Bioorganic & Medicinal Chemistry Letters 19 (2009) 4437–4440

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Bicyclic cyanothiazolidines as novel dipeptidyl peptidase 4 inhibitors

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ARTICLE INFO

Article history:
Received 30 April 2009
Revised 11 May 2009
Accepted 13 May 2009
Available online 18 May 2009

Keywords:
Diabetes
DPP4
Cyanopyrrolidine

ABSTRACT

The synthesis and biochemical evaluation of novel cyanothiazolidine inhibitors of dipeptidyl peptidase 4 (DPP4) is described. Their main structural feature is a constrained bicyclic core that prevents the intramolecular formation of inactive cyclic species. The inhibitors show good to moderate biochemical potency against DPP4 and display distinct selectivity profiles towards DPP7, DPP8 and DPP9 depending on their substitution.

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Diabetes is a major health problem that is approaching pandemic proportions worldwide. The search for new therapies with novel mechanistic approaches and improved safety profiles to control this chronic metabolic disease has come to the forefront of research in medicinal chemistry.[1] Type-2 diabetes mellitus accounts for over 90% of the disease cases and is characterized by high levels of glucose resulting from progressive insulin resistance. Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by intestinal L-cells in response to food intake and is a key player in the modulation of blood glucose levels.[2] GLP-1 stimulates insulin secretion, delays glucose absorption and inhibits glucagon secretion leading to reduced hepatic glucose production. The primary physiological route of GLP-1 degradation is cleavage by dipeptidyl peptidase 4 (DPP4), a ubiquitous and highly specific serine protease that preferentially cleaves N-terminal dipeptides from polypeptides with L-proline or L-alanine at the penultimate position.

DPP4 inhibition, through the preservation of active GLP-1 levels, offers a number of potential advantages over existing diabetes therapies including minimized risk for hypoglycemia and improved β-cell survival.[3] Clinical studies have shown that DPP4 inhibitors are well tolerated, lower blood glucose levels, improve insulin response to oral glucose, and decrease HbA1c levels in patients with type 2 diabetes.[4] The most advanced compound (Sitagliptin, Januvia™) gained FDA approval in 2006 for the treatment of type 2 diabetes.[5]

Most of the DPP4 inhibitors described to date have been dipeptidomimetics resembling the natural substrate preference of the enzyme, in particular compounds containing L-proline mimetics in the P1 position. A widely explored class of these inhibitors includes N-substituted cyanopyrrolidines such as NVP-728 (**1**), LAF-237 (Vildagliptin, **2**), BMS-477118 (Saxagliptin, **3**) and ABT-279 (**4**) (Fig. 1).[6] The cyano group common to all of these inhibitors acts as an electrophilic trap for the Ser 630 of the DPP4 catalytic triad by formation of an imidate adduct.

It is well established that the P-2 site amine of these molecules can intramolecularly attack the carbon of the nitrile group under neutral and basic aqueous conditions to form an inactive cyclic amidine (Fig. 2). In fact, this property has been closely monitored

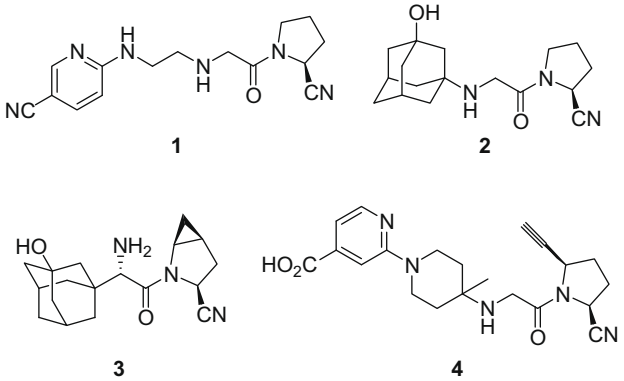

Figure 1. Cyanopyrrolidine inhibitors of DPP4.

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doi:10.1016/j.bmcl.2009.05.048

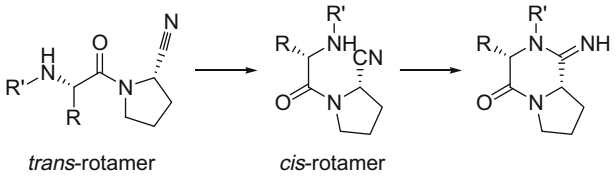


Figure 2. Formation of cyclic amidine adducts.

by the medicinal chemistry groups working with this class of inhibitors in order to minimize the formation of inactive species and increase chemical stability.[7]

While increasing the size of the R or R′ substituent has been a strategy pursued in some DPP4 programs, a straightforward alternative method of avoiding the formation of inactive cyclic species could be the introduction of a short tether between the P-2 α-amino acid and the five-membered P-1 heterocycle (Fig. 3). This tether renders the amine unavailable for the intramolecular nucleophilic attack on the nitrile group. Examination of the published X-ray structures of DPP4 in complex with known inhibitors supported this hypothesis due to the presence of the large S2 enzyme pocket which would permit binding of the tethered molecule.[8] The resulting rigid scaffold would in turn provide an entropically favored platform to study the effect of the introduction of additional substituents on inhibitory potency and selectivity.[9]

