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(3*R*)-3-Amino-4-(2,4,5-trifluorophenyl)-*N*-{4-[6-(2-methoxyethoxy)benzothiazol-2-yl]tetrahydropyran-4-yl}butanamide as a potent dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes

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ABSTRACT

Novel series of 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamides and 3-amino-*N*-(4-aryltetrahydropyran-4-yl)butanamides were synthesized and evaluated as dipeptidyl peptidase IV (DPP-IV) inhibitors. Derivatives incorporating the 6-substituted benzothiazole group showed highly potent DPP-IV inhibitory activity. Oral administration of (3*R*)-3-amino-4-(2,4,5-trifluorophenyl)-*N*-{4-[6-(2-methoxyethoxy)benzothiazol-2-yl]tetrahydropyran-4-yl}butanamide (**12u**) reduced blood glucose excursion in an oral glucose tolerance test.

Dipeptidyl peptidase IV (DPP-IV) is a serine protease cleaving dipeptide derived from the N-terminus of peptides. One of the important roles of DPP-IV is a rapid inactivation of the glucagon-like peptide 1 (GLP-1).[1] GLP-1, which is an incretin secreted after meal ingestion, stimulates insulin biosynthesis and release and inhibits glucagon release. GLP-1 also inhibits gastric emptying and regulates pancreatic β-cell mass.[2] The inhibition of DPP-IV enhances the effects of the endogenous GLP-1; therefore, DPP-IV inhibitors are a new candidate in the treatment of type 2 diabetes without any side effects such as hypoglycemia and exhaustion of β-cells.[3] Several DPP-IV inhibitors including Sitagliptin,[4,5] Vildagliptin,[6] Saxagliptin,[7] and Alogliptin are currently under clinical development.[8] Among them, Sitagliptin have received approval from the FDA (Fig. 1).[9] Our goal was to identify novel inhibitors of DPP-IV with good potency and oral activity.

Merck group reported the β-amino amides as a class of DPP-IV inhibitors.[10] In the course of our modification of the β-amino amide, we found that benzyl piperazine moiety could be replaced by aryl substituted ring which was readily derivatized because of no chiral center. In this study, we report the discovery, structure–activity relationships (SARs), and pharmacological properties of the novel series of 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamides and 3-amino-*N*-(4-aryltetrahydropyran-4-yl)butanamides as potent DPP-IV inhibitors.

The series and analogues of 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamides **12** (Y = SO_2) were prepared by the acylation of amine **4** with (3*R*)-*N*-Boc-β-amino acid using a coupling reagent followed by acidic deprotection (Scheme 1). In case Y was a sulfone, the corresponding sulfide **10** was converted to a sulfone **11** by oxidation using *m*CPBA or tetrapropylammonium perruthenate (TPAP).[11] The preparation of amine **4** from ketone **1** was carried out by three methods. The first was the Ritter reaction: tertiary alcohol **2** derived from ketone **1** was converted to acetamide **3** followed by acidic hydrolysis or deacetylation using titanium tetraisopropoxide and diphenylsilane.[12] The second method involved the use of oxime: the reaction of oxime **6**, which was obtained from ketone

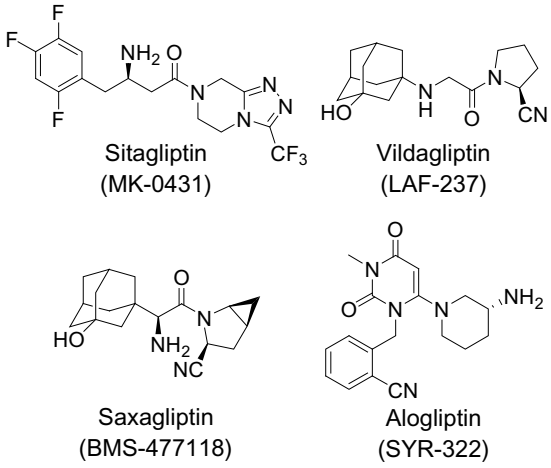

Figure 1. Potent DPP-IV inhibitors.

