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Short communication

Synthesis and evaluation of structurally constrained imidazolidin derivatives as potent dipeptidyl peptidase IV inhibitors

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ABSTRACT

To find potent and selective inhibitors of dipeptidyl peptidase IV (DPP-IV), we synthesized a series of 2-cyanopyrrolidine derivatives with constrained imidazolidin ring and tested their activities against DPP-IV. Most of them exhibited submicromolar inhibitory activities against DPP-IV. The most potent compound among these is (*S*)-1-(2-(2-(3-(3,4-dimethoxyphenyl)-2-oxoimidazolidin-1-yl)ethyl-amino)-acetyl)pyrrolidine-2-carbonitrile (**6n**), which is a 2 nM DPP-IV inhibitor.

1. Introduction

The incretin hormone glucagon-like peptide 1(GLP-1 (7-36 amide or 7-37)) is a 30-amino acid peptide hormone produced by L-cells of the small intestine in response to food intake [1]. GLP-1 has been shown in mammals to stimulate the secretion of insulin in a glucose-dependent manner, inhibit glucagon release, slow gastric emptying, induce satiety, and stimulate the regeneration and differentiation of islet β-cells, with all of these actions promoting the control of glucose homeostasis in patients with type II diabetes [2]. However, active GLP-1 is rapidly degraded to give inactive GLP-1 (9-36 amide or 9-37) by the serine protease dipeptidyl peptidase IV (DPP-IV) which catalyzes the cleavage of dipeptides from the *N*-terminus of proteins with the preference to proline (Pro) or alanine (Ala) as the penultimate (P1) amino acid [3]. DPP-IV is widely distributed in mammalian tissues and body fluids, and is found in great abundance in the kidney, liver, intestinal epithelium, and placenta [4]. Thus, inhibition of DPP-IV could increase the half-life of active GLP-1, lead to the persistence of circulating GLP-1 levels, and prolong the beneficial effects of this incretin hormone, which would enhance insulin secretion and improve glucose tolerance. Therefore much attention has been paid to DPP-IV as a promising new target for drugs. In fact, a clinical investigation has already demonstrated the benefits of DPP-IV inhibition in type II diabetes [5]. A number of small molecule inhibitors of DPP-IV have been described [6] and several of these, including Vildagliptin (LAF-237) [7], Saxagliptin (BMS477118) [8], Sitagliptin (MK-0431) [9] and Alogliptin (SYR-322) [10], Fig. 1 are in late-stage of clinical development or approved by the U.S. Food and Drug Administration. In our laboratory, we focused on substitutions of 2-cyanopyrrolidine, because 2-cyanopyrrolidine derivatives are one of the most potent inhibitors. In this paper, we reported a new series of structurally constrained imidazolidin2-cyanopyrrolidine derivatives **6**.

2. Chemistry

The DPP-IV inhibitors were prepared as described in Scheme 1. Amine (**1**) was heated with triphosgene in toluene to afford isocyanate compound (**2**), then reacted with diethanolamine in dichloromethane at room temperature to provide substituted urea derivatives (**3**) in excellent yield. **3** was brominated with tribromophosphine in dichloromethane under ice-water conditions to yield the desired intermediates (**4**) followed to cyclize at based conditions to obtain the key imidazolidin-2-one derivatives (**5**). **5** was coupled with (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile to provide the desired 2-cyanopyrrolidine derivatives (**6**).

