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Bioorganic & Medicinal Chemistry Letters 17 (2007) 2966–2970
Bioorganic & Medicinal Chemistry Letters

1,3-Disubstituted 4-aminopiperidines as useful tools in the optimization of the 2-aminobenzo[a]quinolizine dipeptidyl peptidase IV inhibitors

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Received 5 February 2007; revised 20 March 2007; accepted 22 March 2007
Available online 25 March 2007

Abstract—In a search for novel DPP-IV inhibitors, 2-aminobenzo[a]quinolizines were identified as submicromolar HTS hits. Due to the difficult synthetic access to this compound class, 1,3-disubstituted 4-aminopiperidines were used as model compounds for optimization. The developed synthetic methodology and the SAR could be transferred to the 2-aminobenzo[a]quinolizine series, leading to highly active DPP-IV inhibitors.

The incretin hormone glucagon-like peptide-1 (GLP-1) is released from the gastrointestinal tract in response to nutrient ingestion, and stimulates insulin secretion from the pancreas in a glucose-dependent manner. GLP-1 is degraded by dipeptidyl peptidase IV (DPP-IV), a multifunctional type II transmembrane serine protease which cleaves a dipeptide from the N-terminus of peptide substrates with either proline or alanine at the penultimate position. Once in circulation, GLP-1 has a half-life of only few minutes. Thus, inhibitors of DPP-IV increase levels of GLP-1 and subsequently insulin, and are therefore promising therapeutic agents for the treatment of type 2 diabetes.[1]

There are both covalent and non-covalent inhibitors of DPP-IV in late clinical development (Fig. 1). The cyanopyrrolidines vildagliptin **1** (Novartis, FDA approval filed)[2] and saxagliptin **2** (Bristol-Myers Squibb, phase 3)[3] bind covalently to the active serine in the S1 pocket of DPP-IV, whereas the non-covalent inhibitors sitagliptin **3** (Merck, launched 2006)[4] and alogliptin **4** (Takeda, phase 3)[5] rely entirely on non-covalent protein–ligand interactions and have substituted phenyl groups as ligands for the S1 pocket of DPP-IV.

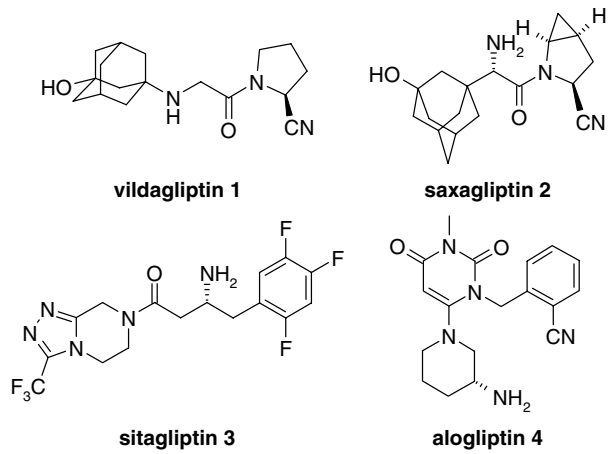

Figure 1. Selected clinically studied DPP-IV inhibitors.

Clinical trials have proven that DPP-IV inhibitors reduce blood glucose and HbA1c levels in diabetic patients and an excellent safety profile with minimal side effects can be achieved.[6]

In a high throughput screening the 2-aminobenzo[a]-quinolizine **5** and the aminopyrimidine derivative **6** were

Keywords: Dipeptidyl peptidase IV; DPP-IV inhibitor; 2-Aminobenzo-[a]quinolizine.
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doi:10.1016/j.bmcl.2007.03.072

identified as inhibitors of DPP-IV (Fig. 2). It turned out that the HTS hits **5** and **6** share a similar structural recognition motif for DPP-IV with sitagliptin **3**, whose structure was not known to us when we performed the research reported in this paper.

The diastereomer **7** of the screening hit **5** had an interesting drug-like profile and was significantly active in an oral glucose tolerance test (OGTT) in fa/fa rats (Table 1).