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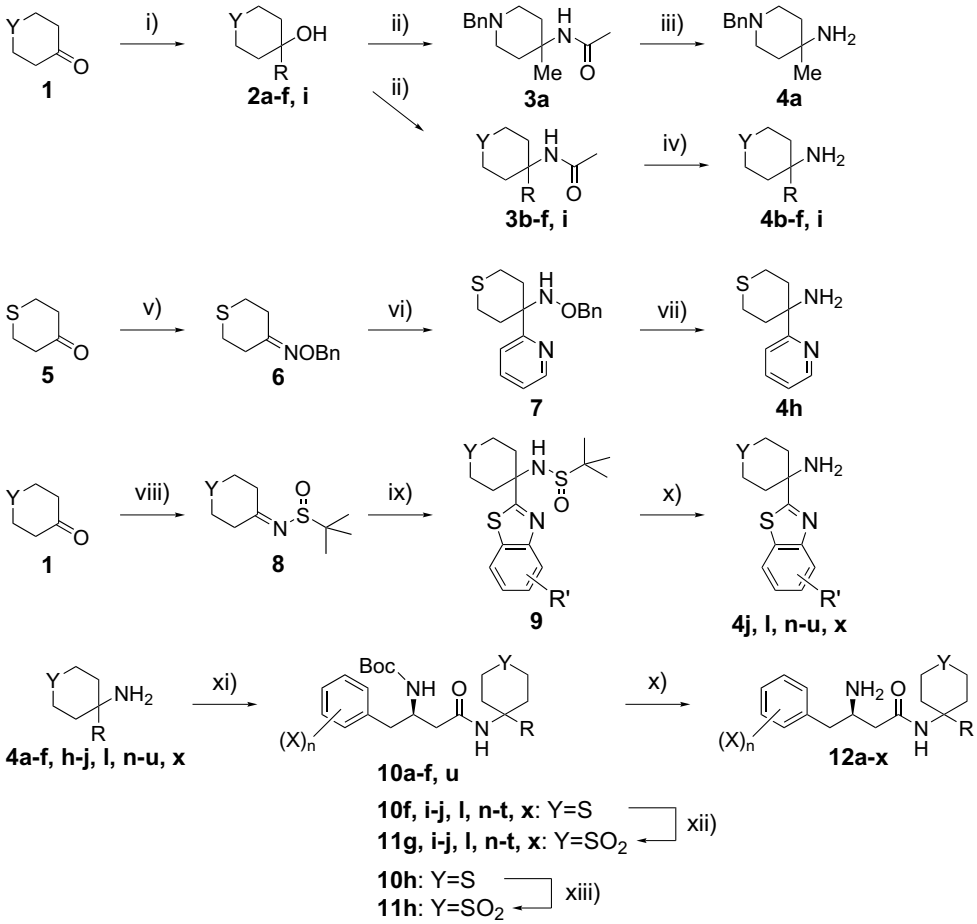
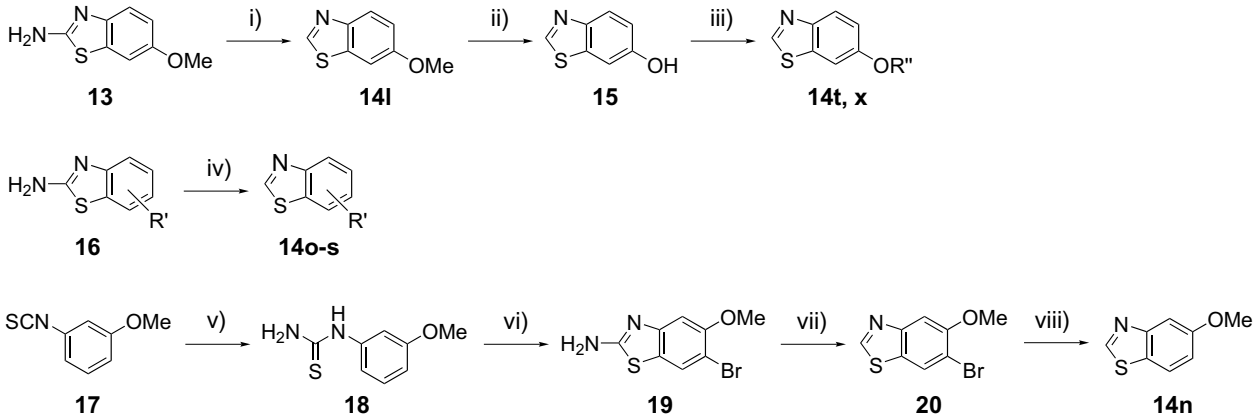


