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Brief Article

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Discovery of Highly Polar β-homophenylalanine Derivatives as Non-systemic Intestine-Targeted Dipeptidyl Peptidase IV Inhibitors

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KEYWORDS Non-systemic intestine-targeted, DPPIV, Inhibitors, β-homophenylalanine, Highly polar

ABSTRACT: Although intensively expressed within intestine, the precise roles of intestinal dipeptidyl peptidase IV (DPPIV) in numerous pathologies remain incompletely understood. Here, we firstly reported a non-systemic intestine-targeted (NSIT) DPPIV inhibitor with β-homophenylalanine scaffold, compound **7**, which selectively inhibited the intestinal rather than plasmatic DPPIV at an oral dosage as high as 30 mg/kg. We expect that compound **7** could serve as a qualified tissues-selective tool to determine undetected physiological or pathological roles of intestinal DPPIV.

INTRODUCTION

Dipeptidyl peptidase IV (DPPIV), also referred as T-cell antigen CD26, is a 110-kDa glycoprotein that exists in two major isoforms, a membrane-anchored form and a soluble form[1]. DPPIV is viewed as a complex protein and plays important roles in many physiological processes[1]. For example, as a serine peptidase, DPPIV can regulate the catabolism of over 40 bioactive peptides, which are related to metabolism, nociception, psychoneuroendocrine regulation, cardiovascular adaptation, and so on[2]. In addition, it is also involved in cell adhesion and the immune response through its non-enzymatic functions via interactions with contiguous membrane or extra-cellular matrix proteins[2]. Furthermore, the extensive distribution of the DPPIV protein further amplifies its complexity. Soluble DPPIV principally circulates in body fluids, such as plasma, cerebrospinal fluid and seminal fluid, while the membrane-bound form can be found in numerous tissues including the lung, intestine, brain, pancreas, kidney, blood vessels, liver, lymph nodes, and spleen, and is predominately situated on the surface of epithelial, endothelial and immune cells[3].

Therefore, because of the variety of its enzymatic and non-enzymatic functions, the panoply of its bioactive substrates and binding partners and the universality of its distribution, changes in DPPIV expression and/or activity are closely associated with a great number of pathological conditions, for example, metabolic disorders[1], tumors[4], autoimmune[5] and inflammatory diseases[2, 6]. Among them, the relationship between DPPIV and glucose homeostasis is extensively studied and more than 12 DPPIV inhibitors are currently approved to treat type 2 diabetes[7, 8]. Research on DPPIV inhibitors' glucoregulatory mechanisms found that changes to the incretin hormones pathway, such as preventing the inactivation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), and several incretin-independent pathways collectively contribute to the antidiabetic actions of DPPIV[1, 9, 10].

Usually, the biological roles of DPPIV will change with its location and the molecular targets it interacts with[2]. However, the specific roles of DPPIV derived from different tissues or cells in many physiological and pathological processes remain inadequately understood. Even for the deeply studied hypoglycemic effect, the precise roles of DPPIV-containing cells and tissues were only identified by Mulvihill and coworkers in 2017, eleven years after the first DPPIV inhibitor was marketed. They found that DPPIV from endothelial and hematopoietic cells contributed to glucose-lowering actions, while enterocyte DPPIV, although representing substantial intestinal DPPIV activity, did not produce significant effects on the plasma DPPIV activity and incretin hormone levels[11]. Furthermore, the precise role of enterocyte DPPIV in other physiological processes is also unknown and previous studies on DPPIV usually employed systemic inhibitors or conventional gene knockout and therefore could not accurately reflect the consequences of DPPIV inhibition in specific tissues. In view of the intensive distribution of the DPPIV enzyme in the intestine and the high involvement of the intestine in a large number of physiological processes[3, 12, 13], we expect to identify a series of non-systemic intestine-targeted (NSIT)

DPPIV inhibitors that could be used as chemical tools to determine the biological functions of intestinal DPPIV.