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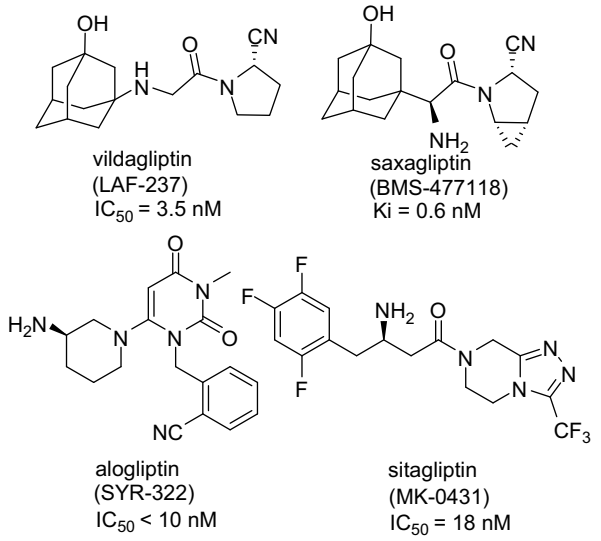
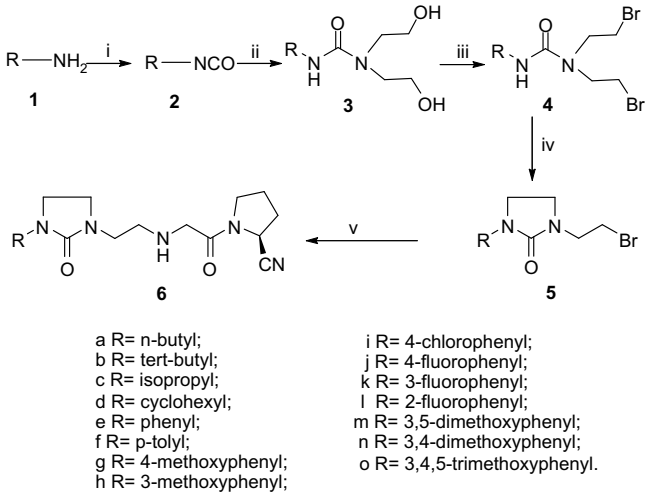


Fig. 1. Structures of selected DPP-IV inhibitors.

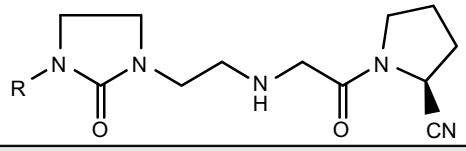
3. Results and discussion

Compounds **6a**–**o** were evaluated *in vitro* for their inhibition of DPP-IV extracted from human plasma (Table 1). LAF-237 was used as a reference compound. Among compounds **6a**–**e**, **6e** showed the most active with an IC_{50} value of 34.8 nM. Changing phenyl to cyclohexyl or *tert*-butyl resulted in a 2-fold decrease in potency (**6e** vs. **6d**; **6e** vs. **6b**). Changing phenyl to *n*-butyl or isopropyl resulted only in a slight decrease in potency, respectively (**6e** vs. **6a**; **6e** vs. **6c**). To increase the inhibitory activity, we introduced several substituents on the phenyl ring. The influence of electron-donating or electron-withdrawing substituents on enzyme inhibition was investigated. The introduction of electron-donating methyl (**6f**) or electron-withdrawing chloro (**6i**) into the 4-position of the phenyl ring causes a slight increase in potency, respectively (**6e** vs. **6f**; **6e** vs. **6i**). However, the introduction of electron-donating methoxyl (**6g**, **6h**) or electron-withdrawing fluoro (**6j**–**l**) into the 2-, 3-or 4-position of the phenyl ring causes a modest decrease in potency, respectively (**6e** vs. **6g**–**h**; **6e** vs. **6j**–**l**). The introduction of two methoxyl substituents into the phenyl ring gave generally more active compounds. For instance, a 3,5-dimethoxyphenyl or a 3,4-dimethoxyphenyl substituent caused a 2-fold (**6e** vs. **6m**) or a 17-fold (**6e** vs. **6n**) improvement in activity, respectively. However, the introduction of three methoxyl substituents into the phenyl ring gave less active compounds. For instance, a 3,4,5-trimethoxyphenyl substituent caused a 5-fold (**6e** vs. **6o**) drop in activity. It was shown that the optimum substituent is 3,4-dimethoxyphenyl substituent which results in an improved, low nanomolar inhibitor (**6n**) with an IC_{50} of 2.0 nM which is 17 times as potent as **6e**.



Scheme 1. Reagent and conditions: (i) triphosgene, toluene, heated, 4-8 h; (ii) diethanolamine, CH_2Cl_2, rt, overnight; (iii) PBr_3, CH_2Cl_2, 10 °C, 5 h; (iv) $NaHCO_3$, PH ≈ 10, overnight; (v) (*S*)-1-(2- aminoacetyl)pyrrolidine-2-carbonitrile, K_2CO_3, CH_3CN, rt, overnight.