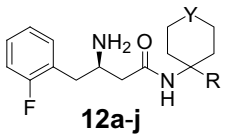
Scheme 1. Reagents and conditions: (i) RLi or RMgBr, THF, −78 °C to rt; (ii) MeCN, concd H_2SO_4, 0 °C to rt; (iii) 6M HCl, reflux; (iv) $Ti(Oi\text{-}Pr)_4$, Ph_2SiH_2, THF, rt; (v) $BnONH_2$·HCl, AcONa, MeOH/H_2O, 90 °C, quant.; (vi) 2-bromopyridine, *n*-BuLi, Et_2O, −78 °C to rt, 75%; (vii) $Mo(CO)_6$, MeCN/H_2O, 100 °C, 31%; (viii) *t*-$BuSONH_2$, $Ti(OEt)_4$, THF, reflux; (ix) *n*-BuLi, benzothiazole 14, −78 °C to rt; (x) HCl/MeOH, rt; (xi) (3*R*)-*N*-Boc-β-amino acid, EDCI, HOBt, *N*–Et morpholine, CH_2Cl_2, rt, or (3*R*)-*N*-Boc-β-amino acid, HATU, *N*–Et morpholine, DMF, rt; (xii) *m*CPBA, CH_2Cl_2, 0 °C to rt; (xiii) TPAP, NMO, MS4A, MeCN, rt, 44%.

5, with lithiated pyridine resulted in benzyloxyamine **7**. Reductive cleavage of the N–O bond was subsequently performed using molybdenumhexacarbonyl.[13] The third was Ellman's method[14,15] *tert*-butylsulfinimide **8** prepared from ketone **1** was treated with a small excess lithiated benzothiazole derivative to yield sulfinamide **9** followed by acidic deprotection. Benzothiazoles **14** were prepared from commercially available 2-aminobenzothiazoles **13**, **16** or arylisothiocyanate **17**, as shown in Scheme 2. Deamination of 2-aminobenzothiazoles **13**, **16** was carried out by diazotization and subsequent treatment with phosphinic acid.[16] Alkylation of benzothiazole **15**, which was prepared from benzothiazole **14l** by demethylation using hydrobromic acid, yielded benzothiazoles



Scheme 2. Reagents and conditions: (i) H_3PO_2 aq, $NaNO_2$ aq, −15 °C to rt, 78%; (ii) HBr aq, reflux, quant; (iii) R″-Br, K_2CO_3, DMF, rt; (iv) AcOH or TFA, 50% H_2SO_4, −15 °C, then H_3PO_2 aq, $NaNO_2$ aq, −15 °C to rt; (v) 7 M NH_3/MeOH, THF, rt; (vi) Br_2, $CHCl_3$, 0 °C to rt; (vii) TFA, 50% H_2SO_4, −15 °C, then H_3PO_2 aq, −15 °C to rt, 43% (from **17**); (viii) *n*-BuLi, THF, −78 °C, then H_2O, 78%.

Table 1
DPP-IV inhibiting activity of compound **12a–j**



12a-j

Compound	Y	R	IC_{50} (nM)
12a	NBn	Me	7800
12b	NBn	Ph	1000
12c	NBn	Bn	2200
12d	NMe	Ph	870
12e	O	Ph	2500
12f	S	Ph	3300
12g	SO_2	Ph	570
12h	SO_2	2-Pyridyl	470
12i	SO_2	2-Thiazolyl	190
12j	SO_2	2-Benzothiazolyl	64

14t, **x**. The preparation of benzothiazole **14n** began with the aminolysis of arylisothiocyanate **17** with ammonia. The resulting thiourea **18** was cyclized using bromine to give 2-aminobenzothiazole **19**,[16c,17] which was then subjected to deamination and subsequent debromination conditions to produce benzothiazole **14n**.

