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Novel series of 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamides as potent and selective dipeptidyl peptidase IV inhibitors

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ABSTRACT

A series of novel 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamides were investigated as dipeptidyl peptidase IV (DPP-4) inhibitors. Introduction of a 4-phenylthiazol-2-yl group showed highly potent DPP-4 inhibitory activity. Among various derivatives, (3*R*)-3-amino-*N*-(4-(4-phenylthiazol-2-yl)-tetrahydro-2*H*-thiopyran-4-yl)-4-(2,4,5-trifluorophenyl)butanamide 1,1-dioxide (**30**) reduced blood glucose excursion in an oral glucose tolerance test by oral administration.

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Glucagon-like peptide 1 (GLP-1),[1] which is an incretin secreted from L cells of the small intestine after ingestion of food, stimulates insulin biosynthesis and release and inhibits glucagon release. It also inhibits gastric emptying and regulates islet β-cell mass.[2] Dipeptidyl peptidase IV (DPP-4) is a serine protease which selectively cleaves dipeptide derived from the *N*-terminus of peptides. One of the important roles of DPP-4 is rapid inactivation of GLP-1. Inhibition of DPP-4 increases the concentration of endogenous GLP-1 and, as a result, increases insulin secretion,[3] which can ameliorate hyperglycemia in type 2 diabetes without any side effects such as hypoglycemia and exhaustion of β-cells.[4]

Among various DPP-4 inhibitors reported, Sitagliptin,[5,6] Vildagliptin,[7] Saxagliptin,[8] Alogliptin,[9] Linagliptin,[10] and Teneligliptin have received approval for the treatment of type 2 diabetes from the FDA and/or the EMEA and/or Japan (Fig. 1).[11,12]

We reported the β-amino amide derivatives as DPP-4 inhibitors.[13] Structurally, DPP-4 is similar to several other proteases, so selectivity against other serine proteases is necessary, especially DPP-8, DPP-9 and QPP.[14] Our DPP-4 inhibitors mentioned above showed good potency but poor selectivity against these enzymes. In order to improve this weakness, our efforts were focused on modification of a heteroaryl group of the β-amino amide derivatives. In this study, we report the synthesis and biological evaluation of another series of 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamides as potent and selective DPP-4 inhibitors.

The series and analogues of 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamides were prepared by the acylation of amine **4** with (3*R*)-*N*-Boc-β-amino acid using a coupling reagent followed by oxidation using *m*CPBA. Subsequently, iodides **5** were converted to biaryls **20–25** by the Suzuki–Miyaura coupling reaction or Migita-Kosugi-Stille coupling reaction with various heteroaromatics

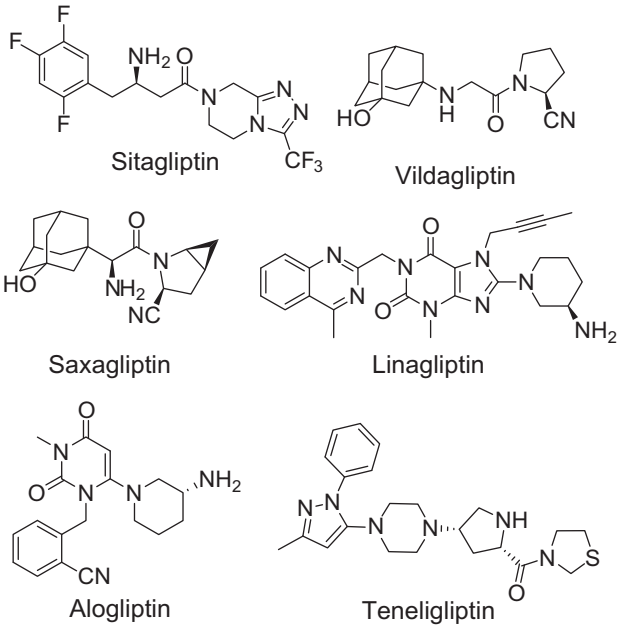

Figure 1. Potent DPP-4 inhibitors.

