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Inhibitors of dipeptidyl peptidase 8 and dipeptidyl peptidase 9. Part 2: Isoindoline containing inhibitors

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ABSTRACT

To obtain selective and potent inhibitors of dipeptidyl peptidases 8 and 9, we synthesized a series of substituted isoindolines as modified analogs of *allo*-Ile-isoindoline, the reference DPP8/9 inhibitor. The influence of phenyl substituents and different P2 residues on the inhibitors' affinity toward other DPPs and more specifically, their potential to discriminate between DPP8 and DPP9 will be discussed. Within this series compound **8j** was shown to be a potent and selective inhibitor of DPP8/9 with low activity toward DPP II.

Proline-selective dipeptidyl peptidases (DPPs) are a group of phylogenetically related serine proteases.[1] Many peptide hormones, chemokines, and neuropeptides contain one or more proline residues that could function as regulatory elements for proteolytic processing. The biological significance of the DPPs is generally deemed to reside in the hydrolytic modification of peptides containing such 'checkpoint'-residues, a property suggesting potential as targets for drug development.[2,3] Dipeptidyl peptidase IV (DPP IV, CD26, EC 3.4.14.5) has become the best-characterized DPP since it was shown that inhibition of the enzyme leads to an increased half-life of the incretin hormone glucagon-like peptide-1.

During the last decade, DPP8 and DPP9 have been described as two members of the S9b subfamily of serine proteases, in which also DPP IV is categorized. As compared to the latter, however, relatively little is known about DPP8 and DPP9. Neither the tertiary structures nor in vivo substrates and corresponding physiological functions of these two enzymes are currently known.[1,4] Similar to DPP8, DPP9 is also ubiquitously expressed and the catalytic efficiencies of both enzymes toward model substrates were found to be very similar.[5]

DPP8 and DPP9 have garnered much attention following research results reported by Lankas et al. suggesting that the use of the DPP8/9 inhibitor *allo*-Ile-isoindoline is associated with severe toxicity in animal models.[6] Whether the observed toxicity was DPP8/DPP9 related, or solely due to compound mediated off-target effects, remains nonetheless to be unambiguously established.[1] In accordance with this remark, a recent study suggests that DPP8/9 inhibition alone cannot account for the gastrointestinal toxic symptoms.[7] Secondly, another report claims the observation that DPP8 and DPP9 are upregulated in experimentally induced asthma and that these peptidases specifically respond to the inflammatory stimulus.[8] Finally, in an in vitro model of T-cell activation, inhibition of DPP8/9 activity attenuated T-cell proliferation.[6] This finding suggests that the previously assumed DPP IV mediated effect on the immune function could at least partially be attributed to DPP8/9.[1] In order to verify whether the reported toxicity observations are related to DPP8/9 inhibition and to further study the clinical relevance of potential enzyme involvement in pathologies

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or T-cell function, there is an urgent need for new inhibitors of these enzymes.

The inhibitors in this report were developed starting from *allo*-Ile-isoindoline **1** as a lead (Fig. 1). Two types of structural modifications were investigated in order to obtain compounds with a maximally optimized DPP8/9 potency-selectivity profile and to explore the possibility of discerning between DPP8 and DPP9 with α-aminoacyl isoindoline derivatives.

The first modification type consists of introducing substituents on the phenyl ring of isoindoline in order to allow for a steric and electronic scan of the P1 pocket of DPPs. Secondly, since we could not conclude from literature data that the P2 *allo*-Ile residue in compound **1** had already been optimized, analogs with different P2 residues were prepared. Isoleucine, lysine, and ε-*N*-*Z*-protected lysine, three amino acids that were identified elsewhere in this issue as useful building blocks for DPP8/9 inhibitors; along with 4-aminoproline were selected for this purpose.[9] The latter can be viewed as a conformationally constrained mimic of dibasic α-amino acids.

For the preparation of structurally modified isoindoline analogs, mainly three synthetic strategies were followed (Schemes 1–3).

Target compounds **8a**–**l** were synthesized starting from commercially available phthalic anhydrides, outlined in Scheme 1. The route starts with the amidation of substituted phthalic anhydrides **2**, using aqueous ammonia in tetrahydrofuran (THF). By thermal dehydration, a ring closure was performed yielding the corresponding phthalimides.[10] Compounds **3** were reduced using borane in THF forming isoindolines **4** followed by an *N*-Boc protection step to allow for chromatographic purification.[11] Acidolytic cleavage using trifluoroacetic acid (TFA) was followed by coupling intermediates **6** with *N*-Boc protected isoleucine or *allo*-isoleucine. Final deprotection of these intermediates with TFA afforded compounds **8a**–**l**.

