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Discovery of β-homophenylalanine based pyrrolidin-2-ylmethyl amides and sulfonamides as highly potent and selective inhibitors of dipeptidyl peptidase IV

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ABSTRACT

Modifications of DPP-4 inhibitor **5**, that was discovered by structure based design, are described and structure–activity relationships discussed. With analogue **7k** one of the most potent non-covalent inhibitors of DPP-4 reported to date (IC_{50} = 0.38 nM) was discovered. X-ray structure of inhibitor **7k** bound to DPP-4 revealed a hydrogen bonding interaction with Q553. First successful efforts in balancing overall properties, as demonstrated by improved metabolic stability, highlight the potential of this series.

Inhibition of dipeptidyl peptidase IV (DPP-4) has recently received intense interest as a novel therapeutic approach to the treatment of type 2 diabetes.[1] DPP-4 is a serine protease that cleaves dipeptides N-terminal from its peptide substrates.[2] The incretin hormone glucagon-like peptide 1 (GLP-1) that is responsible for the secretion of insulin after food intake is rapidly inactivated by DPP-4.[3] Consequently inhibition of DPP-4 prolongs the half-life of endogenous GLP-1 and enhances its beneficial effects in glucose dependent insulin secretion and β-cell restoration.[4]

To date a range of structurally diverse inhibitors of DPP-4 have been reported, some of which have now reached the market or entered advanced stage clinical trials. Among them are Sitagliptin (**1**),[5] Alogliptin (**4**)[6] and the covalent binding pyrrolidine nitriles Vildagliptin (**2**)[7] and Saxagliptin (**3**)[8] (Fig. 1).

We have previously described[9] our approach to the design of DPP-4 inhibitor **5** (Fig. 2) arising from the discovery of a 'reverse' binding to DPP-4 when compared to classical DPP-4 inhibitors such as **6** (P32/98).[10] We now report on the elaboration of a series of DPP-4 inhibitors, based on structure **5**, and the elucidation of structure–activity relationships (SAR) therein.

Based on our findings from the X-ray analysis of inhibitor **5**[9] bound to DPP-4, where the fluorophenyl ring occupied the proline-binding S1 pocket, the amino group was anchored to the glutamates E205 and E206, the pyrrolidine located distal to S1 and the phenylamide had contacts with Y547 and R125, initial sites attractive for SAR exploration were determined to be the phenyl ring of the β-amino acid moiety and the pyrrolidin-2-ylmethyl amide.

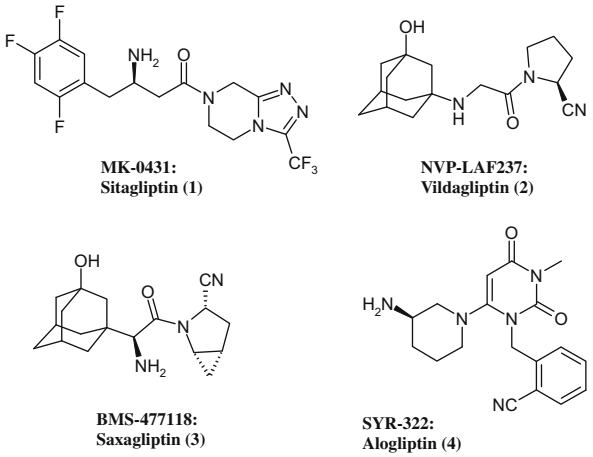

Figure 1. Selected DPP-4 inhibitors.

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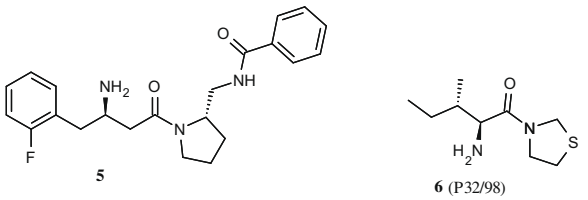


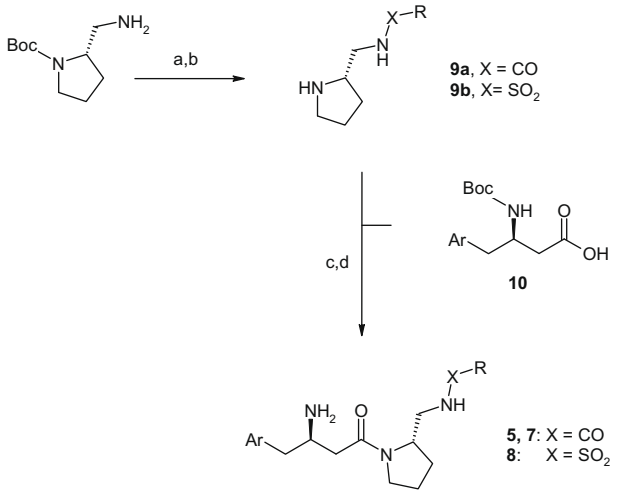
Figure 2. DPP-4 inhibitors.