Particularly, interesting was the 3-butyl substituent in compound **7**, which according to its X-ray complex structure with DPP-IV pointed into the S1 specificity pocket (Fig. 3).[7] The butyl group did not fill the hydrophobic S1 pocket optimally. Synthesis of derivatives with different substituents in 3-position was often cumbersome. Therefore, we looked for a simplified open-chain analogue. The fact that in the 2-aminobenzo[a]quinolizine **8** annulation of a cyclohexyl ring is tolerated suggested 1-substituted 4-amino-3-butylpiperidines **9** as potential DPP-IV inhibitors (Fig. 4).

The 4-aminopiperidines **9** were easily accessible in six steps starting from the commercially available β-ketoester **10** using literature known procedures (Scheme 1).[8] The residue R was introduced through double Hofmann elimination and Michael addition of the amine to the ammonium salt **11**. Oxime-formation and reduction yielded the 4-aminopiperidines **9** as mixtures of

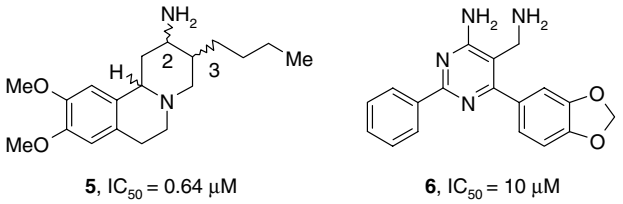
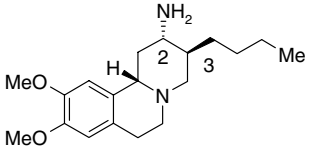


Figure 2. DPP-IV inhibitory activity of selected HTS screening hits.

Table 1. Profile of the 2-aminobenzo[a]quinolizine **7**



DPP-IV IC_{50} (nM)	520
Solubility (mg/L)	>414
log *D* (pH 7.4)	0.8
Permeation coefficient Pe (10^{-6} cm s^{-1}) (PAMPA)	2.5
CL_{micr} (mL/min/mg protein) rat/human	4.7/0.0
IC_{50} CYPs (μM) (2C9, 2D6, 3A4)	>50
OGTT ($\Delta_{glucose}$, 40 min) (%)	−16

PAMPA, parallel artificial membrane permeation assay; CL_{micr}, intrinsic clearance in liver microsome preparations; CYPs, cytochrom P450 enzyme; OGTT, oral glucose tolerance test in fa/fa rats (reduction of glucose levels 40 min after glucose challenge (2 g/kg) compared to non-treated animals; 0.3 mg/kg of **7** given 2 h before glucose challenge).

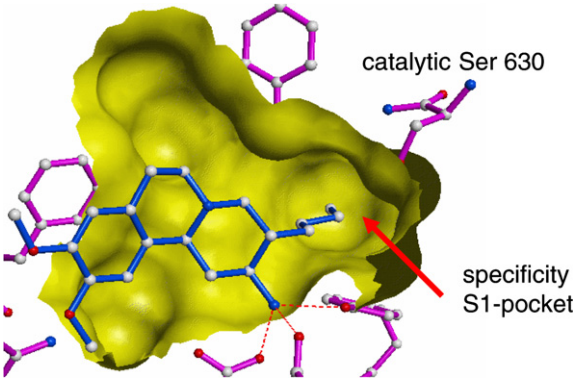


Figure 3. X-ray structure of the binding pocket of the complex of 2-aminobenzo[a]quinolizine **7** with human DPP-IV.