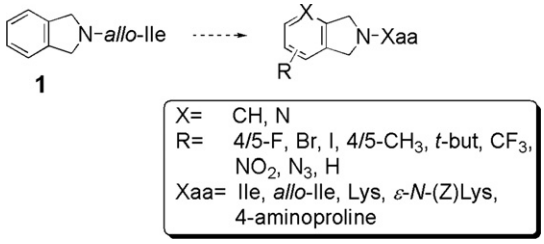
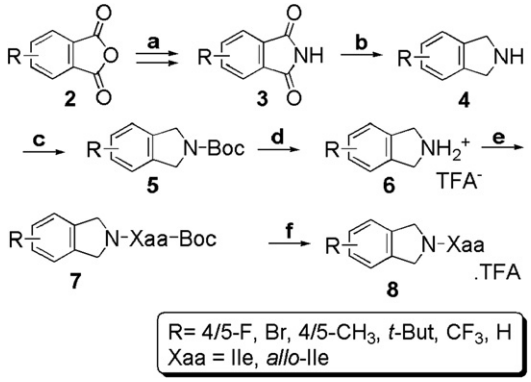
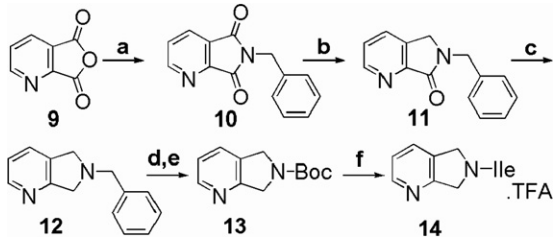


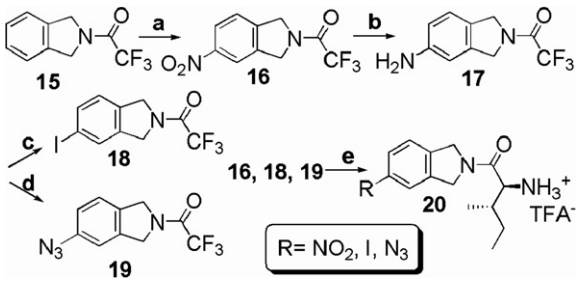
Figure 1. Target compounds derived from lead structure *allo*-Ile-isoindoline (**1**).



Scheme 1. Synthesis of substituted isoindoline inhibitors (**8a**–**l**). Reagents and conditions: (a) NH_4OH, THF followed by thermal dehydration (95–99%); (b) borane, THF; (c) Et_3N, Boc_2O, DCM (b + c 21–33%); (d) TFA, DCM (95–99%); (e) Ile-Boc (**8a–h**) or *allo*-Ile-Boc (**8i–l**) TBTU, Et_3N, DCM (38–80%; (f) TFA, DCM (95–99%).



Scheme 2. Synthesis of 6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridine compound **14**. Reagents and conditions: (a) benzylamine followed by thermal dehydration (95–99%); (b) metallic tin, acetic acid, HCl (37–67%); (c) $LiAlH_4$, THF (13%); (d) H_2, Pd/C; (e) Et_3N, Boc_2O, DCM (d + e 45%); (f) 1—TFA, DCM; 2—Ile-Boc, TBTU, Et_3N, DCM; (1 + 2 56%); 3—TFA, DCM (96%).

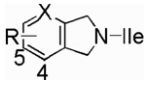


Scheme 3. Synthesis of 5-nitro-, 5-iodo-, and 5-azido-substituted isoindoline inhibitors (**20a**–**c**). Reagents and conditions: isoindoline was protected with a *N*-trifluoroacetyl protecting group using TFA anhydride and Et_3N yielding **15** (70%); (a) nitronium tetrafluoroborate, ACN (70%); (b) H_2, Pd/C, methanol (81%); (c) p-TosOH, ACN; $NaNO_2$/KI, H_2O, 15 °C (65%); (d) $NaNO_2$/NaN_3; TFA (52%); (e) 1—K_2CO_3, H_2O (61–99%); 2—Et_3N, Ile-Boc, TBTU, DCM (28–35%); 3—TFA, DCM (95–99%).

Due to the incompatibility of **14**, **16**, and **19** with the reaction conditions used in the synthesis of the substituted isoindolines, two other synthetic routes were elaborated.