MOLECULAR DESIGN

Currently, NSIT drugs have been designed for various diseases including inflammatory bowel disease, diabetes mellitus and obesity [14]. A number of approaches have been successively developed to obtain NSIT drug candidates, resulting in three categories of NSIT drugs: non-absorbable prodrugs, rapidly metabolized soft drugs and compounds with physicochemical properties outside "Lipinskis Rule of Five"[14]. For instance, compound **2**, an apical sodium-dependent bile acid transporter (ASBT) inhibitor, is an example of the last class (**Figure 1A**)[15]. By introducing a highly polar and zwitterionic aminodiacid moiety to the pharmacophores (compound **1**), the structural units accounting for nearly all of the pharmacological activity, the resulting compound **2** generated moderately increased potency (4-fold) but substantially decreased the cLogP value, cellular permeability and portal vein and systemic drug levels after oral administration (**Figure 1A**)[15]. Consequently, the introduction of highly polar moieties, also known as kinetophores that minimally influence the intrinsic pharmacological activity of pharmacophores but significantly alter specific physicochemical characteristics, facilitate the non-systemic targeting of the intestine[14].

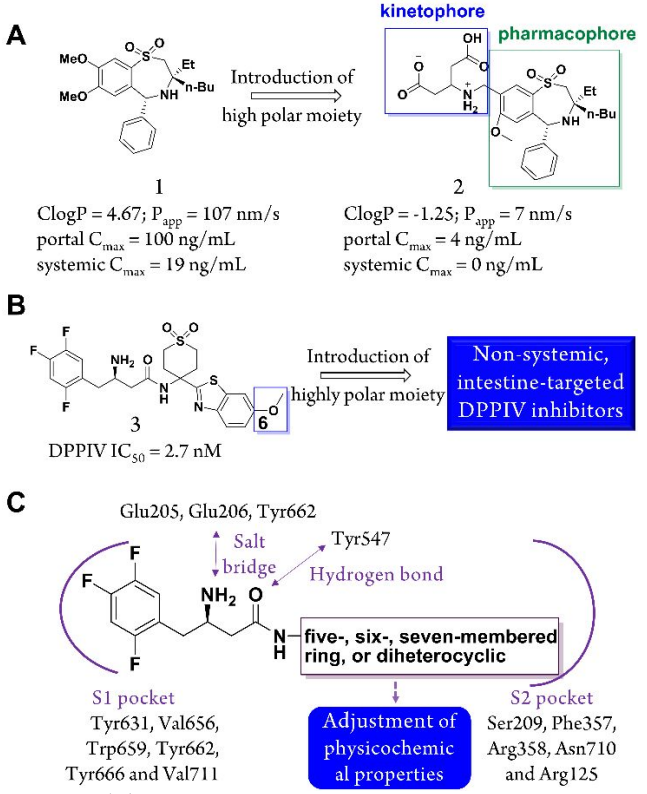
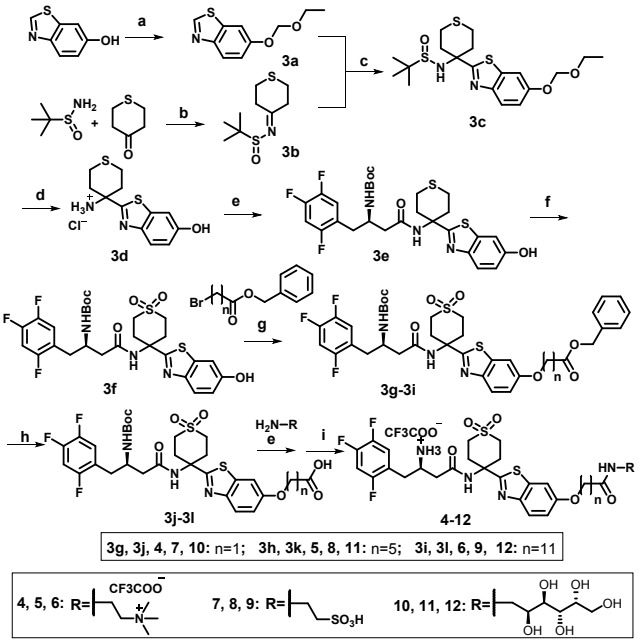


Figure 1. (**A**) The design of NSIT compounds with high polar moieties. (**B**) The design of the NSIT DPPIV inhibitors. The site where the highly polar moieties were introduced was labeled with a blue box. (**C**) The binding mode of β-homophenylalanine derivatives with DPPIV.

