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Original article

Highly potent dipeptidyl peptidase IV inhibitors derived from Alogliptin through pharmacophore hybridization and lead optimization

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ABSTRACT

The superposition of the DPP-IV complex revealed that the butynyl group of Linagliptin can be freely switched with the cyanobenzyl group of Alogliptin. Thus, a pharmacophore hybridization of Alogliptin was initiated and led to a novel DPP-IV inhibitor, **11a**. Although it did not exhibit the desired activity (IC_{50} = 0.2 μM), compound **11a** acts as a lead compound, which triggered a resulting structural optimization and the formation of compound **11m**. A novel series of potent DPP-IV inhibitors represented by compound **11m** (IC_{50} = 0.4 nM) was ultimately obtained with a robust pharmacokinetic profile and superior *in vitro* and *in vivo* efficacy compared to Alogliptin.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that affects more than 371 million people worldwide. Type 2 diabetes (T2D, formerly referred to as non-insulin-dependent or adult-onset diabetes) results from an insufficient response to insulin by the body and comprises over 90% of diabetes patients. Undiagnosed T2D combined with corresponding multisystem complications causes disability of patients and brings with great social burden. Nonetheless, traditional T2D treatments are usually associated with undesired side effects, such as hypoglycemia, edema, and weight gain. Thus, more persistent efforts should be made to meet the medical needs of this disease [1].

Glucagon like peptide-1 (GLP-1) is one of the important incretin hormones that stimulate insulin release after an enteric glucose load [2]. Unlike other incretin hormones, GLP-1 exhibits glucose regulatory actions in diabetes patients. Bioactive GLP-1 has displayed multiple functions, such as an increase in insulin secretion and sensitivity, stimulation of β cell mass, and reduction of glucagon secretion [3]. Further understanding of the GLP-1 inactivation process revealed the essential role of dipeptidyl peptidase IV (DPP-IV) and contributed to the development of DPP-IV inhibitors. Orally bioavailable DPP-IV inhibitors can retain the biological function of GLP-1 by blocking DPP-IV truncation of bioactive GLP-1 [4–6]. Thus, these orally effective inhibitors gradually became the major intervention for type 2 diabetics and are represented by the following marketed drugs: Sitagliptin **1** [7,8], Vildaglipin **2** [9–11], Saxagliptin **3** [12], Alogliptin **4** [13], Linagliptin **5** [14], Gemigliptin **6** [15], and Teneligliptin **7** [16] (Fig. 1).

Previously, we identified several series of DPP-IV inhibitors derived from Alogliptin using multiple classic medicinal chemistry strategies [17–19]. Inspired by the approval of Linagliptin and both its superior potency and longer duration of action in comparison to the other marketed drugs [20], we carefully compared the binding mode of Linagliptin in the DPP-IV enzyme to that of Alogliptin.

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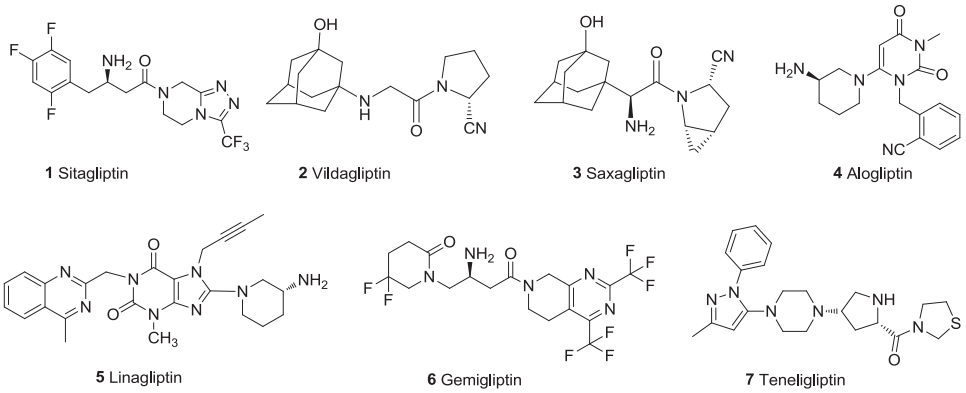


Fig. 1. Marketed DPP-IV inhibitors.

The similarity of the two molecules prompted us to note that the cyanobenzyl group of Alogliptin could be replaced with the butynyl group of Linagliptin. Herein, we reported how we rapidly generated a new lead compound (**11a**, IC_{50} = 198 nM) by pharmacophore hybridization and then generated highly potent DPP-IV inhibitors from structural optimization. Compound **11m** (IC_{50} = 0.4 nM) was ultimately obtained with a robust pharmacokinetic profile and better *in vitro* and *in vivo* efficacy than Alogliptin.

2. Chemistry

The synthesis of compounds **11a**–**o** is outlined in Scheme 1. Generally, the alkylation of commercially available material **8** with 1-bromo-2-butyne produced precursor **9**. Compounds **10a**–**o** were obtained by the *N*-alkylation of compound **9**. The final compounds **11a**–**o** were obtained by the replacement of the chloro group with a 3-(*R*)-amino-piperidinyl group.

3. Results and discussion

3.1. Computational comparison of the binding modes of Alogliptin and Linagliptin

Both Alogliptin and Linagliptin have the same 3-(*R*)-amino-piperidinyl as their functional group, yet Linagliptin is more potent than Alogliptin and other DPP-IV inhibitors on the drug market. We superimposed the crystal structures of the DPP-4 complex with Alogliptin [13] and Linagliptin [20] in Discovery Studio 3.0 (Accelrys, San Diego, CA). The superposition (Fig. 2) revealed that both 3-(*R*)-amino-piperidinyl groups interact with E205 and E206. In addition, the butynyl of Linagliptin and the cyanobenzyl of Alogliptin occupy the same S1 pocket of the DPP-IV enzyme. Thus, the structural superposition indicates that the two functional groups butynyl and cyanobenzyl can be freely switched between Alogliptin and Linagliptin.

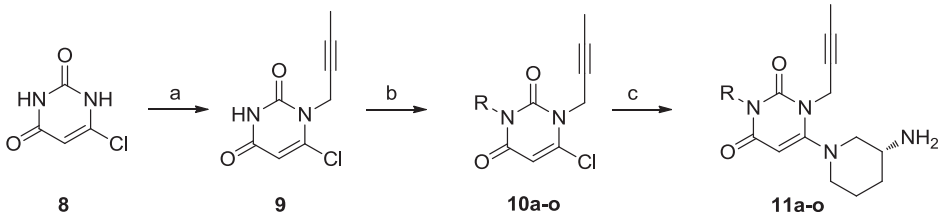
3.2. Generation of lead compound *11a*

We previously synthesized several series of DPP-IV inhibitors solely based on Alogliptin through the scaffold hopping strategy [17]. In this study, we kept the scaffold of Alogliptin constant and replaced the cyanobenzyl group with the butynyl group to immediately generate a novel compound, **11a** (Fig. 3). To our disappointment, compound **11a** did not exert the desired *in vitro* DPP-IV inhibitory activity (IC_{50} = 198 nM). However, encouraged by our previous successful experience of lead optimization of the DPP-IV inhibitor, compound **11a** was put forward for structural optimization towards the discovery of highly potent DPP-IV inhibitors.

