Procedures

**5.2.1 Preparation of Compound 10** 8-Bromo-3-methylxanthine (compound **9**) (100 mg, 0.41 mmol) was dissolved in 10 mL DMF and stirred at room temperature. Diisopropylethylamine (0.1 mL, 0.57 mmol) and a DMF solution of 2-cyanobenzyl bromide (96 mg, 0.49 mmol in 5 mL) were added sequentially. The mixture was stirred at room temperature until TLC indicated complete reaction. Addition of 50 mL water precipitated a white solid, which was washed with water and ether to afford 72 mg of product (58.5% yield). $^1$H NMR (400 MHz, DMSO, ppm) : 11.35 (s, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.66 (t, J = 7.2 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H).

**5.2.2 Preparation of Compound 11** Compound **10** (1.09 g, 3.03 mmol) and 2-bromomethyl-4-methylquinazoline (0.64 g, 3.33 mmol) were dissolved in 30 mL NMP and stirred in an oil bath at 140 °C for 2 hours. Upon TLC confirmation of complete reaction, 100 mL water was added to precipitate a brown solid. After washing with water and ether, 1.37 g of product was obtained (87.8% yield). $^1$H NMR (400 MHz, CDCl , ppm) : 7.95 (d, J = 8.4 Hz, 1H), 7.78-7.70 (m, 2H), 7.62 (d, J = 8.0 Hz, 1H), 7.49-7.44 (m, 2H), 7.37-7.31 (m, 1H), 6.96 (d, J = 7.6 Hz, 1H), 5.76 (s, 2H), 5.47 (s, 2H), 3.56 (s, 3H), 2.81 (s, 3H).

**5.2.3 Preparation of Compound 12** Compound **10** (1.5 g, 4.16 mmol), (R)-2-(3-piperidyl)isoindoline-1,3-dione (1.44 g, 6.25 mmol), and diisopropylethylamine (2.9 mL, 16.64 mmol) were dissolved in 30 mL NMP and stirred in an oil bath at 140 °C for 2 hours. After TLC confirmed completion, the reaction mixture was poured into crushed ice to precipitate a yellow solid. Washing with water and ether afforded 1.95 g of product (90.2% yield). $^1$H NMR (400 MHz, DMSO, ppm) : 7.83-7.80 (m, 2H), 7.72-7.67 (m, 3H), 7.56-7.52 (m, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 5.54 (AB q, J = 24.8 Hz, 16.8 Hz, 2H), 4.49-4.41 (m, 1H), 3.50 (s, 3H), 3.42-3.35 (m, 2H), 3.33-3.30 (m, 1H), 3.03-2.96 (m, 1H), 2.46-2.34 (m, 1H), 1.90-1.87 (m, 1H), 1.80-1.78 (m, 2H).

**5.2.4 Preparation of Compound 13a** Compound **12** (200 mg, 0.39 mmol) was dissolved in 5 mL DMF, and potassium carbonate (86.2 mg, 0.62 mmol) was added. Chloromethylcyclopropane (58.3 mg, 0.5 mmol) was added dropwise at room temperature, and the mixture was stirred overnight. After TLC confirmed completion, the reaction was poured into crushed ice to precipitate a white solid. The filtered cake was washed with n-hexane to afford 130 mg of product (58.8% yield). $^1$H NMR (400 MHz, CDCl , ppm) : 7.83-7.80 (m, 2H), 7.74-7.68 (m, 3H), 7.53 (t, J = 7.6 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 5.59 (AB q, J = 24.0 Hz, 16.8 Hz, 2H), 4.50-4.44 (m, 1H), 3.85 (q, J = 7.2 Hz, 2H), 3.56 (s, 3H), 3.40-3.36 (m, 1H), 3.31-3.28 (m, 1H), 3.03-2.96 (m, 1H), 2.45-2.40 (m, 1H), 1.91-1.88 (m, 1H), 1.82-1.78 (m, 1H), 1.27-1.24 (m, 2H), 0.44-0.33 (m, 4H). MS (ESI): m/z 564.3 [M+H] .