Table 1
DPP-IV inhibitory activity.[a]



Compd	R	IC_{50} (nM)
6a	*n*-Butyl	53.5
6b	*tert*-Butyl	68.1
6c	Isopropyl	47.8
6d	Cyclohexyl	68.6
6e	Phenyl	34.8
6f	p-tolyl	29.3
6g	4-Methoxyphenyl	82.4
6h	3-Methoxyphenyl	65.1
6i	4-Chlorophenyl	25.8
6j	4-Fluorophenyl	44.5
6k	3-Fluorophenyl	60.1
6l	2-Fluorophenyl	133.6
6m	3,5-Dimethoxyphenyl	20.4
6n	3,4-Dimethoxyphenyl	2.0
6o	3,4,5-Trimethoxyphenyl	165.6
LAF-237		16.6

[a] Values are IC_{50} (nM) expressed as the mean of three independent determinations.

Table 2
DPP-IV inhibition and selectivity assays of **6n**.

DPP-IV	DPP-VII	DPP-VIII	DPP-IX
2.0 nM	>100 μM	53 μM	>100 μM

Another criteria for DPP-IV inhibitor development are selectivity against the closely related enzymes. There are a number of other dipeptidyl peptidases in the body, and although the function of most of these peptidases remains unknown at present, there is evidence indicating that selectivity over other peptidases may be important. We screened **6n** for selectivity against DPP-VII (also known as DPP-II and QPP) [11], DPP-VIII [12], DPP-IX [13] (Table 2) **6n** displays more than 10 000-fold selectivity against these enzymes. In addition, the specificity of **6n** was profiled in over 20 receptor and enzyme assays *in vitro* and no significant binding was observed (10 μM).

4. Conclusions

In summary, we have synthesized a series of 2-cyanopyrrolidine derivatives with constrained imidazolidin ring and evaluated their ability to inhibit DPP-IV. All of them exhibited submicromolar inhibitory activities against DPP-IV. The most potent compound is **6n**, which is a 2 nM DPP-IV inhibitor with good selectivity over related proline peptidases. These preliminary results suggest that **6n** needs further study in next step.

5. Experimental

5.1. General

^{1}H NMR and ^{13}C NMR spectra were obtained at 600 MHz on a Bruker Avance 600 instrument using $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane as internal standard. Mass spectra were measured on an Agilent 1946B ESI-MS instrument. All commercially available reagents were used without purification.

5.2. General procedure for the synthesis of **5**

In toluene (200 mL), triphosgene (85 mmol) was dissolved, the solution of **1** (100 mmol) in toluene was added in drop with ice cooling. The temperature was gradually increased. The mixture was stirred for an hour at room temperature, then was heated to 100 °C for 3 h. The mixture was concentrated to about 80 mL, added to the solution of the diethanolamine (200 mmol) in CH_2Cl_2 (200 mL) and continued to stir overnight. The reacting solution was diluted with CH_2Cl_2, washed with brine, dried over Na_2SO_4 and concentrated in vacuo to afford **2**, which can be used for next reaction without purification.

To the solution of **2** in CH_2Cl_2 (200 mL) was added tribromophosphine (120 mmol) in drop in ice-water bath. The mixture was stirred for 3 h at room temperature, followed to be basified with saturated $NaHCO_3$ solution and continued stirring overnight. The organic phase was isolated and the aqueous layer was extracted with CH_2Cl_2 two times. The combined organic phase was washed with brine and dried over Na_2SO_4. Concentration was followed by purification by chromatography using silica gel (eluting sequentially with petroleum ether/ethyl acetate = 10/1 to 3/1) to afford **5**. Rate (four steps): 15%–35%.

5.2.1. 1-(2-Bromoethyl)-3-butylimidazolidin-2-one (**5a**)

^{1}H NMR δ ($CDCl_3$): 3.59 (t, 2H, J = 6.4 Hz); 3.48–3.44 (m, 4H), 3.33 (t, 2H, J = 8.4 Hz); 3.19 (t, 2H, J = 7.4 Hz); 1.51–1.46 (m, 2H); 1.38–1.30 (m, 2H); 0.93 (t, 3H, J = 7.4 Hz). ESI-MS: 249, 251 ($M + H^+$).

5.2.2. 1-(2-Bromoethyl)-3-tert-butylimidazolidin-2-one (**5b**)

^{1}H NMR δ ($CDCl_3$): 3.55 (t, 2H, J = 6.5 Hz); 3.46 (t, 2H, J = 6.2 Hz); 3.34 (s, 4H); 1.35 (s, 9H). ESI-MS: 249, 251 ($M + H^+$).

