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New fluorinated pyrrolidine and azetidine amides as dipeptidyl peptidase IV inhibitors

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Abstract—Cyclohexylglycine amides of various fluorinated pyrrolidines and azetidines were prepared and tested for activity against dipeptidyl peptidase IV and in vivo in the KK mouse model of type 2 diabetes. The tetrafluoropyrrolidide, *cis*-3,4-difluoropyrrolidide and the fluorinated azetidides displayed unexpectedly strong activity.

Glucagon-like peptide-1, an incretin hormone responsible for stimulating insulin release in response to a glucose stimulus, is rapidly degraded by the serine protease dipeptidyl peptidase IV (DPP-IV). Inhibitors of DPP-IV have been proposed as therapeutic agents for type 2 diabetes, a condition characterized by elevated glucose levels, dyslipidemia and other metabolic abnormalities.[1] A number of agents have progressed to clinical trials and positive data have been disclosed with LAF-237 (**1**)[2] and MK-431 (**2**)[3] (see Fig. 1).

Early reports on competitive inhibitors focused on simple amides such as valine pyrrolidide[4] and isoleucine thiazolidide.[5] The SAR indicated that the pyrrolidine and thiazolidine rings fit into a tight binding pocket, since most simple modifications of the ring led to a dramatic decrease in activity.[6] However, 3-fluoropyrrolidine analogs (e.g., **3**) were shown to have activity equal or superior to that of the parent compounds.[6] Later reports disclosed that 3,3-difluoropyrrolidides in the cyclohexylglycine series, such as **4**, had enzymatic potency equivalent to that of the corresponding thiazolidides,[7] while the *trans* vicinal (*R*,*R*)- and (*S*,*S*)-3,4-difluoropyrrolidides were significantly inferior.[7b] Other workers reported a series of compounds containing a fused cyclopropyl ring at either the 3,4- or 4,5-position of the 2-cyanopyrrolidide moiety.[8]

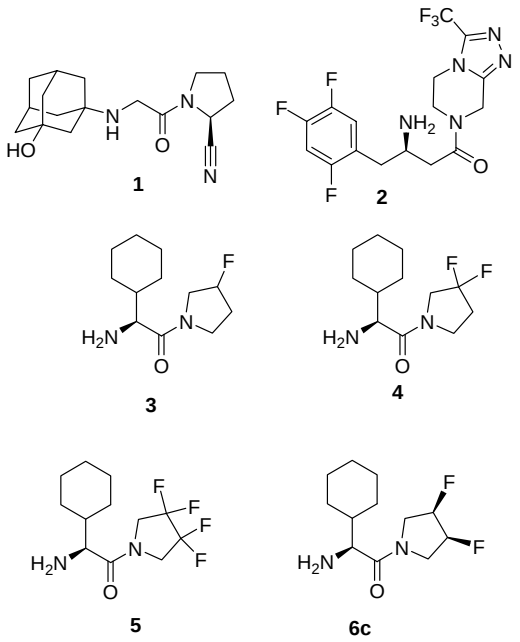

Figure 1. Inhibitors of DPP-IV.

Our initial work on DPP-IV inhibitors has included the synthesis and testing of new fluorinated pyrrolidine and azetidine derivatives of cyclohexylglycine. In this letter, we report an improved preparation of 3,3,4,4-tetrafluoropyrrolidine, a synthesis of the novel building block *cis*-3,4-difluoropyrrolidine and the unexpected strong DPPIV inhibition of the corresponding cyclohexylglycine amides

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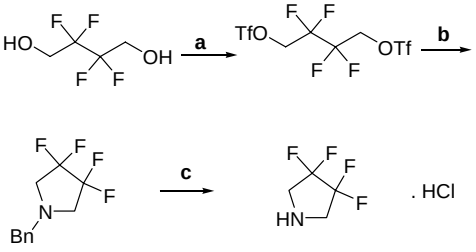
5,[7a,9] **6c**,[7a] as well as the analogous fluorinated azetidine analogs.[7a,10]

The cyclohexylglycine amides were prepared in a straightforward manner by EDC coupling of the amine with *Boc*-(L)-cyclohexylglycine, followed by acid removal of the *Boc* group. 3-Fluoropyrrolidine was obtained from a commercial source while 3,3-difluoropyrrolidine[11] and 3,3-difluoroazetidine[12] were prepared by literature methods. The 3-fluoroazetidine analog **5** was obtained by coupling *Boc*-(L)-cyclohexylglycine with 3-hydroxyazetidine followed by diethylaminosulfur trifluoride (DAST) fluorination and *Boc* deprotection.

