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Development of Potent and Selective Dipeptidyl Peptidase II Inhibitors

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Abstract—Structure–activity investigations of product-like dipeptide analogues lacking the C-terminal carbonyl function resulted in potent and selective dipeptidyl peptidase II (DPP II) inhibitors. Dab-Pip has an $IC_{50} = 0.13$ μM for DPP II and a 7600-fold selectivity with respect to DPP IV. This compound will be highly valuable for the investigation of the biochemical function of DPP II.

Introduction

Dipeptidyl peptidases (DPPs) sequentially release dipeptides from polypeptides. Among those enzymes, DPP II (EC 3.4.14.2) and DPP IV (EC 3.4.14.5) cause the release of N-terminal dipeptides containing proline or alanine at the penultimate position. DPP II and DPP IV are similar with respect to their substrate specificity, but differ in other aspects.

DPP II was first identified in bovine anterior pituitary extracts by McDonald et al.[1] and has also been found in a number of mammalian tissues and body fluids. It is generally localised in lysosomes and has an acidic optimal pH (4.5–6.0). Human quiescent cell proline dipeptidase (QPP), which was recently isolated and cloned from T cells,[2,3] is a 58 kDa glycoprotein existing as a homodimer formed with a leucine zipper motif.[4] Based on the high sequence homology (79%) between rat kidney DPP II and human QPP, and on the identical biochemical properties, it is believed that DPP II and QPP are identical.[5,6]

DPP IV is also expressed ubiquitously in mammalian tissues, but is bound to the cell membrane and has an alkaline pH optimum. In the hematopoietic system it was identified as the leukocyte antigen CD26. The function and properties of DPP IV/CD26 were recently reviewed by several authors.[7,8]

Due to the unique structure of proline, relatively few peptidases are able to cleave peptide bonds containing proline.[9] Post-proline cleaving enzymes are recently emerging as an important protease family with interesting therapeutic potential for their inhibitors. DPP IV/CD26 has been shown to modulate the function of several chemokines,[10] and DPP IV inhibitors are currently under evaluation for the treatment of type 2 diabetes.[7] It has been shown that QPP inhibitors cause apoptosis in quiescent lymphocytes, but not in activated or transformed lymphocytes. This process is believed to be independent of DPP IV/CD26, because both $CD26^+$ and $CD26^-$ Jurkat T cells undergo apoptosis.[2]

However, in order to further investigate the DPP II/QPP function, it is necessary to develop highly specific and potent inhibitors. Due to the similarity in substrate specificity as well as catalytic mechanism between DPP II and DPP IV, this is a challenging task.

The few compounds (Fig. 1) reported to have DPP II inhibitory activity were originally described as DPP IV inhibitors. Pyrrolidinylboronic acids such as Val-boroPro

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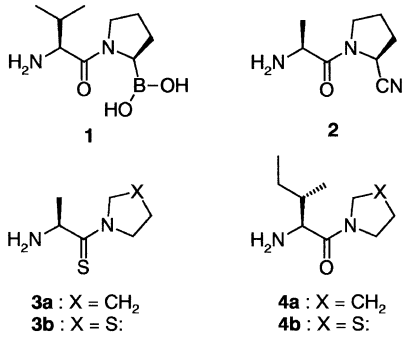


Figure 1. DPP II inhibitors reported in the literature.

Table 1. DPP II inhibitors reported in the literature

Compd	DPP II/QPP inhibition	DPP IV inhibition	SI[a]
1[b]	$K_i = 125$ nM	$K_i = 2$ nM	0.016
2[c]	$IC_{50} = 110$ μM	$K_i = 0.2$ μM	
3a[d]	$K_i = 1.43$ μM	$K_i = 47.6$ μM	33
3b[d]	$K_i = 0.277$ μM	$K_i = 7.88$ μM	28
4a[d]	$K_i = 24.7$ μM	$K_i = 0.218$ μM	0.0088
4b[d]	$K_i = 8.17$ μM	$K_i = 0.126$ μM	0.015

[a]SI = selectivity index = value for DPP IV divided by value for DPP II.
[b]Values taken from ref 3.
[c]Values taken from ref 11.
[d]Values taken from ref 14.

(**1**),[2,3] pyrrolidinylnitriles such as Ala-Pyrr-2-CN (**2**),[11] and aminoacyl pyrrolidines and thiazolidines (**4a**, **4b**) show indeed a higher potency towards DPP IV. The inhibitory potential of these compounds for DPP IV is well described and recently reviewed.[7,12,13] Thioamide analogues of the latter compounds such as Ala-ψ[CS-N]-Pyrr (**3a**) and Ala-ψ[CS-N]-Thia (**3b**) are the only DPP II–DPP IV inhibitors described to have some selectivity towards DPP II[14] (Table 1).

In this paper we report a systematic structure–activity relationship of aminoacyl pyrrolidines and piperidines as inhibitors for DPP II. The thorough investigation of this class of compounds with respect to selectivity between DPP II and DPP IV was done for the first time and aims to indentify lead compounds for the further development of highly selective and potent DPP II inhibitors.

