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Aminomethylpyrimidines as novel DPP-IV inhibitors: A 10^5-fold activity increase by optimization of aromatic substituents

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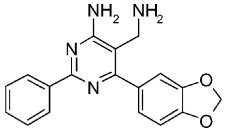
Abstract—The influence of aromatic substitution on a newly discovered class of inhibitors of dipeptidyl peptidase IV was investigated. A 10^5-fold increase in potency was achieved by the optimization of aromatic substituents in a parallel chemistry program. The observed SAR could be explained by an X-ray structure of the protein–ligand complex.

Dipeptidyl peptidase IV (DPP-IV) cleaves and inactivates glucagon-like peptide 1 (GLP-1),[1] which is an important stimulator of insulin secretion.[2] Inhibition of DPP-IV increases the level of circulating GLP-1 and thus increases insulin secretion.[3] An improved insulin secretion could moderate hyperglycaemia in type 2 diabetes. Consequently, DPP-IV inhibition has been proposed as a new treatment of type 2 diabetes.[4] Several series of DPP-IV inhibitors have been published as potential new medicines.[5] Clinical proof of concept has already been established in phase II with the DPP-IV inhibitor NVP-DPP728.[6,7] In a search for novel structures, we have identified 6-methylenedioxyphenyl-aminomethylpyrimidine **1a** as a relatively weak inhibitor of DPP-IV in a high-throughput screen.



1a
HTS hit, IC_{50} = 10μM

With the goal of finding analogues of **1a** with increased potency, we prepared 5-aminomethylpyrimidines **1** with small substituents (Me, Cl, MeO, F and CF_3) in different positions at both phenyl rings. 5-Cyanopyrimidines **4** were obtained in a first step by the reaction of benzylamidines **2** and arylidenemalononitriles **3** under basic conditions (Scheme 1).[8] Yields of **4** were greatly enhanced by treating the reaction mixtures with $KMnO_4$, e.g. from 13% to 52% for **4c**-H. Subsequently, the nitrile functionality was reduced to give the desired 5-aminomethylpyrimidines **1**. The syntheses were carried out in a parallel fashion using disposable polypropylene tubes as reaction vessels and automated preparative, reversed-phase HPLC for purification. The identities and purities of **4** and **1** were assessed by HPLC/MS.[9]

Compounds **1** were evaluated for their ability to inhibit DPP-IV mediated cleavage of Ala-Pro-7-amido-4-trifluoromethylcoumarin in a fluorogenic assay.[10] Table 1 documents the SAR of single substituents at the 6-phenyl ring of **1**. Removal of the methylenedioxy substituent of **1a** gave a reference compound, **1b**-H (R=H, Table 1) with a slightly reduced activity. Introduction of *ortho* methyl (**1b**-*o*-Me), *ortho* chloro (**1b**-*o*-Cl), or *ortho* methoxy (**1b**-*o*-OMe) substituents increased the inhibitory activity by an order of magnitude. A similar potency increase was observed for the introduction of *para* methyl (**1b**-*p*-Me), *para* chloro (**1b**-*p*-Cl), or *para* trifluoromethyl (**1b**-*p*-CF_3) sustituents. The effects of other *ortho* and *para* substituents (**1b**-*o*-F, **1b**-*o*-CF_3, **1b**-*p*-OMe, **1b**-*p*-F) were generally favorable but less

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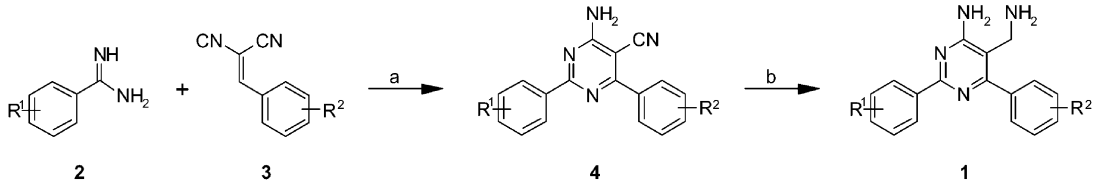
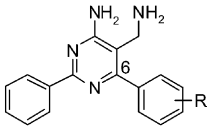