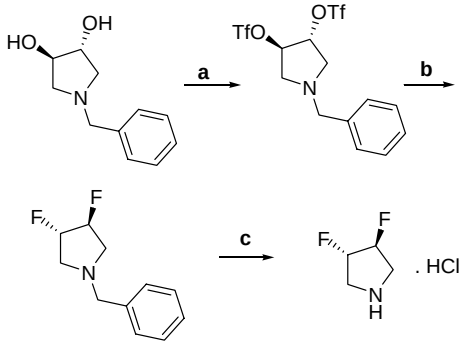
The previously reported route to 3,3,4,4-tetrafluoropyrrolidine utilizes a perfluorosuccinimide intermediate. The perfluorosuccinimide is reduced with lithium aluminum hydride[13] or borane[14] to afford the desired 3,3,4,4-tetrafluoropyrrolidine in moderate yield after purification by sublimation. In the hope of increasing the overall yield and avoiding the sublimation, we decided to try an alternate route.[9] Tetrafluorobutanediol was activated as the *bis*-triflate and converted to the benzyl-protected pyrrolidine by treatment with benzylamine. Hydrogenolysis in acid efficiently provided the hydrochloride salt (Scheme 1). In our hands, this route improved the overall yield from 44% to 81%.

The *trans* 3,4-difluoropyrrolidine isomers were obtained from the available *N*-benzylpyrrolidinediols by a sequence similar to that reported by Caldwell et al.[7b] (Scheme 2).

The hitherto unknown *meso*-3,4-difluoropyrrolidine proved to be more difficult to synthesize. Attempts to use the same route as that used for the *trans* 3,4-difluoropyrrolidine isomers, that is, activation of the hydroxyl groups of *cis*-*N*-benzyl-3,4-pyrrolidinediol to the ditriflate, led to the isolation of *N*-benzyl-3-pyrrolidinone and starting material. The desired *cis* isomer was prepared in four steps by metathesis of *Boc*-diallylamine to *Boc*-pyrroline, which was then epoxidized with potassium peroxymonosulfate. Opening of the epoxide with triethylamine hydrogen fluoride was followed by treatment with diethylaminosulfur trifluoride to provide the *Boc*-protected *cis*-difluoropyrrolidine (Scheme 3). The same sequence could also be carried out from *Cbz*-diallylamine.



Scheme 1. Reagents: (a) Tf_2O, pyridine, CH_2Cl_2; (b) $BnNH_2$, Et_3N, EtOH, 95% over two steps; (c) H_2, Pd–C, EtOH, 85%.

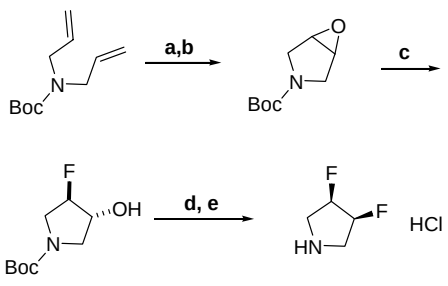


Scheme 2. Reagents: (a) Tf_2O, pyridine, CH_2Cl_2, 75%; (b) Bu_4NF, THF, 76%; (c) H_2, Pd–C, EtOH, 99%.

The compounds were first tested for their ability to inhibit the recombinant DPP-IV enzyme.[15] Selected in vitro active compounds were tested in vivo in an Oral Glucose Tolerance Test (OGTT) in the spontaneously diabetic KK mouse as a qualitative test of their ability to improve glucose tolerance. Table 1 shows the in vitro and in vivo activities of the various fluorinated pyrrolidine and azetidine derivatives of cyclohexylglycine compared to those of the parent compounds.

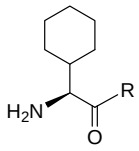
While the respective in vitro potencies of pyrrolidine derivatives **7**, **3r**, **3s**, **6r**, and **6s** are consistent with those previously observed, the newer analogs gave surprising results. The tetrafluoropyrrolidine amide **5** and the *cis*-difluoro compound **6c** are ca. 10-fold more potent than **6r** and **6s**. Both **5** and **6c** are fully active in vivo with 59% and 60% inhibition of the glucose excursion (Fig. 2) at 10 mg/kg in the KK mouse, a model of type 2 diabetes. The introduction of fluorine also has an effect on the azetidine analogs: while the parent azetidide **8** is a weak inhibitor, as expected based on an earlier report,[6] the monofluoro and difluoro compounds show activity equivalent to cyclohexylglycine pyrrolidide (**7**).