**5.2.5 General Procedure for Compounds 13b-f (Exemplified by 13f)** Compound **12** (200 mg, 0.39 mmol) was dissolved in 4 mL NMP, and sodium carbonate (56 mg, 0.52 mmol) was added. 2-Chloromethylquinoline (76.4 mg, 0.43 mmol) was added dropwise at room temperature, and the mixture was heated in an oil bath at 150 °C for 2 hours. After TLC confirmed completion, the reaction was poured into crushed ice to precipitate a yellow solid. The solid was filtered, washed with water and ether, then dissolved in dichloromethane. The organic solution was washed with saturated brine, dried over anhydrous sodium sulfate, filtered, concentrated, and purified by column chromatography on 200-300 mesh silica gel using petroleum ether-ethyl acetate (1:1) to afford 80 mg of product (31.4% yield). $^1$H NMR (400 MHz, CDCl , ppm) : 8.05 (d, J = 8.5 Hz, 1H), 7.99 (d, J = 8.5 Hz, 1H), 7.83-7.82 (m, 2H), 7.75-7.71 (m, 3H), 7.66-7.62 (m, 2H), 7.51 (t, J = 8.0 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.33 (d, J = 7.5 Hz, 1H), 7.27-7.26 (m, 1H), 7.18 (d, J = 8.0 Hz, 1H), 5.60 (AB q, J = 31.0 Hz, 16.5 Hz, 2H), 5.47 (s, 2H), 4.51-4.46 (m, 1H), 3.58 (s, 3H), 3.45-3.43 (m, 1H), 3.39-3.33 (m, 2H), 3.06-3.03 (m, 1H), 2.45-2.41 (m, 1H), 2.38-2.35 (m, 1H), 2.03-2.00 (m, 1H), 1.92-1.89 (m, 1H). MS (ESI): m/z 651.1 [M+H] .

**5.2.6 Preparation of Compound 13g** Compound **11** (91.5 mg, 0.236 mmol) and (R)-2-(3-piperidyl)isoindoline-1,3-dione (81.4 mg, 0.354 mmol) were dissolved in 10 mL NMP. Diisopropylethylamine (0.2 mL, 3 mL) was added at

room temperature, and the mixture was heated in an oil bath at 140 °C for 2 hours. After TLC confirmed completion, the reaction was poured into crushed ice to precipitate a yellow solid, which was washed with water and ether to afford 102 mg of product (80.3% yield). $^1$H NMR (400 MHz, CDCl , ppm) : 8.00 (d, J = 8.0 Hz, 1H), 7.87-7.76 (m, 4H), 7.73-7.71 (m, 2H), 7.65 (d, J = 7.6 Hz, 1H), 7.51 (dd, J = 14.8 Hz, 7.6 Hz, 2H), 7.32 (t, J = 7.6 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 5.61 (AB q, J = 27.2 Hz, 16.8 Hz, 2H), 5.52 (s, 2H), 4.53-4.45 (m, 1H), 3.60 (s, 3H), 3.46-3.32 (m, 3H), 3.06-2.99 (m, 1H), 2.87 (s, 3H), 1.92-1.89 (m, 1H). MS (ESI): m/z 666.3 [M+H] .

**5.2.7 General Procedure for Compounds 8a-g (Exemplified by 8a)** Compound **13a** (130 mg, 0.23 mmol) was suspended in 2 mL toluene and stirred in an oil bath at 80 °C. Ethanolamine (0.2 mL) was added dropwise, and after addition, the temperature was increased to 90 °C. Stirring continued until TLC indicated complete reaction. The cooled reaction mixture was diluted with 50 mL water and extracted three times with 150 mL dichloromethane. The combined organic phases were dried over anhydrous sodium sulfate and concentrated to afford a yellow solid (78.9 mg, 85.8% yield).

**8a:** Light yellow solid, 85.8% yield. $^1$H NMR (400 MHz, CDCl , ppm) : 7.69 (d, J = 7.6 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 5.58 (AB q, J = 21.2 Hz, 16.8 Hz, 2H), 3.84 (q, J = 7.2 Hz, 2H), 3.56 (s, 3H), 3.34-3.31 (m, 1H), 3.19-3.16 (m, 1H), 2.96-2.86 (m, 2H), 2.71-2.66 (m, 1H), 1.94-1.89 (m, 1H), 1.76-1.71 (m, 1H), 1.66-1.56 (m, 1H), 1.27-1.18 (m, 2H), 0.43-0.33 (m, 4H). $^{13}$C NMR (125 MHz, CDCl , ppm) : 156.97, 154.55, 151.64, 147.99, 140.57, 133.28, 132.87, 128.09, 127.02, 116.85, 110.50, 104.92, 58.46, 50.73, 47.13, 47.02, 45.32, 33.36, 29.64, 23.19, 10.09, 3.67. MS (ESI): m/z 434.2 [M+H] .

