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Synthesis, SAR, and X-ray structure of novel potent DPPIV inhibitors: Oxadiazolyl ketones

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Abstract—Synthesis of a novel series of DPPIV inhibitors with 1,2,4- and 1,3,4-oxadiazolyl ketone derivatives and its structure–activity relationships are discussed. Compound **18h** showed good inhibitory activity against DPPIV and favorable pharmacokinetic properties. In vivo pharmacodynamic efficacy and co-crystal structure of compound **18h** with DPPIV is also described.

GLP-1 (glucagon-like peptide 1) has been intensively studied as a treatment of type 2 diabetes mellitus (T2DM).[1] GLP-1 is an incretin hormone secreted by intestinal L-cells in response to food intake. However GLP-1 is rapidly degraded in plasma by the action of dipeptidyl peptidase IV (DPPIV). Inhibition of DPPIV has been reported to increase the level of GLP-1[2] which increases pancreatic β-cell proliferation and survival. For this reason, DPPIV inhibitors are emerging as new potential drugs of T2DM,[3] with Sitagliptin **1**[4] already in the market. Extensive research has resulted in a series of potent DPPIV inhibitors, and several DPPIV inhibitors are under late-stage clinical development including Vildagliptin **2**[5] (Fig. 1).

In a recent study, DPPIV inhibitors utilizing (*S*)-2-cyanopyrrolidine as a key scaffold showed good activity against dipeptidyl peptidase IV. Our search for a unique scaffold led us to keto aryl pyrrolidines, which were used as a common surrogate among serine protease inhibitors.[6] Same applied for DPPIV inhibitors.[6b] We report the synthesis of oxadiazolyl ketone derivatives that are highly effective and exhibit long-acting in vivo pharmacodynamic activity.

Synthesis of the compounds listed in Tables 1–3 is outlined in Schemes 1–3.

A series of 1,2,4-oxadiazolyl ketone pyrrolidines was synthesized according to Scheme 1. 1,2,4-Oxadiazolyl ketone substituted compounds **10** were prepared from hydroxy proline **3** as starting material (Scheme 1). Fluorination of compound **3** using (diethylamino)sulfur trifluoride (DAST) in the presence of dichloromethane gave fluoropyrrolidine **4**. Reduction of compound **4** with DIBAL-H, followed by cyanide addition, provided cyanohydrin **5**. Sequential treatment of cyanohydrin **5** with ethyl vinyl ether in the presence of PPTS and hydroxyl amine produced amidoxime **6**, which was reacted with proper carboxylic acid 'R^1CO_2H' to give 5-alkyl-oxadiazol-3-yl pyrrolidine **7**.[7] Removal of ethoxyethyl group of pyrrolidine **7** in the presence of PPTS and Dess–Martin oxidation of the resultant alcohol gave 1,2,4-oxadiazolyl ketone **8**. Conversion of the *tert*-butoxy residue of **8** to a bromoacetyl bromide residue to produce **9** was carried out by treatment with hydrochloric acid in ethyl acetate, followed by coupling reaction of the deprotected pyrrolidine nitrogen with bromoacetyl bromide. And the subsequent reaction of bromide **9** with proper amine 'R^2NH_2' in the presence of potassium carbonate gave the desired compounds **10a**–**i**.

The inhibitors **13a**–**13e** were also prepared as shown in Scheme 2. In this case, adamantyl amine groups were first introduced from compound **4** in Scheme 1 as in the usual method. Then compound **11** was converted to compound **13** in a manner similar to Scheme 1. 1,3,4-Oxadiazolyl compounds **18a**–**i** were synthesized from ester **14** as shown in Scheme 3. Hydrolysis of the

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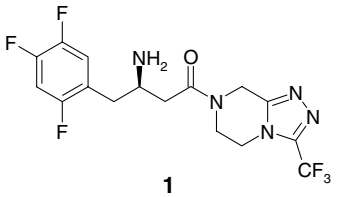


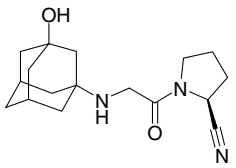
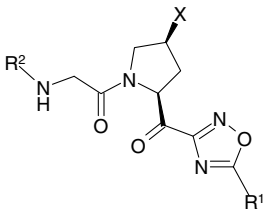
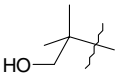
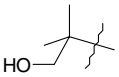
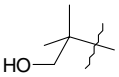
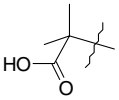
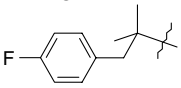


Figure 1. Known DPPIV inhibitors.

