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Ketopyrrolidines and ketoazetidines as potent dipeptidyl peptidase IV (DPP IV) inhibitors

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Abstract—In this paper, the synthesis and structure–activity relationships (SAR) of two classes of electrophile-based dipeptidyl peptidase IV (DPP IV) inhibitors, the ketopyrrolidines and ketoazetidines, is discussed. The SAR of these series demonstrate that the 2-thiazole, 2-benzothiazole, and 2-pyridylketones are optimal S1′ binding groups for potency against DPP IV. In addition, both cyclohexyl glycine (CHG) and octahydroindole carboxylate (OIC) serve as the most potent S2 binding groups within each series. Stereochemistry at the α-position of the central ring is relevant to potency within the ketopyrrolidines series, but not in the ketoazetidine series. Finally, the ketoazetidines display enhanced stability over the corresponding ketopyrrolidines, while maintaining their potency. In fact, certain stabilized ketoazetidines can maintain their in vitro potency and inhibit DPP IV in the plasma for up to 6 h.

1. Introduction

Dipeptidyl peptidase IV (DPP IV) is a proline-specific serine protease responsible for the in vitro and in vivo cleavage and degradation of many endogenous peptides and neuropeptides.[1] The most prominent substrates for this enzyme are GLP-1, neuropeptide Y, substance P, and casmorphin.[2] The inhibition of DPP IV and prevention of the degradation of these substrates can ultimately produce pharmacological effects in therapeutic areas as wide ranging as diabetes,[3] pain,[4] and cognition enhancement.[5]

A common strategy for the inhibition of serine proteases is to use substrate based analogs containing a chemically reactive electrophile to covalently bind the catalytic serine at the active site. Examples of several electrophile- and non-electrophile-based inhibitors of DPP IV are outlined in Figure 1. These inhibitors can be divided into three major categories based on their mode of inhibition: (1) competitive inhibitors such as pyrrolidine **1a** (IC_{50} = 125 nM)[6] and thiazolidine **1b** (IC_{50} = 150 nM); (2) irreversible inhibitors such as phosphonic esters **2** (K_i = 15 nM);[7] (3) slow binding, reversible inhibitors such as boronic acid **3** (K_i = 2 nM),[6] and nitrile **4** (IC_{50} = 8 nM).[8] In general, the incorporation of an electrophilic, serine binding group into this proline-derived pharmacophore can increase the potency by as much as an order of magnitude over the unsubstituted pyrrolidines **1a** and **1b**.[7,9]

Aryl and heteroaryl ketones have also been used effectively as reversible inhibitors of serine proteases.[10] In the following text we will discuss two series of inhibitors, the ketopyrrolidines (**5a**–**n**) and ketoazetidines (**7a**–**i**), as potent DPP IV inhibitors. Structure–activity relationships as well as stability studies and ex vivo DPP IV inhibition experiments will be discussed below.

The preparation of ketopyrrolidines **5** is outlined in Scheme 1. Commercially available Weinreb amide **8** was alkylated with either an aryl lithium or an alkyl Grignard reagent to afford the ketones **11a**–**h** in 31–72% yield. The deprotection and subsequent coupling of the amino ketones **11a**–**h** with various Boc-amino acids **17**–**20** (Fig. 2) led to the Boc-protected ketopyrrolidines **14a**–**n** in moderate yields (26–79%). The rapid deprotection of compounds **14a**–**n** followed by removal of the solvent, triturating with Et_2O, and filtration

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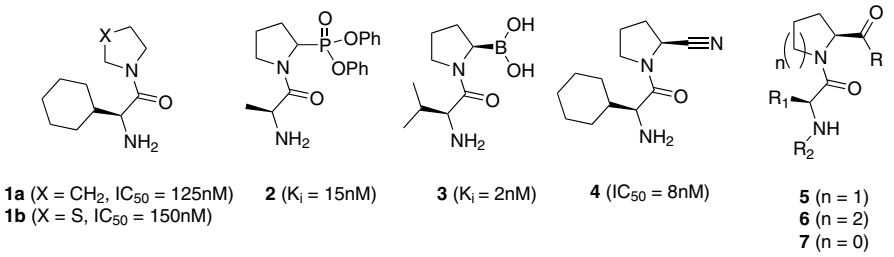
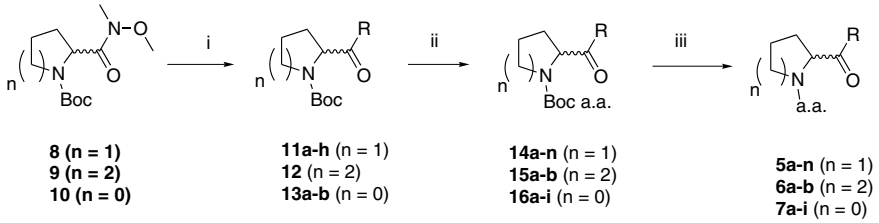


Figure 1. Examples of electrophile- and non-electrophile-based DPP IV inhibitors.