Preparation of the 6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridine **14**, as illustrated in Scheme 2, began with protection of the imide by reacting the starting product with benzylamine followed by thermal dehydration. The protected pyrrolopyridine-5,7-dione **10** was reduced in two steps to the corresponding amine **12** using metallic tin in acetic acid followed by reduction of the amide with lithium aluminum hydride.[12,13] The benzyl group was removed via catalytic hydrogenation yielding the free amine. Coupling to isoleucine

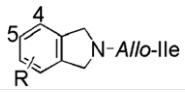
Table 1
IC_{50} values of substituted isoindolines (**8a**–**h**, **20a**–**c**, **14**)



Compound[a]	R	IC_{50} (μM)			
		DPP8	DPP9	DPP IV	DPP II
8a	H	1.3 ± 0.3	4.3 ± 0.1	115 ± 11	43 ± 11
8b	4-F	7.3 ± 1.3	8.6 ± 0.4	>100	12.2 ± 1.6
8c	4-CH_3	>100	>100	>100	>100
8d	5-F	1.2 ± 0.1	0.54 ± 0.02	80.1 ± 4.6	23 ± 2.5
8e	5-CH_3	4.6 ± 0.4	7.6 ± 0.6	>100	>100
8f	5-*t*-But	>100	>100	>100	>100
8g	5-CF_3	>100	>100	>100	13.0 ± 1.3
8h	5-Br	4.9 ± 0.5	4.1 ± 0.4	>100	7.4 ± 0.4
20a	5-I	>25	>50	>100	2.6 ± 0.2
20b	5-NO_2	>50	>25	>50	66 ± 5
20c	5-N_3	>100	96 ± 8	>100	>50
14	H	56.9 ± 7.4	82.8 ± 8.7	>100	>100

[a] X = N (**14**), X = CH (**8a**–**h**, **20a**–**c**).

Table 2
IC_{50} values of substituted isoindolines (**1**, **8i–l**)



Compound	R	IC_{50} (μM)			
		DPP8	DPP9	DPP IV	DPP II
1	H	0.12 ± 0.01	0.29 ± 0.02	90 ± 4	29 ± 1
8i	4-F	0.8 ± 0.1	1.2 ± 0.05	>100	7.1 ± 0.8
8j	5-F	0.16 ± 0.016	0.07 ± 0.04	>100	21.5 ± 3.2
8k	5-CH_3	2.8 ± 1.4	1.1 ± 0.1	>100	47 ± 4.4
8l	5-CF_3	>100	>100	>100	12.6 ± 2.5

with TBTU was followed by acidolytic cleavage of the Boc-functionality, furnishing target compound **14**.

Scheme 3 summarizes the synthesis of the P1 5-nitro-substituted isoindoline residue, obtained by regioselective nitration of *N*-protected-isoindoline using nitronium tetrafluoroborate.[14] Hydrogenation of **16** followed by iodination or transfer azidation with sodium azide resulted in compounds **18** and **19**, respectively.[15,16] After deprotection, the isoindoline derivatives were elaborated into final products **20** using identical protocols as shown for intermediates **6**.

All inhibitors were tested for their inhibitory activity against DPP II, DPP IV, DPP8, and DPP9.[17]

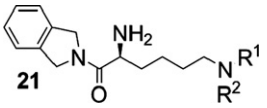
Tables 1 and 2 document the SAR of inhibitors containing 4- or 5-substituted isoindolines as the P1 fragment (**8a–l**, **20a–c**). In general, the compounds tested showed very limited affinity for DPP IV, comparable to what was observed for reference compound **1**. In terms of DPP8/9 potency, introduction of a 4-substituent (**8b**, **8c**, **8i**) proved to be less favorable than introducing the same substituent at the 5-position (**8d**, **8e**, **8j**). Further, a general trend in these data consists of a decrease in potency toward DPP8 and 9 with increasing substituent sizes for both DPP8 and DPP9, but not for DPP II. Halogenated inhibitors **8d**, **8h**, and **20a** might serve as illustrative examples thereof. The iodinated isoindoline residue in **20a** can even be envisaged as a novel, selectivity conferring building P1-block for the development of dipeptide-derived DPP II inhibitors.

The sterically most demanding *t*-butyl and azide substituents (**8f** and **20c**), however, do not seem to be accommodated efficiently by DPP II either. Next, *allo*-isoleucine clearly outperforms isoleucine as a P2 fragment in inhibitors of both DPP8 and 9: upon comparing, for example, compound **8a** (Table 1) with Ref.1 (Table 2), a 10-fold increase in DPP8/9 potency and selectivity toward DPP II can be observed. Finally, the most active compound prepared in this part of the study was found to be **8j**, bearing a P1 5-fluoroisoindoline. Although its inhibitory potential toward DPP8 is similar to that of parent structure **1**, there is a modest, fourfold increase in potency toward DPP9 compared to the latter. In addition, the presence of a fluorine atom might lead to improved biopharmaceutical properties. Nevertheless optimization of the P2 residue is required to obtain inhibitors which are able to discriminate between DPP8 and DPP9.