3.3. N-3 position variations on compound *11a*

The crystal complex of Linagliptin and Alogliptin also suggests that there is a large space at the N-3 position of Alogliptin. Thus, a substitution variation at the N-3 position of compound **11a** was conducted and generated some of the DPP-IV inhibitors listed in Table 1. Although the butynyl group is a very important part of the pharmacophore, it (compound **11b**) did not exert activity as a functional group at the N-3 position. The replacement of benzyl **11c** at N-3 also led to the loss of activity. The addition of heterocyclic rings (compounds **11d**–**g**) immediately resulted in good activity and important selectivity over DPP-8/9 [21–23]. These data suggest that N-3 methylheteroaromatic group could improve DPP-IV activity and selectivity due to hydrogen bond effect.

Compound **11f** has an IC_{50} value of 3.1 nM and good pharmacokinetic properties with 63% oral bioavailability as well as 4.2 h of oral half-life in male Sprague Dawley (SD) rats. However, **11f**



Scheme 1. Synthesis of compounds **11a**–**o**. Reagents: (a) 1-bromo-2-butyne, DIEA, DMF, rt; (b) RX (X = Cl, Br, I), NaH, LiBr, DMF; (c) 3-(*R*)-aminopiperidine, $NaHCO_3$, 120 °C, $NaHCO_3$, EtOH.

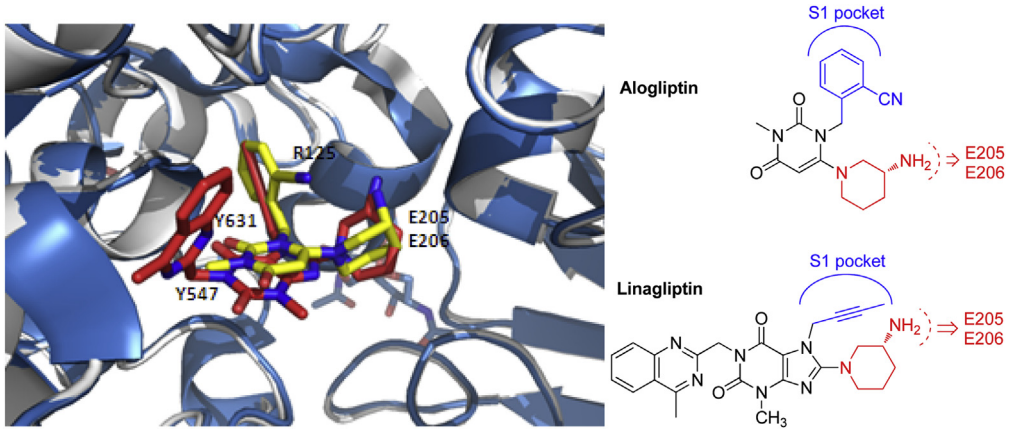


Fig. 2. Structural superposition of crystal structures of DPP-IV binding Alogliptin (PDB ID. 3G0B, blue and yellow) and Linagliptin (PDB ID. 2RGU, gray and red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

exhibits little inhibition on cytochrome P450 3A (IC_{50} = 97.9 μM), which may suggest a lower drug–drug interaction risk.

3.4. Further optimization of compound *11f*

Although compound **11f** exhibited the desired *in vitro* activity, selectivity and other related properties, we were still curious about its further optimization. Eight close analogs (**11h**–**o**, Table 2) of compound **11f** substituted on the quinoline ring were synthesized and screened. All of the compounds exhibited good activity and selectivity against DPP-IV, DPP-8/9 and CYP 3A. The analysis of the 6-halogen compounds **11h**–**j** demonstrated a good activity trend for bromo, chloro and fluoro as 6-substituents, which indicated that larger substituents are not suitable for this position. The effect of chloro substitution at the 6 (**11i**), 7 (**11m**), and 4 (**11o**) positions was closely compared and revealed that the 7 position is among the best. Thus we introduced 7-chloro group (compound **11m**) while keeping position 4 and 6 unsubstituted. Finally, compound **11m** was found to be the most active inhibitor in Table 2 and selected for further studies, since 7-fluroroquinolinylmethyl compound **11n** did not display a higher *in vitro* potency.

3.5. Pharmacological evaluations of compound *11m*

As described above, compound **11m** exhibits good activity and selectivity against DPP-IV (Table 2) and has a low risk of drug–drug interaction and heart QT interval prolongation with an IC_{50} value of 46.2 μM against CYP 3A and of over 100 μM against hERG. We conducted molecular docking to simulate the potential binding sites of DPP-IV by Discovery Studio 3.0 (Accelrys, San Diego, CA, Fig. 4). Compound **11m** (IC_{50} = 0.4 nM) interacts with serine 630 and glutamic acid 205, 206, which is much like the binding mode of Alogliptin and Linagliptin. This similarity of the binding mode revealed the feasibility of rapid generation of drug-like derivatives through molecular operation based on market drugs.

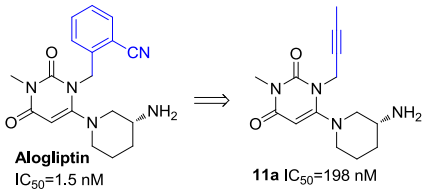


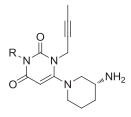
Fig. 3. Generation of **11a** by pharmacophore hybridization strategy.

In regard to its *in vivo* evaluations, compound **11m** showed 73.3% oral bioavailability and over 5 h of half-life (Table 3, Fig. 5). In a 24 h *in vivo* DPP-IV activity test in ICR mice, **11m** displayed stronger and longer inhibition of DPP-IV than Alogliptin at 1 mg/kg and 3 mg/kg (Fig. 6). The corresponding inhibition rates, listed in Table 4, were calculated and were consistent with the *in vitro* activity and *in vivo* half-life between Alogliptin and compound **11m** (Alogliptin: IC_{50} = 1.5 nM, $T_{1/2}$ = 2 h; **11m**: IC_{50} = 0.4 nM, $T_{1/2}$ = 5 h).

4. Conclusion

The accumulation of DPP-IV inhibitors as market drugs provides more elaborate information for drug design with classic medicinal strategies. The similarity of the binding modes of Alogliptin and Linagliptin prompted us to conduct a molecular operation between the two market drugs with pharmacophore hybridization and structural optimization. The replacement of the cyanobenzyl group of Alogliptin with the butynyl group of Linagliptin immediately generated the novel compound **11a** (IC_{50} = 198 nM) as a lead, which

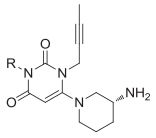
Table 1
DPP inhibitory profiles of compounds **11b**–**g**.

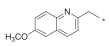
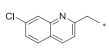


No.	R	IC_{50}		
		DPP-IV (nM)	DPP-8 (μM)	DPP-9 (μM)
11b		2177.5	>100	>100
11c		>10,000	>100	>100
11d		3.4	>100	>100
11e		22.0	>100	>100
11f		3.1	>100	>100
11g		31.3	>100	>100

Data represent means of at least two independent experiments.

Table 2
Inhibitory profiles of compounds **11h–o**.