Results and Discussion

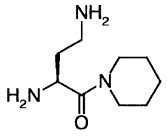
The inhibitors were synthesised in parallel using a polymer-assisted solution-phase approach as described recently.[15] In a first synthetic round a representative set of aminoacyl pyrrolidines (**4a**, **5**–**17**, Table 2) were used to investigate the importance of the P-2 position. Lys-Pyrr (**17**) with an $IC_{50} = 9.9$ μM and His-Pyrr (**16**) exhibiting an $IC_{50} = 1.16$ μM came out as the most active DPP II inhibitors. These two compounds were also the most selective with respect to DPP IV. From this set of pyrrolidines we can conclude that basic (Lys, **17**) and neutral amino acids at P-2 are preferable over acidic amino acids. An acidic amino acid at this position seems not to be tolerated (Asp, **14**), which is in agreement with the reported substrate specificity.[16]

Having established lysine and histidine as good P-2 amino acids, we could improve considerably the potency and selectivity by replacing pyrrolidine with piperidine. As reported earlier this replacement gave a serious decrease in potency for DPP IV inhibitors.[17] However, with respect to DPP II, piperidine seemed

Table 2. Inhibitory activities and selectivity index for DPP II and DPP IV for the described compounds

		DPP II inhibition IC_{50} (μM)	DPP IV inhibition IC_{50} (μM)	SI[a]
5	Gly-Pyrr	>1000	>1000	1
6	Ala-Pyrr	179±15	41±6	0.23
7	Val-Pyrr	223±13	4±0.4	0.018
4a	Ile-Pyrr	110±7	4±0.9	0.036
8	Cha-Pyrr[b]	42±3	17±2	0.40
9	Pro-Pyrr	>500	15±2	<0.03
10	ThiaPro-Pyrr	>500	>500	1
11	Phe-Pyrr	79±29	21±4	0.27
12	Tyr-Pyrr	150±24	14±2.4	0.093
13	Ser-Pyrr	65±30	190±120	2.9
14	Asp-Pyrr	>500	122±2	<0.24
15	Asn-Pyrr	152±50	188±6	1.2
16	His-Pyrr	1.16±0.06	23.21±0.98	20
17	Lys-Pyrr	9.9±1.3	39±2	3.94
18	Ile-Pip	62±27	67±11	1.1
19	Cha-Pip[b]	9.9±0.9	217±13	22
20	His-Pip	0.328±0.06	213±42	704
21	Lys-Pip	1.6±0.3	247±20	154
22	Lys(Z)-Pip	2.10±0.15	134.9±1.21	64
23	Orn-Pip	0.45±0.08	>500	>1111
24	Dab-Pip[c]	0.13±0.008	>1000	>7592
25	Dap-Pip[d]	1.84±0.13	>1000	>544

[a]SI = selectivity index = value for DPP IV divided by value for DPP II.
[b]Cha = cyclohexylalanine.
[c]Dab = 2,4-diaminobutyric acid.
[d]Dap = 2,3-diaminopropionic acid.



24

Figure 2. Structure of Dab-Pip.

tolerable.[3] Indeed, comparison of compounds **18**–**21** with **4a**, **8**, **16** and **17** respectively indicates a 2–6-fold increase in DPP II inhibition, whereas inhibition of DPP IV decreased simultaneously with a factor between 6 and 17. Therefore, changing pyrrolidine to piperidine results in a considerable increase in potency and selectivity for DPP II. Blocking the ε-amino function in Lys-Pip with benzyloxycarbonyl (**22**) afforded only a slight decrease in potency, whereas selectivity was reduced by a factor 2–4. This could indicate that basic amino acids in P-2 are not necessary for DPP II inhibition but might be important to introduce selectivity.

Finally, investigation of the side chain length ($(CH_2)_nNH_2$) was done by replacing the P-2 amino acid lysine ($n=4$) (**21**) with respectively ornithine ($n=3$) (**23**), 2,4-diaminobutyric acid ($n=2$) (**24**) and 2,3-diaminopropionic acid ($n=1$) (**25**). Decreasing the side chain length to $n=2$ enhanced the DPP II inhibitory potency. Further decrease of the side chain revealed a reduction in potency (**25**, $n=1$). Also selectivity was significantly improved since inhibition of DPP IV declined tremendously with decreasing side chain length. Dab-Pip (**24**, Fig. 2) with an $IC_{50}=0.13$ μM and a selectivity index of more than 7000 is the most active and by far the most selective DPP II inhibitor reported to date.

Among the investigated pyrrolidines and piperidines, Val-Pyrr (**7**) is the most potent DPP IV inhibitor. It is also the most selective pyrrolidine with a 56-fold lower IC_{50} value for DPP IV than for DPP II. Val-Pyrr (**7**) is frequently used in animal models for type II diabetes,[7] but one can argue if its selectivity is sufficient to study the role of DPP IV in biological systems. The Pro-Pro diaryl phosphonates reported by our group as irreversible inhibitors of DPP IV have very low DPP II inhibitory activity,[18] and are therefore more selective DPP IV inhibitors.