Inspired by the design of compound **2**, we attempted to obtain NSIT DPPIV inhibitors by introducing highly polar kinetophores to a known systemic DPPIV inhibitor, namely, pharmacophore. According to the binding modes of DPPIV with known inhibitors, the S2 binding pocket, formed by Phe357, Ser209, and Arg358, is large enough to tolerate multifarious changes in its substituents without inducing significantly adverse effect on the pharmacological activity [16]. Therefore, we selected a β-homophenylalanine derivative, compound **3**[17], as the starting point for the NSIT DPPIV inhibitors discovery program (**Figure 1B**), and focused exploration on the benzothiazole moiety that is in the S2 pocket (**Figure 1C**)[16]. There are three reasons for this choice: first, β-homophenylalanine is a reliable and widely studied scaffold among DPPIV inhibitors, as sitagliptin, the first marketed DPPIV inhibitor, is a β-homophenylalanine derivative[7]; second, these derivatives are convenient to prepare and the β-homophenylalanine moiety is commercially available; finally, position 6 of the benzothiazole moiety seems to be a preferable choice to introduce the kinetophores (**Figure 1B**), and a previous study revealed that this position could tolerate relatively large substituents such as a 2-morpholinoethyoxyl group[17]. Collectively, we adjusted the pharmacokinetic behavior of compound **3** to meet the need of high intestinal retention by introducing highly polar kinetophores to position 6 of the benzothiazole moiety (labeled by a blue box in **Figure 1B**).

CHEMISTRY

Scheme 1. Synthesis of Compounds **4**-**12**[a].



[a] **Reagents and conditions:** (a) NaH, dry THF, rt; (b) dry THF, rt; (c) nBu-Li, dry THF, -78°C; (d) acetylchloride, EtOH, rt; (e) HATU, DIPEA, DMF, rt; (f) mCPBA, DCM; (g)K_2CO_3, DMF, rt; (h) Pd/C, H_2, MeOH, rt; (i) DCM/TFA (v/v = 5:1), rt.

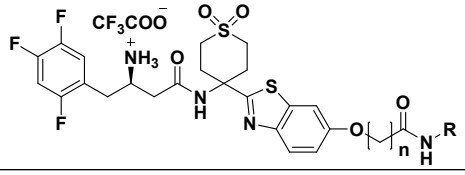
The designed compounds **4**–**12** were synthesized by using the route outlined in **Scheme 1**. Briefly, the attack of 6-(ethoxymethoxy) benzo[*d*]thiazole (**3a**) to the sulfinamide intermediate (**3b**) generated the intermediate **3c**. After the deprotection of **3c** and condensation with β-homophenylalanine, the pharmacophore (**3f**) was obtained from the oxidization of the above condensation product, **3e**. Subsequently, the intermediates **3g**-**3i** were obtained by a nucleophilic substitution reaction between **3f** and benzyl aliphatic esters containing bromide substitution. The carboxyl group was exposed under reductive conditions, and then condensed with different highly polar moieties to obtain compounds **4**-**12**. Detailed

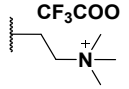
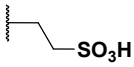
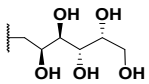
synthetic procedures and structural characterization are provided in the Supporting Information.

RESULTS

In vitro DPPIV inhibitory potency, aqueous solubility and Caco-2 permeability assays. To cover multiple structural types, we characterized the kinetophores with sulfonic acid, quaternary ammonium salt and saccharide fragments, which will present anion, cation and neutral forms, respectively, under the physiological conditions. In addition, to minimize the potential adverse effect on the pre-existing inhibitory activity, these three hydrophilic fragments were extended far from the benzothiazole moiety by an aliphatic chain containing 2, 6, or 12 carbon atoms. Consequently, we synthesized nine compounds in these three categories and tested their ability to inhibit the DPPIV activity of mouse plasma *in vitro*.

Table 1. The results of the inhibitory potency of compounds on mouse plasma DPPIV *in vitro*.