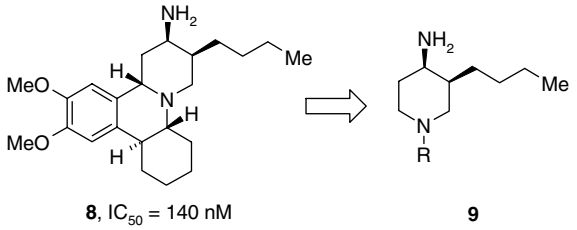
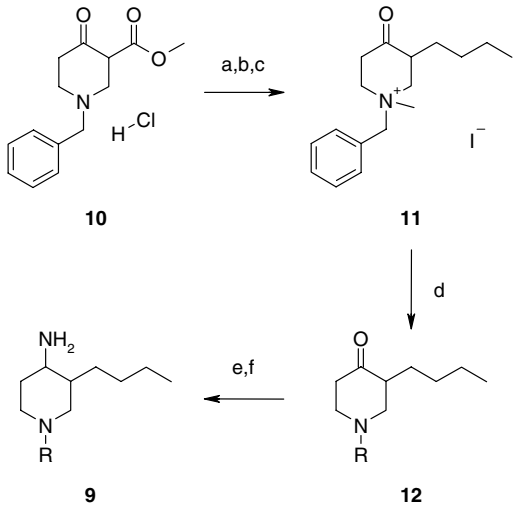


Figure 4. Simplification of the 2-aminobenzo[a]quinolizine structure.

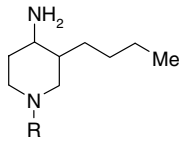


Scheme 1. Reagents and conditions: (a) 1.9 equiv BuI, 4.5 equiv K_2CO_3, 1 equiv *i*-Pr_2NEt, acetone, reflux, 12 h; (b) 20% aq HCl, reflux, 24 h, 67% over two steps; (c) 1.2 equiv MeI, acetone, rt, 3 h, 78%; (d) 0.8 equiv RNH_2, 0.13 equiv K_2CO_3, EtOH, reflux, 3 h, 57–100%; (e) 1.1 equiv NH_2OH*HCl, 1.1 equiv NaOAc, EtOH, rt, 2 h; (f) Ra–Ni alloy, EtOH, H_2O, NaOH, rt, 2 h, 23–71% over two steps.

cis/*trans*-diastereomers, which could be often separated by silica gel chromatography.[9]

A phenyl substituent (**9c**) was more active than a cyclohexyl (**9a**) or a benzylic substituent (**9b**) (Table 2).[10] Generally, the *cis*-diastereomer was more potent than the *trans*-diastereomer. Various substitutions with electron-rich (**9j**) as well as electron-poor (**9i**) residues on the aryl moiety were allowed. The most active derivative

Table 2. DPP-IV inhibitory activity of 4-aminopiperidine derivatives **9**



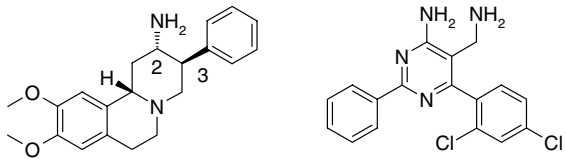
Compound	R[a]	IC_{50} (μM)		
		cis	*trans*	*cis*/*trans*
9a	c-Hex			45.2
9b	Ph_2CH			18.6
9c	Ph	3.5	35.7	
9d	4-PhOPh	6.3	15.0	
9e	4-Tol	5.2	94.7	
9f	3,5-$(CF_3)_2$–Ph			13.4
9g	3-MeO-5-CF_3–Ph	2.1		8.4
9h	3-Ph-4-MeO–Ph	5.8	142	
9i	4-Cl-3-CF_3–Ph	2.8		2.6
9j	3,4-$(MeO)_2$–Ph	4.9	26	
9k	3,4,5-$(MeO)_3$–Ph	2.3	18.4	
9l	3,4-Cl_2–Ph	3.3		
9m	2-Naphthyl	1.5	9.4	
9n	5,6,7,8-Tetrahydro-naphthalen-1-yl			13.6
9o	1-Naphthyl			5.3

[a] The numbering refers to the position of the substituent on the corresponding aromatic ring.

was the 2-naphthyl-compound ***cis*-9m**. Because of the relatively flat SAR we decided to keep the more polar 3,4-dimethoxyphenyl group constant and vary the substituent in 3-position.