Table 1. DPPIV inhibitors with 1,2,4-oxadiazolyl ketone



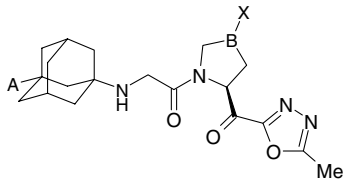
Compound	R^2	X	R^1	IC_{50}[a] (μM)			
				DPPIV	DPPII	DPPVIII	Elastase
10a		H	CH_3	0.077	5.47	67.3	>500
10b		F	*t*-Bu	0.067	70.1	69.9	>400
10c		H	*t*-Bu	0.223	10	6.59	—
10d		H	CH_3	7.939	—	—	—
10e		H	H	0.035	0.20	1.45	—
10f		F	*t*-Bu	0.063	35.1	5.72	338
10g		H	*p*-Fluorophenyl	0.015	<0.39	<0.39	>400
10h		H	1-Methylcyclohexyl	0.095	<0.39	<0.39	>400
10i	Adamantyl	H	Me	0.21	—	—	—

[a] All IC_{50} were measured with human DPPIV enzyme.

proline ester **14,** followed by coupling with *N*,*O*-dimethyl hydroxylamine, gave Weinreb amide **15**. After preparation of Grignard reagent of compound **15a**,[7] Weinreb amide **15** was added to the resultant solution to give 1,3,4-oxadiazolyl ketone **16**. The compounds **18a**–**i** were synthesized in a similar manner as shown in Scheme 1 from compound **16**.

Biological evaluation of novel compounds in Tables 1–3 was done based on IC_{50} values for inhibition of DPPIV. Generally in Table 1, DPPIV activities are good except when R^2 has carboxylic acid substituent (**10d**) or adamantyl substituent (**10i**). Also, selectivities against elastase are excellent (at least >300 μM). In case where R^2 is *tert*-butyl group, *p*-fluorophenyl (**10g**) and 1-methylcyclohexyl (**10h**) substituents at R^1 provided less selectivity compared to *tert*-butyl (**10f**) substituents. Fluorinated pyrrolidine compound **10b** displayed better DPPII and DPPVIII selectivities than compounds **10a**,**c** which have no fluorine on pyrrolidine ring. When aromatic ring is substituted at R^2 (**10e**) it showed good DPPIV activity but exhibited unfavorable selectivity against DPPII and DPPVIII enzymes. Compound **10b** (hydroxyl on *tert*-butyl substituent) and compound **10f**

Table 2. Adamantyl derivatives with 1,3,4-oxadiazolyl ketone



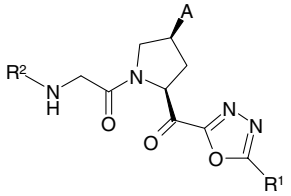
Compound	A	B	X	Inhibition, IC_{50} (μM)		
				DPPIV	DPPII	DPPVIII
13a	H	S	—	0.224	3.24	5.8
13b	H	C	H	0.084	2.07	0.808
13c	H	C	F (*cis*)	0.053	2.67	1.44
13d	H	C	di-F	0.680	1.25	3.26
13e	OH	C	H	0.339	10	4.4

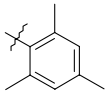
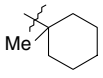
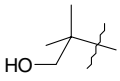
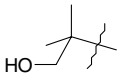
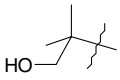
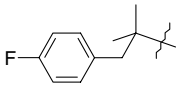
(no hydroxyl on *tert*-butyl substituent) showed similar activity. However selectivity against DPPII and DPPVIII is higher of compound **10b** than that of compound **10f**. Most 1,2,4-oxadiazolyl substituted inhibitors in Table 2 showed good activity and selectivity especially against DPPVIII.

