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Rational design of a novel, potent, and orally bioavailable cyclohexylamine DPP-4 inhibitor by application of molecular modeling and X-ray crystallography of sitagliptin

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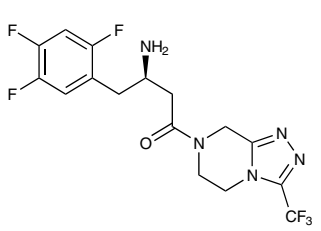
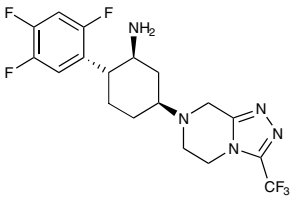
Abstract—Molecular modeling was used to design a rigid analog of sitagliptin **1**. The X-ray crystal structure of sitagliptin bound to DPP-4 suggested that the central β-amino butyl amide moiety could be replaced with a cyclohexylamine group. This was confirmed by structural analysis and the resulting analog **2a** was synthesized and found to be a potent DPP-4 inhibitor (IC_{50} = 21 nM) with excellent in vivo activity and pharmacokinetic profile.

Diabetes is a major health problem that is growing rapidly. It is estimated that there are over 170 million patients with type 2 diabetes worldwide.[1] The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) play important roles in glucose homeostasis.[2,3] GLP-1 and GIP have been evaluated as potential antidiabetic agents.[4,5] However, both hormones are inactivated rapidly in vivo through the action of dipeptidyl peptidase IV (DPP-4), a serine exopeptidase which cleaves a dipeptide from the N-terminus.[6,7] Development of DPP-4 resistant GLP-1 agonists, such as exenatide,[8] circumvents this problem. An alternative approach is to use small-molecule inhibitors of DPP-4 to prolong the beneficial effects of endogenous GLP-1,[9,10] as well as to stabilize GIP.[11] Human clinical trials of several small molecule DPP-4 inhibitors, including sitagliptin[12–14] and vildagliptin,[15] have shown improved glucose tolerance in patients with diabetes. In addition, DPP-4 inhibitors are not likely to cause hypoglycemia or body-weight gain, and could potentially alter the progression of diabetes by restoring β-cell function in the pancreas.

Sitagliptin (**1**), the first DPP-4 inhibitor approved by the FDA for the treatment of diabetes, has a trifluorophenyl group linked to a β-amino butanoyl moiety coupled to a triazolopiperazine. We were interested in replacing the



sitagliptin **1**



2a

central β-amino butanoyl group with a rigid analog. However, in the absence of structural information as to the mode of binding of sitagliptin to the DPP-4 binding site, choice of an appropriate ring system

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was not obvious. As soon as the crystal structure of sitagliptin in the DPP-4 binding site was solved (PDB entry 1×70), it appeared that cyclohexylamine could be an appropriate replacement of the middle β-amino butanoyl portion of the molecule, providing ring constrained analogs such as **2a**. This was later confirmed by molecular modeling and by solving the structure of **2a** bound to DPP-4 (PDB entry 2P8S; Fig. 1A). Overlaying of **2a** (yellow) onto sitagliptin (magenta) in the DPP-4 binding site shows that the cyclohexylamine group was very well accommodated. The interactions made by sitagliptin with the protein are retained in the new class of inhibitors: the cyclohexylamine nitrogen hydrogen bonds to the side chains of Glu205, Glu206, and Tyr662, and the hydrophobic interactions with the side chain of Phe357 are also retained. Interestingly, the structure also showed that the triazolopiperazine moiety in **2a** is disordered, that is, has multiple conformations in the binding site, although only two conformers were modeled in the current structure (Fig. 1B). In one of the two conformers (yellow, also shown in panel A), the triazole is stacked over the side chain of Phe357, and the trifluoromethyl group is loosely interacting with the side chain of Arg358 and Ser209. In the second conformer (orange), the piperazine is making a side-to-face hydrophobic interaction with Phe357, and one of the triazole nitrogens is part of a hydrogen bonding network that includes a water molecule, the side chain of His126, and the main chain oxygen of Glu205 (red lines in figure). In this paper, we will describe the synthesis and activity of the novel cyclohexylamine lead **2a**.