Compds	R	n	$IC_{50} \pm SD$ (nM)	ClogP[a]
4		1	1.4 ± 0.5	-1.9
5		5	3.0 ± 0.8	-0.8
6		11	115.7 ± 32.7	2.4
7		1	1.1 ± 0.3	-2.4
8		5	2.0 ± 0.9	-1.3
9		11	81.8 ± 22.3	1.9
10		1	2.2 ± 0.4	0.7
11		5	3.5 ± 1.4	1.7
12		11	168.1 ± 50.4	4.9
Sitagliptin	-	-	14.7 ± 1.8	0.4

[a]Calculated by ChemDraw Professional 16.0.

As shown in **Table 1**, compounds with the sulfonic acid substituent (**7**-**9**) showed the best inhibitory potency of mouse plasma DPPIV compared with the compounds containing the other two high polar substituents (compounds **4**-**6** and **10**-**12**) when linked by an aliphatic chain of the same length. The 2 carbon atom-length aliphatic chain (compounds **4**, **7**, and **10**) might be the optimal linker, as the inhibitory activity decreased as the length of the chain increased. In addition, the compounds with short linkers exhibited negative ClogP values (compounds **4**, **5**, **7**, and **8**), suggesting a high hydrophilcity (**Table 1**). We then determined the aqueous solubility of the six compounds with good inhibitory activity under three different conditions (**Table 2**). The results show that compounds **4**, **7**, and **10**, whose highly polar moiety was linked by 2 carbon atoms, exhibited decent aqueous solubility under all three conditions, while a longer aliphatic chain would interfere with the solubility to some extent (compounds **8** and **11**). Collectively, the compounds linked a polar moiety by 2 carbon atoms possessed preferable DPPIV inhibitory potency and aqueous solubility.

A further Caco-2 permeability evaluation found that the above six compounds had a much low apparent permeability coefficient (Papp A to B less than 0.4 nm/s) compared to sitagliptin with moderate permeability, suggesting a poor ability to penetrate the membrane of Caco-2 cells (**Table 2**). A longer linker might facilitate the corresponding compound become a substrate of efflux transporters, resulting in a high efflux ratio (compounds **5**, **8**, and **11**). On the contrary, compound **10** showed the lowest efflux ratio among tested compounds, indicating that it might be a suitable substrate of uptake transporters, which might be easy to be absorbed into the blood. Taken together, these results showed that compounds **4** and **7** owned excellent potency, decent aqueous solubility and poor membrane permeability, preferably meeting the designing goals. Then, they were selected for further study.

Table 2. The result of Caco-2 permeability

Compds	Solubility			Mean Papp (nm/s)		Efflux Ratio
	pH 6.8 (μM)	pH 7.4 (μM)	pH 7.0 (μM)	Apical to Basal	Basal to Apical	
4	> 50	> 50	> 50	0.2	0.04	0.2
5	> 50	> 50	> 50	0.2	1.1	6
7	> 50	> 50	> 50	0.4	0.1	0.3
8	20 ~ 50	> 50	> 50	0.02	5.9	295
10	> 50	> 50	> 50	0.2	0.01	0.05
11	> 50	20 ~ 50	20 ~ 50	0.2	2.7	13
Sitagliptin	-	-	-	2.5	122.4	48.7

In vitro stability evaluation in human and mouse intestinal S9 fraction. To explore whether there are the metabolites that generated within the intestine and could enter systemic circulation and inhibit systemic DPPIV, we evaluated the intestinal stability of compounds **4** and **7** in by commercially human and mouse intestinal S9 fraction. Compared to the positive controls, testosterone and 7-hydroxycoumarin (7-HC), we were delighted to find that compounds **4** and **7** showed decent metabolic stability against both phase I and phase II enzymes in human and mouse intestinal S9 fractions (**Table S1**), indicating the low possibility of generating systemic metabolites.

In vivo pharmacokinetic study and plasmic and intestinal DPPIV activity determination. We subsequently determined the plasma exposure of compounds **4** and **7** after oral administration at 30 mg/kg. Surprisingly, although compounds **4** and **7** had similar Caco-2 cell permeability, they showed great difference in plasma concentrations after oral administration. As shown in **Figure S1**, compound **4** possessed 45-fold and 38-fold higher plasma concentration than that of compound **7** at the 2 h and 4 h time points, respectively.

