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Discovery of potent and selective β-homophenylalanine based dipeptidyl peptidase IV inhibitors

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Abstract—Modification of in-house screening lead β-aminoacyl proline **8** gave an equipotent thiazolidide **9**. Extensive SAR studies on the phenyl ring of **9** led to the discovery of a novel series of potent and selective DP-IV inhibitors. Introduction of a fluorine at the 2-position proved to be crucial for the potency of this series. The 2,5-difluoro (**22q**) and 2,4,5-trifluoro (**22t**) analogues were potent inhibitors of DP-IV (IC_{50} = 270, 119 nM, respectively).

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that is released from the gut during meals and serves as an enhancer of glucose stimulated insulin release from pancreatic β-cells. Chronic infusion of GLP-1 to patients with type 2 diabetes resulted in significant decreases in both blood glucose and hemoglobin A_{1c} levels,[1] however, GLP-1 is rapidly degraded in plasma by the serine protease dipeptidyl peptidase IV (DP-IV). Inhibition of DP-IV increases the levels of endogenous intact circulating GLP-1. Thus, inhibition of DP-IV is rapidly emerging as a novel therapeutic approach to the treatment of type 2 diabetes.[2]

A range of DP-IV inhibitors have been reported, some of which are illustrated in Figure 1. The (*S*)-2-cyanopyrrolidine derivatives **1** and **2** are potent inhibitors of DP-IV, but both compounds contain an electrophilic nitrile group.[3] A systemic study to improve the chemical stability of such nitrile-containing compounds has been reported recently.[4] Inhibitors lacking an electrophile, such as **3** and **4**, are generally of only modest intrinsic potency.[5] Earlier reports from our laboratories described 4-amino cyclohexylglycine analogues **5** and **6**

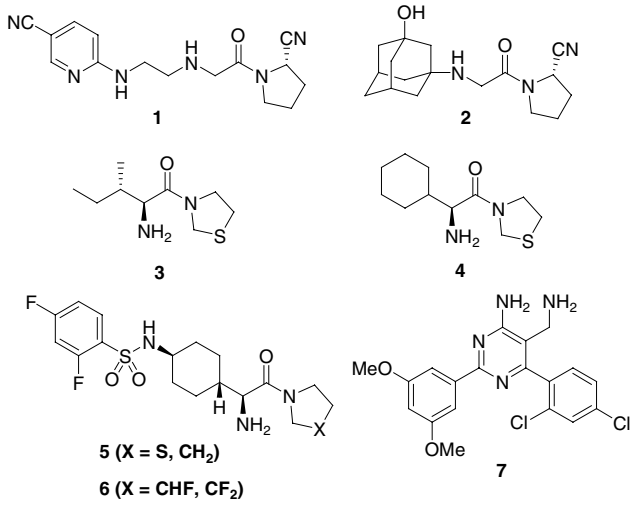

Figure 1. Published DP-IV inhibitors.

as potent DP-IV inhibitors lacking an electrophile.[6,7] More recently, 4-aminomethylpyrimidine **7** was reported as a very potent DP-IV inhibitor (IC_{50} = 0.1 nM).[8] In an effort to find novel structural classes, we have identified β-aminoacyl proline **8** (Fig. 2) from the Merck sample collection as a novel lead. It was soon discovered that the right-hand side of **8** could be

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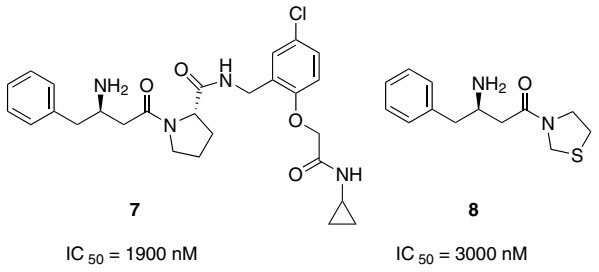
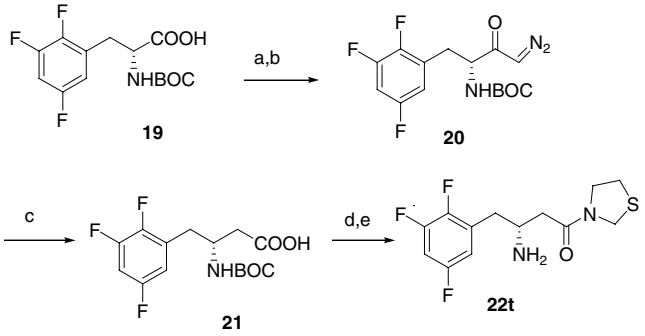


Figure 2. Initial leads.

replaced by a thiazolidine without a significant loss in potency while reducing the molecule weight of the initial lead by half. The work described here summarizes our initial efforts at optimizing the left-hand side of this novel series of β-homophenylalanine based DP-IV inhibitors.

The β-amino acid derived inhibitors were prepared by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-mediated coupling of β-amino acids with thiazolidine. Noncommercially available β-amino acids were readily accessible via the Arndt–Eistert homologation of α-amino acids (Scheme 1), which were either commercially available or prepared using Evans' asymmetric azidation[9] or Schöllkopf's bis-lactim methodology.[10] A representative example of the synthesis of these inhibitors is shown in Scheme 1. Treatment of α-amino acid **19** with isopropyl chloroformate followed by reaction with an excess of diazomethane afforded diazo ketone **20**. Sonication of the diazo ketone in 1,4-dioxane in the presence of silver benzoate resulted cleanly in the formation of β-amino acid **21**.[11] An EDC-mediated coupling of **21** with thiazolidine followed by deprotection of the *tert*-butylcarbamate provided the desired compound **22t**.[12]

Inhibitors were tested for their selectivity profiles against a variety of DP-IV homologues and proline-specific enzymes including quiescent cell proline dipeptidase (QPP/DPP-II), prolyl endopeptidase (PEP), amino peptidase P (APP), and prolidase. Since significant off-target activity was only observed with QPP (<100,000 nM), inhibition data are presented for DP-IV and QPP only.[13]

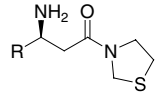


Scheme 1. Reagents: (a) Et_3N, *i*-PrO(CO)Cl, THF; (b) CH_2N_2, ether; (c) $C_6H_5CO_2$–Ag^+, H_2O, dioxane, sonication, rt; (d) thiazolidine, EDC, HOBT, DIEA, DMF; (e) trifluoroacetic acid, CH_2Cl_2, 1 h.