5.2.3. 1-(2-Bromoethyl)-3-isopropylimidazolidin-2-one (**5c**)

^{1}H NMR δ ($CDCl_3$): 4.14 (m, 1H); 3.59 (t, 2H, J = 6.6 Hz); 3.47 (t, 2H, J = 6.4 Hz); 3.43 (t, 2H, J = 7.4 Hz); 3.29 (t, 2H, J = 7.2 Hz); 1.12 (d, 6H, J = 6.8 Hz). ESI-MS: 235, 237 ($M + H^+$).

5.2.4. 1-(2-Bromoethyl)-3-cyclohexylimidazolidin-2-one (**5d**)

^{1}H NMR δ ($CDCl_3$): 3.72–3.67 (m, 1H); 3.59 (t, 2H, J = 6.5 Hz); 3.47 (t, 2H, J = 6.7 Hz); 3.42 (t, 2H, J = 8.7 Hz); 3.31 (t, 2H, J = 8.4 Hz); 1.79 (d, 2H, J = 12 Hz); 1.72 (d, 2H, J = 9.5 Hz); 1.65 (d, 2H, J = 13 Hz); 1.40–1.29 (m, 4H); 1.10–1.04 (m, 1H). ESI-MS: 275, 277 ($M + H^+$).

5.2.5. 1-(2-Bromoethyl)-3-phenylimidazolidin-2-one (**5e**)

^{1}H NMR δ ($CDCl_3$): 7.54 (d, 2H, J = 8.7 Hz); 7.33 (t, 2H, J = 7.4 Hz); 7.04 (t, 1H, J = 7.4 Hz); 3.86 (t, 2H, J = 7.6 Hz); 3.71 (t, 2H, J = 6.4 Hz); 3.65 (t, 2H, J = 7.5 Hz); 3.54 (t, 2H, J = 6.2 Hz). MS: 269, 271 ($M + H^+$).

5.2.6. 1-(2-Bromoethyl)-3-p-tolylimidazolidin-2-one (**5f**)

^{1}H NMR δ ($CDCl_3$): 7.42 (d, 2H, J = 8.6 Hz); 7.14 (d, 2H, J = 8.6 Hz); 3.83 (t, 2H, J = 7.6 Hz); 3.71 (t, 2H, J = 6.4 Hz); 3.63 (t, 2H, J = 7.6 Hz); 3.54 (t, 2H, J = 6.4 Hz); 2.31 (s, 3H). ESI-MS: 283, 285 ($M + H^+$).

5.2.7. 1-(2-Bromoethyl)-3-(4-methoxyphenyl)imidazolidin-2-one (**5g**)

^{1}H NMR δ ($CDCl_3$): 7.43 (d, 2H, J = 9.0 Hz); 6.88 (d, 2H, J = 9.2 Hz); 3.81 (t, 2H, J = 7.5 Hz); 3.78 (s, 3H); 3.70 (t, 2H, J = 6.4); 3.62 (t, 2H, J = 7.6 Hz); 3.53 (t, 2H, J = 6.4 Hz). ESI-MS: 298, 300 ($M + H^+$).

5.2.8. 1-(2-Bromoethyl)-3-(3-methoxyphenyl)imidazolidin-2-one (**5h**)

^{1}H NMR δ ($CDCl_3$): 7.33 (t, 1H, J = 2.4 Hz); 7.22 (t, 1H, J = 8.0 Hz); 7.00 (dd, 1H, J = 8.2, 2.0 Hz); 6.61 (dd, 1H, J = 8.2, 2.3 Hz); 3.84 (t, 2H, J = 8.4 Hz); 3.82 (s, 3H); 3.71 (t, 2H, J = 6.5 Hz); 3.64 (t, 2H, J = 8.5 Hz); 3.54 (t, 2H, J = 6.5 Hz). ESI-MS: 209,301 ($M + H^+$).

5.2.9. 1-(2-Bromoethyl)-3-(4-chlorophenyl)imidazolidin-2-one (**5i**)

^{1}H NMR δ ($CDCl_3$): 7.49 (d, 2H, J = 9.0 Hz); 7.29 (d, 2H, J = 8.8 Hz); 3.83 (t, 2H, J = 8.4 Hz); 3.71 (t, 2H, J = 6.4 Hz); 3.66 (t, 2H, J = 8.7 Hz); 3.54 (t, 2H, J = 6.1 Hz). ESI-MS: 302, 304, 306 ($M + H^+$).