Furthermore, we characterized the alteration of plasmic and intestinal DPPIV activity after oral administration of compounds **4** and **7**, taking sitagliptin as a positive comparison. Although the dosage was as high as 30 mg/kg, the plasmic DPPIV activity of the compound **7**-treated group showed a trend similar to that of the control group, indicating a negligible impact on the plasma DPPIV enzyme (**Figure 2A**). However, compound **4** and sitagliptin could significantly inhibit the plasma DPPIV activity even at the 8 h time point after oral administration (**Figure S3A**). Based on the results of oral plasma exposure, we supposed that the significant plasma

DPPIV inhibition of compound **4** might result from its high concentration in the plasma. In the intestine, both the compounds **4**-treated, **7**-treated and sitagliptin-treated groups showed intensive DPPIV inhibition in all three selected parts of the intestine (**Figure 2B** and **Figure S3B**). Notably, the significant intestinal DPPIV inhibition of compound **7** could also be reflected by its high concentration within the intestine (**Figure S2**).

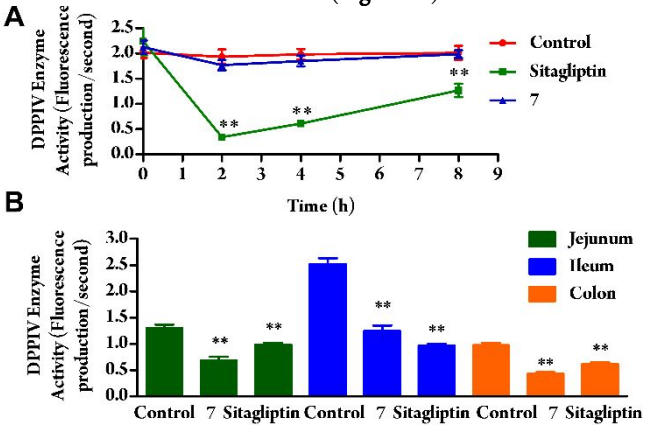


Figure 2. The relative DPPIV activity in plasma (**A**) and the intestine (**B**) after oral administration 30 mg/kg of sitagliptin and compound **7** to mice. The intestinal DPPIV activity was tested at 8h after oral administration. (n = 6). The data are expressed as the mean ± SEM. **P < 0.01, compared with control groups. Student's *t*-test statistical analysis was used.

Selectivity and preliminary toxicity investigations. As a qualified chemical tool, isozyme selectivity is essential. After determining the inhibitory effects of compound **7** on DPP8 and DPP9, we were delighted to find that compound **7** showed a greater than 2000-fold selectivity towards these two isozymes (**Table S2**).

To evaluate the cytotoxic effect of compound **7**, human hepatocellular carcinoma cell line (HepG2), human embryonic kidney 293 cell line (HEK293) and mouse primary hepatocytes were treated with compound 7 for 24 h, and cell viability was measured using the MTT assay. As shown in **Figure S4**, compound **7** exhibited no obvious cytotoxicity after 24 h of treatment up to a concentration of 200 μM. For the acute toxicity study, ICR mice were treated with single doses of compound **7** (1000 mg/kg). All of the animals survived the 4 days of the experimental period (**Figure S5A**). General conditions, such as body weight and clinical and behavioral symptoms, were recorded during this period, and no obvious change or death attributable to the toxicity of compound **7** was observed (**Figure S5B**). Compound **7** was found to be safe at the dosage of 1000 mg/kg employed in the present study.

CONCLUSION AND DISCUSSION

DPPIV possesses multiple physiological functions and is widely distributed in body tissues[9, 11, 18]. The discriminatory contribution of DPPIV with different cellular sites on glucose homeostasis indicates that the biological roles of DPPIV are cell or tissues specific[11]. However, studies regarding DPPIV were rarely performed depending on its cellular sites or originating tissues, probably due to the lack of tissue-selective DPPIV inhibitors. Here, by introducing high polar moieties to a systemic DPPIV inhibitor, we discovered a NSIT DPPIV inhibitor, compound **7**, which was distributed throughout the intestine and could selectively inhibit intestinal DPPIV activity while having no significant effect on the plasmatic DPPIV activity after oral administration at a dose as high as 30 mg/kg. In addition, compared with systemic compounds, NSIT molecules might harbor low toxicity risks as a result of their extremely poor systemic exposure. Therefore, coupled with the good performance of compound **7** in isozyme selectivity and preliminary toxicity investigations, we believed that it could serve as a qualified NSIT DPPIV inhibitor to determine the precise roles of intestinal DPPIV in various physiological and pathological processes.