The phenyl substituted 2-aminobenzo[a]quinolizine **13** exhibited a nice inhibitory activity of 200 nM (Fig. 5). The modeled overlay with the co-crystal structure of DPP-IV with inhibitor **14**,[11] an optimized compound from a previous lead series identified from the HTS hit **6**, clearly showed that a substantial improvement in affinity could be expected by the introduction of the correct substitution pattern on the 3-aryl substituent of the 4-aminopiperidines, respectively, the 2-aminobenzo-[a]quinolizines (Fig. 6).

The employed synthetic route to **13** was not generally applicable for the variation of the 3-aryl substituent. Therefore, we decided to study the direct introduction of an aryl substituent into the ketone-precursor. As a model system we choose the 4-aminopiperidines. Several methods such as the direct arylation of the copper-enolate, generated from **15** with LDA and CuCN, with a mixed bis aryl iodonium salt,[12] gave



13, IC_{50} = 200 nM **14**, IC_{50} = 10 nM

Figure 5. DPP-IV inhibitors.

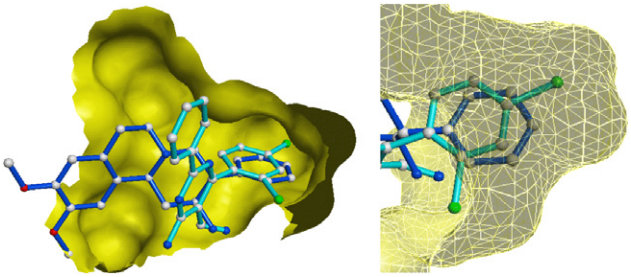
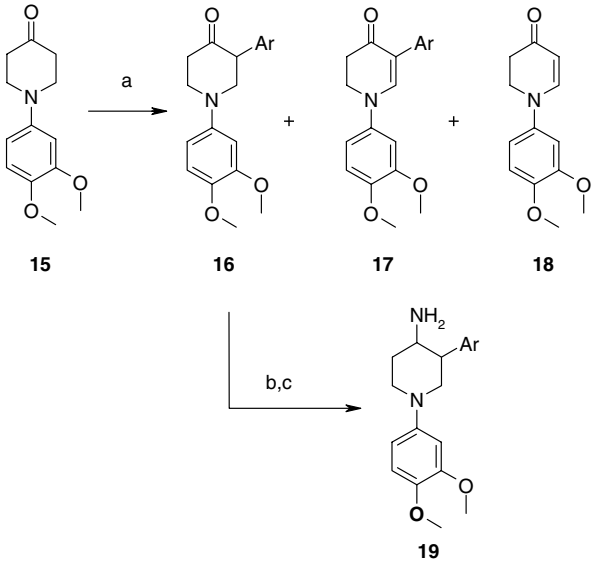


Figure 6. Modeled overlay of 2-aminobenzo[a]quinolizine **13** with the co-crystal structure of DPP-IV with inhibitor **14** illustrating the asymmetric shape of the S1 pocket.

only low yields (5%). The Pd-catalyzed arylation[13] of **15** was successful, however furnished **16** in low yield (Scheme 2) because of the formation of the dehydro by-products **17** and **18**.

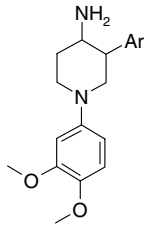
The SAR revealed that a small lipophilic group such as methyl or possibly a halogen substituent in the meta-position of the aromatic residue was favored (Table 3). The toluene derivative **19c** had a IC_{50} value of 600 nM combined with a low molecular weight. It was still a diastereomeric mixture.