Conclusion

In conclusion, it is shown that Dab-Pip (**24**) is a potent inhibitor for DPP II. It is the first selective DPP II inhibitor ever reported, with a selectivity with respect to DPP IV that is much higher than reported for the thioamides.[14] Dab-Pip (**24**) promises to be a useful compound to establish the physiological and biochemical role of DPP II as well as its potential as a therapeutic target. Its high selectivity will enable to differentiate between DPP II and DPP IV in biological systems. Dab-Pip (**24**) will also serve as a lead compound for the further development of DPP II inhibitors in our laboratory.

Biological Evaluation

DPP IV was purified from human seminal plasma as described previously.[19] DPP II was isolated from the same source using techniques described previously for purification of the enzyme from porcine seminal plasma,[20] supplemented with adenosine deaminase affinity chromatography to eliminate contaminating DPP IV.[19] Enzyme activity was measured kinetically with the chromogenic substrates Gly-Pro-*p*-nitroanilide at pH 8.3 and Lys-Ala-*p*-nitroanilide at pH 5.5 for DPP IV and DPP II respectively. Test compounds were dissolved and diluted in DMSO (final concentrations during assay 5% v/v). IC_{50} value was defined as the inhibitor concentration which caused a 50% decrease of the activity under assay conditions.

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References and Notes

1. McDonald, J. K.; Leibach, F. H.; Grindeland, R. E.; Ellis, S. *J. Biol. Chem.* **1968**, *243*, 4143.
2. Chiravuri, M.; Schmitz, T.; Yardley, K.; Underwood, R.; Dayal, Y.; Huber, B. T. *J. Immunol.* **1999**, *163*, 3092.
3. Underwood, R.; Chiravuri, M.; Lee, H.; Schmitz, T.; Kabcenell, A. K.; Yardley, K.; Huber, B. T. *J. Biol. Chem.* **1999**, *274*, 34053.
4. Chiravuri, M.; Lee, H.; Mathieu, S. L.; Huber, B. T. *J. Biol. Chem.* **2000**, *275*, 26994.
5. Araki, H.; Li, Y.-H.; Yamamoto, Y.; Haneda, M.; Nishi, K.; Kikkawa, R.; Ohkubo, I. *J. Biochem.* **2001**, *129*, 279.
6. Fukasawa, K. M.; Fukasawa, K.; Higaki, K.; Shiina, N.; Ohno, M.; Ito, S.; Otogoto, J.; Ota, N. *Biochem. J.* **2001**, *353*, 283.
7. Villhauer, E. B.; Coppola, G. M.; Hughes, T. E. In *Annual Reports in Medicinal Chemistry*, Academic Press, 2001, Vol. 36, pp 191–200.
8. De Meester, I.; Korom, S.; Van Damme, J.; Scharpé, S. *Immunol. Today* **1999**, *20*, 367.
9. Vanhoof, G.; Goossens, F.; De Meester, I.; Hendriks, D.; Scharpé, S. *Faseb J.* **1995**, *9*, 736.
10. Lambeir, A.-M.; Proost, P.; Durinx, C.; Bal, G.; Senten, K.; Augustyns, K.; Scharpé, S.; Van Damme, J.; De Meester, I. *J. Biol. Chem.* **2001**, *276*, 29839.
11. Li, J.; Wilk, E.; Wilk, S. *Arch. Biochem. Biophys.* **1995**, *323*, 148.
12. Augustyns, K.; Bal, G.; Thonus, G.; Belyaev, A.; Zhang, X. M.; Bollaert, W.; Lambeir, A. M.; Durinx, C.; Goossens, F.; Haemers, A. *Curr. Med. Chem.* **1999**, *6*, 311.
13. Schön, E.; Born, I.; Demuth, H. U.; Faust, J.; Neubert, K.; Steinmetzer, T.; Barth, A.; Ansorge, S. *Biol. Chem. Hoppe-Seyler* **1991**, *372*, 305.
14. Stöckel-Maschek, A.; Mrestani-Klaus, C.; Stiebitz, B.; Demuth, H.-U.; Neubert, K. *Biochim. Biophys. Acta* **2000**, *1479*, 15.

15. Senten, K.; Van der Veken, P.; Bal, G.; Haemers, A.; Augustyns, K. *Tetrahedron Lett*. **2001**, *42*, 9135.
16. Mentlein, R.; Struckhoff, G. *J. Neurochem*. **1989**, *52*, 1284.
17. Augustyns, K.; Lambeir, A.-M.; Borloo, M.; De Meester, I.; Vedernikova, I.; Vanhoof, G.; Hendriks, D.; Scharpé, S.; Haemers, A. *Eur. J. Med. Chem*. **1997**, *32*, 301.
18. Belyaev, A.; Zhang, X. M.; Augustyns, K.; Lambeir, A.-M.; De Meester, I.; Vedernikova, I.; Scharpé, S.; Haemers, A. *J. Med. Chem*. **1999**, *42*, 1041.
19. De Meester, I.; Vanhoof, G.; Lambeir, A.-M.; Scharpé, S. *J. Immunol. Meth*. **1996**, *189*, 99.
20. Huang, K.; Takagaki, M.; Kani, K.; Ohkubo, I. *Biochim. Biophys. Acta* **1996**, *1290*, 149.