**8b:** Light yellow solid, 94.1% yield. $^1$H NMR (400 MHz, CDCl , ppm) : 7.66 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 5.54 (AB q, J = 21.6 Hz, 16.8 Hz, 2H), 3.78 (d, J = 7.2 Hz, 2H), 3.52 (s, 3H), 3.34-3.30 (m, 1H), 3.18-3.14 (m, 1H), 2.94-2.84 (m, 2H), 2.70-2.64 (m, 1H), 1.91-1.87 (m, 1H), 1.76-1.68 (m, 2H), 1.63-1.54 (m, 2H). $^{13}$C NMR (125 MHz, CDCl , ppm) : 157.01, 154.65, 151.61, 147.91, 140.55, 133.25, 132.86, 128.07, 127.07, 116.83, 110.48, 104.80, 58.43, 50.71, 47.09, 46.70, 36.45, 33.35, 30.61, 29.66, 26.28, 25.75, 23.19. MS (ESI): m/z 476.2 [M+H] .

**8c:** Light yellow solid, 89.1% yield. $^1$H NMR (400 MHz, CDCl , ppm) : 7.69 (d, J = 7.5 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.42-7.38 (m, 3H), 7.28-7.20 (m, 3H), 7.13 (d, J = 7.5 Hz, 1H), 5.59 (AB q, J = 25.0 Hz, 17.0 Hz, 2H), 5.14 (s, 2H), 3.54 (s, 3H), 3.37-3.33 (m, 1H), 3.21-3.18 (m, 1H), 2.92-2.88 (m, 2H), 2.71-2.67 (m, 1H), 2.04-1.99 (m, 1H), 1.92-1.89 (m, 1H), 1.74-1.71 (m, 1H), 1.64-1.59 (m, 2H). $^{13}$C NMR (125 MHz, CDCl , ppm) : 157.06, 154.26, 151.43, 148.11, 140.44, 137.46, 133.29, 132.88, 128.60, 128.20, 128.12, 127.24, 127.04, 116.82, 110.50, 104.81, 58.25, 50.62, 47.07, 44.06, 33.22, 29.68, 29.57, 23.13. MS (ESI): m/z 470.2 [M+H] .

**8d:** Light yellow solid, 89.65% yield. $^{1}$H NMR (400 MHz, CDCl , ppm) : 7.69 (d, J = 7.6 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.43-7.38 (m, 3H), 7.11 (d, J = 8.0 Hz, 1H), 6.93 (t, J = 8.4 Hz, 2H), 5.57 (AB q, J = 22.0 Hz, 16.8 Hz, 2H), 5.08 (s, 2H), 3.53 (s, 3H), 3.35-3.32 (m, 1H), 3.20-3.17 (m, 1H), 2.94-2.86 (m, 2H), 2.70-2.65 (m, 1H), 1.93-1.89 (m, 1H), 1.75-1.69 (m, 1H), 1.64-1.54 (m, 2H). $^{13}$C NMR (125 MHz, CDCl , ppm) : 163.02 (0.5C), 161.07 (0.5C), 157.15, 154.17, 151.41, 148.18, 140.43, 133.28, 132.92, 130.69, 130.63, 128.16, 127.00, 116.80, 115.06, 114.88, 110.54, 104.80, 58.36, 50.61, 47.12, 47.10, 43.35, 33.35, 29.70, 23.17. MS (ESI): m/z 488.3 [M+H] .

**8e:** Light yellow solid, 59.3% yield. $^{1}$H NMR (400 MHz, CDCl , ppm) : 7.64 (d, J = 7.6 Hz, 1H), 7.51-7.47 (m, 3H), 7.35 (t, J = 7.6 Hz, 1H), 7.15-7.12 (m, 2H), 7.08 (d, J = 8.0 Hz, 1H), 5.45 (AB q, J = 26.0 Hz, 16.8 Hz, 2H), 5.36 (s, 2H), 3.48 (s, 3H), 3.38-3.36 (m, 1H), 3.15-3.12 (m, 1H), 2.97-2.92 (m, 1H), 2.88-2.81 (m, 1H), 2.77-2.71 (m, 1H), 1.91-1.88 (m, 1H), 1.72-1.68 (m, 1H), 1.59-1.50 (m, 1H), 1.31-1.18 (m, 1H). $^{13}$C NMR (125 MHz, CDCl , ppm) : 157.12, 153.87, 151.48, 149.97, 148.39, 140.04, 133.38, 132.96, 128.25, 127.24, 122.39, 116.82, 110.34, 104.78, 57.27, 50.56, 47.08, 46.89, 38.43, 32.43, 29.79, 22.92. MS (ESI): m/z 510.2 [M+H] .