For example, the relationship between DPPIV and inflammatory bowel disease (IBD) has been determined in a vast number of publications[2, 19-22]. However, the defined role of DPPIV in the process of IBD remains unclear, and several mechanisms have been proposed including the changing of the immune response at systemic and local levels[20, 23] and the degradation of glucagon-like peptide-2 (GLP-2)[19, 24], a potent and specific gastrointestinal growth factor. Although there are still some inconsistencies, most studies observed that DPPIV inhibition could lead to the alleviation of IBD[6, 24-26], suggesting a potential and novel approach to treat IBD. However, DPPIV inhibition in all of these studies was accomplished by a systemic DPPIV inhibitor or nonspecific *DPPIV* gene knockout, and, as far as we know, no selective intestinal DPPIV inhibitor was applied. Because the nidus of IBD is located within the intestine, illuminating the role of intestinal DPPIV in the progress of IBD is beneficial for understanding the pathological process or even for developing a new treatment for IBD.

A NSIT DPPIV inhibitor could also be used to comprehensively understand the impact of the DPPIV inhibitor on the gut microbiota. Recently, Olivares *et al.* found that vildagliptin, a systemic DPPIV inhibitor, could prevent the disruption of intestinal homeostasis in association with modulation of the gut microbiota[27]. Very recently, Liao *et al.* revealed an important alteration of gut microbiota induced by sitagliptin treatment, indicating a new hypoglycemic mechanism and an additional benefit of DPPIV inhibitors[28]. Considering that sitagliptin and vildagliptin decreased both systemic and intestinal DPPIV activity[27], we could not exclude that this beneficial effects or the alteration in the composition of the gut microbiota resulted from the changes of the systemic environment induced by vildagliptin. In this context, comparing the effects of systemic and intestinal DPPIV inhibitors on the gut microbiota is conducive for determing the precise regulatory mechanisms for gut dysfunctions. Furthermore, with increasing knowledge of the relationships between intestinal microbiota and numerous human pathologies, managing the microbial composition will gradually become an attractive therapeutic approach to different diseases[29-32]. Therefore, clarifying the effects of the NSIT DPPIV inhibitor on the gut microbiota might provide a new method to regulate the gut microbiota, which may opened new therapeutic approaches to different dysfunctions.

Finally, whether the marketed DPPIV inhibitors could reduce the cardiovascular (CV) events and mortality in type 2 diabetic patients is a pending question[33-35]. Except for linagliptin, most marketed DPPIV inhibitors could not produce significantly beneficial effects on the risk of CV events, and even worse, saxagliptin was found to be responsible for an increased risk of heart failure[36, 37]. Since CV complications are the main cause of death in type 2 diabetic patients, it is worthwhile to study the potential risks of DPPIV inhibitors thoroughly [38]. Currently, several mechanisms have been suggested but all are focused on the consequences of systemic DPPIV inhibition, such as the increased level of stromal cell-derived factor-1 (SDF-1) in circulatory system[38], the sympathetic activation[39] and

the inhibition of luminal sodium-hydrogen exchanger 3, (NHE3)[40]. However, as shown in **Figure 2**, the systemic DPPIV inhibitor simultaneously inhibited plasmic and intestinal DPPIV activity even at the 8 h time point after oral administration, indicating that it is hard to distinguish the effects of plasmic and intestinal DPPIV on the risk of CV. Therefore, compound **7**, a NSIT DPPIV inhibitor, might contribute to the determination of the specific roles of plasmic and intestinal DPPIV in the pathology of CV or other disorders.

Collectively, by adjusting the physicochemical properties of a systemic DPPIV inhibitor out of the Lipinski's rule of five, we firstly reported a NSIT DPPIV inhibitor, compound **7**. And, the studies regarding the application of this compound are undergoing.