No.	R	IC_{50}			
		DPP-IV (nM)	DPP-8 (μM)	DPP-9 (μM)	CYP 3A (μM)
11h		10.8	>100	>100	79.36 ± 14.78
11i		6.6	>100	>100	91.93 ± 30.21
11j		2.0	>100	>100	58.28 ± 9.47
11k		9.1	>100	>100	46.24 ± 8.07
11l		1.9	>100	>100	42.19 ± 6.19
11m		0.4	>100	>100	46.18 ± 6.80
11n		0.7	>100	>100	54.65 ± 7.25
11o		1.2	>100	84.5	14.08 ± 1.95
Alogliptin		1.5			>30 [13]

Data represent nominalized means of at least two independent experiments.

triggered a further structural modification. Variations on the N-3 position of compound **11a** resulted in a series of novel DPP-IV inhibitors with activity in the nanomolar range. The most potent inhibitor, **11m** (IC_{50} = 0.4 nM, F = 77%, $T_{1/2}$ = 5 h), was proven to bear a better and longer duration of *in vivo* DPP-IV activity than Alogliptin. This study demonstrated that the employment of classic medicinal chemistry strategies on marketed type 2 diabetes drugs is an efficient way to generate novel DPP-IV inhibitors.

5. Experimental section

5.1. Chemistry

All commercially available compounds and solvents were of reagent grade and were used without further treatment unless otherwise noted. Reactions were monitored by TLC using Qing Dao Hai Yang 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 fuming with an iodine steam. Silica gel column chromatography was performed on silica gel (200–300 mesh) from Qing Dao Hai Yang. NMR spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) or a Bruker NMR AVANCE 500 (500 MHz). Chemical shifts (δ) were recorded in ppm and coupling constants (J) 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). MS data were measured on an Agilent MSD-1200 ESI-MS system.

Table 3
Selected pharmacokinetic parameters for compound **11m** in male Sprague Dawley rats.

Dose (mg/kg) i.v./p.o.	Iv $T_{1/2}$ (h)	Oral $T_{1/2}$ (h)	poAUC$_{0-t}$ (μg h mL^{-1})	CLp (L h^{-1} kg^{-1})	$V_{Z/F}$ (L kg^{-1})	F%
5/25	5.65 ± 1.13	5.02 ± 1.39	17.58 ± 2.82	1.05 ± 0.15	8.65 ± 2.43	73

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

5.1.1. 1-(But-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**9**)

1-Bromo-2-butyne (9.4 mL, 0.11 mol) was added to a mixture of 6-chloropyrimidine-2,4(1*H*,3*H*)-dione (14.6 g, 0.1 mol), ethyldiisopropylamine (15 mL, 0.15 mol) and 250 mL of *N*,*N*-dimethylformamide. The reaction mixture was stirred overnight at ambient temperature. For work-up, the reaction mixture was diluted with approximately 300 mL of water. The light precipitate formed was suction filtered and washed with water. The filter cake was washed with diethyl ether and dried to give 1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1*H*,3*H*)-dione (**9**) as a yellow powder (17 g, yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (s, 1H), 4.75 (d, J = 2.0 Hz, 2H), 1.82 (t, J = 2.0 Hz, 3H). ESI-MS calculated for ($C_8H_8ClN_2O_2$) $[M + H]^+$, 199.03, found 199.0.

5.1.2. 1-(But-2-yn-1-yl)-6-chloro-3-methylpyrimidine-2,4(1H,3H)-dione (***10a***)

A mixture of **9** (500 mg, 2.5 mmol) and 5 mL of DMF was added to NaH (60% in oil, 2e q) at 0 °C and LiBr (3 eq) after being stirred for 5 min. The reaction mixture was subsequently cooled to 0 °C after being stirred for 20 min, and CH_3I (900 mg, 6.2 mmol) was added at 0 °C. The reaction mixture was stirred overnight at room temperature. The mixture was poured into 50 mL of water and extracted with ethyl acetate. The combined organic layers were washed with water and brine and dried over magnesium sulfate. After filtration, the solvent was removed, and the crude residue was purified by silica gel column chromatography to give 525 mg (98%) of the title

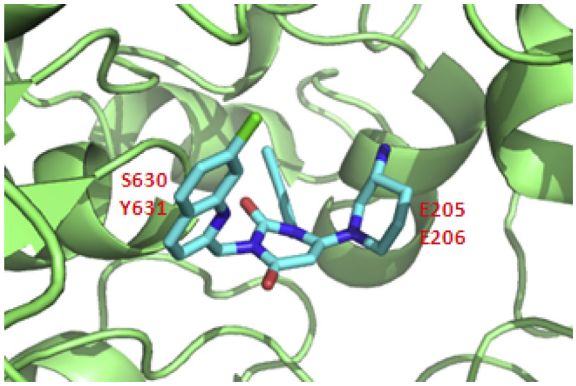


Fig. 4. Molecular docking of **11m** and DPP-IV enzyme (PDB ID. 3G0B).

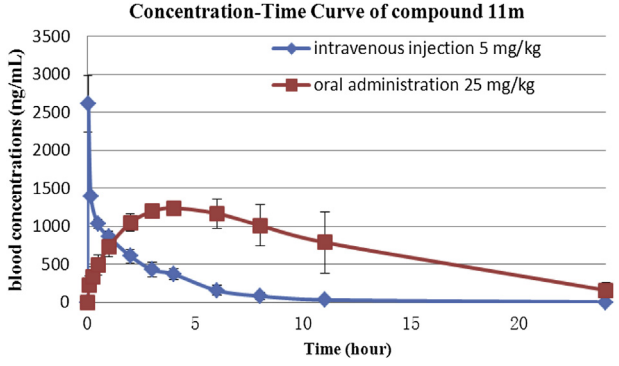


Fig. 5. Concentration-time curve of compound **11m** in a pharmacokinetic study in SD rats.

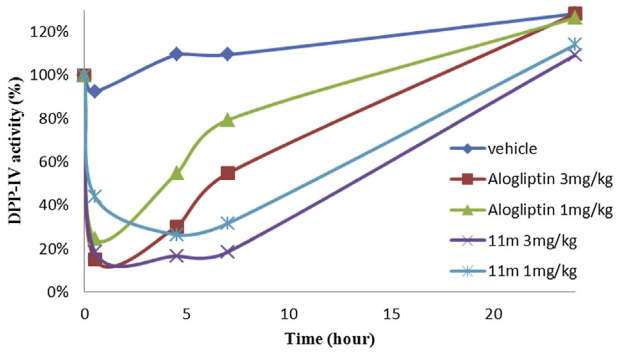


Fig. 6. Effect of oral administration of compound **11m** and Alogliptin on plasma DPP-IV in ICR mice at 1, 3 mg/kg.

compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 (s, 1H), 4.77 (d, J = 2.4 Hz, 2H), 3.33 (s, 3H), 1.81–1.80 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_9H_{10}ClN_2O_2$) $[M + H]^+$, 213.04, found 213.0.

*5.1.3. (R)-6-(3-Aminopiperidin-1-yl)-1,3-di(but-2-yn-1-yl) pyrimidine-2,4(1H,3H)-dione (**10b**)*

The title compound was prepared from 1-bromobut-2-yne in 90.0% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (s, 1H), 4.80–4.78 (q, J = 2.4 Hz, 2H), 4.64–4.62 (q, J = 2.4 Hz, 2H), 1.82–1.81 (t, J = 2.4 Hz, 3H), 1.78–1.77 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{12}H_{12}ClN_2O_2$) $[M + H]^+$, 251.06, found 251.0.

*5.1.4. 3-Benzyl-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**10c**)*

The title compound was prepared from (bromomethyl)benzene in 76.0% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.38 (m, 2H), 7.26–7.14 (m, 3H), 5.86 (s, 1H), 4.99 (s, 2H), 4.65–4.64 (d, J = 2.4 Hz, 2H), 1.73–1.72 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{15}H_{14}ClN_2O_2$) $[M + H]^+$, 289.07, found 289.1.

