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Aryl- and heteroaryl-substituted aminobenzo[*a*]quinolizines as dipeptidyl peptidase IV inhibitors

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ABSTRACT

Synthesis and SAR are described for a structurally distinct class of DPP–IV inhibitors based on aminobenzo[*a*]quinolizines bearing (hetero-)aromatic substituents in the S1 specificity pocket. The *m*-(fluoromethyl)-phenyl derivative (*S,S,S*)-**2g** possesses the best fit in the S1 pocket. However, (*S,S,S*)-**2i**, bearing a more hydrophilic 5-methyl-pyridin-2-yl residue as substituent for the S1 pocket, displays excellent in vivo activity and superior drug-like properties.

Dipeptidyl peptidase IV (DPP–IV) inhibitors have emerged as a new therapeutic option to treat patients with type 2 diabetes.[1,2] The pharmacological action is based mainly on the ability to prevent cleavage and inactivation of glucagon-like peptide (GLP–1), an incretin hormone which stimulates insulin secretion and inhibits glucagon secretion.

Several DPP–IV inhibitors have advanced into late stage clinical studies.[2–4] Of these, sitagliptin[5] (**1a**, Fig. 1), vildagliptin[6] and saxagliptin[7] have been approved and launched in several countries. While DPP–IV inhibitors encompass a remarkable structural diversity, with a primary or secondary amino group being the sole recurring motif, they all possess substituents which are able to occupy the S1 specificity pocket of the target. For instance, the trifluorophenyl subunit of **1a** fully occupies the S1 pocket of DPP–IV.

We have recently reported the discovery of aryl-substituted aminobenzo[*a*]quinolizines **2a–c** (Fig. 1), a structurally distinct class of DPP–IV inhibitors.[8] These inhibitors were derived from high-throughput screening hit **3**, a legacy compound with favourable molecular properties, which was synthesised in the 1950s as one member of a series of emetine analogues. X-ray structural information was used together with structure-activity relationships obtained for simplified 1,3-substituted 4-aminopiperidines and aminomethyl-pyrimidines[9] such as **4a** as the key elements in the lead identification process culminating in **2b** and **2c**, the first highly potent representatives of this class. Like **1a** and **4a**, **2b** and **2c** possess a substituted benzene ring as ligand for the S1 pocket. Here, we report the further evolution of the class culminating in (*S,S,S*)-**2i**, a highly potent compound with a more balanced profile.

Having established that a lipophilic substituent at the *meta*-position of the aromatic ring (R^1 in compound **2**) brings about a significant improvement in activity by occupying the lipophilic niche

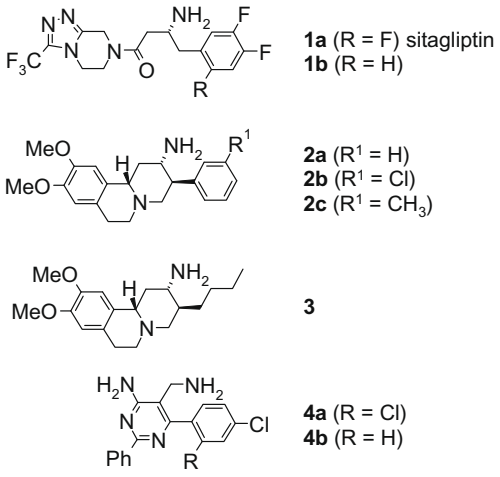

Figure 1. Selected DPP–IV inhibitors.

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at the back of the S1 specificity pocket, we sought to explore alternative substituents on the aromatic ring. Also, there is room at the aromatic position *para* to R^1 (substituent X in compound **2**, Scheme 1) that could be occupied, either by a lipophilic substituent or by a heteroatom.

Therefore, we synthesised a set of aryl- and heteroaryl derivatives of the general formula **2** according to Scheme 1.[10] Thus, tricyclic ketone **5**[11] was regio- and diastereoselectively arylated with halides **6** under palladium catalysis,[12] affording only the 3β,11bβ–stereoisomer **7**. Conversion of the keto group of **7** to the oxime followed by reduction produced mixtures of the 2α and 2β amine epimers in a ratio of ca 8:1. The 2α–aminobenzo[*a*]quinolizines **2a–j** were separated from the less active 2β–epimers[8] by column chromatography.