Scheme 1. Reagents and conditions: (i) RLi or RMgBr, THF, −78 to −40 °C, 31–72%; (ii) (1) TFA (2) HATU, **17**–**20**, DMA, DIEA, 26–79%; (iii) 4.0 M HCl in dioxane, 53–91%. See Supplementary data for experimental details.

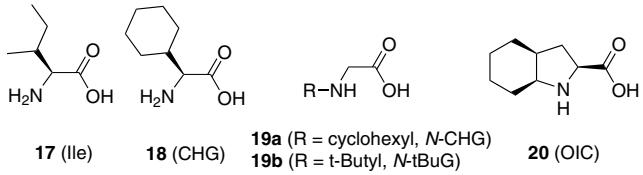


Figure 2. Representative hydrophobic amino acids.

afforded the ketopyrrolidines **5a**–**n**.[11] The ketopiperidines **6a**–**b** and ketoazetidines **7a**–**i** were synthesized in a similar manner to the ketopyrrolidines except that the Weinreb amides **9** and **10** were synthesized from either (*R*)- or (*S*)-Boc-azetidine-2-carboxylic acid or (*R*)- or (*S*)-Boc pipecolic acid according to the literature.[12]

2. Biological evaluation and SAR discussion

The binding mode for these proline-derived inhibitors is outlined in Figure 3 based on previous studies with other proline mimetics.[7,13] The electrophile portion (E) of the molecule resides in the S1′ pocket while the central ring lies in a very tight proline binding pocket (S1). The hydrophobic amino acid side chain occupies the spacious S2 pocket of the enzyme. Based on this binding mode, three variables were taken into account to optimize the enzymatic potency: (1) the nature of the ketone R group bound to the S1′ site; (2) the hydrophobic S2 binding group; (3) the size and stereochemistry in the central ring, binding to the S1 site. Synthetic efforts systematically addressed each of these factors in turn and the results are outlined in Tables 1–3.

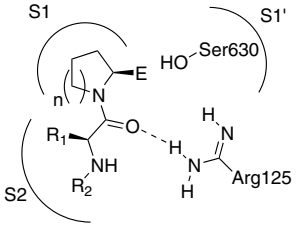
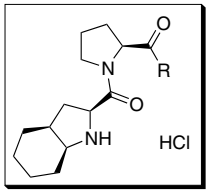


Figure 3. Binding mode for proline-based DPP IV inhibitors.

Potency data for several alkyl and aryl pyrrolidinyl ketones are outlined in Table 1. The S2 binding group and the ring size and stereochemistry (S1 binding group) were kept constant while the ketone substituent was altered. These results indicated that the heteroaryl ketones (i.e., 2-thiazole **5a**, 2-benzothiazole **5b**, and 2-pyridyl **5c**) were necessary to gain substantial potency. The simple methyl ketone **5d** was inactive as was the phenyl derivative **5e**. Interestingly, the 3-pyridyl derivative **5f** was over an order of magnitude less potent than the 2-pyridyl

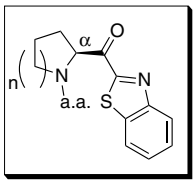
Table 1. Optimization of S1′ binding groups for DPP IV inhibition



Compounds	R Group	IC_{50} (nM)[a]
5a	2-Thiazole	**44** (±**8**)[b]
5b	2-Benzothiazole	**30** (±**5**)[b]
5c	2-Pyridiyl	2000
5d	Methyl	>100,000
5e	Phenyl	>100,000
5f	3-Pyridyl	>100,000
5g	2-Thiophenyl	50,000
5h	*N*-Methyl imidazole	>100,000

[a] See Supplementary data for details of the DPP IV inhibitory assay.
[b] Average of three values.

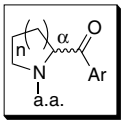
Table 2. Optimization of S2 binding groups for DPP IV inhibition



Compounds	*n*	Isomer[a]	Amino acid group	IC_{50} (nM)[b]
5i	1	*S*	Ile	100
5b	1	*S*	OIC	44 (±8)[c]
5j	1	*S*	CHG	50
5k	1	*S*	*N*-CHG	300
7a	0	*S*	Ile	200
7b	0	*S*	OIC	80 (±14)[c]
7c	0	*S*	CHG	42 (±4)[c]
7d	0	*S*	*N*-CHG	190 (±10)[c]

[a] Denotes the stereochemistry at the α-position.
[b] See Supplementary data for details of the DPP IV inhibitory assay.
[c] Average of three values.