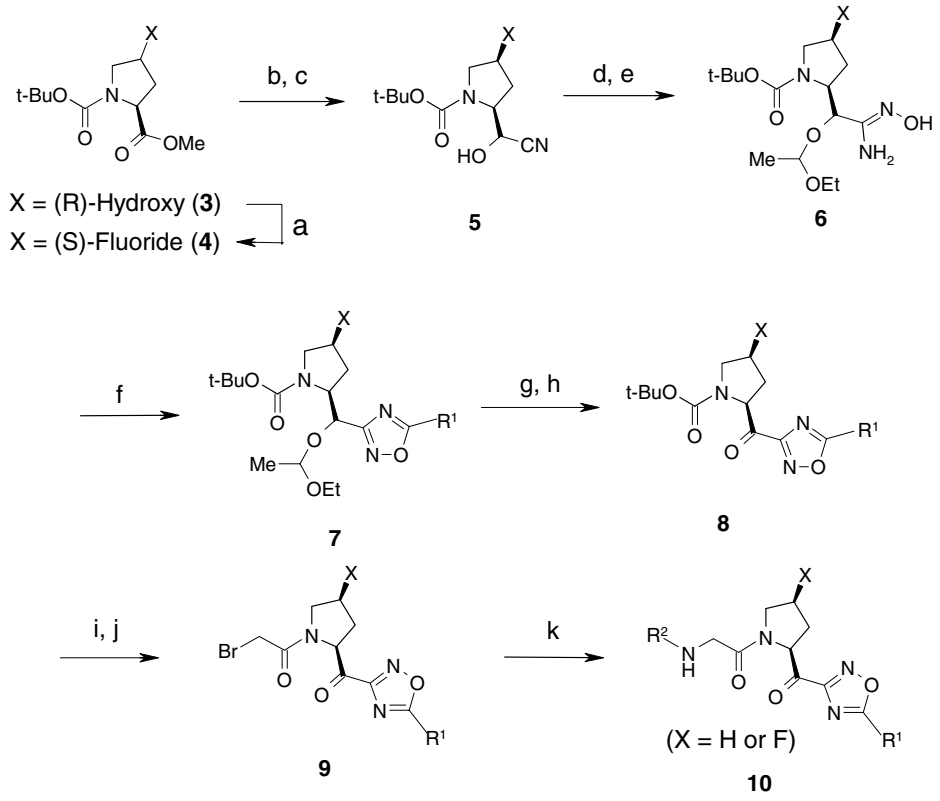
In Table 2, adamantyl derivatives of 1,3,4-oxadiazolyl substituted compounds **13a–e** showed acceptable DPPIV activities but displayed low selectivity against DPPII and DPPVIII enzymes. For example, investigation of thiazolidine **13a**, difluoropyrrolidine **13d**, and hydroxyadamantyl **13e** led to insufficient inhibition over DPPIV enzyme. Pyrrolidine **13b** and *cis*-fluoropyrrolidine **13c** showed good DPPIV inhibitory effect but relatively lower selectivity against DPPII and DPPVIII. These results suggested that the further optimization should be concentrated on the modification of the adamantyl substituent and of the 5-membered 1,3,4-oxadiazole derivatives (see Table 2).

Most of the compounds in Table 3 showed good activity against DPPIV and exhibited excellent selectivity against elastase. A variety of substituents at R^1 (**18a–e**) resulted in no differences in DPPIV inhibition. While phenyl (**18c**) or cyclohexyl substituents (**18d**) at R^1 gave low

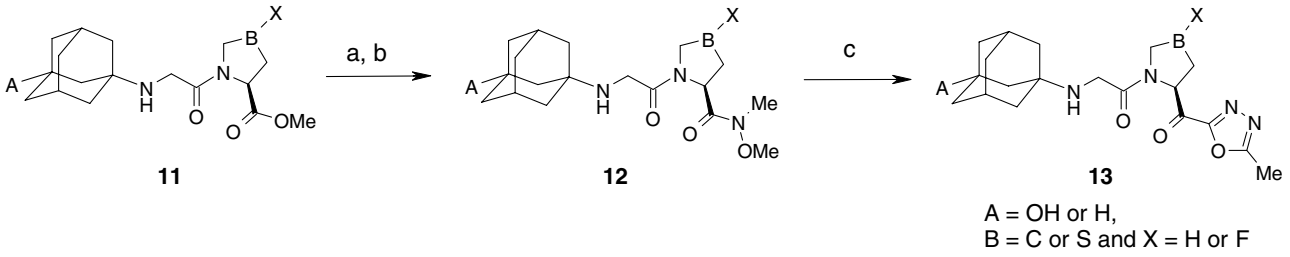
Table 3. DPPIV inhibitors with 1,3,4-oxadiazolyl ketones



Compound	R^2	A	R^1	Inhibition, IC_{50} (μM)			
				DPPIV	DPPII	DPPVIII	Elastase
18a		F		0.020	97.2	<0.39	222
18b		H	*t*-Bu	0.056	>10	1.29	—
18c		H		0.017	<0.39	<0.39	>400
18d		F		0.030	<0.39	<0.39	>400
18e		H		0.010	8.66	<0.39	238
18f		H	Me	0.255	1.38	3.10	—
18g		H	*t*-Bu	0.047	60	0.93	>400
18h		F	*t*-Bu	0.022	145.8	1.15	227.8
18i		F	*t*-Bu	0.025	1.71	1.09	—