With the goal of finding analogues of thiazolidide **9** (Fig. 2) with increased potency, initially we looked at replacement of the phenyl group in **9** to see if it was essential for the activity in this series. Table 1 summarizes the DP-IV inhibitory properties of these β-amino thiazolidides. Lengthening or shortening the distance between the phenyl group and the amino group was not tolerated (**10**, **11**). Replacement of the phenyl group with a heterocycle usually resulted in a decrease in potency. However, the bioisosteric thiophene **15** was only 2-fold less potent. Replacement of the phenyl group with a cyclopentyl or cyclohexyl group resulted in 10-fold decrease in potency. We also prepared the enantiomer of **9**, which was inactive (DP-IV IC_{50} > 100,000 nM). At that point, we decided to examine substitution on the phenyl ring in **9**.

Table 2 summarizes the SAR of these derivatives. In most cases, mono-substitution on the phenyl ring had little effect or was detrimental to activity. However, addition of a fluorine or methyl group at the 2-position of the phenyl ring increased potency by 3-fold (**22a**, **22b**). The 3,4-difluoro analogue **22n** was also very interesting because it was more potent than 3-fluoro analogue **22e** and 4-fluoro analogue **22h**. We observed the beneficial additive effect of substitution at both the 3- and 4-position on the phenyl ring. Therefore, we

Table 1. Inhibitory property of β-aminoacid thiazolidides



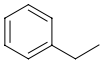
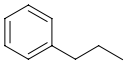
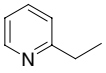
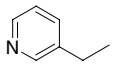
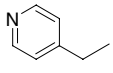
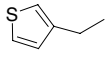
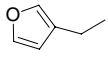
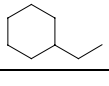
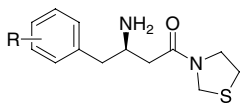
Compd	R	DP-IV IC_{50}, nM
9		3000
10		>100,000
11		16,480
12		39,640
13		44,860
14		51,000
15		5700
16		46,910
17		27,470
18		33,000

Table 2. Inhibitory properties of β-aminoacid thiazolidides



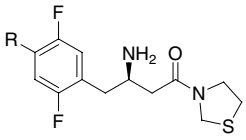
Compds	R	DP-IV IC_{50}, nM	QPP IC_{50}, nM
9	2-H	3000	>100,000
22a	2-F	931	49,700
22b	2-Me	981	92,060
22c	2-CN	2519	>100,000
22d	2-CF_3	2263	82,120
22e	3-F	2026	48,510
22f	3-CN	8951	29,750
22g	3-CF_3	3611	24,570
22h	4-F	3279	67,630
22i	4-Cl	3028	36,190
22j	4-I	10,700	5775
22k	4-CF_3	7688	ND
22l	4-CN	14,860	ND
22m	4-NO_2	1552	24,330
22n	3-F, 4-F	1700	33,890
22o	2-F, 3-F (*rac*)	9400	ND
22p	2-F, 4-F	589	56,050
22q	**2-F**, **5-F**	**270**	77,560
22r	2-F, 6-F (*rac*)	8476	ND
22s	3-Cl, 4-Cl	2026	48,510
22t	**2-F**, **4-F**, **5-F**	**119**	25,380

decided to look at further effects of disubstitution of fluorine on the phenyl ring. A combination of 2-fluoro and 4-fluoro on the phenyl ring increased potency by 5-fold (**22p**). A 10-fold increase in potency was observed with 2,5-difluoro analogue **22q**. Given the additive beneficial effects of substitution of fluorine at the 3- and 4-position on the phenyl ring, we prepared the corresponding 2,4,5-trifluoro analogue **22t**. A dramatic 25-fold increase was observed with **22t** (IC_{50} = 119 nM). Both **22q** and **22t** exhibited >200-fold selectivity over QPP.

The pharmacokinetic properties of compounds **22q** and **22t** were determined in rats. These compounds suffered from high clearance, short half-life and poor oral bioavailability, which precluded further development of this series (Table 3).

In summary, modification of an in-house screening lead β-aminoacyl proline **8**, gave an equipotent thiazolidide **9**. SAR studies on the phenyl ring of **9** led to the discovery of a novel series of potent and selective DP-IV inhibitors. Introduction of a fluorine at the 2-position of the phenyl ring proved to be crucial for the potency of these compounds. The 2,5-difluoro (**22q**) and 2,4,5-trifluoro (**22t**) analogues were the most potent DP-IV inhibitors, exhibiting DP-IV IC_{50}s of 270 and 119 nM, respectively. Incorporation of the fluorine containing β-homophenylalanine derivatives described herein into a novel series of piperazine containing DP-IV inhibitors will be described in the following paper.[14]

Table 3. Pharmacokinetic properties (1 mpk IV, 2 mpk PO) of selected DP-IV inhibitors in rats



Compds	R	Clp (mL/min/kg)	$t_{1/2}$ (h)	*F* (%)
22q	H	110	0.5	2
22t	F	120	1.1	3

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