Table 3. Optimization of S1 binding group for DPP IV inhibition



Compounds	*n*	Isomer[a]	Aryl group	Amino acid group	IC_{50} (nM)[b]
5l	1	*S*	Thiazole	CHG	42
5m	1	*R*	Thiazole	CHG	1000
6a	2	*S*	Thiazole	CHG	>100,000
6b	2	*R*	Thiazole	CHG	>100,000
7e	0	*S*	Thiazole	CHG	30
7f	0	*R*	Thiazole	CHG	40
7b	0	*S*	Benzothiazole	OIC	80 (±14)[c]
7g	0	*R*	Benzothiazole	OIC	115

[a] Denotes the stereochemistry at the α-position of the ring.
[b] See Supplementary data for details of the DPP IV inhibitory assay.
[c] Average of three values.

derivative **5c**. In addition, the 2-thiophenyl derivative **5g** was three orders of magnitude less potent than the thiazole derivative **5a**. Taken together, these results indicate the necessity of a nitrogen atom in the 2-position of the aryl group in order to maintain optimal potency.

This critical finding can be rationalized by examining a model of the complex of **5a** bound in the active site of DPP IV (Fig. 4).[13] The side chain of Arg125 forms a hydrogen bond to the 2-thiazole nitrogen, as well as a hydrogen bond to the prolyl amide oxygen. The formation of this extra hydrogen bond with the thiazolidine could explain the difference in potency between the 2-thiazole and 2-thiophenyl derivatives **5a** and **5g**, as well as the 2- and 3-pyridyl derivatives **5c** and **5f**. Two other prominent features seen in this protein–ligand model are as follows: (1) the proximity of the ketone with the catalytic serine (Ser630) and (2) the interaction the ketone oxygen with residues in the oxyanion hole of DPP IV (i.e., Tyr547 side chain and Tyr631 backbone).

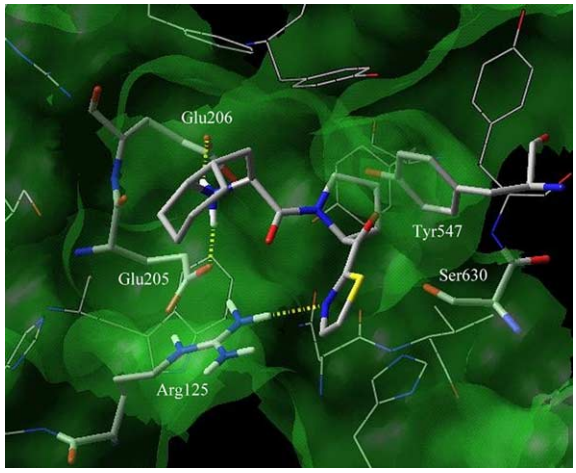


Figure 4. A model depicting the predicted binding mode of compound **5a**.

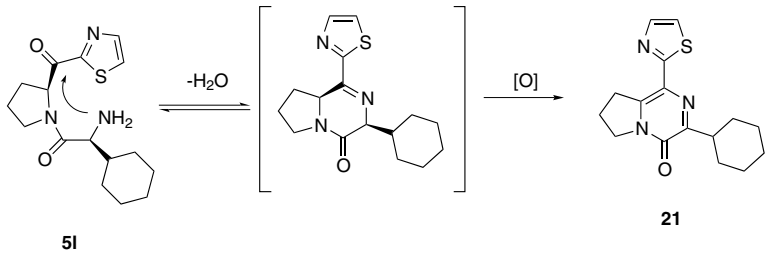
Based on previous SAR studies of nitrile based DPP IV inhibitors,[7,8] a panel of Boc-protected hydrophobic amino acids were coupled to the central ring. Compounds **17**–**20** (Fig. 2) are representative hydrophobic amino acids since they have the substituents on the α-carbon, for example, **17** (Ile) and **18** (CHG), on the amino acid nitrogen, for example, **19a** and **19b** (*N*-CHG and *N*-*t*-BuG) and on both the α-carbon and the nitrogen, for example, **20** (OIC).

The inhibitory data outlined in Table 2 indicates that the two optimal amino acid groups for potency are OIC and CHG. The most potent ketopyrrolidine derivatives are **5b** and **5j** while the most potent ketoazetidine derivatives are **7b** and **7c**.