*5.1.5. 1-(But-2-yn-1-yl)-6-chloro-3-(pyrimidin-2-ylmethyl) pyrimidine-2,4(1H,3H)-dione (**10d**)*

The title compound was prepared from 2-(chloromethyl)pyrimidine in 71.0% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, J = 4.8 Hz, 2H), 7.09 (t, J = 4.8 Hz, 1H), 5.94 (s, 1H), 5.28 (s, 2H), 4.72–4.71 (m, 2H), 1.73 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{13}H_{12}ClN_4O_2$) $[M + H]^+$, 291.06, found 291.0.

*5.1.6. 3-((1H-Benzo[d]imidazol-2-yl)methyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**10e**)*

The title compound was prepared from 2-(chloromethyl)-1*H*-benzo[*d*]imidazole in 27.6% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59–7.56 (m, 2H), 7.24–7.21 (m, 2H), 5.89 (s, 1H), 5.40 (s, 2H), 4.75 (d, J = 2.4 Hz, 2H), 1.79 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{16}H_{14}ClN_4O_2$) $[M + H]^+$, 329.08, found 329.1.

Table 4
DPP-IV inhibition of compound **11m** in ICR mice.

	Dose	DPP-IV inhibition			
	mg/kg	0.5 h	4.5 h	7 h	24 h
Alogliptin	3	83.8%	71.5%	50.9%	0.8%
	1	72.6%	49.4%	27.6%	0.5%
11m	3	80.4%	84.7%	82.3%	13.5%
	1	51.8%	76.0%	71.6%	11.5%

*5.1.7. 1-(But-2-yn-1-yl)-6-chloro-3-(quinolin-2-ylmethyl) pyrimidine-2,4(1H,3H)-dione (**10f**)*

The title compound was prepared from 2-(chloromethyl)quinoline in 23.9% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.67–7.63 (m, 1H), 7.50–7.46 (m, 1H), 7.32 (d, J = 8.4 Hz, 1H), 6.05 (s, 1H), 5.43 (s, 2H), 4.82–4.80 (m, 2H), 1.82 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{18}H_{15}ClN_3O_2$) $[M + H]^+$, 340.08, found 340.1.

*5.1.8. 3-(Benzo[f]quinolin-3-ylmethyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**10g**)*

The title compound was prepared from 3-(chloromethyl)benzo[*f*]quinoline in 33.1% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (d, J = 8.8 Hz, 1H), 8.58 (d, J = 8.0 Hz, 1H), 7.95–7.90 (m, 3H), 7.69–7.60 (m, 2H), 7.49 (d, J = 8.4 Hz, 1H), 6.06 (s, 1H), 5.48 (s, 2H), 4.81 (d, J = 2.4 Hz, 2H), 1.83 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{22}H_{17}ClN_3O_2$) $[M + H]^+$, 390.10, found 390.1.

*5.1.9. 3-((6-Bromoquinolin-2-yl)methyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1H,3H)-dione (**10h**)*

The title compound was prepared from 6-bromo-2-(chloromethyl)quinoline in 32.4% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.8 Hz, 1H), 7.93 (d, J = 2.0 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.72–7.69 (m, 1H), 7.34 (d, J = 8.4 Hz, 1H), 6.05 (s, 1H), 5.40 (s, 2H), 4.81–4.80 (m, 2H), 1.82 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{18}H_{14}BrClN_3O_2$) $[M + H]^+$, 418.00,419.99, found 419.0, 421.00.

*5.1.10. 1-(But-2-yn-1-yl)-6-chloro-3-((6-chloroquinolin-2-yl) methyl)pyrimidine-2,4(1H,3H)-dione (**10i**)*

The title compound was prepared from 6-chloro-2-(chloromethyl)quinoline in 53.7% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 7.58–7.56 (m, 1H), 7.34 (d, J = 8.4 Hz, 1H), 6.04 (s, 1H), 5.40 (s, 2H), 4.81–4.79 (m, 2H), 1.82 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{18}H_{14}Cl_2N_3O_2$) $[M + H]^+$, 374.05, 376.04, found 374.0, 376.0.

*5.1.11. 1-(But-2-yn-1-(but-2-yn-1-yl))-6-chloro-3-((6-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**10j**)*

The title compound was prepared from 2-(chloromethyl)-6-fluoroquinoline in 58.9% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 8.4 Hz, 1H), 7.99 (dd, J = 5.2 Hz, 1H), 7.44–7.34 (m, 3H), 6.05 (s, 1H), 5.41 (s, 2H), 4.82–4.80 (m, 2H), 1.82 (t, J = 2.0 Hz, 3H). ESI-MS calculated for ($C_{18}H_{14}ClFN_3O_2$) $[M + H]^+$, 358.08, found 358.1.

*5.1.12. 1-(But-2-yn-1-yl)-6-chloro-3-((6-methylquinolin-2-yl) methyl)pyrimidine-2,4(1H,3H)-dione (**10k**)*

The title compound was prepared from 2-(chloromethyl)-6-methylquinoline in 51.9% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.87 (d, J = 1.6 Hz, 1H), 7.80–7.77 (m, 1H), 7.26 (d, J = 8.4 Hz, 1H), 5.99 (s, 1H), 5.25 (s, 2H), 4.77–4.75 (m, 2H), 2.72 (s, 3H), 1.80 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{19}H_{17}ClN_3O_2$) $[M + H]^+$, 354.10, found 354.1.

*5.1.13. 1-(But-2-yn-1-yl)-6-chloro-3-((6-methoxyquinolin-2-yl) methyl)pyrimidine-2,4(1H,3H)-dione (**10l**)*

The title compound was prepared from 2-(chloromethyl)-6-methoxyquinoline in 55.1% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 9.2 Hz, 1H), 7.31–7.27 (m, 2H), 7.03 (d, J = 2.8 Hz, 1H),

6.04 (s, 1H), 5.39 (s, 2H), 4.81–4.79 (m, 2H), 3.90 (s, 3H), 1.82 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{19}H_{17}ClN_3O_3$) $[M + H]^+$, 370.10, found 370.1.

*5.1.14. 1-(But-2-yn-1-yl)-6-chloro-3-((7-chloroquinolin-2-yl) methyl)pyrimidine-2,4(1H,3H)-dione (**10m**)*

The title compound was prepared from 7-chloro-2-(chloromethyl)quinoline in 58.8% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.4 Hz, 1H), 8.00 (s, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.43–7.41 (m, 1H), 7.32 (d, J = 8.4 Hz, 1H), 6.04 (s, 1H), 5.40 (s, 2H), 4.81–4.80 (m, 2H), 1.83 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{18}H_{14}Cl_2N_3O_2$) $[M + H]^+$, 374.05, 376.04, found 374.0, 376.0.

*5.1.15. 1-(But-2-yn-1-yl)-6-chloro-3-((7-fluoroquinolin-2-yl) methyl)pyrimidine-2,4(1H,3H)-dione (**10n**)*

The title compound was prepared from 2-(chloromethyl)-7-fluoroquinoline in 42.7% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 8.8 Hz, 1H), 7.77–7.73 (m, 1H), 7.63–7.60 (m, 1H), 7.30–7.25 (m, 2H), 6.05 (s, 1H), 5.41 (s, 2H), 4.82–4.81 (m, 2H), 1.83 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{18}H_{14}ClFN_3O_2$) $[M + H]^+$, 358.08, found 358.1.