EXPERIMENTAL SECTION

General Chemistry. All reagents were purchased from commercial suppliers and used without further drying or purification unless otherwise stated. Yields were not optimized. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC400 or a Bruker AC500 NMR spectrometer using tetramethylsilane ($CDCl_3$) or deuterium reagent itself (DMSO-d_6) as an internal reference. Low-resolution mass spectra were determined on an Agilent liquid-chromatography mass spectrometer system that consisted of an Agilent 1260 infinity LC coupled to Agilent 6120 Quadrupole mass spectrometer (ESI). High-resolution mass spectra were conducted on a triple TOF 5600+ MS/MS system (AB Sciex, Concord, Ontario, Canada) in the negative or positive ESI mode. The purity of test compounds are determined by HPLC (Agilent ChemStation, Purospher® STAR RP-18 endcapped (2 μm), 2.1×50 mm, 30 °C, UV 240 nM) and the mobile phase was method A in **Table S3**. All the assayed compounds are purified by preparative liquid chromatograph (Instrument: Unimicro Easysep-1010 series LC, UV 254 nm, 25 °C; Column: Agilent Prep-C18 10 μm, 21.2 × 250 mm, the mobile phase was methods B-D in **Table S3**) and possess a more than 95% purity. Column chromatography was performed on silica gel (200–300 mesh) or with pre-packed silica cartridges (4-40g) from Bonna-Agela Technologies Inc. (Tianjin, China) and eluted with a CombiFlash@ Rf 200 from Teledyne Isco, and preparative TLC was performed on HSGF 254 (0.4–0.5 mm thickness; Yantai Jiangyou Company, Yantai, Shangdong, China).

(R)-4-((1,1-dioxido-4-(6-(2-oxo-2-((2-(trimethylammonio)ethyl)amino)ethoxy)benzo[d]thiazol-2-yl)tetrahydro-2H-thiopyran-4-yl)amino)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-aminium trifluoroacetate (4). To a solution of **3j** (1 g, 1.5 mmol) in DMF was successively added the HATU (1.1 g, 3.0 mmol), DIPEA (784 μL, 4.5 mmol) and the trifluoroacetate of 2-amino-*N,N,N*-trimethylethan-1-aminium trifluoroacetate (759 mg, 2.3 mmol). The mixture was stirred for 2h at room temperature. The solvent was directly evaporated in vacuo and the residue was redissolved by the mixed solution of DCM and TFA (V/V = 5:1). After stirring overnight, the mixture was directly evaporated in vacuo and the residue was purified by preparative liquid chromatograph to give **4** (white solid, 464 mg, yield 35%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.52 (t, *J* = 5.9 Hz, 1H), 8.06 – 7.95 (m, 3H), 7.88 (d, *J* = 8.9 Hz, 1H), 7.61 (d, *J* = 2.6 Hz, 1H), 7.58 (td, *J* = 10.3, 7.0 Hz, 1H), 7.53 – 7.46 (m, 1H), 7.18 (dd, *J* = 9.0, 2.6 Hz, 1H), 4.60 (s, 2H), 3.69 (dq, *J* = 12.7, 6.9 Hz, 1H), 3.58 (q, *J* = 6.1 Hz, 2H), 3.52 – 3.41 (m, 4H), 3.24 – 3.16 (m, 2H), 3.09 (s, 9H), 2.99 – 2.74 (m, 4H), 2.70 – 2.52 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.60 , 170.45 , 168.18 , 157.99 (q, *J* = 31.5 Hz), 156.14 (dd, *J* = 243.1, 9.3 Hz), 155.50 , 148.33 (dt, *J* = 248.2, 14.5 Hz), 147.31 , 145.98 (ddd, *J* = 242.7, 13.6, 3.9 Hz), 135.75 , 123.37 , 119.91 (d, *J* = 5.9 Hz), 119.64 (dd, *J* = 19.4, 5.1 Hz), 117.09 (q, *J* = 299.3 Hz), 115.89 , 106.18 (dd, *J* = 29.0, 20.2 Hz), 106.16 , 67.52 , 63.68 , 56.73 , 52.61 , 52.58 , 52.56 , 47.28 , 46.50 , 46.46 , 36.72 , 35.12 , 33.60 , 33.31 , 32.99 . HRMS (ESI): m/z [M-CF_3COOH-CF_3COO^-]$^+$ calculated for $C_{29}H_{37}F_3N_5O_5S_2^+$, 656.2183; found, 656.2192. HPLC purity, 96%; t_R, 4.86 min.