The activities of **2a–j** as DPP–IV inhibitors are summarised in Table 1. As anticipated from the rigid nature of the S1 specificity pocket, the structure-activity relationship at R^1 is rather steep. A chlorine atom or methyl group improve the activity by 40–50-fold (**2b**, **c** vs **2a**).[8] However, the advantage over the unsubstituted phenyl residue in **2a** is partly lost when R^1 is a slightly larger alkyl group [ethyl (**2d**), cyclopropyl (**2c**)]. Even larger R^1 groups, such as isopropyl (**2f**), are not tolerated at all. On the other hand, one order of magnitude improvement in activity over **2c** is gained by addition of a single fluorine atom (**2g**). Introduction of a methyl group at position X leads to a slight improvement in activity (ca two-fold for 2,5-dimethylphenyl derivative **2h**). This effect is significant but smaller than that observed for electronegative groups like the ortho-chlorine in **4a**[9] (140-fold improvement over **4b**) or for the ortho-fluorine in **1a** (seven-fold improvement over **1b**)[5] which benefit from a favourable electrostatic interaction with the positively charged side chain of Arg125.[13] The polar environment afforded by the side chains of Arg125 and Asn710 also tolerates the incorporation of an *ortho*-pyridine. In fact, the pyridine derivatives **2i** and **2j** are only slightly less active than the benzene counterparts **2c** and **2g** although they are around one order of magnitude less lipophilic.

We were able to solve the X-ray complex structure of the fluoromethyl-substituted inhibitor **2g** bound to human DPP–IV to a resolution of 2.8 Å (Fig. 2). The binding mode of the aminobenzo[a]quinolizine subunit is nearly identical to that of the HTS hit **1**,[13,16] in that the aromatic ring makes a favourable, parallel-shifted π–π interaction with Phe357, and the positively charged amino group is engaged in a tight H-bonding network with Glu205, Glu206, and Tyr662. The hydrophobic S1 pocket is fully occupied by the (fluoromethyl)-benzene moiety of **2g**. In particular, the CH_2F group nicely fits the asymmetry of the S1 pocket, with five *van der Waals* contacts to the side chains of Tyr631, Val656, Trp659, and Tyr666. The *van der Waals* interaction strength for the fluorine is about twice that of the hydrogen, thus rationalising the affinity improvement of **2g** in comparison with **2c**.

Compound **2c** is a highly potent compound with a favourable set of drug-like properties. It is highly soluble and permeable, metabolically stable, and has only a minimal potential for drug–drug interaction. In vivo, it is active at an oral dose of 0.3 mg kg^{-1} in the oral glucose tolerance test (OGTT) in Zucker fatty (*fa/fa*) rats, reducing glucose levels by 41% at a time-point of 40 min after the glucose challenge.[8] On the downside, in vivo clearance and volume of distribution are very high (Table 2), and brain uptake is extensive, which is not a desirable property for a compound

Table 1
Structure–activity relationship of racemic compounds **2**

Compd	X	R^1	IC_{50}[a] (nM)	log $D_{7.4}$[b]	in vitro PL[c]
2a	CH	H	200[d]	0.8	5
2b	CH	Cl	4.2[d]	1.7	n.t.
2c	CH	CH_3	4.6[d]	1.3	2.5
2d	CH	CH_2CH_3	61	1.7	n.t.
2e	CH	Cyclopropyl	38	1.8	n.t.
2f	CH	Isopropyl	5500	2.0	n.t.
2g	CH	CH_2F	0.5	1.6	n.t.
2h	C–CH_3	CH_3	1.7	1.6	n.t.
2i	N	CH_3	19	0.3	15
2j	N	CH_2F	1.2	0.1	n.t.

[a] For a description of experimental details for the determination of DPP-IV inhibition see: Ref. 8.
[b] Distribution coefficient at pH 7.4.[14]
[c] Lowest concentration [μM] to induce phospholipidosis in vitro (test range 2.5–20 μM).[15]
[d] Data from Ref. 8.

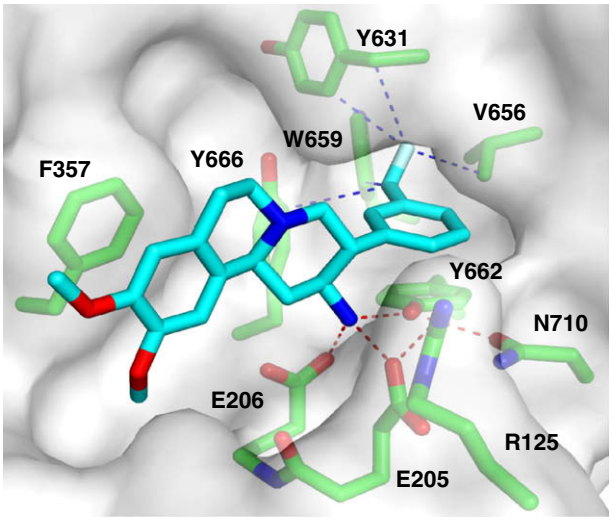
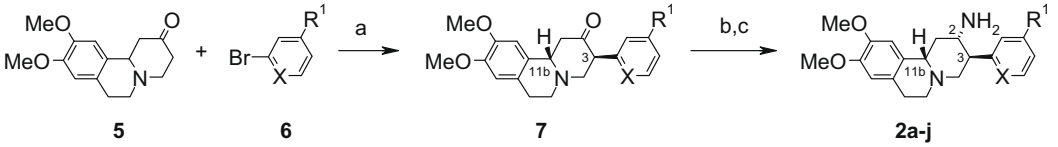