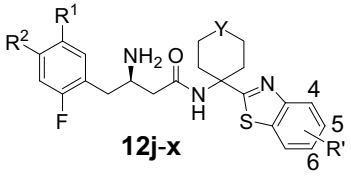
The synthesized compounds were evaluated for the inhibition of DPP-IV derived from human colonic carcinoma cells (Caco-2).[18] The results are shown in Tables 1 and 2. The potencies of compounds having various Y groups were in the following order: sulfide (compound **12f**) < ether (compound **12e**) < benzylamino (compound **12b**) < methylamino (compound **12d**) < sulfone (compound **12g**). As the substituent R, the compounds with an aryl group were more potent than those with a methyl group (compound **12a**); the maximum potency was displayed by a compound having a 2-benzothiazolyl group (compound **12j**) (IC_{50} = 64 nM). In the series of compounds possessing a substituted benzothiazolyl group, the use of 5 or 6-substituents resulted in an increase in the inhibitory effects of DPP-IV. On the contrary, the use of 4-substituents resulted in a decrease in the inhibitory effects (compounds **12o**, **q**, and **s**).

Compounds **12v**, **x** were the most potent inhibitors and their IC_{50} values were of a subnanomolar concentration (compound **12v**: IC_{50} = 0.64 nM, compound **12x**: IC_{50} = 0.79 nM). Based on the previously reported SAR of the phenyl moiety in β-amino acid, it can be said that trifluoro derivatives (compounds **12k**, **m**) are more potent than nonsubstituted ones (compounds **12j**, **l**).[19] Chlorofluoro derivatives (compounds **12v**, **w**) showed stronger inhibitory effects than trifluoro derivatives (compounds **12t**, **u**). To the best of our knowledge, these results are new information on SAR.

Some compounds showing high DPP-IV inhibitory effects were tested in vitro for human, rat, and mouse cytochromes P450 (CYP) metabolism.[20] As shown in Table 2, some differences based on species were observed between the human, rat, and mouse metabolism of these compounds. As a general trend, in comparison with testosterone, the readily metabolizable reference, the metabolism of a mouse was steady for the compounds **12t**, **u**, **v**, **w**, and **x**, while that of a rat was stable only in the case of compounds **12t**, **v**, and **x**. Moreover, compound **12x**, one of the most potent inhibitors, was metabolized in humans as rapidly as testosterone (CL_{int} = 0.0928 mL/min/mg). Of the assessed compounds, compound **12u** was most stable in vitro during human CYP metabolism (CL_{int} = 0.0219 mL/min/mg). Rat plasma DPP-IV activity was measured after oral administration of compound **12u**,[21] which was metabolically stable in humans and exhibited potent DPP-IV inhibition. The compound inhibited the plasma DPP-IV activity in a dose-dependent manner (ID_{50} = 2.7 mg/kg). When 30 mg/kg of compound **12u** was administered orally to an ICR mouse, blood glucose excursion in an oral glucose tolerance test (OGTT) was reduced (Fig. 2).[22]

In conclusion, the novel series of 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamides and 3-amino-*N*-(4-aryltetrahydropyran-4-yl)butanamides exhibited profound DPP-IV inhibitory effects. The compounds having a 6-substituted-2-benzothiazolyl group were the most potent. Oral administration of the compound **12u**,

Table 2
DPP-IV inhibiting activity and microsomal stability of compound **12j–x**



12j-x

Compound	R^1	R^2	Y	R′	Human DPP-IV IC_{50} (nM)	CL_{int} (mL/min/mg)[a]		
						Human	Rat	Mouse
Testosterone	—	—	—	—	—	0.1093	0.3756	0.3126
12j	H	H	SO_2	H	64	0.0346	0.0734	ND
12k	F	F	SO_2	H	13	0.0399	0.0644	ND
12l	H	H	SO_2	6-OMe	12	0.0262	0.0515	ND
12m	F	F	SO_2	6-OMe	2.7	0.0323	0.0423	ND
12n	F	F	SO_2	5-OMe	3.3	ND	ND	ND
12o	H	H	SO_2	4-OMe	270	ND	ND	ND
12p	H	H	SO_2	6-Cl	35	ND	ND	ND
12q	H	H	SO_2	4-Cl	130	ND	ND	ND
12r	H	H	SO_2	6-Me	70	ND	ND	ND
12s	H	H	SO_2	4-Me	350	ND	ND	ND
12t	F	F	SO_2	6-OCH_2CH_2OMe	1.0	0.0466	0.0219	0.0341
12u	F	F	O	6-OCH_2CH_2OMe	3.6	0.0219	0.0496	0.0221
12v	Cl	H	SO_2	6-OCH_2CH_2OMe	0.64	0.0638	0.0315	0.0330
12w	Cl	H	O	6-OCH_2CH_2OMe	1.9	0.0342	0.0981	0.0286
12x	F	F	SO_2	6-OCH_2CH_2-morpholino	0.79	0.0928	0.0260	0.0345

[a] ND, no data.