**8f:** Dark yellow solid, 76.6% yield. $^{1}$H NMR (400 MHz, CDCl , ppm) : 8.05 (d, J = 8.5 Hz, 1H), 7.98 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.66-7.61 (m, 2H), 7.51 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.36 (t, J = 7.5 Hz, 1H), 7.27 (d, J = 6.5 Hz, 1H), 7.18 (d, J = 7.5 Hz, 1H), 5.59 (AB q, J = 25.0 Hz, 17.0 Hz, 2H), 5.47 (s, 2H), 3.58 (s, 3H), 3.39-3.35 (m, 1H), 3.24-3.22 (m, 1H), 2.95-2.91 (m, 2H), 2.74-2.70 (m, 1H), 2.04-1.99 (m, 1H), 1.93-1.91 (m, 1H), 1.76-1.69 (m, 1H). $^{13}$C NMR (125 MHz, CDCl , ppm) : 157.04, 156.82, 154.30, 151.60, 148.31, 147.61, 140.32, 136.45, 133.28, 132.81, 129.12 (2C), 128.07, 127.32, 127.09, 125.87, 118.78, 116.80, 110.40, 104.79, 58.10, 50.62, 47.05, 46.24, 33.08, 29.76, 29.55, 23.09. MS (ESI): m/z 521.3 [M+H] .

**8g:** Dark yellow solid, 66.2% yield. $^{1}$H NMR (400 MHz, CDCl , ppm) : 7.98 (d, J = 8.4 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.77-7.73 (m, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.52-7.47 (m, 2H), 7.34-7.31 (m, 1H), 7.17 (d, J = 7.6 Hz, 1H), 5.58 (AB q, J = 22.0 Hz, 16.8 Hz, 2H), 5.50 (s, 2H), 3.57 (s, 3H), 3.42-3.40 (m, 1H), 3.20-3.17 (m, 1H), 3.06-3.01 (m, 1H), 2.95-2.89 (m, 1H), 2.85 (s, 3H), 2.82-2.79 (m, 1H), 1.94-1.92 (m, 1H), 1.76-1.73 (m, 1H), 1.65-1.57 (m, 1H), 1.34-1.32 (m, 1H). $^{13}$C NMR (125 MHz, CDCl , ppm) : 168.47, 160.72, 156.17, 154.50, 151.64, 149.75, 147.89, 140.18, 133.38, 133.21, 132.90, 128.62, 128.17, 127.44, 126.68, 124.78, 123.00, 117.06, 110.47, 105.04, 54.14, 53.36, 51.21, 46.84, 46.22, 29.82, 29.57, 22.08, 21.66. MS (ESI): m/z 536.2 [M+H] .

### 5.3 Biological Activity Assay

Test compounds were dissolved in DMSO and prepared as 8-10 different concentration stock solutions ( 10  M final concentration). Various concentrations of test compound solutions were added to 100  L reaction systems contain-

ing human DPP-IV/DPP-VIII/DPP-IX enzymes (0.1 nM/L final concentration). After pre-incubation at 37 °C for 10 minutes, the highly sensitive fluorogenic substrate Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; Sigma-Aldrich, 10  M final concentration) was added to initiate the reaction. Fluorescence intensity was measured at excitation and emission wavelengths of 400 nm and 505 nm, respectively. Inhibition constants (IC ) were calculated from enzyme kinetic curves using standard mathematical models [**?**, **?**].