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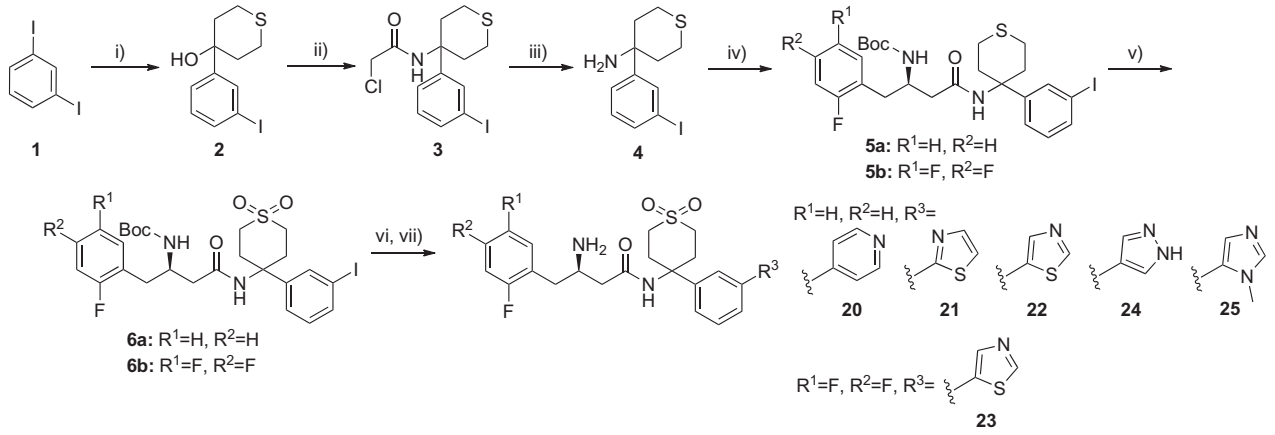
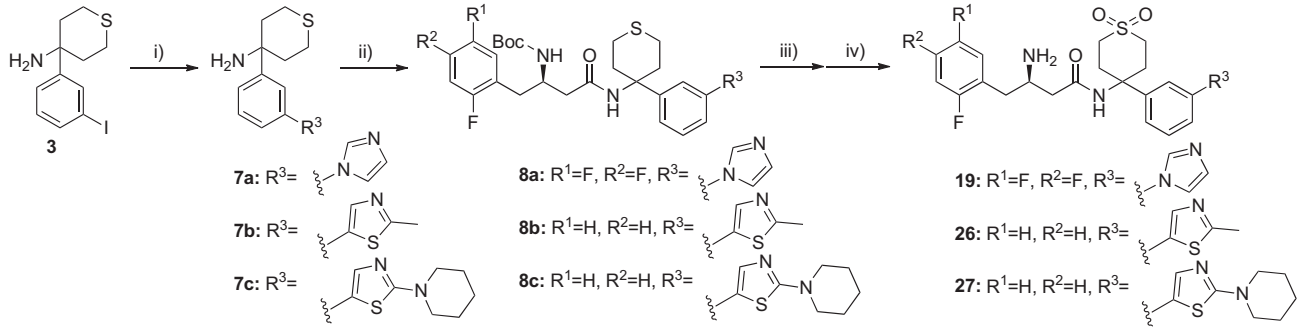
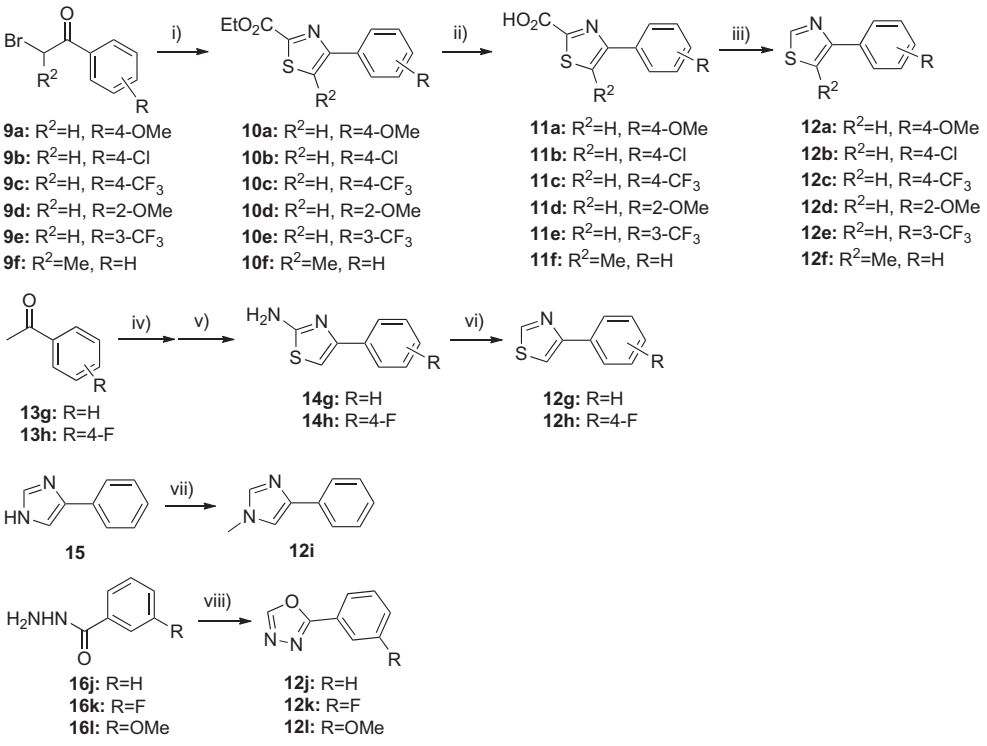