Scheme 1. Reactions and conditions: (a) DAST, CH_2Cl_2, −78 °C; (b) DIBAL-H, THF, 0 °C; (c) $NaHSO_3$, NaCN, H_2O, 0 °C; (d) ethyl vinyl ether, PPTS, CH_2Cl_2; (e) $NH_2OH{\cdot}HCl$, MeOH, $NaHCO_3$, reflux; (f) R $^1CO_2H$, CDI, DMF, 90 °C; (g) PPTS, EtOH, 40 °C; (h) Dess–Martin reagent, CH_2Cl_2; (i) 4 N HCl in EtOAc; (j) $BrCH_2COBr$, *i*-Pr_2NEt, CH_2Cl_2, 0 °C; (k) R $^2NH_2$, K_2CO_3, CH_2Cl_2.

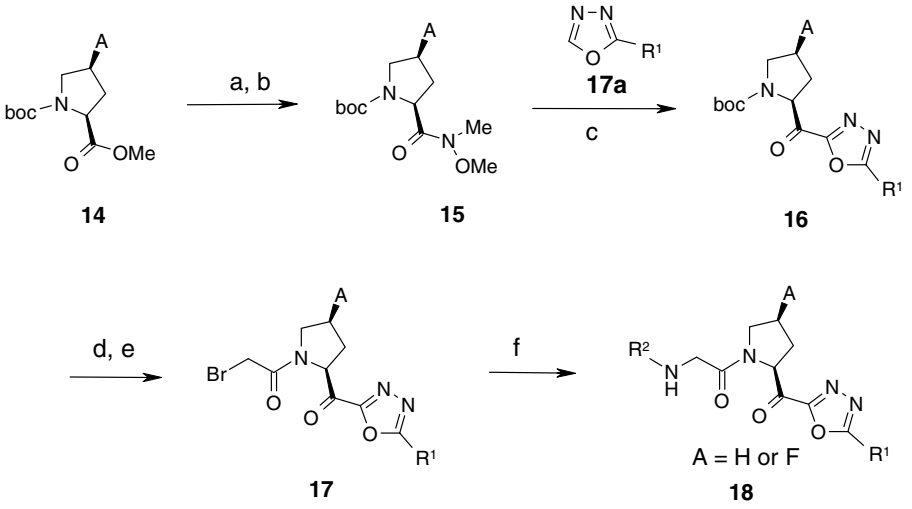


Scheme 2. Reactions and conditions: (a) LiOH, MeOH, THF, H_2O; (b) $HNMe(OMe){\cdot}HCl$, EDC, HOBt, Et_3N, DMF; (c) *n*-BuLi, 2-methyl-1,3,4-oxadiazole, $MgBr_2{\cdot}OEt_2$, THF, −78 °C.

selectivities against DPPII and DPPVIII, bulkier substituents **20a** or **20e** at R^1 exhibited high selectivity against DPPII but low selectivity against DPPVIII. Similar to compound **10e** in Table 4, phenyl substituted compound **18i** at R^2 also gave low selectivity. Fluorinated pyrrolidine compound **18h** gave not only better activity against DPPIV, but also displayed better DPPII and DPPVIII selectivity than compounds **18f**,**g** which lack the fluorine on pyrrolidine ring.

Compound **18h** was selected for pharmacokinetic screening since it showed excellent activity and selectivity (Table 4). Compound **18h** exhibited good pharmacokinetic profiles across the species in rat, dog, and monkey. Bioavailability of **18h** was good especially on dogs. Plasma clearance and volume of distribution of compound **18h** were much reduced in monkeys compared to rats and dogs. Based on these data and long half-lives in monkey, even though it is very difficult to predict human PK profiles, **18h** is expected for once-daily dosing in human.

In pharmacodynamic studies, plasma DPPIV activity of **18h** was evaluated in Sprague–Dawley rats (Fig. 2). Upon the oral administration of the compound **18h**, plasma DPPIV activity was kept suppressed by more than 50% for 24 h after dosing even at 0.03 mg/kg (Fig. 2). This long duration of activity can be explained by the $k_{on} - k_{off}$ rate and X-ray crystallography.



Scheme 3. Reactions and conditions: (a) LiOH, MeOH, THF, H_2O; (b) HNMe(OMe)·HCl, EDC, HOBt, Et_3N, DMF; (c) *n*-BuLi, **17a**, $MgBr_2{\cdot}OEt_2$, THF, −78 °C, then added **15**; (d) HCl in EtOAc; (e) $BrCH_2COBr$, Et_3N, 0 °C; (f) R $^2NH_2$, K_2CO_3, CH_2Cl_2.