(R)-4-((1,1-dioxido-4-(6-(2-oxo-2-((2-sulfoethyl)amino)ethoxy)benzo[d]thiazol-2-yl)tetrahydro-2H-thiopyran-4-yl)amino)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-aminium trifluoroacetate (7). Starting with **3j** (1 g, 1.5 mmol) and 2-aminoethane-1-sulfonic acid (288 mg, 2.3 mmol), **7** was obtained by using the process of the preparation of compound **4** (white solid, 41%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.41 – 8.30 (m, 1H), 7.85 (d, *J* = 8.9 Hz, 1H), 7.69 – 7.25 (m, 6H), 7.14 (d, *J* = 8.7 Hz, 1H), 4.53 (s, 2H), 3.69 – 3.63 (m, 1H), 3.46 – 3.34 (m, 6H), 3.18 (d, *J* = 11.7 Hz, 2H), 3.01 – 2.78 (m, 4H), 2.67 – 2.57 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.71 , 170.83 , 167.08 , 158.47 (q, *J* = 31.1 Hz), 156.17 (dd, *J* = 243.3, 9.9 Hz), 155.59 , 148.50 (dt, *J* = 248.2, 13.9 Hz), 147.34 , 146.04 (dd, *J* = 242.0, 11.8 Hz), 135.79 , 123.36 , 120.11 (d, *J* = 18.4 Hz), 119.71 (dd, *J* = 19.3, 5.2 Hz), 117.21 (d, *J* = 299.8 Hz), 116.06 , 106.24 , 106.01 (dd, *J* = 29.0, 21.4 Hz), 67.66 , 56.79 , 50.11 , 47.55 , 46.60 , 46.56 , 37.11 , 35.23 , 33.59 , 33.42 , 31.36 . HRMS (ESI): m/z [M-CF_3COO^-]$^+$ calculated for $C_{26}H_{30}F_3N_4O_8S_3^+$, 679.1172; found, 679.1179. HPLC purity, 98%; t_R, 5.37 min.

The detailed experimental experiment procedures and other designed compounds were synthesized by following a similar procedure (Supporting Information).

ASSOCIATED CONTENTS

Supporting Information

The materials are available free of charge via the Internet at http://pubs.acs.org.

DPPIV inhibitory effects of compounds *in vitro*, the solubility test, Caco-2 permeability assay, the stability test in human and mouse intestinal S9 fraction, *in vivo* pharmacokinetic study, determination of plasma and intestinal DPPIV activity *in vivo*, DPP8 and DPP9 inhibitory effect of compounds *in vitro*, *in vitro* cytotoxicity test by MTT assay, acute toxicity study of compound 7 in mice, synthetic chemistry and abbreviations. (PDF)

Molecular formula strings (CSV)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Notes

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ABBREVIATIONS

IC_{50}, half maximal inhibitory concentration; DCM, dichloromethane; EA, ethyl acetate; THF, tetrahydrofuran; DMF, *N,N*-dimethyl formamide; NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography; ESI, electron spray ionization; CH_3CN, acetonitrile; UV, under voltage; FA, formic acid; TLC, thin-layer chromatography; NaH, sodium hydride; THF, tetrahydrofuran; rt, room temperature; nBu-Li, n-butyllithium; EtOH, ethanol; HATU, 2-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; DIPEA, *N,N*-Diisopropylethylamine; mCPBA, 3-chloroperoxybenzoic acid; K_2CO_3, potassium carbonate; MeOH, methanol; TFA, trifluoroacetic acid; NH_4Cl, ammonium chloride; $MgSO_4$, magnesium sulfate; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, dimethylaminopyridine.

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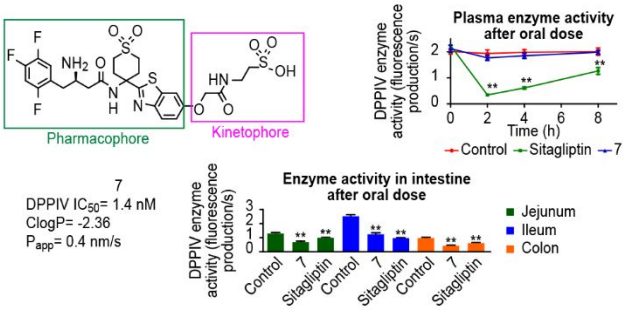

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