Compounds **21**, **22**, and **23** bearing a substituted lysine or a substituted 4-aminoproline at the P2 position are listed in Tables 3–5. These inhibitors were synthesized by coupling isoindoline to substituted lysines (**21**) or substituted 4-amino-prolines (**22–23**), respectively. Naphthyl and benzyl substituents were introduced relying on a reductive amination protocol optimized earlier.[18] Substituted 4-aminoproline building blocks were synthesized following a literature procedure.[19]

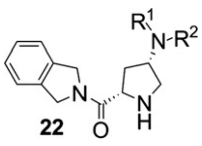
As discussed in our preceding paper the side chains of Ile, *allo*-Ile, Lys, and Lys(Z) can be selected as useful P2 fragments for dipep-

Table 3
IC_{50} values of substituted lysine isoindolines (**21a–f**)



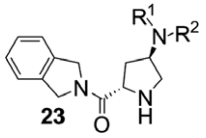
Compound	R^1	R^2	IC_{50} (μM)			
			DPP8	DPP9	DPP IV	DPP II
21a	H–	H–	0.20 ± 0.01	0.5 ± 0.03	161± 8	1.78 ± 0.08
21b	H–	Z–	0.20 ± 0.02	n.a.	245 ± 85	2.5 ± 0.3
21c	Bn–	H–	0.111 ± 0.006	0.2 ±0.006	>50	0.117 ± 0.007
21d	Bn–	Bn–	0.666 ± 0.064	2.39 ± 0.14	>62.5	0.365 ± 0.021
21e	2-Naphthyl	H–	0.043 ± 0.002	0.169 ± 0.014	178 ± 14	0.316 ± 0.025
21f	2-Naphthyl	2-Naphthyl	0.479 ± 0.045	3.67 ± 0.27	>31.25	0.416 ± 0.021

Table 4
IC_{50} values of substituted 4-aminoproline isoindolines (**22a–e**)



Compound	R^1	R^2	IC_{50} (μM)			
			DPP8	DPP9	DPP IV	DPP II
22a	Bn–	H–	2.9 ± 0.2	13.1 ± 1.3	>250	3.4 ± 0.4
22b	1-Naphthyl	H–	0.71 ± 0.03	41 ± 3	4.97 ± 0.45	0.86 ± 0.02
22c	1-Naphthyl	1-Naphthyl	12 ± 2	16.1 ± 1.1	n.i.	1.7 ± 0.3
22d	2-Naphthyl	H–	0.91 ± 0.1	5.77 ± 0.34	>100	1.3 ± 0.2
22e	2-Naphthyl	2-Naphthyl	10.2 ± 1.4	6.69 ± 0.6	n.a.	>100

Table 5
IC_{50} values of substituted 4-aminoproline isoindolines (**23a–d**)



Compound	R^1	R^2	IC_{50} (μM)			
			DPP8	DPP9	DPP IV	DPP II
23a	H–	H–	24 ± 0.6	67.8 ± 3.7	>500	41 ± 2.2
23b	Bn–	H–	5.9 ± 0.3	29.8 ± 2.38	>1000	3.5 ± 0.6
23c	Bn–	Bn–	13 ± 0.7	>25	>500	4.5 ± 0.9
23d	2-Naphthyl	H–	0.29 ± 0.02	2.65 ± 0.27	>250	0.38 ± 0.04

tide-derived DPP8 or DPP9 inhibitors.[9] However, substitution of *allo*-Ile in compound **1** for lysine (**21a**) or Z-protected lysine (**21b**) is shown not to be beneficial, as it reduces selectivity toward DPP II. Introduction of mono- or di- ε-substitution such as benzyl (**21c**,**d**) or naphthyl (**21e**,**f**) increased potency for DPP8/9; however, significant DPP II inhibition was also observed.

As depicted in Tables 4 and 5 substitution of the *allo*-Ile moiety by a 4-aminoprolyl residue afforded some compounds with high nM activity against DPP II (**22b** and **23d**). Unfortunately the activity against DPP9 decreased about 10 times for the most active compound **23d** compared to **1**. No correlation between conversion in stereochemistry at the 4-amino group and change in potency or selectivity was observed.