Scheme 1. Reagents and conditions: (i) 1 equiv *n*-BuLi/hex, THF, −78 °C, then 4-oxothiane, −78 °C → rt, 85%; (ii) chloroacetonitrile, concd H_2SO_4, AcOH, rt; (iii) thiourea, EtOH/AcOH (5:1), reflux; (iv) *N*-Boc-β-aminoacid, TsCl, *N*-methylimidazole, CH_3CN, 0 °C-rt, then **4**; (v) *m*CPBA, CH_2Cl_2, 0 °C; (vi) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) pyridine, $PdCl_2$ (dppf), Cs_2CO_3, dioxane, 80 °C, or 2–3 equiv Het-$SnBu_3$, $Pd(PPh_3)_4$, CH_3CN, 80 °C or 2–3 equiv Het-$SnBu_3$, Pd-C/CuI/$AsPh_3$, CH_3CN, 80 °C, (vii) HCl/MeOH, rt.

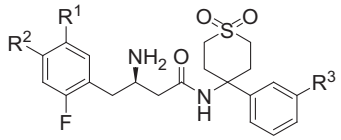


Scheme 2. Reagents and conditions: (i) imidazole, CuI, Cs_2CO_3, dioxane, 1,10-phenanthroline, 110 °C, or 1 equiv Het-$SnBu_3$, $Pd(PPh_3)_4$, CH_3CN, 80 °C; (ii) *N*-Boc-β-aminoacid, TsCl, *N*-methylimidazole, CH_3CN, 0 °C → rt, then **7**; (iii) *m*CPBA, CH_2Cl_2, 0 °C; (iv) HCl/MeOH, rt.



Scheme 3. Reagents and conditions: (i) ethyl 2-amino-2-thioxoacetate, EtOH, reflux; (ii) NaOH aq., EtOH, rt; (iii) concd HCl, 1,4-dioxane, reflux; (iv) $PhMe_3NBr_3$, THF, rt; (v) Thiourea, EtOH, reflux; (vi) 50% H_2SO_4, TFA, $NaNO_2$ aq, −15 °C, then H_3PO_2 aq, −15 °C → rt; (vii) NaH, MeI, DMF, rt, 59%; (viii) $CH(OEt)_3$, 145 °C.

Table 1
DPP-4 inhibiting activity and selectivity of compound



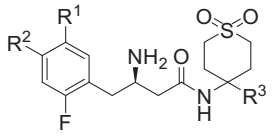
Compound	R^1	R^2	R^3	IC_{50}[a] (μM)			
				DPP-4	QPP	DPP-8	DPP-9
17	H	H	H	0.57	28.4	49.0	9.0
18	F	F	H	0.083	6.7	13.6	3.0
19	F	F	1-Imidazolyl	0.012	4.6	5.0	1.7
20	H	H	4-Pyridyl	0.49	ND	ND	ND
21	H	H	2-Thiazolyl	0.39	ND	ND	ND
22	H	H	5-Thiazolyl	0.041	ND	ND	ND
23	F	F	5-Thiazolyl	0.0078	1.7	6.3	0.94
24	H	H	4-Pyrazolyl	0.33	ND	ND	ND
25	H	H	1-Methyl-5-imidazolyl	0.084	34.6	45.4	11.2
26	H	H	2-Methyl-5-thiazolyl	0.28	ND	ND	ND
27	H	H	2-Piperidyl-5-thiazolyl	0.25	ND	ND	ND