For the sake of synthetic ease, the thiazolidine ring (X = S) was chosen as the P-1 proline mimetic. Initial efforts focused on determining the appropriate ring size required for activity (Scheme 1). Since it was previously reported that the DPP4 enzyme could tolerate both natural and unnatural amino acids in its S2 pocket,[10] compounds **5** through **8** were synthesized following the methodology developed by Baldwin et al. for related systems.[11,12] The hexahydropyrrolo[2,1-*b*]thiazole **7** possessing the unnatural stereochemistry in the P-2 amino acid exhibits the best potency against DPP4.[13]

These preliminary results prompted us to explore lead optimization for the series. As exemplified by compounds **1**, **2** and **4**, one of the most common strategies within the cyanopyrrolidine series has been the alkylation of the amino group. Inhibitors **9** and **10** were prepared by reductive alkylation of amine **7** with the corresponding carbonyl compounds.[14] However, when evaluated against DPP4, they showed a complete lack of activity (Scheme 2). As a result, N-alkylated analogs were not further pursued in our series.

In order to expand our SAR, we prepared a series of compounds bearing a wide range of substituents in the α-position of the P-2 amino acid. The desired compounds were synthesized according to the procedure exemplified in Scheme 3 for the phenyl analog. The benzaldimine of L-phenyl glycine methyl ester **12** was treated with *t*BuOK and alkylated with allyl bromide. After hydrolysis of the imine and protection of the resulting amine by a Boc group, ozonolysis of the olefin followed by reductive work-up with PPh_3 provided the corresponding hemiacetal.[15] Condensation of **14** with L-cysteine methyl ester hydrochloride gave a circa 1.6:1 mixture of two pairs of the diastereomeric thiazolidines **15**. In order to increase the yield of isomer **16,** a systematic investigation of coupling conditions was carried out. The most efficient method involved refluxing the mixture of thiazolidines in toluene in the presence of pTSA.[16] Aminolysis of methyl ester **16** followed by dehydration of the resulting amide with TFAA yielded nitrile **17**. Final deprotection of the Boc group provided the free amine that was isolated as its hydrochloride salt.

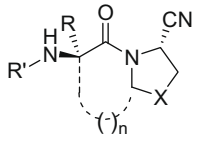
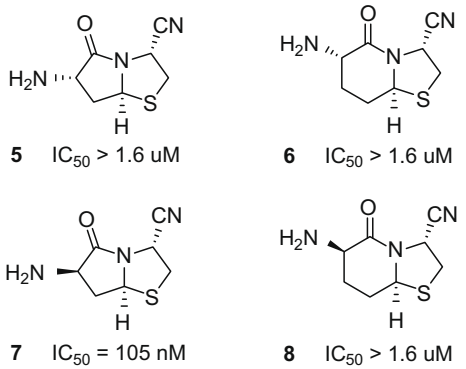
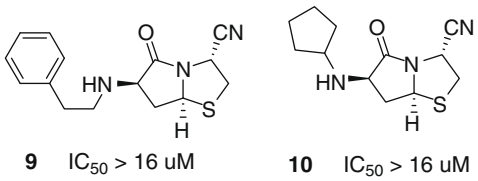


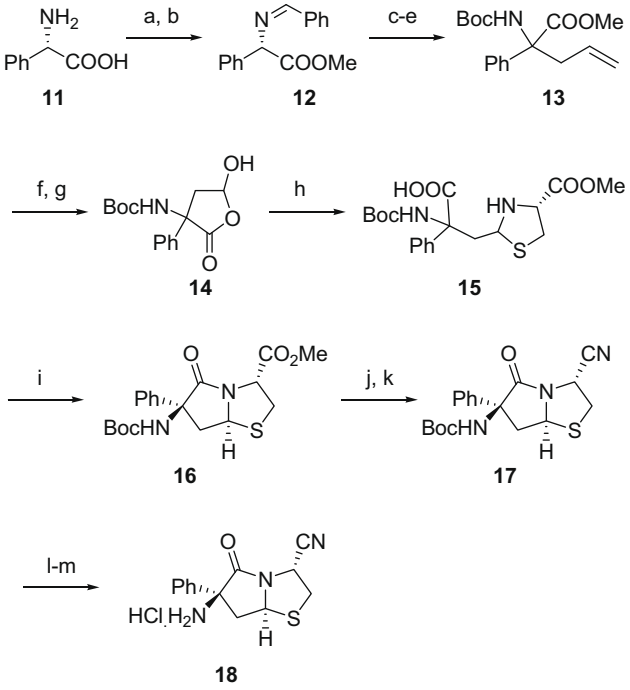
Figure 3. Constrained cyano derivatives.



Scheme 1. DPP4 activity of bicyclic cyanothiazolidine inhibitors.



Scheme 2. N-alkylated cyanothiazolidine inhibitors.