In conclusion, fluorinated compound **8j**, (2*S*,3*R*)-2-amino-1-(5-fluoroisoindolin-2-yl)-3-methylpentan-1-one was found to exhibit the best balance of potency and selectivity. The introduction of substituents larger than fluorine results in a negative effect on potency and selectivity. Further optimization of the P2 position might provide selective DPP8/9 inhibitors that will be able to discriminate between these two closely related dipeptidyl dipeptidases. Finally, this study attests the potential of substituted isoindolines as useful building blocks for structurally novel, selective DPP II inhibitors.

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Supplementary data

Enzymatic assay conditions are described in the supplementary information of this article. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.079.

References and notes

1. Van der Veken, P.; Soroka, A.; Brandt, I.; Chen, Y.-S.; Maes, M.-B.; Lambeir, A.-M.; Chen, X.; Haemers, A.; Scharpe, S.; Augustyns, K.; De Meester, I. *Curr. Top. Med. Chem.* **2007**, *7*, 621.
2. Lee, H.-J.; Chen, Y.-S.; Chou, C.-Y.; Chien, C.-H.; Lin, C.-H.; Chang, G.-G.; Chen, X. *J. Biol. Chem.* **2006**, *281*, 38653.
3. Rosenblum, J. S.; Kozarich, J. W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 496.
4. Lambeir, A.-M.; Durinckx, C.; Scharpé, S.; De Meester, I. *Crit. Clin. Lab. Sci.* **2003**, *40*, 194.
5. Bjelke, J. R.; Christensen, J.; Nielsen, P. F.; Branner, S.; Kanstrup, A. B.; Wagtmann, N.; Rasmussen, H. B. *Biochem. J.* **2006**, *396*, 391.
6. Lankas, G. R.; Leiting, B.; Roy, R. S.; Eiermann, G. J.; Beconi, M. G.; Biftu, T.; Chan, C. C.; Edmondson, S.; Feeney, W. P.; He, H. B.; Ippolito, D. E.; Kim, D.; Lyons, K. A.; Ok, H. O.; Patel, R. A.; Petrov, A. N.; Pryor, K. A.; Qian, X. X.; Reigle, L.; Woods, A.; Wu, J. K.; Zaller, D.; Zhang, X. P.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes* **2005**, *54*, 2988.
7. Rosemblum, J. S.; Minimo, L. C.; Liu, Y.; Wu, J.; Kozarich, J. W. *Abstract of Posters*, National Meeting of the ADA; Diabetes, 2007; Abstract 138.
8. Schade, J.; Stephan, M.; Schmiedl, A.; Wagner, L.; Niestroj, A. J.; Demuth, H.-U.; Frerker, N.; Klemann, C.; Raber, K. A.; Pabst, R.; von Hörsten, S. *J. Histochem. Cytochem.* **2008**, *56*, 147.
9. Van der Veken, P.; De Meester, I.; Dubois, V.; Soroka, A.; Van Goethem, S.; Maes, M.-B.; Brandt, I.; Lambeir, A.-M.; Chen, X.; Haemers, A.; Scharpé, S.; Augustyns, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4154.
10. Noyes, W. A.; Porter, P. K. *Org. Synth.* **1941**, *1*, 457.
11. Gawley, R. E.; Chemburkar, S. R.; Smith, A. L.; Anklekar, T. V. *J. Org. Chem.* **1988**, *53*, 5381.
12. Gadekar, S. M.; Frederick, J. L.; Semb, J.; Vaughan, J. R. *J. Am. Chem. Soc.* **1961**, *26*, 468.
13. Barnes, R. A.; Godfrey, J. C. *J. Am. Chem. Soc.* **1957**, *22*, 1043.
14. Dwyer, C. L.; Holzapfel, C. W. *Tetrahedron* **1998**, *54*, 7843.
15. Krasnokutskaya, E. A.; Semenischeva, N. I.; Filimonov, V. D.; Knochel, P. *Synthesis* **2007**, *1*, 81.
16. Mendenhall, G. D.; Smith, A. S. *Org. Synth.* **1973**, *5*, 829.
17. Dubois, V.; Lambeir, A.-M.; Van der Veken, P.; Augustyns, K.; Creemers, J.; Chen, X.; Scharpé, S.; De Meester, I. *Front. Biosci.* **2008**, *13*, 3558.
18. Senten, K.; Van der Veken, P.; De Meester, I.; Lambeir, A.-M.; Scharpé, S.; Haemers, A.; Augustyns, K. *J. Med. Chem.* **2004**, *47*, 2906.
19. Curran, T. P.; Marques, K. A.; Bilimoria, S. I. *Lett. Org. Chem.* **2005**, *2*, 15.