These results indicate that fluorine has a beneficial effect on the activity of the pyrrolidide or azetidides as long as strict geometrical constraints are enforced. The weak activity of the *trans*-difluoropyrrolidides **6r** and **6s** initially suggested that in the pyrrolidine-binding pocket vicinal fluorine atoms at C-3 and C-4 are not tolerated. This is contradicted however by the activity of the tetrafluoropyrrolidide **5** and the *meso*-difluoropyrrolidide **(6c).** The vicinal fluorines on the pyrrolidine ring can boost the activity as long as they are in a *syn* stereochemical relationship (**6c**). These discrepancies are diffi-



Scheme 3. Reagents: (a) $Cl_2(PCy)_3Ru$ = CH–Ph, benzene 92%; (b) oxone®, CF_3COCF_3, EDTA, 100%; (c) HF–TEA, 45%; (d) DAST, 42%; (e) HCl–dioxane, 100%.

Table 1. DPP-IV inhibitor constants and in vivo activity of compounds **3**–**10**



Compound	R	DPP-IV K_i (nM ± SEM)[a]	OGTT[b] % inhibition at 10 mg/kg
7	Pyrrolidine	121 ± 2	69
3r	(*R*)-3-Fluoropyrrolidine	128 ± 4	
3s	(*S*)-3-Fluoropyrrolidine	56 ± 1	
4	3,3-Difluoropyrrolidine	28 ± 3	78
6r	(*R*,*R*)-3,4-Difluoropyrrolidine	504 ± 37	
6s	(*S*,*S*)-3,4-Difluoropyrrolidine	1267 ± 41	
6c	*cis*-3,4-Difluoropyrrolidine	61 ± 7	60
5	Tetrafluoropyrrolidine	81 ± 3	59
8	Azetidine	3350 ± 1080	
9	3-Fluoroazetidine	109 ± 14	70
10	3,3-Difluoroazetidine	117 ± 4	109

[a] K_is are calculated from the IC_{50}s using the Cheng–Prussoff equation for competitive inhibitors (Ref. 16). IC_{50}s were obtained from a standard dose–inhibition curve.

[b] Oral Glucose Tolerance Test. A 10 mg/kg oral dose of the compound was administered to fasted mice, followed 15 min later by a 1 g/kg oral glucose load. Blood glucose levels were measured 30 min later. The effect of the compound is expressed as percent reduction in blood glucose concentration relative to that measured in animals dosed with an inert vehicle rather than active compound.

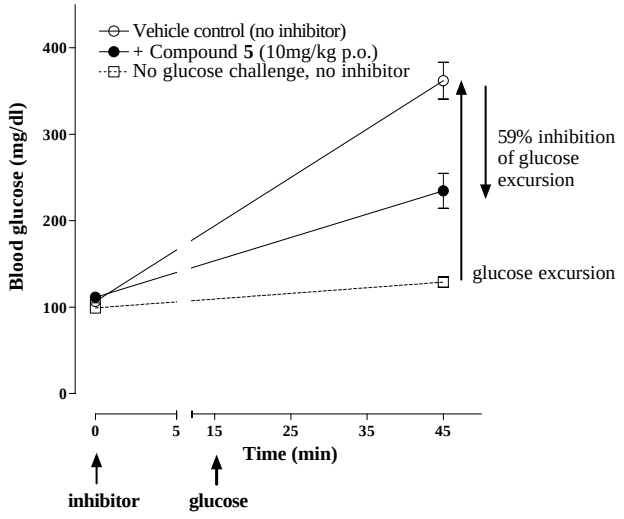


Figure 2. Oral Glucose Tolerance Test of **5** in KK mice at 10 mg/kg.

cult to explain without the benefit of structural information. Molecular modeling based on the published DPP-IV/valine pyrrolidide complex[17] failed to elucidate the binding site's ability to discriminate between *cis* and *trans* vicinal difluoropyrrolidides, as well as the ca. 30-fold boost of activity upon mono- or difluorination of the azetidine ring. Fluorine is known to occupy little more space than hydrogen and to generally increase lipophilicity by a small degree, however, the magnitude of the increase in potency and the equivalent potencies of **9** and **10** make it clear that other effects are at work, possibly involving interactions between the fluorine atoms and the residues of the binding cavity.[18]

In summary, we have shown that *cis*-3,4-difluoropyrrolidides, 3,3,4,4-tetrafluoropyrrolidides, and fluorinated azetidides can be easily prepared and are potent inhibitors of DPP-IV, thereby offering alternatives to the usual pyrrolidides, thiazolidides, and 3,3-difluoropyrrolidides.

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