5.2.10. 1-(2-Bromoethyl)-3-(4-fluorophenyl)imidazolidin-2-one (**5j**)

^{1}H NMR δ ($CDCl_3$): 7.50–7.47 (m, 2H); 7.05–7.00 (m, 2H); 3.83 (t, 2H, J = 8.6 Hz); 3.71 (t, 2H, J = 6.4 Hz); 3.64 (t, 2H, J = 8.4 Hz); 3.54 (t, 2H, J = 6.4 Hz). ESI-MS: 287, 289 ($M + H^+$).

5.2.11. 1-(2-Bromoethyl)-3-(3-fluorophenyl)imidazolidin-2-one (**5k**)

^{1}H NMR δ ($CDCl_3$): 7.44 (d, 1H, J = 11.8 Hz); 7.28–7.23 (m, 2H); 6.73 (t, 1H, J = 8.8 Hz); 3.84 (t, 2H, J = 8.0 Hz); 3.72 (t, 2H, J = 6.4 Hz); 3.66 (t, 2H, J = 8.4 Hz); 3.54 (t, 2H, J = 6.2 Hz). ESI-MS: 287, 289 ($M + H^+$).

5.2.12. 1-(2-Bromoethyl)-3-(2-fluorophenyl)imidazolidin-2-one (**5l**)

^{1}H NMR δ ($CDCl_3$): 7.53(t, 1H, J = 7.9 Hz); 7.18–7.08 (m, 3H); 3.89 (t, 2H, J = 7.4 Hz); 3.71 (t, 2H, J = 6.4 Hz); 3.66 (t, 2H, J = 7.6 Hz); 3.55 (t, 2H, J = 6.4 Hz). ESI-MS: 287, 289 ($M + H^+$).

5.2.13. 1-(2-Bromoethyl)-3-(3,5-dimethoxyphenyl)imidazolidin-2-one (**5m**)

^{1}H NMR δ ($CDCl_3$): 6.81 (d, 2H, J = 2.2 Hz); 6.18 (t, 1H, J = 2.2 Hz); 3.82 (t, 2H, J = 8.5 Hz); 3.79 (s, 6H); 3.70 (t, 2H, J = 6.4); 3.63 (t, 2H, J = 8.3 Hz); 3.53 (t, 2H, J = 6.4). ESM-MS: 329, 331 ($M + H^+$).

5.2.14. 1-(2-Bromoethyl)-3-(3,4-dimethoxyphenyl)imidazolidin-2-one (**5n**)

^{1}H NMR δ ($CDCl_3$): 7.63 (d, 1H, J = 2.6 Hz); 6.82 (d, 1H, J = 8.6 Hz), 6.65 (dd, 1H, J = 8.6, 2.4 Hz); 3.90 (s, 3H); 3.85 (s, 3H); 3.83 (t, 2H, J = 7.4 Hz); 3.71 (t, 2H, J = 6.4 Hz); 3.62 (t, 2H, J = 7.8 Hz); 3.54 (t, 2H, J = 6.2 Hz). ESI-MS: 351, 353 ($M + H^+$).

5.2.15. 1-(2-Bromoethyl)-3-(3,4,5-trimethoxyphenyl)imidazolidin-2-one (**5o**)

^{1}H NMR δ ($CDCl_3$): 6.93 (s, 2H); 3.8 (t, 2H, J = 7.5 Hz); 3.74 (s, 6H); 3.71 (t, 2H, J = 6.4 Hz); 3.68 (s, 3H); 3.62 (t, 2H, J = 7.8 Hz); 3.54 (t, 2H, J = 6.2 Hz). ESI-MS: 381, 383 ($M + H^+$).

5.3. General procedure for the synthesis of **6**

(*S*)-1-(2-Aminoacetyl)pyrrolidine-2-carbonitrile trifluoroacetate (24 mmol) was dissolved in acetonitrile, K_2CO_3 (64 mmol) was added, the mixture stirred for 30 min to liberate free amine. Then **5** (8 mmol) was added and continued stirring for 48 h at room temperature. The mixture was filtered to remove insoluble substance

and concentrated in vacuo. Purification by flash column chromatography (eluted with $CHCl_3$/MeOH = 10/1) yielded **6** as a viscous oil. The free base was dissolved in isopropanol, the solution of oxalic acid in isopropanol was added in drop, and precipitate occurred. Filtration was followed dried in vacuo over P_2O_5. Rate: 15%–30%.