Table 3 outlines the differences in potency upon changing the size of the central ring (S1 binding group) and the stereochemistry at the α-position. The six-membered piperidines **6a** and **6b** were notably less potent than the ketopyrrolidines **5l**–**m**. The ketoazetidine **7e**, however, displayed a similar potency to the five-membered pyrrolidine **5l**. The stereochemistry at the α-position is necessary for potency within the ketopyrrolidine series as the 2-(*R*) derivative **5m** was less potent than its diastereomer **5l**. Consequently, both the size and stereochemistry results were mirrored in a similar series of DPP IV inhibitors, the cyanopyrrolidines, and cyanopiperidines.[7] Interestingly, the diastereomer of **7e**, the 2-(*R*) azetidine **7f**, were equipotent, indicating that there was no difference in potency between the 2-(*R*) and 2-(*S*) diastereomers within the ketoazetidine series. Testing the diastereomers **7b** and **7g**, both of which had similar potencies against DPP IV, reconfirmed this observation.

3. Stability data and ex vivo experiments

Despite the potency of these amino ketones, major issues involving their stability had to be addressed. A simple intramolecular cyclization can occur between the amine and ketone forming an imine bond. This



Scheme 2. Cyclization/oxidation of ketopyrrolidine **5l**.

Table 4. DPP IV inhibition of sterically hindered ketones **7g** and **7h**

Compounds	*n*	Aryl group[a]	Amino acid group	Time at rt (h)	Estimated % ketone[b]	IC_{50} (nM)[c]
5l	1	Tzl	CHG	0.25	95	30
5l	1	Tzl	CHG	24	<5	>20,000
21					0	>100,000
7g	0	Bntzl	OIC	0.25	99	80
7g	0	Bntzl	OIC	24	75	250
7g	0	Bntzl	OIC	168	55	500
7h	0	Bntzl	*N*-*t*-BuG	0.25	99	100
7h	0	Bntzl	*N*-*t*-BuG	168	75	200
7h	0	Bntzl	*N*-*t*-BuG	504	70	900

[a] Tzl = thiazole, Bntzl = benzothiazole.
[b] Measured by ^{1}H NMR integration.
[c] See Supplementary data for details of the DPP IV inhibitory assay.

intermediate can either revert back to the parent ketone in a reversible manner or oxidize into the dihydroketopyrazine as outlined in Scheme 2. The major product (>90%) of the decomposition of **5l** is indeed the dihydroketopyrazine **21** as evidenced by ^{1}H NMR, MS, and CHN (IC_{50} > 100 μM). This conversion appears to be almost stoichiometric with minimal byproducts visible by ^{1}H NMR and HPLC. Consequently, the azetidinyl derivative **7g** displayed a minimal increase in stability with an estimated cyclization at 24 h of 55% (vs 95% for compound **5l**, Table 4). This improvement was further amplified by the *N*-*t*-BuG derivative **7h**. The bulky secondary *tert*-butyl amine reacts very sluggishly with the internal aryl ketone and DPP IV inhibitory potency is maintained over several days at room temperature (Table 4).

Ex vivo experiments were done in order to determine the relative potency of these derivatives in the plasma and brain of Sprague-Dawley rats. Animals were dosed p.o. with compound **7g** (50 mg/kg). One animal was sacrificed at each time point followed by collection of both plasma and brain samples. As Figure 5 illustrates, compound **7g** displays sufficient inhibitory potency against DPP IV over a 6 h period in the plasma.

In conclusion, structure–activity relationships of the ketopyrrolidines and ketoazetidines have determined that the 2-thiazole, 2-benzothiazole, and 2-pyridylketones are optimal S1′ binding groups for potency against DPP IV. This finding can be rationalized from the docking studies and crystal structure model. In addition, both cyclohexyl glycine and octahydroindole carboxylate serve as potent S2 binding groups. Stereochemistry at the α-position of the central ring is relevant to potency in the ketopyrrolidine series, but not in the ketoazetidine series. Finally, certain stabilized ketoazetidines such as **7g** can maintain their in vitro potency and inhibit DPP IV in the plasma for up to 6 h.

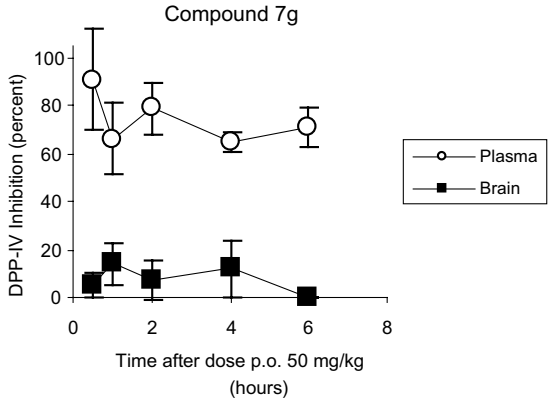


Figure 5. Ex vivo experiment with compound **7g**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.08.057.

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