*5.1.16. 1-(But-2-yn-1-yl)-6-chloro-3-((4-chloroquinolin-2-yl) methyl)pyrimidine-2,4(1H,3H)-dione (**10o**)*

The title compound was prepared from 4-chloro-2-(chloromethyl)quinoline in 58.0% yield according to the procedure for example **10a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.72–7.68 (m, 1H), 7.60–7.56 (m, 1H), 7.41 (s, 1H), 6.05 (s, 1H), 5.39 (s, 2H), 4.81–4.80 (m, 2H), 1.82 (t, J = 2.4 Hz, 3H). ESI-MS calculated for ($C_{18}H_{14}Cl_2N_3O_2$) $[M + H]^+$, 374.05, 376.04, found 374.0, 376.0.

*5.1.17. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-methylpyrimidine-2,4(1H,3H)-dione (**11a**)*

A mixture of **10a** (200 mg, 0.94 mmol), (*R*)-3-aminopiperidine dihydrochloride (244 mg, 1.41 mmol) and sodium bicarbonate (395 mg, 4.7 mmol) was stirred with 100 mg of activated MS (4A) in dry ethanol (10 mL) at 100 °C for 2 h. The reaction was concentrated in vacuo and purified by flash chromatography to yield 169 mg (65.2%) of the title compound. ^{1}H NMR (400 MHz, MeOD) δ 5.32 (s, 1H), 4.67–4.57 (m, 2H), 3.38–3.36 (m, 2H), 3.32 (s, 3H), 3.05–2.98 (m, 1H), 2.84–2.79 (m, 1H), 2.61–2.56 (m, 1H), 2.08–2.04 (m, 1H), 1.96–1.91 (m, 1H), 1.87 (t, J = 2.4 Hz, 3H), 1.83–1.74 (m, 1H), 1.44–1.35 (m, 1H); ^{13}C NMR (125 MHz, MeOD) δ 165.41, 161.55, 153.75, 89.32, 80.97, 74.80, 59.41, 52.82, 48.49, 36.62, 33.47, 28.12, 24.33, 3.07. ESI-MS calculated for ($C_{14}H_{21}N_4O_2$) $[M + H]^+$, 277.17, found 277.2.

*5.1.18. (R)-6-(3-Aminopiperidin-1-yl)-1,3-di(but-2-yn-1-yl) pyrimidine-2,4(1H,3H)-dione (**11b**)*

The title compound was prepared from 1,3-di(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1*H*,3*H*)-dione (**10b**) in 68.2% yield according to the procedure for example **11a**. ^{1}H NMR (400 MHz, MeOD) δ 5.32 (s, 1H), 4.47–4.58 (m, 4H), 3.41–3.40 (m, 1H), 3.37–3.36 (m, 1H), 3.05–2.98 (m, 1H), 2.86–2.81 (m, 1H), 2.63–2.58 (m, 1H), 2.08–2.04 (m, 1H), 1.97–1.93 (m, 1H), 1.87 (t, J = 2.0 Hz, 3H), 1.81 (t, J = 2.0 Hz, 3H), 1.78–1.74 (m, 1H), 1.45–1.35 (m, 1H); ^{13}C NMR (125 MHz, MeOD) δ 164.15, 161.83, 152.98, 89.32, 81.17, 78.93, 74.73, 74.55, 59.37, 52.79, 48.47, 36.77, 33.45, 31.57, 24.31, 3.11, 3.05. ESI-MS calculated for ($C_{17}H_{23}N_4O_2$) $[M + H]^+$, 315.18, found 315.2.

*5.1.19. (R)-6-(3-Aminopiperidin-1-yl)-3-benzyl-1-(but-2-yn-1-yl) pyrimidine-2,4(1H,3H)-dione (**11c**)*

The title compound was prepared from 3-benzyl-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1*H*,3*H*)-dione (**10c**) in 67.0% yield according to the procedure for example **11a**. ^{1}H NMR (400 MHz, MeOD) δ 7.35–7.31 (m, 2H), 7.29–7.22 (m, 3H), 5.30 (s, 1H), 5.07 (s, 2H), 4.56 (t, J = 18.4 Hz, 2H), 3.37–3.33 (m, 2H), 2.96–2.93 (m, 1H), 2.81–2.73 (m, 1H), 2.57–2.52 (m, 1H), 2.02–1.99 (m, 1H), 1.89–1.86 (m, 1H), 1.81 (s, 3H), 1.76–1.71 (m, 1H), 1.38–1.31 (m, 1H); ^{13}C NMR (125 MHz, MeOD) δ 165.10, 161.72, 153.66, 138.42, 129.35(2C), 129.15(2C), 128.41, 89.37, 81.10, 74.71, 59.36, 52.75, 48.45, 45.22, 36.83, 33.45, 24.31, 3.06. ESI-MS calculated for ($C_{20}H_{25}N_4O_2$) $[M + H]^+$, 353.20, found 353.2.

*5.1.20. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-(pyrimidin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**11d**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-(pyrimidin-2-ylmethyl)pyrimidine-2,4(1*H*,3*H*)-dione (**10d**) in 69.4% yield according to the procedure for example **11a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 4.8 Hz, 2H), 7.09 (t, J = 4.8 Hz, 1H), 5.32 (s, 2H), 5.25 (s, 1H), 4.51 (d, J = 2.0 Hz, 2H), 3.60–3.33 (m, 1H), 3.25–3.22 (m, 1H), 3.01–2.97 (m, 1H), 2.73–2.71 (m, 1H), 2.55–2.45 (m, 1H), 1.97–1.93 (m, 1H), 1.86–1.82 (m, 1H), 1.76 (s, 3H), 1.68–1.60 (m, 1H), 1.30–1.25 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.36, 163.07, 159.52, 157.17, 152.26, 119.30, 89.04, 80.25, 73.64, 56.72, 51.65, 47.50, 46.10, 35.78, 31.07, 22.94, 3.63; ESI-MS calculated for ($C_{18}H_{23}N_6O_2$) $[M + H]^+$, 355.19, found 355.2.

*5.1.21. (R)-3-((1H-Benzo[d]imidazol-2-yl)methyl)-6-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (**11e**)*

The title compound was prepared from 3-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-1-(but-2-yn-1-yl)-6-chloro pyrimidine-2,4(1*H*,3*H*)-dione (**10e**) in 35.1% yield according to the procedure for example **11a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57–7.41 (m, 2H), 7.19–1.12 (m, 2H), 5.31 (s, 2H), 5.18 (s, 1H), 4.40 (s, 2H), 3.22–3.19 (m, 3H), 3.01–2.95 (m, 1H), 2.66–2.59 (m, 1H), 2.46–2.40 (m, 1H), 1.98–1.88 (m, 1H), 1.75 (s, 3H), 1.64–1.55 (m, 1H), 1.32–1.26 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$ + MeOH) δ 162.87, 159.64, 151.96, 149.77, 137.81, 122.36, 114.73, 88.57, 80.37, 73.26, 56.36, 51.30, 49.82, 47.13, 35.71, 30.82, 22.63, 3.25; ESI-MS calculated for ($C_{21}H_{25}N_6O_2$) $[M + H]^+$, 393.20, found 393.20.