---

## 6. References

[1] (a) Savkur RS, Miller AR. Investigational PPAR-gamma agonists for the treatment of Type 2 diabetes [J]. Expert Opinion on Investigational Drugs, 2006, 15 (7): 763-778; (b) Li YQ, Feng ZQ, Song HR, et al. Advances in the study of glucokinase and small molecule glucokinase activators [J]. Acta Pharmaceutica Sinica (药学学报), 2006, 41 (5): 390-394.
[2] Alvaro F. Current treatment of diabetic nephropathy in patients with type II diabetes mellitus. Most recent progress [M]. Nefrologia: publicacion oficial de la Sociedad Espanola Nefrologia, 2001, 21 Suppl 3: 76-82.
[3] Tang L, Yang YS, Ji RY. Progress in studies on antidiabetic agents [J]. Acta Pharmaceutica Sinica (药学学报), 2001, 36 (9): 711-715.
[4] Ahren B, Schmitz O. GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes [J]. Hormone and Metabolic Research, 2004, 36 (11-12): 875-881.
[5] Thornberry NA, Weber AE. Discovery of JANUVIA (Sitagliptin), a selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes [J]. Current Topics in Medicinal Chemistry, 2007, 7 (6): 557-568.
[6] Ristic S, Bates PC. Vildagliptin: a novel DPP-4 inhibitor with pancreatic islet enhancement activity for treatment of patients with type 2 diabetes [J]. Drugs of Today, 2006, 42 (8): 519-531.
[7] Augeri DJ, Robl JA, Betebenner DA, et al. Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes [J]. Journal of Medicinal Chemistry, 2005, 48 (15): 5025-5037.
[8] Feng J, Zhang Z, Wallace MB, et al. Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV [J]. Journal of Medicinal Chemistry, 2007, 50 (10): 2297-2300.
[9] Eckhardt M, Langkopf E, Mark M, et al. 8-(3-(R)-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione (BI 1356), a highly potent, selective, long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes [J]. Journal of Medicinal Chemistry, 2007, 50 (26): 6450-6453.
[10] Noh YH, Lim HS, Jin SJ, et al. Effects of ketoconazole and rifampicin on the pharmacokinetics of gemigliptin, a dipeptidyl peptidase-IV inhibitor: a crossover drug-drug interaction study in healthy male Korean volunteers [J].

Clinical Therapeutics, 2012, 34 (5): 1182-1194.
[11] Yoshida T, Akahoshi F, Sakashita H, et al. Discovery and preclinical profile of teneligliptin (3-[(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl]pyrrolidin-2-ylcarbonyl]thiazolidine): a highly potent, selective, long-lasting and orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes [J]. Bioorganic & Medicinal Chemistry, 2012, 20 (19): 5705-5719.
[12] Zeng SG, Xie H, Zeng LL, et al. Discovery of potent dipeptidyl peptidase IV inhibitors through pharmacophore hybridization and hit-to-lead optimization [J]. Bioorganic & Medicinal Chemistry, 2013, 21 (7): 1749-1755.
[13] (a) Lloyd DG, Buenemann CL, Todorov NP, et al. Scaffold hopping in de novo design. Ligand generation in the absence of receptor information [J]. Journal of Medicinal Chemistry, 2004, 47 (3): 493-496; (b) Schneider G, Neidhart W, Giller T, et al. "Scaffold-Hopping" by Topological Pharmacophore Search: A Contribution to Virtual Screening [J]. Angewandte Chemie, 1999, 38 (19): 2894-2896.
[14] Deng JF, Peng L, Zhang GC, et al. The highly potent and selective dipeptidyl peptidase IV inhibitors bearing a thienopyrimidine scaffold effectively treat type 2 diabetes [J]. European Journal of Medicinal Chemistry, 2011, 46 (1): 71-76.
[15] Xie H, Zeng LL, Zeng SG, et al. Novel pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors based on pharmacokinetic property-driven optimization [J]. European Journal of Medicinal Chemistry, 2012, 52: 205-212.
[16] Deng JF, Peng L, Zhang GC, et al. The highly potent and selective dipeptidyl peptidase IV inhibitors bearing a thienopyrimidine scaffold effectively treat type 2 diabetes [J]. European Journal of Medicinal Chemistry, 2011, 46: 71-76.
[17] (a) Guo ZR. Strategy of molecular drug design: hits, leads and drug candidates [J]. Acta Pharmaceutica Sinica (药学学报), 2008, 43 (9): 898-904; (b) Guo ZR. Strategy of molecular design of drugs: the unification of macro-properties and micro-structures of a molecule [J]. Acta Pharmaceutica Sinica (药学学报), 2008, 43 (3): 227-233; (c) Guo ZR. Strategy of molecular drug design: activity and druggability [J]. Acta Pharmaceutica Sinica (药学学报), 2010, 45 (5), 539-547.
[18] Scharpe S, De Meester I, Vanhoof G, et al. Assay of dipeptidyl peptidase IV in serum by fluorometry of 4-methoxy-2-naphthylamine [J]. Clinical Chemistry, 1988, 34 (11): 2299-2301.

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