Figure 2. X-ray complex crystal structure of human DPP–IV with compound (*S,S,S*)–**2g**. Hydrogen bonds are displayed in red and van der Waals interactions (d < ∑ r_vdW + 0.5 Å) of the CH_2F moiety with DPP-IV are highlighted in blue.[17]

Table 2
Pharmacokinetic parameters (Wistar rat) for selected compounds

Compd	Cl[a,b]	V_{ss}[a,c]	$t_{1/2}$[a,d]	F[e]	B/P[f]
2c	87	42	6.9	56	5.8
(*S,S,S*)-**2i**	118	12	1.4	50	0.7

[a] Dose: 1 mg kg^{-1} i.v.
[b] Clearance [mL min^{-1} kg^{-1}].
[c] Volume of distribution at steady state, [L kg^{-1}].
[d] Half life [h].
[e] Oral bioavailability [%] at 3 mg kg^{-1}.
[f] Brain to plasma ratio.



Scheme 1. Reagents and conditions: (a) $Pd(OAc)_2$, P^tBu_3, NaO^tBu, THF, 21–51%; (b) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, 83–99%; (c) H_2, Raney Ni, aq NH_3, MeOH, THF, 34–85%.

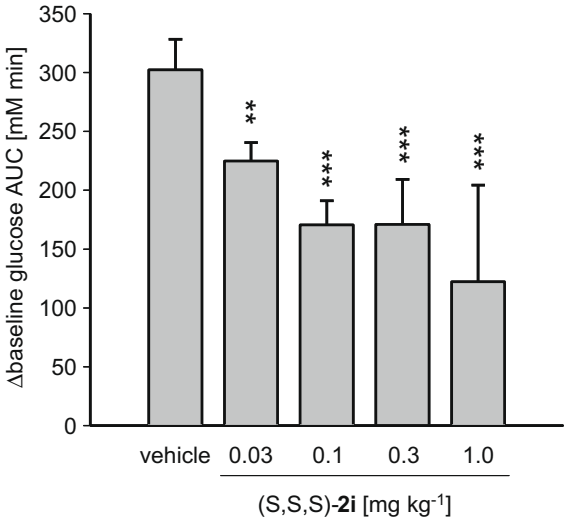


Figure 3. Dose-dependent effect of (*S,S,S*)-**2i** on glucose levels (area under the curve, $t = 0 \rightarrow 120$ min) during an oral glucose tolerance test in *fa/fa* rats. The compound was applied 2 h before the glucose challenge (2 g kg^{-1}). $^{**}p \leqslant 0.01$, $^{***}p \leqslant 0.001$, ANOVA followed by Dunnett's PostHoc test versus vehicle.

intended for a peripheral mode-of-action. Finally, **2c** strongly induces phospholipidosis in cultured fibroblasts[15] at the lowest tested concentration of 2.5 μM.

A proven strategy to reduce the risk of phospholipidosis is to minimise the free energy of amphiphilicity, $\Delta\Delta G_{am}$.[18,19] Indeed, the pyridine analogue **2i** ($\Delta\Delta G_{am}$ = -6.02 kJ mol^{-1}) is significantly less amphiphilic than **2c** ($\Delta\Delta G_{am}$ = -6.41 kJ mol^{-1}). Accordingly, the lowest concentration of **2i** to induce phospholipidosis in vitro is 15 μM. Fluorine substitution on the pyridine methyl of **2i** enhances the potency further (**2j**), but this compound unfortunately tested positive in the Ames test for mutagenicity[20] and in the micronucleus test for clastogenicity[21] and was thus not considered further.

The racemate **2i** was separated into its enantiomers using chiral HPLC (Chiralpak® AD). The (*S,S,S*)-enantiomer is potent (IC_{50} = 11 nM), whereas the (*R,R,R*)-enantiomer is virtually inactive (IC_{50} = 53,000 nM).

In comparison with lead compound **2c**, (*S,S,S*)-**2i** exhibits superior pharmacokinetic properties, having a lower volume of distribution, less brain penetration, and a shorter half life (Table 2). In vivo, (*S,S,S*)-**2i** reduces glucose levels during an oral glucose tolerance test (OGTT) in Zucker fatty (*fa/fa*) rats in a dose-dependent manner (Fig. 3). Indeed, the compound already shows appreciable glucose reduction at a dose as low as 0.03 mg kg^{-1}.

In summary, the X-ray structure of DPP–IV suggested that potency and molecular properties in the aryl-aminobenzo[a]quinolizine series can be modulated through optimisation of the S1 substituent. Compound (*S,S,S*)-**2g**, bearing a *meta*-(fluoromethyl)-phenyl subunit, has the best fit in the S1 pocket. However, improved drug-like properties were obtained by installing a more polar pyridine ring in the S1 ligand position, leading to (*S,S,S*)-**2i**, a highly potent DPP–IV inhibitor with more balanced properties.

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