*5.1.22. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-(quinolin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**11f**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-(quinolin-2-ylmethyl)pyrimidine-2,4 (1*H*,3*H*)-dione (**10f**) in 89.7% yield according to the procedure for example **11a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.8 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.2 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 8.4 Hz, 1H), 5.40 (s, 2H), 5.28 (s, 1H), 4.53 (d, J = 2.4 Hz, 2H), 3.35–3.32 (m, 1H), 3.24–3.21 (m, 1H), 3.02–2.98 (m, 1H), 2.73–2.71 (m, 1H), 2.55–2.46 (m, 1H), 1.97–1.93 (m, 1H), 1.79–1.76 (m, 1H), 1.72 (s, 3H), 1.71–1.61 (m, 1H), 1.31–1.23 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.04, 159.51, 156.49, 152.39, 147.65, 136.56, 129.26, 129.21, 127.37, 127.25, 126.02, 119.09, 89.05, 80.15, 73.68, 58.42, 51.48, 47.47, 46.47, 35.65, 32.44, 23.09, 3.59; ESI-MS calculated for ($C_{23}H_{26}N_5O_2$) $[M + H]^+$, 404.21, found 404.2.

*5.1.23. (R)-6-(3-Aminopiperidin-1-yl)-3-(benzo[f]quinolin-3-ylmethyl)-1-(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (**11g**)*

The title compound was prepared from 3-(benzo[f]quinolin-3-ylmethyl)-1-(but-2-yn-1-yl)-6-chloropyrimidine-2,4(1*H*,3*H*)-dione (**10g**) in 85.8% yield according to the procedure for example **11a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, J = 8.8 Hz, 1H), 8.46 (d, J = 8.0 Hz, 1H), 7.88–7.80 (m, 3H), 7.58–7.50 (m, 2H), 7.41 (d, J = 8.4 Hz, 1H), 5.44 (s, 2H), 5.24 (s, 1H), 4.48 (d, J = 2.0 Hz, 2H), 3.26–3.23 (m, 1H), 3.17–3.14 (m, 1H), 2.92–2.87 (m, 1H), 2.64–2.59 (m, 1H), 2.43–2.39 (m, 1H), 1.88–1.84 (m, 1H), 1.77–1.76 (m, 1H), 1.73 (s, 3H), 1.62–1.53 (m, 1H), 1.22–1.16 (m, 1H); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 163.08, 159.48, 156.06, 152.35, 147.63, 131.46, 131.25, 130.58, 129.51, 128.53, 128.19, 126.90, 124.15, 122.47, 119.13, 89.05, 80.19, 73.66, 57.83, 51.51, 47.46, 46.29, 35.70, 31.98, 23.02, 3.61; ESI-MS calculated for ($C_{27}H_{28}N_5O_2$) $[M + H]^+$, 454.22, found 454.2.

*5.1.24. (R)-6-(3-Aminopiperidin-1-yl)-3-((6-bromoquinolin-2-yl) methyl)-1-(but-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (**11h**)*

The title compound was prepared from 3-((6-bromoquinolin-2-yl)methyl)-1-(but-2-yn-1-yl)-6-chloro pyrimidine-2,4(1*H*,3*H*)-dione (**10h**) in 86.3% yield according to the procedure for example **11a**. ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.8 Hz, 1H), 7.88–7.84 (m, 2H), 7.68–7.65 (m, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 5.37 (s, 2H), 5.28 (s, 1H), 4.54 (d, *J* = 2.4 Hz, 2H), 3.36–3.34 (m, 1H), 3.25–3.22 (m, 1H), 3.04–2.98 (m, 1H), 2.75–2.70 (m, 1H), 2.54–2.51 (m, 1H), 1.98–1.94 (m, 1H), 1.88–1.83 (m, 1H), 1.77 (t, *J* = 2.4 Hz, 3H), 1.74–1.64 (m, 1H), 1.33–1.27 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.99, 159.51, 156.92, 152.23, 146.03, 135.50, 132.58, 130.79, 129.31, 128.22, 119.99, 119.70, 88.83, 80.14, 73.55, 57.58, 51.43, 47.36, 46.25, 35.68, 31.77, 22.91, 3.52; ESI-MS calculated for ($C_{23}H_{25}BrN_5O_2$) $[M + H]^+$, 482.12, 484.12, found 482.1, 484.1.

*5.1.25. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((6-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**11i**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((6-chloroquinolin-2-yl)methyl) pyrimidine-2,4(1*H*,3*H*)-dione (**10i**) in 82.9% yield according to the procedure for example **11a**. ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.70 (d, *J* = 2.4 Hz, 1H), 7.55–7.52 (m, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 5.37 (s, 2H), 5.28 (s, 1H), 4.53 (d, *J* = 2.4 Hz, 2H), 3.35–3.32 (m, 1H), 3.24–3.21 (m, 1H), 3.03–2.97 (m, 1H), 2.74–2.69 (m, 1H), 2.53–2.50 (m, 1H), 1.97–1.93 (m, 1H), 1.87–1.82 (m, 1H), 1.76 (t, *J* = 2.4 Hz, 3H), 1.71–1.64 (m, 1H), 1.31–1.26 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.98, 159.44, 156.71, 152.15, 145.76, 135.57, 131.50, 130.59, 130.00, 127.64, 125.91, 119.96, 88.76, 80.11, 73.49, 57.06, 51.40, 47.29, 46.17, 35.67, 31.34, 22.82, 3.47; ESI-MS calculated for ($C_{23}H_{25}ClN_5O_2$) $[M + H]^+$, 438.17, found 438.2.

*5.1.26. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((6-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**11j**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((6-fluoroquinolin-2-yl)methyl) pyrimidine-2,4(1*H*,3*H*)-dione (**10j**) in 88.1% yield according to the procedure for example **11a**. ^1H NMR (400 MHz, $CDCl_3$) δ 7.99–7.96 (m, 2H), 7.40–7.29 (m, 3H), 5.37 (s, 2H), 5.28 (s, 1H), 4.53 (d, *J* = 2.4 Hz, 2H), 3.35–3.32 (m, 1H), 3.24–3.21 (m, 1H), 3.03–2.98 (m, 1H), 2.73–2.68 (m, 1H), 2.52–2.50 (m, 1H), 1.97–1.93 (m, 1H), 1.87–1.82 (m, 1H), 1.77 (t, *J* = 2.0 Hz, 3H), 1.71–1.61 (m, 1H), 1.31–1.26 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$ + MeOH) δ 163.12, 159.99, 159.52, 155.65, 152.15, 144.30, 136.11, 131.02, 127.67, 119.74, 119.43, 110.30, 88.76, 80.22, 73.26, 57.17, 51.42, 47.18, 46.04, 35.57, 31.41, 22.76, 3.27; ESI-MS calculated for ($C_{23}H_{25}FN_5O_2$) $[M + H]^+$, 422.20, found 422.2.

*5.1.27. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((6-methylquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**11k**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((6-methylquinolin-2-yl)methyl) pyrimidine-2,4(1*H*,3*H*)-dione (**10k**) in 82.1% yield according to the procedure for example **11a**. ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.4 Hz, 1H), 7.92–7.87 (m, 2H), 7.82–7.79 (m, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 5.23–5.22 (m, 3H), 4.49 (d, *J* = 1.2 Hz, 2H), 3.31–3.28 (m, 1H), 3.20–3.17 (m, 1H), 3.01–2.94 (m, 1H), 2.68 (s, 3H), 2.48–2.46 (m, 1H), 1.96–1.92 (m, 1H), 1.85–1.80 (m, 1H), 1.78 (t, *J* = 2.4 Hz, 3H), 1.71–1.59 (m, 2H), 1.29–1.27 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.86, 159.23, 158.70, 152.02, 146.98, 136.11, 134.27, 130.53, 128.27, 127.65, 125.98, 121.87, 88.83, 80.14, 73.48, 57.97, 51.29, 47.29, 43.98, 35.52, 32.08, 25.04, 22.91, 3.47; ESI-MS calculated for ($C_{24}H_{28}N_5O_2$) $[M + H]^+$, 418.22, found 418.2.