*5.3.1. (S)-1-(2-(2-(3-Butyl-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6a**)*

^{1}H NMR δ (DMSO-d_6): 4.83 (dd, 1H, J = 7.4, 3.8 Hz); 4.04–4.01 (m, 2H); 3.61–3.57 (m, 1H); 3.43–3.39 (m, 1H); 3.60 (t, 2H, J = 5.8 Hz); 3.29 (s, 4H); 3.08–3.05 (m, 4H); 2.20–2.17 (m, 2H); 2.07–2.03 (m, 2H). ESI-MS: 322 ($M + H^+$).

*5.3.2. (S)-1-(2-(2-(3-Tert-butyl-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6b**)*

^{1}H NMR δ (DMSO-d_6): 4.77 (dd, 1H, J = 4.4, 7.2 Hz); 4.09–4.03 (m, 2H); 3.61–3.57 (m, 1H); 3.46–3.40 (m, 5H); 3.29–3.26 (m, 4H); 2.28–2.24 (m, 2H); 2.14–2.09 (m, 2H); 1.26 (s, 9H). ESI-MS: 322 ($M + H^+$).

*5.3.3. (S)-1-(2-(2-(3-Isopropyl-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6c**)*

^{1}H NMR δ (DMSO-d_6): 4.82 (dd, 1H, J = 7.4,3.8 Hz); 3.95–3.88 (m, 3H); 3.59–3.56 (m, 1H); 3.43–3.39 (m, 1H); 3.32 (t, 2H, J = 5.9 Hz); 3.27 (t, 2H, J = 7.3 Hz); 3.22 (t, 2H, J = 7.1 Hz); 3.00 (t, 2H, J = 5.5 Hz); 2.18–2.16 (m, 2H); 2.06–2.01 (m, 2H); 1.04 (d, 6H, J = 6.9 Hz). ESI-MS: 308 ($M + H^+$).

*5.3.4. (S)-1-(2-(2-(3-Cyclohexyl-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6d**)*

^{1}H NMR δ (DMSO-d_6): 4.83 (dd, 1H, J = 7.1, 4.0 Hz); 4.00–3.90 (m, 2H); 3.60–3.57 (m, 1H); 3.51–3.46 (m, 1H); 3.43–3.39 (m, 1H); 3.34 (t, 2H, J = 6.0 Hz); 3.27 (s, 4H); 3.03 (t, 2H, J = 5.1 Hz); 2.20–2.17 (m, 2H); 2.07–2.00 (m, 2H); 1.74 (d, 2H, J = 12.9 Hz); 1.58 (d, 3H, J = 9.7 Hz); 1.39–1.33 (m, 2H); 1.30–1.24 (m, 2H); 1.09–1.03 (m, 1H). ESI-MS: 348 ($M + H^+$).

*5.3.5. (S)-1-(2-(2-(2-Oxo-3-phenylimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6e**)*

^{1}H NMR δ (DMSO-d_6): 7.59 (d, 2H, J = 8.0 Hz); 7.33 (t, 2H, J = 7.9 Hz); 7.01 (t, 1H, J = 7.4 Hz); 4.82 (dd, 1H, J = 7.1, 4.2 Hz); 4.05–3.93 (m, 2H); 3.81 (t, 2H, J = 7.9 Hz); 3.62–3.59 (m, 1H); 3.51 (t, 4H, J = 6.0 Hz); 3.44–3.40 (m, 1H); 3.10 (t, 2H, J = 5.0 Hz); 2.21–2.16 (m, 2H); 2.05–2.00 (m, 2H). ESI-MS: 342 ($M + H^+$).

*5.3.6. (S)-1-(2-(2-(2-Oxo-3-p-tolylimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6f**)*

^{1}H NMR δ (DMSO-d_6): 7.45 (d, 2H, J = 8.5 Hz); 7.13 (d, 2H, J = 8.5 Hz); 4.82 (dd, 1H, J = 7.3, 4.0 Hz); 4.03–3.94 (m, 2H); 3.78 (t, 2H, J = 7.5 Hz); 3.61–3.58 (m, 1H); 3.50–3.47 (m, 4H); 3.44–3.40 (m, 1H); 3.10 (t, 2H, J = 5.9 Hz); 2.25 (s, 3H); 2.20–2.16 (m, 2H); 2.07–2.00 (m, 2H). ESI-MS: 356 ($M + H^+$).