We were able to solve the X-ray complex structure of **19c** with DPP-IV to a resolution of 2.8 Å (Fig. 7).[14] The binding mode revealed clear electron density for the (3*R*,4*S*)-enantiomer of the *cis*-isomer. The protonated primary amine is engaged in the three characteristic hydrogen bonds with Glu 205, Glu 206, and Tyr 662, as seen with other primary amines, such as sitagliptin **3**. The favorable 3-methyl phenyl substituent points into the S1 pocket and perfectly occupies the small hydrophobic niche in the back. Some residual electron density



Scheme 2. Reagents and conditions: (a) 1 equiv ArBr, 3 equiv NaO*t*-Bu, 10 mol% $Pd(OAc)_2$, 10 mol% Pd*t*-Bu_3, THF, rt, overnight, 10–27% (b) 1.1 equiv NH_2OH*HCl, 1.1 equiv NaOAc, EtOH, rt, 2 h; (c) Ra–Ni alloy, EtOH, H_2O, NaOH, rt, 2 h, 28–96% over two steps.

Table 3. DPP-IV inhibitory activity of 4-aminopiperidine derivatives **19**



Compound	Ar[a]	IC_{50} (μM) *cis*/*trans*
19a	4-Me–Ph	14.8
19b	3,4-Me_2–Ph	31.3
19c	3-Me–Ph	0.6
19d	3-MeO–Ph	25.6
19e	2-Pyridyl	8.9

[a] The numbering refers to the position of the substituent on the corresponding aromatic ring.

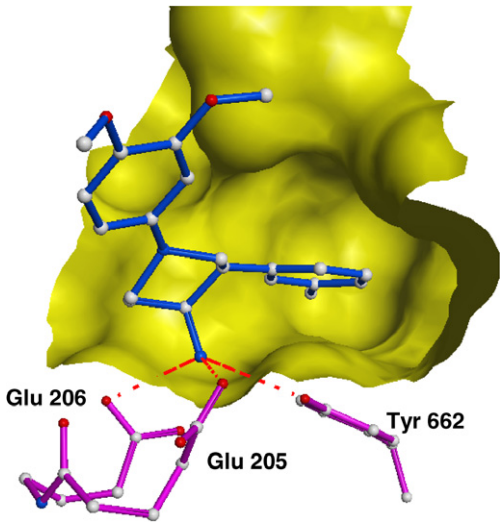
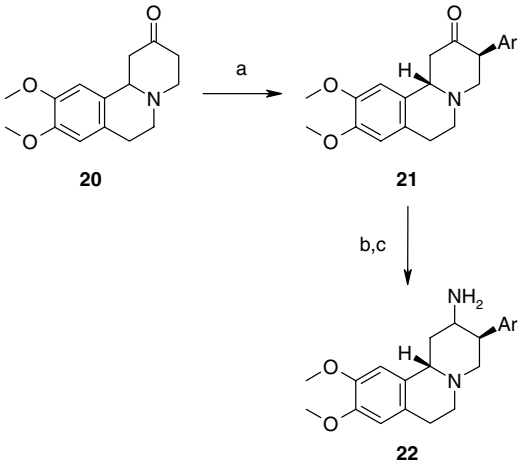


Figure 7. X-ray complex crystal structure of human DPP-IV with compound **19c** (*cis*, (3*R*,4*S*)-enantiomer). Protein residues engaged in hydrogen bonds (dashed red lines) with the ligand are shown.

is, however, seen for a second ligand conformation in which the 3-methyl phenyl substituent is rotated by 180° around the bond connecting the P1 substituent with the piperidine ring.

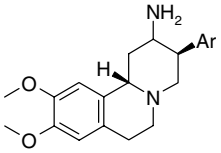
The developed methodology could be nicely transferred to the 2-aminobenzo[a]quinolizines (Scheme 3). Phenyl iodides in general and electron-poor aromatic bromides were not successful in the arylation of the ketone **20**. Aryl chlorides sometimes produced the products **22** in low yields, when the corresponding aryl bromides and iodides failed.