[a] ND = no data.

followed by acidic deprotection (Scheme 1).[15] Amine **3** from 1,3-diiodobenzene **1** was prepared by the Ritter reaction: tertiary alcohol **2** derived from 4-oxothiane was converted to chloroacetamide **3** followed by dechloroacylation using thiourea.[16] On the other hand, amine **4** was directly converted to biarylamines **7** by the Buchwald reaction with imidazole or the Migita-Kosugi-Stille coupling reaction with stannyl thiazoles followed by acylation, oxidation and deprotection (Scheme 2). The analogues **28**–**42** having a direct bond with dioxothiane and the azoles were prepared from 4-oxothiane and substituted phenyl azoles **12**, as shown in Scheme 3 followed by the synthetic route reported previously.[13] Phenylthiazols **12a–h** were prepared by two methods from commercially available 2-bromo-1-phenylethanones **9** or acetophenones **13**. De-esterification of ethyl 4-phenylthiazole-2-carboxylates **10** was carried out by hydrolysis followed by decarboxylation. Deamination of 4-phenylthiazol-2-amines **14** was carried out by the Sandmeyer reaction.[17,18] Imidazole **12i** and oxadiazoles **12j–l** were prepared by methylation of **15** and cyclization of benzohydrazides **16**, respectively.[19]

Firstly, the synthesized compounds were evaluated for the inhibition of DPP-4 derived from human colonic carcinoma cells (Caco-2).[20] The results of 4-phenyl-1,1-dioxothiane derivatives

Table 2
DPP-4 inhibiting activity and selectivity compound



Compound	R^1	R^2	R^3	IC_{50}[a] (μM)			
				DPP-4	QPP	DPP-8	DPP-9
17	H	H	Ph	0.57	28.4	49.0	9.0
28	H	H	2-Thiazolyl	0.19	85.2	45.8	37.6
29	H	H	4-Phenylthiazol-2-yl	0.060	22.0	9.6	8.6
30	F	F	4-Phenylthiazol-2-yl	0.016	2.7	4.1	7.7
31	H	H	4-(3-Methoxyphenyl)thiazol-2-yl	0.024	8.6	16.0	7.0
32	H	H	4-(4-Methoxyphenyl)thiazol-2-yl	0.67	ND	ND	ND
33	F	F	4-(2-Methoxyphenyl)thiazol-2-yl	0.059	ND	ND	ND
34	F	F	4-(4-Chlorophenyl)thiazol-2-yl	1.1	ND	ND	ND
35	F	F	4-(4-(Trifluoromethyl)phenyl)thiazol-2-yl	0.11	ND	ND	ND
36	F	F	4-(3-(Trifluoromethyl)phenyl)thiazol-2-yl	0.19	ND	ND	ND
37	H	H	4-(4-Fluorophenyl)thiazol-2-yl	0.10	29.4	9.2	8.8
38	H	H	5-Methyl-4-phenylthiazol-2-yl	0.072	45.4	20.9	17.4
39	F	F	5-Phenyl-1,3,4-oxadiazol-2-yl	0.094	ND	ND	ND
40	F	F	5-(3-Fluorophenyl)-1,3,4-oxadiazol-2-yl	0.055	ND	ND	ND
41	F	F	5-(3-Methoxyphenyl)-1,3,4-oxadiazol-2-yl	0.050	ND	ND	ND
42	F	F	1-Methyl-4-phenyl-1H-imidazol-2-yl	0.16	ND	ND	ND

[a] ND = no data.