*5.1.28. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((6-methoxyquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**11l**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((6-methoxyquinolin-2-yl)methyl) pyrimidine-2,4(1*H*,3*H*)-dione (**10l**) in 88.4% yield according to the procedure for example **11a**. ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.4 Hz, 1H), 7.89 (d, *J* = 9.2 Hz, 1H), 7.28–7.24 (m, 2H), 7.00 (d, *J* = 2.4 Hz, 1H), 5.37 (s, 2H), 5.28 (s, 1H), 4.53 (d, *J* = 1.6 Hz, 2H), 3.86 (s, 3H), 3.34–3.32 (m, 1H), 3.24–3.21 (m, 1H), 3.03–2.99 (m, 1H), 2.73–2.68 (m, 1H), 2.53–2.50 (m, 1H), 1.98–1.94 (m, 1H), 1.86–1.83 (m, 1H), 1.78 (s, 3H), 1.69–1.65 (m, 1H), 1.29–1.27 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.06, 159.39, 157.35, 153.88, 152.29, 143.63, 135.34, 130.48, 128.13, 121.84, 119.42, 104.93, 88.97, 80.11, 73.63, 57.47, 55.36, 51.46, 47.39, 46.27, 35.65, 31.68, 22.94, 3.55; ESI-MS calculated for ($C_{24}H_{28}N_5O_3$) $[M + H]^+$, 434.22, found 434.2.

*5.1.29. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((7-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**11m**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((7-chloroquinolin-2-yl)methyl) pyrimidine-2,4(1*H*,3*H*)-dione (**10m**) in 66.4% yield according to the procedure for example **11a**. ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.41–7.39 (m, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 5.39 (s, 2H), 5.30 (s, 1H), 4.56 (d, *J* = 2.0 Hz, 2H), 3.38–3.36 (m, 1H), 3.27–3.24 (m, 1H), 3.06–3.02 (m, 1H), 2.77–2.72 (m, 1H), 2.56–2.54 (m, 1H), 1.99–1.96 (m, 1H), 1.90–1.86 (m, 1H), 1.79 (s, 3H), 1.75–1.70 (m, 1H), 1.34–1.29 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.97, 159.53, 157.48, 152.35, 147.94, 136.27, 134.96, 128.57, 128.25, 126.97, 125.56, 119.40, 88.96, 80.13, 73.62, 58.45, 51.47, 47.44, 46.16, 35.61, 32.47, 23.07, 3.55; ESI-MS calculated for ($C_{23}H_{25}ClN_5O_2$) $[M + H]^+$, 438.17, found 438.2.

*5.1.30. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((7-fluoroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**11n**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((7-fluoroquinolin-2-yl)methyl) pyrimidine-2,4(1*H*,3*H*)-dione (**10n**) in 92.5% yield according to the procedure for example **11a**. ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.8 Hz, 1H), 7.75–7.71 (m, 1H), 7.64–7.61 (m, 1H), 7.28–7.22 (m, 2H), 5.40 (s, 2H), 5.31 (s, 1H), 4.56 (d, *J* = 1.6 Hz, 2H), 3.38–3.36 (m, 1H), 3.27–3.24 (m, 1H), 3.06–3.02 (m, 1H), 2.77–2.72 (m, 1H), 2.56–2.54 (m, 1H), 2.00–1.97 (m, 1H), 1.90–1.86 (m, 1H), 1.80 (s, 3H), 1.75–1.72 (m, 1H), 1.34–1.29 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.96, 162.85, 159.50, 157.52, 152.30, 148.52, 136.36, 129.31, 124.16, 118.40, 116.35, 112.80, 88.91, 80.10, 73.59, 58.11, 51.44, 47.40, 46.22, 35.61, 32.19, 23.00, 3.51; ESI-MS calculated for ($C_{23}H_{25}FN_5O_2$) $[M + H]^+$, 422.20, found 422.2.

*5.1.31. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-3-((4-chloroquinolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**11o**)*

The title compound was prepared from 1-(but-2-yn-1-yl)-6-chloro-3-((4-chloroquinolin-2-yl)methyl) pyrimidine-2,4(1*H*,3*H*)-dione (**10o**) in 71.9% yield according to the procedure for example **11a**. ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.4 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.38 (s, 1H), 5.37 (s, 2H), 5.30 (s, 1H), 4.55 (s, 2H), 3.38–3.35 (m, 1H), 3.26–3.24 (m, 1H), 3.08–2.97 (m, 1H), 2.82–2.67 (m, 1H), 2.60–2.47 (m, 1H), 1.98–1.96 (m, 1H), 1.88–1.87 (m, 1H), 1.79 (s, 3H), 1.75–1.65 (m, 1H), 1.34–1.26 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.87, 159.59, 156.62, 152.35, 148.49, 142.98, 130.19, 129.63, 127.05, 125.47, 123.80, 119.16, 89.01, 80.28, 73.58, 58.68, 51.49, 47.49, 46.22, 35.72, 32.66,

23.12, 14.06, 3.60; ESI-MS calculated for ($C_{23}H_{25}ClN_5O_2$) $[M + H]^+$, 438.17, found 438.17.

5.2. In vitro inhibition of DPP-IV, DPP-8 and DPP-9

Solutions of test compounds at varying concentrations (final concentration ≤10 mM) 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 min at ambient temperature before the reaction was initiated by the addition of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin, Sigma–Aldrich, 10 μM final concentration). The total volume of the reaction mixture was 100 μL. The kinetics of the reaction was monitored (excitation at 400 nm, emission at 505 nm) for 5–10 min, or an endpoint was measured after 10 min. Inhibition constants (IC_{50}) 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 the test compounds dissolved in distilled water at a single dose of 25 mg/kg by oral administration and 5 mg/mL by injection. Blood samples of 100–200 μL were collected from the orbit at 11 time points within 24 h. The blood concentration of the test compounds was determined by LC-MS/MS. The PK parameters were obtained from the pharmacokinetic software DAS. 2.0.

5.4. In vivo efficacy study in ICR mice

Adult male ICR mice (at least $n = 4$ per group) were orally gavaged with the test compounds dissolved in distilled water at a single dose of 1 mg/kg or 3 mg/kg. Blood samples of 20–25 μL were collected from the orbit at the time points indicated in Fig. 6 for 24 h and the plasma fraction was kept frozen until DPP-IV activity measurement. The plasma DPP-IV activity was determined by 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 that was composed of 25 mM tris(hydroxymethyl)-aminomethane HCl (PH 7.4), 140 mM NaCl, 10 mM KCl and 0.1% bovine serum albumin, the fluorescence was determined by using Thermo Scientific Fluoroskan Ascent FL (excitation at 400 nm and emission at 505 nm). One unit of activity is defined as the amount of enzyme that produces 1 μM products per minute. The DPP-IV relative activity in plasma was described as activity (indicated time points)/activity (initial point).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.08.010.