*5.3.7. (S)-1-(2-(2-(3-(4-Methoxyphenyl)-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6g**)*

^{1}H NMR δ (DMSO-d_6): 7.48 (d, 2H, J = 9.2 Hz); 6.92 (d, 2H, J = 9.2 Hz); 4.83 (dd, 1H, J = 7.2, 4.0 Hz); 4.02–3.92 (m, 1H); 3.77 (t, 2H, J = 7.1 Hz); 3.72 (s, 3H); 3.61–3.58 (m, 1H); 3.49–3.47 (m, 4H); 3.46–3.42 (m, 1H); 3.10 (t, 2H, J = 5.6 Hz); 2.18–2.16 (m, 2H); 2.07–2.00 (m, 2H). ESI-MS: 372 ($M + H^+$).

*5.3.8. (S)-1-(2-(2-(3-(3-Methoxyphenyl)-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6h**)*

^{1}H NMR δ (DMSO-d_6): 7.28 (s, 1H); 7.22 (t, 1H, J = 8.3 Hz); 7.09 (d, 1H, J = 7.3 Hz); 6.60 (d, 1H, J = 8.2 Hz); 4.82 (s, 1H); 4.05–3.94 (m, 2H) 3.81–3.76 (t, 2H, J = 8.34 Hz); 3.73 (s, 3H); 3.60 (s, 1H); 3.49 (s, 4H); 3.44–3.40 (m, 1H); 3.09 (s, 2H); 2.17 (s, 2H); 2.08–2.00 (m, 2H). ESI-MS: 372 ($M + H^+$).

*5.3.9. (S)-1-(2-(2-(3-(4-chlorophenyl)-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6i**)*

^{1}H NMR δ (DMSO-d_6): 7.61 (d, 2H, J = 9.0 Hz); 7.37 (d, 2H, J = 9.0 Hz); 4.81 (dd, 1H, J = 7.4, 4.0 Hz); 4.00–3.91 (m, 2H); 3.80 (t, 2H, J = 7.4 Hz); 3.61–3.57 (m, 1H); 3.51–3.47 (m, 4H); 3.43–3.39 (m, 1H); 3.80 (t, 2H, J = 5.6 Hz); 2.17–2.15 (m, 2H); 2.06–1.99. ESI-MS: 376, 378 ($M + H^+$).

*5.3.10. (S)-1-(2-(2-(3-(4-Fluorophenyl)-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6j**)*

^{1}H NMR δ (DMSO-d_6): 7.60–7.58 (m, 2H); 7.17 (t, 2H, J = 9 Hz); 4.83 (dd, 1H, J = 6.2, 4.6 Hz); 4.05–3.95 (m, 2H); 3.80 (t, 2H, J = 7.4 Hz); 3.72–3.67 (m, 1H); 3.51–3.48 (m, 4H); 3.45–3.40 (m, 1H); 3.11 (s, 2H); 2.20–2.16 (m, 2H); 2.10–2.00 (m, 2H). ESI-MS: 360 ($M + H^+$).

*5.3.11. (S)-1-(2-(2-(3-(3-Fluorophenyl)-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6k**)*

^{1}H NMR δ (DMSO-d_6): 7.44 (d, 1H, J = 12.4 Hz); 7.38–7.34 (m, 1H); 7.31 (d, 1H, J = 8.1 Hz); 6.81 (t, 1H, J = 7.8 Hz); 4.82 (dd, 1H, J = 7.2, 4.0 Hz); 4.01–3.91 (m, 2H); 3.83 (t, 2H, J = 7.8 Hz); 3.62–3.59 (m, 1H); 3.51 (t, 4H, J = 8.8 Hz); 3.44–3.40 (m, 1H); 3.09 (t, 2H, J = 5.8 Hz); 2.20–2.16 (m, 2H); 2.07–2.02 (m, 2H). ESI-MS: 360 ($M + H^+$).