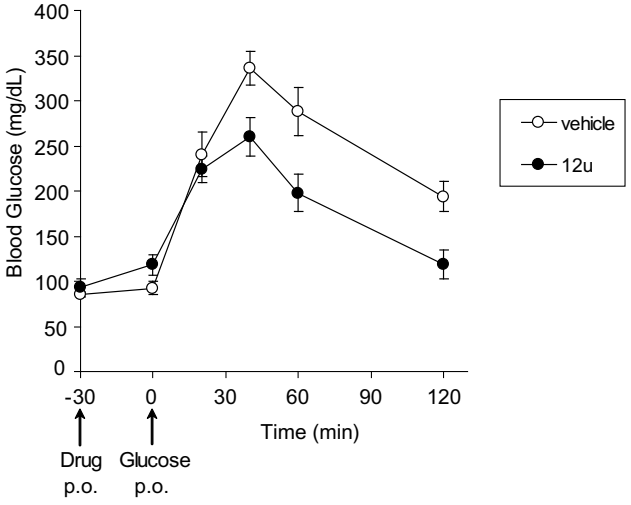


Figure 2. Effect of **12u** in OGTT in ICR mice.

which was stable during in vitro human CYP metabolism, reduced the blood glucose excursion in OGTT. Further optimization of the derivatives is now being investigated.

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18. An extract from Caco-2 was used as the source of DPP-IV in the assay. The cell extract was prepared from cells solubilized in lysis buffer (10 mM, Tris–HCl (pH 8.0), 0.15 M NaCl, 0.04 U aprotinin, 0.50% nonidet-P40) that were then centrifuged at 18,500*g* for 1 h at 4 °C to remove the cell debris. The assay was conducted by adding 5 μg of solubilized Caco-2 protein, diluted to a final volume of 135 μL in an assay buffer (25 mM Tris–HCl (pH 7.4), 0.14 M NaCl, 10 mM KCl, 1% (w/v) BSA) to 96-well flat-bottom plates. The reaction was initiated by adding 15 μL of 0.4 mM substrate (Ala-Pro-AFC). The reaction was run for 20 min at 37 °C, and then 10 μL of 25% acetic acid was added to stop the reaction. Fluorescence was measured using Fusionα (excitation 380 nm; emission 485 nm). The test compounds and solvent controls were added to the assay buffer.
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21. Male SD rats (7–8 weeks of age) were starved overnight. The rats were orally administered a vehicle (distilled water, 5 mL/kg) or **12u** (1, 3, 10 mg/kg; 5 mL/kg); then, blood samples were taken from the jugular vein at intervals of 30 min, 2 h, and 4 h after treatment. Plasma samples were centrifuged at 1400*g* for 10 min at 4 C. The assay was conducted by adding 20 μL of rat plasma to 96-well flat-bottom plates. The reaction was initiated by adding 20 μL of 0.4 mM substrate (Ala-Pro-AFC, diluted in 200 mM Hepes, 0.2 mg/mL BSA, pH 7.5). The reaction was run for 15 min at room temperature and then 6 μL of 25% acetic acid was added to stop the reaction. Fluorescence was measured using Fusionα (excitation 380 nm; emission 485 nm). Male ICR mice (6 weeks of age) were starved overnight.
22. The mice were orally administered a vehicle (distilled water, 10 mL/kg) or **12u** (30 mg/kg; 10 mL/kg). The blood glucose concentration was determined by a glucometer from blood taken from a nick in the tail, 30 min after the treatment. The mice were then orally challenged with glucose (2 g/kg; 10 mL/kg). The blood glucose levels were determined from tail bleeds taken at intervals of 20, 40, 60, and 120 min after the glucose challenge.