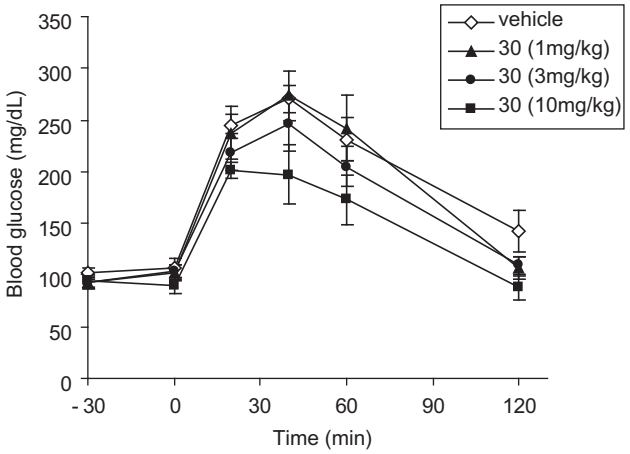


Figure 2. Effect of **30** in OGTT in ICR mice.

are shown in Table 1. Compounds **19** (IC_{50} = 0.012 μM) and **25** (IC_{50} = 0.084 μM) with imidazolyl substituents at the phenyl group were more potent than unsubstituent derivatives **18** (IC_{50} = 0.083 μM) and **17** (IC_{50} = 0.57 μM) respectively. Thiazol-5-yl derivatives **22** (IC_{50} = 0.041 μM) and **23** (IC_{50} = 0.0078 μM) resulted in an increase in the inhibitory effects of DPP-4 against thiazol-2-yl derivative **21** (IC_{50} = 0.39 μM). For compounds **20** (IC_{50} = 0.49 μM), **24** (IC_{50} = 0.33 μM), **26** (IC_{50} = 0.28 μM) and **27** (IC_{50} = 0.25 μM), a substitution on the phenyl group did not exhibit sufficient effects.

Table 2 shows the evaluation results of 4-heteroaryl-1,1-dioxothiane derivatives. Exchange of the phenyl group of compound **17** for the 2-thiazolyl group resulted in compound **28** (IC_{50} = 0.19 μM) and improved DPP-4 inhibition potency. Addition of phenyl substituent to the thiazole ring of **28** exhibited excellent DPP-4 potency of compound **29** (IC_{50} = 0.060 μM). As for the SAR reported previously,[13] the 2,4,5-trifluoro group on the left phenyl ring of **29** had very effective inhibitory activity (**30**: IC_{50} = 0.016 μM). Introduction of substitutions on the 4-phenylthiazol-2-yl group was examined in selected compounds **31**–**38**. Compound **31** with a 3-methoxy substituent on the phenyl group exhibited some improvements in potency over unsubstituted and other substituted derivatives (**31**: IC_{50} = 0.024 μM); however, it showed low DPP-4 inhibitory activity in human plasma in vitro (data not shown). Oxadiazole derivatives **39**–**41** and an imidazole derivative **42** did not exhibit very good activity.

Secondly, since inhibition of DPP-8 and DPP-9 was suggested to be connected to toxicity,[21] some inhibitors showing high DPP-4 inhibitory effects were tested for their selectivity profiles against the DPP-4 homologues DPP-8, DPP-9 and also QPP. These data are presented in Tables 1 and 2. Among the tested compounds, selectivity for DPP-4 of **19**, **23** and **25** in Table 1, and **28**–**31** and **38** in Table 2 over the related enzymes exceeded 100-fold.

Thirdly, mouse plasma DPP-4 activity was measured after oral administration of compounds **19**, **23** and **30**, which was metabolically stable in humans and mice (data not shown) and exhibited potent DPP-4 inhibition and high selectivity. Compounds **19** and **23** did not inhibit plasma DPP-4 activity at 100 mg/kg and 30 mg/kg, respectively, because the bioavailability of both **19** and **23** was lower (data not shown). On the other hand, DPP-4 inhibitory activity of compound **30** in mouse plasma was observed in a dose-dependent manner. Finally, when 1, 3, or 10 mg/kg of compound **30** was administered orally to ICR mice, blood glucose excursion in an oral glucose tolerance test (OGTT) was reduced in a dose-dependent manner (Fig. 2).[22]

In conclusion, the novel series of 3-amino-*N*-(4-aryl-1,1-dioxothian-4-yl)butanamidesexhibited marked DPP-4 inhibitory activity and selectivity against the other proteases. Among these derivatives, oral administration of compound **30** reduced the blood glucose excursion in OGTT. Further optimization of the derivatives is now being investigated.

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20. An extract from Caco-2 was used as the source of DPP-4 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), which 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-bottomed 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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22. The mice were orally administered a vehicle (distilled water, 10 mL/kg) or **30** (10, 3, 1 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.