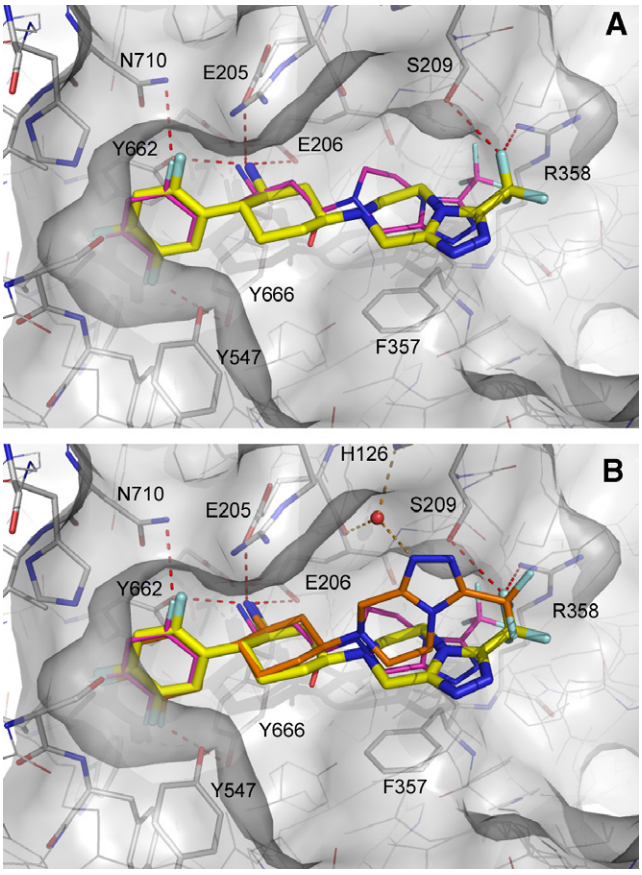
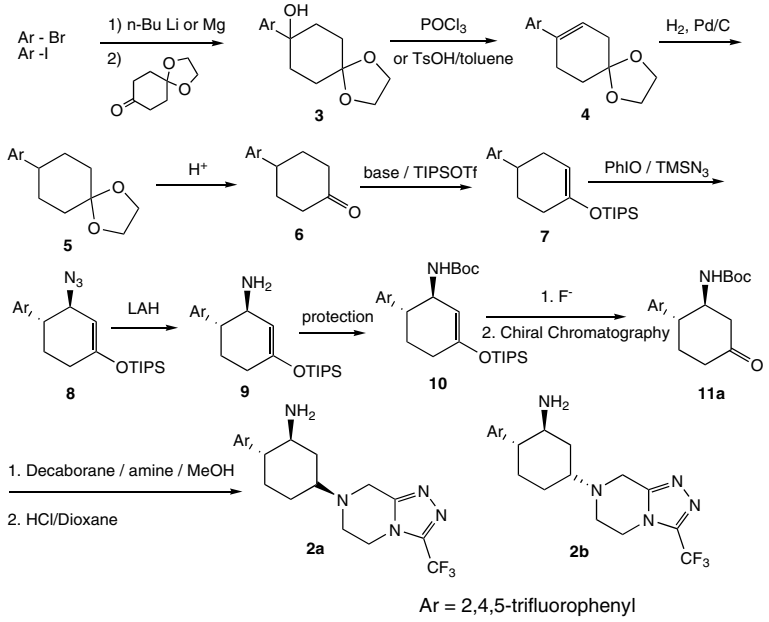


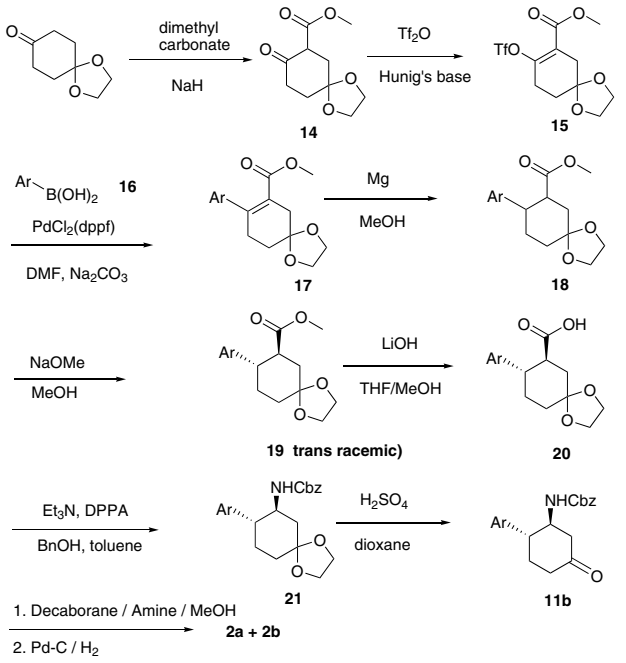
Figure 1. X-ray co-crystal structure of compound **2a** bound to DPP-4 overlaid with sitagliptin.