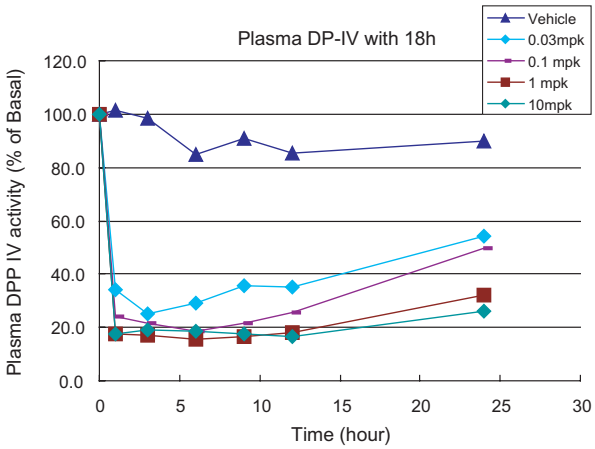
Table 4. Pharmacokinetic data of compound **18h**

	Rat	Dog	Monkey
Dosage[a] (mg/kg)	5	5	5
Dosage[b] (mg/kg)	5	5	5
*C*max[b] (μg/ml)	0.03	0.64	0.21
Cl_p (ml/min/kg)	241	71	18
Vdss (ml/kg)	14027	4867	2421
$t_{1/2}$ [b] (min)	249	88	278
Bioavailability (%)	39	87	35

[a] iv.
[b] po.

Crystal structure of **18h** with DPPIV is shown in Figure 3.[10] The ketone group of the compound **18h** makes a covalent bond with the hydroxyl group of Ser630. The activated carbonyl oxygen atom is stabilized through the interaction with the hydroxyl group of Tyr547. The proline is located in the S1 pocket and its *trans*-4-fluoride atom makes close contacts with the side chain of Tyr631. Two nitrogen atoms of the oxadiazole group make hydrogen bonds to the side chains of Arg125 and His740, respectively. The nitrogen atom of the secondary amine group makes hydrogen bonds with the side chains of Glu205 and Glu206.

The kinetics of DPPIV with **18h** was measured using BiaCore 300 (Table 5). It showed relatively small K_d, which suggested the slow release of **18h** from DPPIV.



Dose (mg/kg)	Inhibition from basal plasma DPP IV Activity (%)	
	1 hr after dosing	24 hr after dosing
0.03	66	46
0.1	76	50
1	82	68
10	82	74

Figure 2. Effects of **18h** on plasma DPPIV activity in male Sprague–Dawley rats.[8]

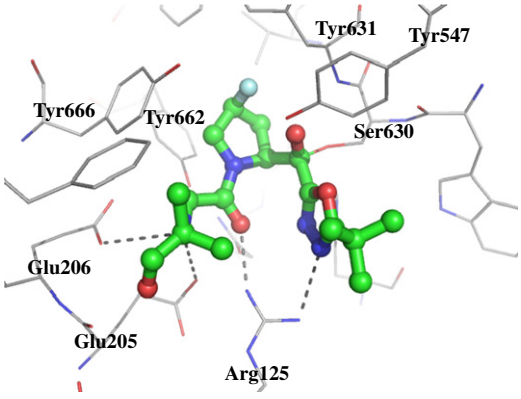


Figure 3. Crystal structure of DPPIV complexed with compound **18h**. Compound **18h** is shown in green; residues within 4 Å of the compound are labeled.[9]

Table 5. Kinetics data of compound **18h**

	18h
K_a (1/Ms)	2.31E+05
K_d (1/s)	5.69E−05
K_A (1/M)	4.05E+09
K_D (M)	2.47E−10
Chi	0.165

K_i of **18h** is 0.247 nM calculated by K_d/K_a which is equal to k_{off}/k_{on}.

A novel series of DPPIV inhibitors with oxadiazolyl ketone have been discovered. They are highly potent DPPIV inhibitors. Representative inhibitor **18h** has good potency against DPPIV, and good selectivity against DPPII, DPPVIII, and elastase. The compound **18h** also resulted in good pharmacokinetic properties especially on monkeys compared to rats and dogs. It has shown good pharmacodynamic profile in SD rats. Further studies on compounds **18h** will be reported elsewhere.

Acknowledgments

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8. Each animal received its oral dosing solution at time 0 h. Vehicle was water. Blood samples were collected through tail vein just before (time 0) and 1, 3, 6, 12, 24, and 48 h after dosing. Data are means ± SE ($n = 3$).
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