Scheme 3. Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C, 2 h, 98%; (b) PhCHO, TEA, $MgSO_4$, CH_2Cl_2, rt, 18 h, 97%; (c) *t*BuOK, CH_2CHCH_2Br, THF, rt, 18 h; (d) 6 N HCl, EtOAc, 1 h, 64%; (e) Boc_2O, THF, reflux, 18 h, 87%; (f) O_3, PPh_3, CH_2Cl_2, −78 °C, 36%; (g) LiOH, THF, 1 h, 95%; (h) L-Cys-OMe·HCl, $NaHCO_3$, EtOH, 16 h, 51%; (i) pTsOH, Toluene, reflux, 1 h, 23%; (j) NH_3, MeOH, rt, 2 h, 97%; (k) TFAA, TEA, CH_2Cl_2, rt, 1 h, 84%; (l) TFA, CH_2Cl_2, rt, 1 h, 14% after preparative HPLC; (m) HCl (g), CH_2Cl_2/Et_2O, 2 h, 53%.

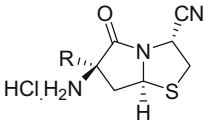
The substituted cyanothiazolidines **18–28** were evaluated for DPP4 inhibitory activity by a fluorescence assay using Gly-Pro-AMC as substrate.[17] Inhibitors were also assayed for their selectivity profiles against a variety of DPP4 homologues including quiescent prolyl peptidase (QPP/DPPII/DPP7), DPP8 and DPP9 (Table 1).[18]

The substituted cyanothiazolidines exhibited potencies against DPP4 ranging from moderate to good in the double digit nanomolar range, with the phenyl substituted compound **18** displaying the best potency at 26 nM. Selectivity profiles against the homologous proteases were clearly modulated by the substitution present on the lactam ring, with small alkyl groups simultaneously displaying improved selectivities against DPP7 and DPP8. In general, the cyanothiazolidines show the least selectivity against DPP9. This is also the case for other cyanopyrrolidines described in the literature such as LAF-237 (**2**) and BMS-477118 (**3**). Larger substituents on the lactam ring show decreased selectivity against DPP9. In fact, compounds **25–27** exhibit greater potency against DPP9 than DPP4. Though the X-ray structure of DPP9 is not available, detailed homology models have recently been established to rationalize the selectivity of known inhibitors against DPP4, DPP8 and DPP9.[19] These studies show that the P2 pocket of DPP9 is larger than that on DPP4. The SAR presented here for the cyanothiazolidines reinforces that observation. The phenyl substituted analog **18** shows the best balance of potency and selectivity within this series. But even small changes such as the introduction of a halogen substitution (compound **28**) on the aromatic ring have a negative impact on the overall profile of the compound.

Although compounds **18**–**28** display different degrees of activity against the other proteases in the DPP family, they did not exhibit significant inhibition against fibroblast activation protein (FAP) (IC_{50} >10 μM), another proline-specific enzyme.

In summary, a novel series of bicyclic cyanothiazolidine inhibitors of dipeptidyl peptidase 4 has been developed.[20] The constrained inhibitors were designed to avoid the formation of inactive cyclic amidine species. They show good to moderate biochemical potency and depending on their substitution exhibit different selectivity profiles against closely related serine proteases. The incorporation of a phenyl group in the P-2 amino acid provides the greatest potency combined with increased selectivity. While substitution in the α-position of the P-2 amino acid is well tolerated, N-alkylation had a negative effect on potency.

Table 1
DPP4 inhibition and selectivity profile of substituted bicyclic cyanothiazolidines



Compounds	R	DPP4 IC_{50} nM	DPP7 SI[a]	DPP8 SI[a]	DPP9 SI[a]
2[b]		3	>16,000	835	13
3[b]		3	>3333	114	22
7	H	105	>476	40	3
18	Ph	26	101	100	10
19	Et	56	129	30	3
20	^{i}Pr	33	277	31	4
21	^{i}Bu	63	6	23	2
22	^{s}Bu(*S*)	53	59	15	3
23	Bn	109	23	41	36
24	Cp	89	32	7	1
25	Cy	59	35	4	0.5
26	$BnCH_2$	205	3	5	0.2
27	$CyCH_2$	118	1	6	0.4
28	4-FPh	152	17	44	4

[a] Selectivity Index (SI) determined by dividing enzyme IC_{50} by DPP4 IC_{50}.
[b] Data generated in our laboratories. Though absolute numbers differ from published data, the relative selectivity and ranking order is the same.

Acknowledgements

The authors would like to thank Drs. Michael Hepperle and Marie O'Farrell for reviewing the manuscript.

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wavelength of 460 nm. Reaction buffers were 25 mM (2-(4-Morpholino)-Ethane Sulfonic Acid), pH 5.5 for DPP7; 25 mM Tris, pH 8, 1% Triton X-100, 100 mM NaCl for DPP8; and 25 mM Tris pH 8 for DPP9 and FAP. IC_{50} and IC_{90} calculations were performed by nonlinear regression analysis using Prism software (GraphPad Software, Inc., San Diego, CA).

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