*5.3.12. (S)-1-(2-(2-(3-(2-Fluorophenyl)-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6l**)*

^{1}H NMR δ (DMSO-d_6): 7.51 (t, 1H, J = 9.1 Hz); 7.28–7.18 (m, 3H); 4.83 (dd, 1H, J = 7.4, 4.0 Hz); 4.09–4.00 (m, 2H); 3.80 (t, 2H, J = 7.6 Hz); 3.62–3.59 (m, 1H); 3.52 (t, 2H, J = 7.9 Hz); 3.49 (t, 2H, J = 6.0 Hz); 3.44–3.42 (m, 1H); 3.10 (t, 2H, J = 6.0 Hz); 2.20–2.17 (m, 2H); 2.09–2.01 (m, 2H). ESI-MS: 360 ($M + H^+$).

*5.3.13. (S)-1-(2-(2-(3-(3,5-Dimethoxyphenyl)-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6m**)*

^{1}H NMR δ (DMSO-d_6): 6.81 (d, 2H, J = 2.0 Hz); 6.19 (t, 1H, J = 2.0 Hz); 4.82 (dd, 1H, J = 7.0, 4.0 Hz); 4.01–3.92 (m, 2H); 3.79–3.75 (m, 3H); 3.72 (s, 3H); 3.61–3.59 (m, 1H); 3.50–3.46 (m, 4H); 3.44–3.42 (m, 1H); 3.09 (t, 2H, J = 5.82 Hz); 2.19–2.16 (m, 2H); 2.07–2.00 (m, 2H). ESI-MS: 402 ($M + H^+$).

*5.3.14. (S)-1-(2-(2-(3-(3,4-Dimethoxyphenyl)-2-oxoimidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6n**)*

^{1}H NMR δ (DMSO-d_6): 7.46 (d, 1H, J = 2.4 Hz); 6.91 (d, 1H, J = 8.6 Hz); 6.85 (dd, 1H, J = 8.7, 2.6 Hz); 4.82 (dd, 1H, J = 7.0, 4.0 Hz); 4.08–4.00 (m, 2H); 3.78 (t, 2H, J = 8.2 Hz); 3.74 (s, 3H); 3.71 (s, 3H); 3.62–3.58 (m, 1H); 3.52–3.48 (m, 4H); 3.44–3.40 (m, 1H); 3.09 (t, 2H, J = 5.0 Hz); 2.19–2.16 (m, 2H); 2.08–1.98 (m, 2H). ^{13}C NMR δ (DMSO-d_6): 165.7, 164.8, 158.1, 149.2, 144.7, 134.9, 119.4, 112.7, 109.3, 103.6, 56.3, 55.9, 47.9, 46.7, 45.9, 45.2, 43.0, 41.8, 41.1, 29.9, 25.1. ESI-MS: 402 ($M + H^+$). HRMS (ES^+): 402.2136 ($M + H^+$), calcd. for $C_{20}H_{28}N_5O_4$.

*5.3.15. (S)-1-(2-(2-(2-Oxo-3-(3,4,5-trimethoxyphenyl)imidazolidin-1-yl)ethylamino)acetyl)pyrrolidine-2-carbonitrile oxalate (**6o**)*

^{1}H NMR δ (DMSO-d_6): 6.90 (s, 2H); 4.80 (dd, 1H, J = 7.0, 4.4 Hz); 3.98–3.86 (m, 2H); 3.8 (t, 2H, J = 7.5 Hz); 3.74 (s, 6H); 3.60 (s, 3H); 3.46–3.38 (m, 6H); 3.06 (s, 2H); 2.12–2.14 (m, 2H); 2.04–1.99 (m, 2H). ESI-MS: 432 ($M + H^+$).

5.4. Biological evaluation

The assay was conducted by adding 20 μg of DPP-IV (SIGMA), diluted to a final volume of 125 μL in assay buffer (25 mM HEPES, 140 mM NaCl, 1% bovine serum albumin, 80 mM $MgCl_2$) to 96-well flat-bottom microtiter plates. The reaction was initiated by adding 50 μM substrate (Gly-Pro-AMC; AMC is 7-amino-4-methyl-coumarin, SIGMA). The reaction was run at room temperature for 20 min, AMC as an indicator of DPP-IV activity was detected at 355/460 nm (Ex/Em) by Fluorometer. A standard curve of free AMC was generated using 0–100 μM solutions of AMC. The curve generated, which was linear, was used for interpolation of catalytic activity. IC_{50} was calculated by Xlfit Software.

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