A series of analogues was prepared according to Scheme 1.[11] Commercial Boc-protected (S)-2-aminomethylpyrrolidine was coupled with a carboxylic acid giving intermediate **9a**. Subsequently the *tert*-butoxycarbonyl protection group was removed under standard conditions, then the pyrrolidine moiety coupled with acid **10** and the resulting intermediate deprotected to yield compounds **7a–l**. Compounds **8a–i** were synthesized in a similar manner employing a sulfonylchloride to furnish the sulfonamide intermediate **9b**.

Since SAR on the β-amino acid moiety corresponds with earlier reports for related compounds,[12] only findings relevant for the work reported here are discussed. In comparison to the initial 2-fluoro substitution (compound **5**, IC_{50} DPP-4 = 90 nM)[9] the 3-chloro analogue (**7a**) was found to be threefold more potent. Further improvement can be achieved with a 2,4,5-trifluorophenyl moiety, which accounts for a fivefold increase in potency or more.[12]

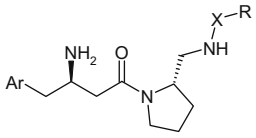
Exploration of the pyrrolidin-2-ylmethyl amide SAR was initiated with respect to potency and basic in vitro ADME properties. Aiming for DPP-4 inhibitors that exhibit a long plasma half-life, we first determined the metabolic stability of **5** towards rat liver microsomes.[13] A 76% turnover of **5** after 60 min indicated metabolic instability. We reasoned that the unsubstituted phenyl ring may be one potential site for metabolism. Thus we synthesized a range of derivatives where we introduced more metabolically robust side-chain groups. The intention to reduce the gross lipophilicity of the compound (**5**: *c* Log *P* 2.82[14]) as a general concept to increase metabolic stability directed our efforts. In addition, a variation from amide (Table 1, **7a–m**) to sulfonamide (Table 1, **8a–i**), thus aiding the 'depeptidisation' of **5**, was investigated.

Within the aryl amide derivatives explored, fluoro-phenyl or pyridyl gave little to no improvement in potency over **5** (Table 1). Substituents of this kind in the 2-position of the aryl amide



Scheme 1. Synthesis of inhibitors **5**, **7** and **8**. Reagents: (a) RCO_2H, EDC, HOBt, DIEA, DMF or RSO_2Cl, TEA, DCM; (b) TFA, DCM; (c) **10**, EDC, HOBt, DIEA, DMF; (d) TFA, DCM.

Table 1
Amide-derived and sulfonamide-derived DPP-4 inhibitors



Compound	Ar	R	X	DPP-4 IC_{50} (nM)[9]
5	2-F–Ph	Ph	CO	90
7a	3-Cl–Ph	Ph	CO	27
7b	2-F–Ph	2-F–Ph	CO	49
7c	2-F–Ph	3-F–Ph	CO	92
7d	2-F–Ph	4-F–Ph	CO	92
7e	2-F–Ph	2-Py	CO	47
7f	2-F–Ph	3-Py	CO	87
7g	2-F–Ph	4-Py	CO	66
7h	2-F–Ph	4-(SO_2Me)–Ph	CO	77
7i	2-F–Ph	2-(SO_2Me)–Ph	CO	27
7j	3-Cl–Ph	2-(SO_2Me)–Ph	CO	14
7k	3-Cl–Ph	3-(SO_2Me)–Ph	CO	0.38
7l	3-Cl–Ph	Cyclopropyl	CO	12
7m	2,4,5-Tri-F–Ph	Cyclopropyl	CO	9
8a	2-F–Ph	Ph	SO_2	34
8b	3-Cl–Ph	Ph	SO_2	8
8c	2-F–Ph	2-F–Ph	SO_2	53
8d	2-F–Ph	3-F–Ph	SO_2	94
8e	2-F–Ph	4-F–Ph	SO_2	27
8f	2-F–Ph	4-Cl–Ph	SO_2	20
8g	2-F–Ph	2,5-Di-Cl–Ph	SO_2	24
8h	3-Cl–Ph	Cyclopropyl	SO_2	30
8i	2,4,5-Tri-F–Ph	Cyclopropyl	SO_2	8

seem to be slightly preferred over 3- or 4-substitution. However, incorporation of a hydrogen bond acceptor motif in the 3-position provided an order of magnitude increase in potency. The reason for this boost in affinity was revealed by X-ray crystallography[15] (Fig. 3). The sulfone group of **7k** forms a hydrogen bonding interaction with the N–H Atom of Q553 in DPP-4. It can be speculated that the huge energy gain is due to the replacement of a water molecule, which is frequently found in this position of DPP-4