As shown in Schemes 1 and 2, compounds **2a** and **2b** were prepared by reductive amination of ketone **11** and trifluoromethyltriazolopiperazine used in sitagliptin.[14]

1-Halo-2,4,5-trifluorobenzene was treated with magnesium to form the corresponding Grignard reagent or lithiated with *n*-butyl lithium and then treated with 1,4-dioxaspiro[4.5]decan-8-one to form the alcohol **3**. Alcohol **3** was dehydrated by treatment with phosphorus oxychloride or by treatment with *p*-toluenesulfonic acid in toluene with azeotropic removal of water, to provide styrene **4**. Reduction by treatment with hydrogen in



Scheme 1. Synthesis of cyclohexylamine DPP-4 inhibitors.



Scheme 2. Synthesis of cyclohexylamine DPP-4 inhibitors.

the presence of palladium on carbon as a catalyst gave the 4-aryl substituted cyclohexane **5**. Deprotection under acidic condition gave the cyclohexanone **6**, which was then converted to triisopropylsilyl enol ether **7**. The enol ether **7** upon treatment with iodosobenzene and trimethylsilyl azide formed the azido cyclohexene **8**, which upon reduction to amine with lithium aluminum hydride yielded amine **9**, as a mixture of *cis* and *trans* isomers. The desired *trans* isomer was separated by chromatography on silica. Protection of the resulting amine as its BOC derivative by treatment with di-*tert*-butyl dicarbonate gave **10**. Treatment of **10** with a source of fluoride anion removed the silyl group and gave the racemic *trans*-**11a** which was resolved on chiral column to yield the desired 1*S*,2*R* isomer. The disposition of substituents around the cyclohexyl ring (axial vs equatorial) was deduced from the vicinal ^{1}H–^{1}H splitting and was confirmed by NOE data. The NMR signal assignments were obtained by 2D NOESY, COSY, and HSQC experiments.[16]

An alternative method to prepare Intermediate **11b** is shown in Scheme 2. The commercially available 1,4-dioxaspiro[4.5]decan-8-one was treated with dimethyl carbonate to form the keto ester **14**, which was then transformed into the enol triflate **15** by treatment with trifluoromethanesulfonic anhydride. Treatment of **15** with 3,4,5-trifluorophenylboronic acid **16** gave the aryl cyclohexene **17**. Reduction of **17** was readily achieved with magnesium in methanol and provided the ester **18** as a mixture of *cis* and *trans* isomers. Conversion to the thermodynamically more stable *trans* isomer **19** was effected by treatment with a base such as sodium methoxide in methanol. Hydrolysis of the ester with lithium hydroxide to form the acid **20** followed by Curtius rearrangement in the presence of benzyl alcohol gave the amine **21**, as its benzylcarbamate derivative. Deprotection of the ketal by treatment with sulfuric acid in dioxane provided Intermediate **11b**. Compounds **2a** and **2b** were prepared by reductive amination of ketone **11b** and trifluoromethyltriazolopiperazine used in sitagliptin[14] and deprotection with hydrogen in the presence of palladium on carbon.

Compounds **2a** and **2b** were evaluated in vitro for their inhibition of DPP-4 activity[17] and selectivity against DASH family members.[18] Among them, selectivity against DPP-8 and DPP-9 was of particular concern since safety studies using a selective DPP-8/9 dual inhibitor suggested that inhibition of DPP-8 and/or DPP-9 is associated with profound toxicity in preclinical species.[19] DPP-8, DPP-9, FAP, and quiescent cell proline dipeptidase (QPP, also known as DPP-7)[17,20] inhibition data are also given in Table 1.

As shown in Table 1, isomer **2a** where all substituents are in equatorial positions has similar potency (IC_{50} = 21 nM) to sitagliptin (IC_{50} = 18 nM) and was also selective in the counter-screening assays. However, when the triazolopiperazine group is in the axial position, **2b** was about an order of magnitude (IC_{50} = 140 nM) less potent than sitagliptin. The two left-hand side rings are similar in both molecules, and that explains the partial activity of **2b**.