References

[1] P.A. Hollander, P. Kushner, Type 2 diabetes comorbidities and treatment challenges: rationale for DPP-4 inhibitors, Postgraduate Medicine 122 (2010) 71–80.
[2] B. Kreymann, G. Williams, M.A. Ghatei, S.R. Bloom, Glucagon-like peptide-1 7-36: a physiological incretin in man, Lancet 2 (1987) 1300–1304.
[3] B. Ahren, DPP-4 inhibitors, Best Practice & Research: Clinical Endocrinology & Metabolism 21 (2007) 517–533.
[4] T. Abel, J. Feher, A new therapeutic possibility for type 2 diabetes: DPP-4 inhibitors (sitagliptin), Orvosi Hetilap 151 (2010) 1012–1016.
[5] C.F. Deacon, J.J. Holst, R.D. Carr, Glucagon-like peptide-1: a basis for new approaches to the management of diabetes, Drugs of Today (Barcelona, Spain) 35 (1999) 159–170.
[6] B. Gallwitz, Diabetes therapy—novel drugs to come (DPP-4 inhibitors, GLP-1 agonists, SGLT-2 inhibitors), MMW – Fortschritte der Medizin 152 (2010) 43–44.
[7] D. Kim, L. Wang, M. Beconi, G.J. Eiermann, M.H. Fisher, H. He, G.J. Hickey, J.E. Kowalchick, B. Leiting, K. Lyons, F. Marsilio, M.E. McCann, R.A. Patel, A. Petrov, G. Scapin, S.B. Patel, R.S. Roy, J.K. Wu, M.J. Wyvratt, B.B. Zhang, L. Zhu, N.A. Thornberry, A.E. Weber, (2*R*)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes, Journal of Medicinal Chemistry 48 (2005) 141–151.
[8] D. Kim, J.E. Kowalchick, L.L. Brockunier, E.R. Parmee, G.J. Eiermann, M.H. Fisher, H. He, B. Leiting, K. Lyons, G. Scapin, S.B. Patel, A. Petrov, K.D. Pryor, R.S. Roy, J.K. Wu, X. Zhang, M.J. Wyvratt, B.B. Zhang, L. Zhu, N.A. Thornberry, A.E. Weber, Discovery of potent and selective dipeptidyl peptidase IV inhibitors derived from beta-aminoamides bearing substituted triazolopiperazines, Journal of Medicinal Chemistry 51 (2008) 589–602.
[9] E.B. Villhauer, J.A. Brinkman, G.B. Naderi, B.E. Dunning, B.L. Mangold, M.D. Mone, M.E. Russell, S.C. Weldon, T.E. Hughes, 1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(*S*)-pyrrolidinecarbon itrile: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties, Journal of Medicinal Chemistry 45 (2002) 2362–2365.
[10] E. Bosi, R.P. Camisasca, C. Collober, E. Rochotte, A.J. Garber, Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin, Diabetes Care 30 (2007) 890–895.
[11] V. Fonseca, A. Schweizer, D. Albrecht, M.A. Baron, I. Chang, S. Dejager, Addition of vildagliptin to insulin improves glycaemic control in type 2 diabetes, Diabetologia 50 (2007) 1148–1155.
[12] D.J. Augeri, J.A. Robl, D.A. Betebenner, D.R. Magnin, A. Khanna, J.G. Robertson, A. Wang, L.M. Simpkins, P. Taunk, Q. Huang, S.P. Han, B. Abboa-Offei, M. Cap, L. Xin, L. Tao, E. Tozzo, G.E. Welzel, D.M. Egan, J. Marcinkeviciene, S.Y. Chang, S.A. Biller, M.S. Kirby, R.A. Parker, L.G. Hamann, 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, Journal of Medicinal Chemistry 48 (2005) 5025–5037.
[13] J. Feng, Z. Zhang, M.B. Wallace, J.A. Stafford, S.W. Kaldor, D.B. Kassel, M. Navre, L. Shi, R.J. Skene, T. Asakawa, K. Takeuchi, R. Xu, D.R. Webb, S.L. Gwaltney 2nd, Discovery of Alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV, Journal of Medicinal Chemistry 50 (2007) 2297–2300.
[14] M. Eckhardt, E. Langkop, M. Mark, M. Tadayyon, L. Thomas, H. Nar, W. Pfrengle, B. Guth, R. Lotz, P. Sieger, H. Fuchs, F. Himmelsbach, 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, Journal of Medicinal Chemistry 50 (2007) 6450–6453.
[15] Y.H. Noh, H.S. Lim, S.J. Jin, M.J. Kim, Y.H. Kim, H.R. Sung, H.Y. Choi, K.S. Bae, 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, Clinical Therapeutics 34 (2012) 1182–1194.
[16] T. Yoshida, F. Akahoshi, H. Sakashita, H. Kitajima, M. Nakamura, S. Sonda, M. Takeuchi, Y. Tanaka, N. Ueda, S. Sekiguchi, T. Ishige, K. Shima, M. Nabeno, Y. Abe, J. Anabuki, A. Soejima, K. Yoshida, Y. Takashina, S. Ishii, S. Kiuchi, S. Fukuda, R. Tsutsumiuchi, K. Kosaka, T. Murozono, Y. Nakamaru, H. Utsumi, N. Masutomi, H. Kishida, I. Miyaguchi, Y. Hayashi, Discovery and preclinical profile of teneligliptin (3-[(2*S*,4*S*)-4-[4-(3-methyl-1-phenyl-1*H*-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, Bioorganic & Medicinal Chemistry 20 (2012) 5705–5719.
[17] J. Deng, L. Peng, G. Zhang, X. Lan, C. Li, F. Chen, Y. Zhou, Z. Lin, L. Chen, R. Dai, H. Xu, L. Yang, X. Zhang, W. Hu, The highly potent and selective dipeptidyl peptidase IV inhibitors bearing a thienopyrimidine scaffold effectively treat type 2 diabetes, European Journal of Medicinal Chemistry 46 (2011) 71–76.
[18] H. Xie, L. Zeng, S. Zeng, X. Lu, G. Zhang, X. Zhao, N. Cheng, Z. Tu, Z. Li, H. Xu, L. Yang, X. Zhang, M. Huang, J. Zhao, W. Hu, Novel pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors based on pharmacokinetic property-driven optimization, European Journal of Medicinal Chemistry 52 (2012) 205–212.

[19] S. Zeng, H. Xie, L.L. Zeng, X. Lu, X. Zhao, G.C. Zhang, Z.C. Tu, H.J. Xu, L. Yang, X.Q. Zhang, W. Hu, Discovery of potent dipeptidyl peptidase IV inhibitors through pharmacophore hybridization and hit-to-lead optimization, Bioorganic & Medicinal Chemistry 21 (2013) 1749–1755.
[20] L. Thomas, M. Eckhardt, E. Langkopf, M. Tadayyon, F. Himmelsbach, M. Mark, (*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylm ethyl)-3,7-dihydro-purine-2,6-dione (BI 1356), a novel xanthine-based dipeptidyl peptidase 4 inhibitor, has a superior potency and longer duration of action compared with other dipeptidyl peptidase-4 inhibitors, Journal of Pharmacology and Experimental Therapeutics 325 (2008) 175–182.
[21] Z. Pei, From the bench to the bedside: dipeptidyl peptidase IV inhibitors, a new class of oral antihyperglycemic agents, Current Opinion in Drug Discovery & Development 11 (2008) 512–532.
[22] P.E. Wiedeman, DPPIV inhibition: promising therapy for the treatment of type 2 diabetes, Progress in Medicinal Chemistry 45 (2007) 63–109.
[23] B.D. Green, P.R. Flatt, C.J. Bailey, Inhibition of dipeptidylpeptidase IV activity as a therapy of type 2 diabetes, Expert Opinion on Emerging Drugs 11 (2006) 525–539.