Scheme 1. Reagents and conditions: (a) K_2CO_3, MeOH, 50 °C, 3 h, then, after evaporation of solvent, $KMnO_4$, acetone, rt, 3 h (yield for **4c**-H (R^1 = H; R^2 = 2,4-Cl_2): 52%); (b) $LiAlH_4$, THF, 40 °C, 3 h (yield for **1c**-H (R^1 = H; R^2 = 2,4-Cl_2): 40%).

Table 1. Structure–activity relationship of 6-phenyl substituents on **1b**: DPP-IV—IC_{50} data (μM)



1b

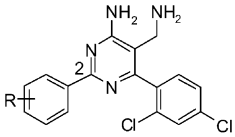
R = H: 42

R =	Me	Cl	OMe	F	CF_3
ortho	1.5	2.5	1.5	14	14
meta	20	31	80	40	170
para	1.0	1.4	47	18	1.1

pronounced. In contrast, *meta* substituents decreased activity (**1b**-*m*-OMe, **1b**-*m*-CF_3) or had little influence (**1b**-*m*-Me, **1b**-*m*-Cl, **1b**-*m*-F).

The obvious assumption that a combination of *ortho* and *para* substituents should further increase activity led to the 2,4-dichloro substituted compound **1c**-H (R=H, Table 2). The activity of **1c**-H surpassed our anticipations with a 1000-fold increase in activity as compared to screening hit **1a**! Taking **1c**-H as a basis for further improvement, we investigated the effects of substitution of the 2-phenyl ring. *ortho* Substitution gave less active compounds (**1c**-*o*-Me, **1c**-*o*-OMe, **1c**-*o*-F, Table 2). A single *meta* fluoro (**1c**-*m*-F) or a single *para* fluoro (**1c**-*p*-F) substituent increased the activity into the picomolar range. A combination of these substituents in one molecule (**1d**) was less efficient. Single *meta* methoxy substitution (**1c**-*m*-OMe) led to a drop of activity. Surprisingly, the introduction of a second *meta* methoxy substituent gave a compound with an outstanding inhibitory activity, **1e**. No clear-cut SAR emerged from these and other (**1c**-*m*-Me, **1c**-*m*-Cl, **1c**-*m*-CF_3, **1c**-*p*-Me, **1c**-*p*-Cl, **1c**-*p*-OMe, **1c**-*p*-CF_3) *meta*- and *para*-substituted derivatives.

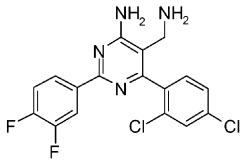
Table 2. Structure–activity relationship of 2-phenyl-substituents on **1c**: DPP-IV—IC_{50} data (μM)



1c

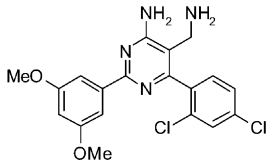
R = H: 0.01

R =	Me	Cl	OMe	F	CF_3
ortho	1.75	—	0.35	0.047	—
meta	0.0009	0.24	0.34	0.0002	0.13
para	0.090	0.053	0.10	0.0002	0.18



1d

IC_{50} = 0.003μM



1e

IC_{50} = 0.0001μM

X-ray crystal structure determination (Fig. 1)[11,12] revealed that **1e** binds to the active site of DPP-IV. The 2,4-dichlorophenyl motif of the inhibitor occupies very well the hydrophobic S1 pocket of the enzyme explaining the beneficial effect of *ortho* and *para* substituents in this part of the molecule. The aminomethyl group of the ligand forms a symmetrical hydrogen bonding network with a tyrosine (Y662) and two glutamate (E205, E206) residues of the protein. This interaction substitutes the binding of the N-terminus of the substrates to this key recognition motif of the active site. Other interactions include an additional hydrogen bond from the aromatic amino function of **1e** to a backbone amide carbonyl