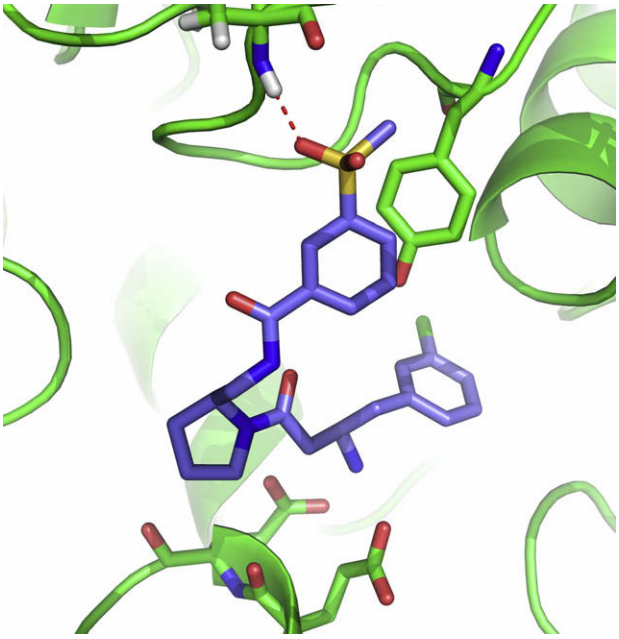


Figure 3. Co-crystal structure of compound **7k**; the new hydrogen bond from one of the sulfone oxygens to the backbone carbonyl of Q553 is highlighted in red dashes. The figure was generated using PYMOL version 0.98 (Delano Scientific, www.pymol.org).

Table 2
Selectivity data for selected DPP-4 inhibitors[21]

Compound	DPP-4 IC_{50} (μM)	DPP-7 IC_{50} (μM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)
7j	0.014	6.3	22	8.8
7k	0.00038	8.1	5.7	5.1
7l	0.012	11	4.0	3.4
7m	0.009	3.4	5.2	2.9
8a	0.034	17	0.7	0.08
8b	0.008	19	0.2	0.1
8h	0.030	12	4.2	2.5
8i	0.008	10	7.3	1.2

co-crystal X-ray structures.[16] Concomitant with this polar interaction, the phenyl ring is placed favorably for a π-stacking interaction with Y547. Similar contacts with DPP-4 have been reported for an α-amino acid derived inhibitor.[17] However it is worthy of note, that the interactions of **7k** with Y547 and Q553 are observed instead of those interactions with Y547 and R125 reported recently for the closely related compound **5**.[9]

Compound **7k** (IC_{50} = 380 pM, Table 1) is one of the most potent non-covalent inhibitors reported so far. As a result of that, the compound provided a much higher selectivity against related enzymes in comparison with similar compounds, even though binding affinity of **7k** for DPP-8 and DPP-9 did not change (Table 2). This is in line with our recently reported findings from homology modeling.[18] It has been suggested that high selectivity, in particular over DPP-8 and DPP-9, may be important for an optimal safety profile.[19]

SAR on the corresponding sulfonamides proved to be similar but not to run entirely parallel to that of the amides (Table 1).

To accommodate an increase in molecular weight and lipophilicity accompanied by the introduction of the potent 3-Cl- or 2,4,5-trifluorophenyl moieties we aimed for a reduction in size and lipophilicity of the side chain amide or sulfonamide. Gratifyingly, incorporation of small, metabolically robust cyclopropyl groups (**7l**; **7m**; **8h**; **8i**) provided potent and selective inhibitors of DPP-4. Overall, balancing any increase of molecular weight and gross lipophilicity (**7m**: *c* Log *P* 1.66; **8i**: *c* Log *P* 1.82) resulted in significantly improved stability in rat liver microsomes (**7m**, **8i**: $t_{1/2}$ > 2 h).[20]

Table 2 provides selectivity data against DPP-7, DPP-8 and DPP-9 for selected compounds which indicate the excellent selectivities achievable in our DPP-4 inhibitor series. The inhibitors were also tested against Seprase (FAP) and POP. None of the compounds showed any relevant inhibition of these related enzymes.

In summary, a novel series of non-covalent DPP-4 inhibitors with excellent selectivities over DPP-7, DPP-8 and DPP-9 has been discovered. Amongst the compounds described is one of the most potent, non-covalent inhibitors reported to date (**7k**), benefiting from an unexpected polar interaction that was revealed by X-ray analysis. First successful efforts in balancing overall properties as demonstrated by improved metabolic stability highlight the potential of this series. The results lay the foundation for the further optimization regarding ADME and pharmacokinetic properties.

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