The pharmacokinetic properties of **2a** in male Sprague–Dawley rats, dogs, and rhesus monkeys are summarized in Table 2. Cyclohexylamine **2a** has low to moderate plasma clearance, excellent oral bioavailability, and long half-life in rats, dogs, and monkeys.

Table 2. Pharmacokinetic parameters of compound **2a**

Species	Cl_p (mL/min/kg)	$t_{1/2}$ (h)	F_{oral} (%)	C_{max} (μM)	AUC (μM h kg mg)
Rat	7.6	6.6	100	1.4	6.8
Dog	3.9	18	95	0.93	10.1
Monkey	9.5	14	94	0.56	3.9

Cl_p, plasma clearance; F_{oral}, oral bioavailability; C_{max}, peak plasma concentration after indicated oral dosing. Pharmacokinetic parameters were obtained following an IV (1 mg/kg) or po (2 mg/kg) dose (amorphous dihydrochloride salt) in water.

Table 1. Activities of cyclohexylamine DPP-4 inhibitors

Compound	DPP-4 IC_{50} (nM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)	QPP IC_{50} (μM)	FAP IC_{50} (μM)
1	18	48	>100	>100	>100
2a	21	>100	>100	>100	60
2b	140	>100	>100	>100	>100

In pharmacodynamic studies, oral glucose tolerance tests (OGTT) in lean male mice were conducted to determine the efficacy of **2a**. The glucose AUC was determined from 0 to 120 min. Percent inhibition values for each treatment were generated from the AUC data normalized to the water-challenged controls. Compound **2a** significantly reduced blood glucose excursions in a dose-dependent manner as follows: 0.03 mg/kg (26% inhibition), 0.1 mg/kg (48% inhibition), 0.3 mg/kg (53% inhibition), 1 mg/kg (60% inhibition), 3 mg/kg (54% inhibition). The mouse DPP-4 IC_{50} is 8 nM. In a separate OGTT of **2a**, plasma DPP-4 inhibition, compound concentration, and active GLP-1 level were measured 20 min after dextrose challenge. At a dose of 0.1 mg/kg, the corresponding plasma concentration of **2a** reached 27 nM, and 94% inhibition of plasma DPP-4 activity was observed, resulting in a 3.5-fold increase in active GLP-1 levels, and full efficacy in glucose reduction.

In summary, compound **2a**, designed by molecular modeling of the X-ray crystal structure of sitagliptin, has similar potency to sitagliptin, excellent pharmacokinetic properties across species, and an excellent profile in an OGTT assay. Further investigation of this lead is continuing.

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16. (a) All new compounds were characterized by ^{1}H NMR and LC–MS prior to submission for biological evaluation. (b) All multiple determinations of the IC_{50} values were within 1.5-fold of the reported average. Compound **12**: ^{1}H NMR (600 MHz, CD_3OD) δ 7.41 (m, 1H), 7.22 (m, 1H), 4.22 (t, 2H, J = 5.5 Hz), 4.11 (AB, 2H, J = 15.6 Hz), 3.59 (m, 1H), 3.19 (m, 2H), 3.04 (tt, 1H, J = 11.9, 3.4 Hz), 2.97 (br m, 1H), 2.38 (dm, 1H, J = 11.8 Hz), 2.09 (dm, 1H, J = 12.3 Hz), 2.00 (dq, 1H, J = 13.8, 3.6 Hz), 1.76 (m, 1H), 1.67 (q, 1H, J = 11.8 Hz), 1.58 (dq, 1H, J = 3.4, 12.3 Hz). Compound **13**: ^{1}H NMR (600 MHz, CD_3OD) δ 7.35 (m, 1H), 7.16 (m, 1H), 4.28 (m, 2H), 4.04 (d, 1H, J = 15.7 Hz), 3.96 (d, 1H, J = 15.7 Hz), 3.77 (dt, 1H, J = 2.7, 11.8 Hz), 3.16 (m, 1H), 3.09 (br, 1H), 3.06 (m, 1H), 2.88 (quintet, 1H, J = 3.0 Hz), 2.54 (dq, 1H, J = 14.0, 2.9 Hz), 2.28 (dm, 1H, J = 14.1 Hz), 1.98 (qm, 1H, J = 12.4 Hz), 1.78 (ddd, 1H, J = 2.6, 13.5, 14.4 Hz), 1.74 (m, 1H), 1.70 (m, 1H).
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