Small lipophilic groups in meta-position, such as methyl or chlorine, were the best substituents, providing compounds with an IC_{50} value of 4 nM (*trans*-**22f** and *trans*-**22g**, Table 4). *Trans*-**22f** reduced glucose levels in the oral glucose tolerance test (OGTT) in fa/fa rats by 41% (Table 5) 40 min after the glucose challenge at a



Scheme 3. Reagents and conditions: (a) 1 equiv ArBr/Cl, 3 equiv NaO*t*Bu, 10 mol% $Pd(OAc)_2$, 10 mol% Pd*t*-Bu_3, THF, rt, overnight, 11–41%; (b) 1.1 equiv NH_2OH*HCl, 1.1 equiv NaOAc, EtOH, rt, 2 h; (c) Ra–Ni alloy, EtOH, H_2O, NaOH, rt, 2 h, 52–73% over two steps.

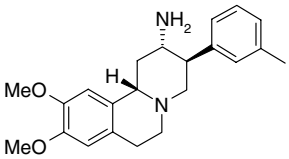
Table 4. DPP-IV inhibitory activity of 2-aminobenzo[a]quinolizines **22**



Compound	Ar[a]	IC_{50} (nM)		
		cis	*trans*	*cis*/*trans*
22a	Ph	270	200	220
22b	2-Pyridyl	990	1520	
22c	4-Me–Ph	10590	370	
22d	3,4-Me_2–Ph	9380	3730	
22e	3-MeO–Ph	5130	650	
22f	3-Me–Ph	32	4	13
22g	3-Cl–Ph	50	4	

[a] The numbering refers to the position of the substituent on the corresponding aromatic ring.

Table 5. Profile of the 2-aminobenzo[a]quinolizine *trans*-**22f**



DPP-IV IC_{50} (nM)	4.6
Solubility (mg/L)	>469
log *D* (pH 7.4)	1.3
Permeation constant Pe (10^{-6} cm s^{-1}) (PAMPA)	3.4
Cl_{micr} (mL/min/mg protein) (rat/human)	1.0/3.0
IC_{50} CYPs (μM) (2C9, 2D6, 3A4)	15, 11, >50
OGTT ($\Delta_{glucose}$, 40 min) (%)	−41

PAMPA, parallel artificial membrane permeation assay; CL_{micr}, intrinsic clearance in liver microsome preparations; CYPs, cytochrom P450 enzyme; OGTT, oral glucose tolerance test in fa/fa rats (reduction of glucose levels 40 min after glucose challenge (2 g/kg) compared to non-treated animals; 0.3 mg/kg of **22f** given 2 h before glucose challenge).

dose of 0.3 mg/kg (applied 120 min before glucose challenge).

In summary, we describe a novel series of 4-aminopiperidine DPP-IV inhibitors. A methodology for the introduction of 3-aryl substituents could be developed. An aromatic residue with a small lipophilic group in meta-position yielded the most active inhibitors. The developed SAR and the synthetic procedures could be applied to the 2-aminobenzo[a]quinazoline series, leading to highly active DPP-IV inhibitors, with *trans*-**22f** and **g** as the best compounds (Table 4).

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9. All new compounds were characterized by ^{1}H NMR and MS prior to submission for biological evaluation. For detailed synthetic procedures, see (a) Boehringer, M.; Hunziker, D.; Kuhn, B.; Loeffler, B. M.; Luebbers, T.; Ricklin, F. WO2006066747; (b) Boehringer, M.; Kuhn, B.; Luebbers, T.; Mattei, P.; Narquizian, R.; Wessel, H. P. WO2005000846.
10. DPP-IV inhibitors were measured for their ability to inhibit DPP-IV mediated cleavage of Ala-Pro-7-amido-4-trifluoromethylcoumarin in a fluorogenic assay. All compounds were measured in triplicate at 5–7 concentrations in the range of 100 μM to 100 pM. IC_{50} values were calculated with a nonlinear 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 ± SD, $n = 12$) at 50 μM substrate concentration and a K_i of 11 ± 3 nM determined at substrate concentration range of 10–600 μM. IC_{50} values of unknown compounds were accepted when the IC_{50} (x) measured for NVP-DPP728 in the assay was $11 < x < 19$ nM.
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14. The coordinates of the X-ray complex structure of human DPP-IV with compound **19c** were deposited to the Protein Data Bank (PDBid: 2ijd).