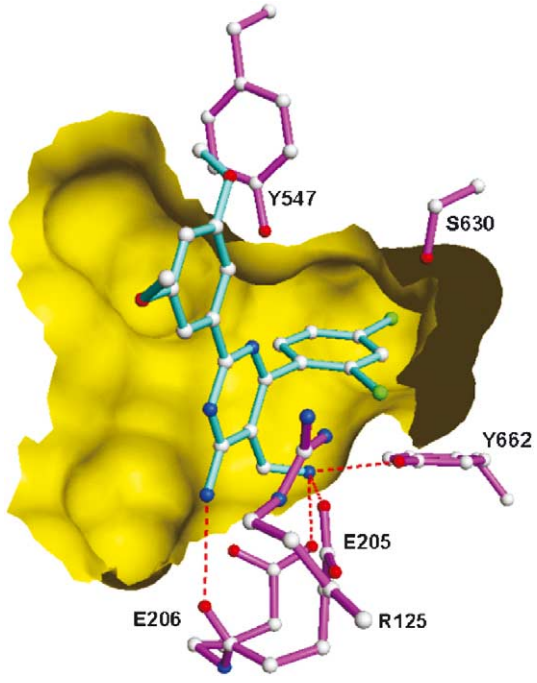


Figure 1. X-ray structure by co-crystallization of **1e** (bonds are colored cyan) and DPP-IV (magenta). Dashed lines indicate hydrogen bonds between protein and ligand.

(E205), and a cation-π-interaction between an arginine residue (R125) and the inhibitor's pyrimidine core. The 3,5-dimethoxyphenyl residue points mainly into the solvent beside some interaction of one methoxy substituent with the side chain of a tyrosin (Y547). No interaction with the catalytic serine (S630) is observed.

In summary, a series of novel DPP-IV inhibitors **1** was derived from screening hit **1a**. We focussed on the derivatization of the 2-phenyl ring and the 6-phenyl ring of **1**. A 2,4-disubstitution pattern at the 6-phenyl ring appears to be highly favored. This is exemplified by **1c**-H with a 1000-fold increase in activity as compared to the original screening hit **1a**. Picomolar compounds could be obtained by the introduction of fluoro and methoxy substituents into the *meta* and *para* positions of the 2-phenyl ring. By optimizing aromatic substituents at both phenyl rings, a 100,000-fold increase in activity was achieved (**1e** vs **1a**).

References and notes

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10. DPP-IV inhibitors were measured in triplicate at 5 to 7 concentrations in the range of 100 μM to 100 pM. IC_{50} values were calculated with a non-linear best fit regression model. All assays were calibrated with NVP-DPP728 as internal standard inhibitor. NVP-DPP728 under the conditions of the assay showed an IC_{50} of 15 ± 4 nM ($M\pm SD$, $n=12$) at 50 μM substrate concentration and a K_i of 11 ± 3 nM determined at substrate concentration range of 10 μM to 600 μM. IC_{50} values of unkonwn compounds were accepted when the IC_{50} (×) measured for NVP-DPP728 in the assay was $11<\times<19$ nM.
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12. Data collection, processing and refinement statistics: resolution range: 15.0–2.2 Å; measured reflections: 216,345; unique reflections: 79,169; completeness: 84.3%; R_{sym}: 8.7%; no. of protein atoms: 11 962; no. of waters 98; no. of ligand atoms: 54; r.m.s. distances: 0.010 Å; r.m.s. bond angles: 1.8; R_{cryst}: 23.8%; R_{free} (5% of data): 28.9%; mean B-factor, protein: 43.4 Å^2; mean B-factor, ligand: 43.6 Å^2; mean B-factor, waters: 40.4 Å^2. The coordinates of this structure have been deposited in the Protein Data Bank (accession code 1RWQ).