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Fluoropyrrolidine amides as dipeptidyl peptidase IV inhibitors

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Abstract—Amides derived from fluorinated pyrrolidines and 4-substituted cyclohexylglycine analogues have been prepared and evaluated as inhibitors of dipeptidyl dipeptidase IV (DP-IV). Analogues which incorporated (*S*)-3-fluoropyrrolidine showed good selectivity for DP-IV over quiescent cell proline dipeptidase (QPP). Compound **48** had good pharmacokinetic properties and was orally active in an oral glucose tolerance test in lean mice.

Type-2 diabetes mellitus, also known as non-insulin-dependent diabetes mellitus, accounts for >90% of the cases of diabetes. This condition is characterized by high levels of glucose resulting from progressive insulin resistance and, at later stages of the disease, impairment of insulin secretion.[1] Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by intestinal L-cells in response to food intake. The active form GLP-1[7-36] amide is a 30-amino acid peptide which stimulates insulin release while inhibiting hepatic glucose production.[2] Continuous subcutaneous infusion of active GLP-1 in diabetic patients for 6 weeks has resulted in reduction of blood glucose and hemoglobin A_{1c} levels.[3] GLP-1[7-36] amide is rapidly converted into the inactive form GLP-1[9-36] amide by dipeptidyl peptidase IV (DP-IV). This enzyme is a serine protease which cleaves an N-terminal dipeptide from polypeptides, with a preference for substrates having a proline or alanine residue at the P_1 site.[1,2]

The use of DP-IV inhibitors to increase levels of endogenous GLP-1[7-36] amide has potential as a therapeutic treatment for diabetic patients.[4] The cyanopyrrolidine derivatives **1** and **2** (Fig. 1) are potent inhibitors of DP-IV, but related compounds lacking the electrophilic nitrile group show a sharp decrease in activity.[5] Additional selected examples of DP-IV inhibitors include (*S*)-isoleucine thiazolidide (**3**) and cyclohexylglycine pyrrolidide (**4**). Clinical trials of compound **3**, which has moderate potency versus DP-IV, resulted in improved glucose tolerance in diabetic patients and healthy

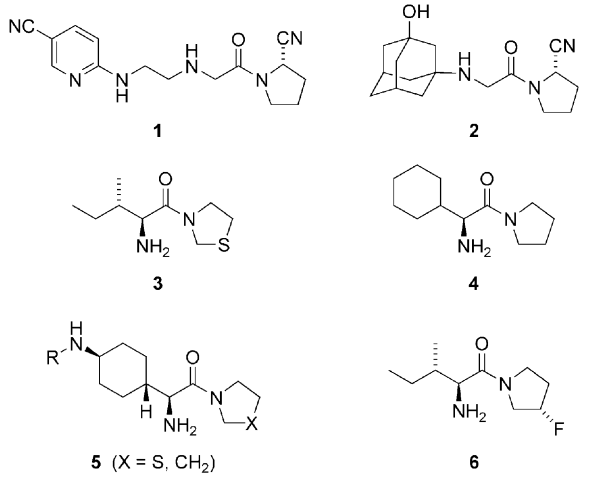

Figure 1. Inhibitors of DP-IV.

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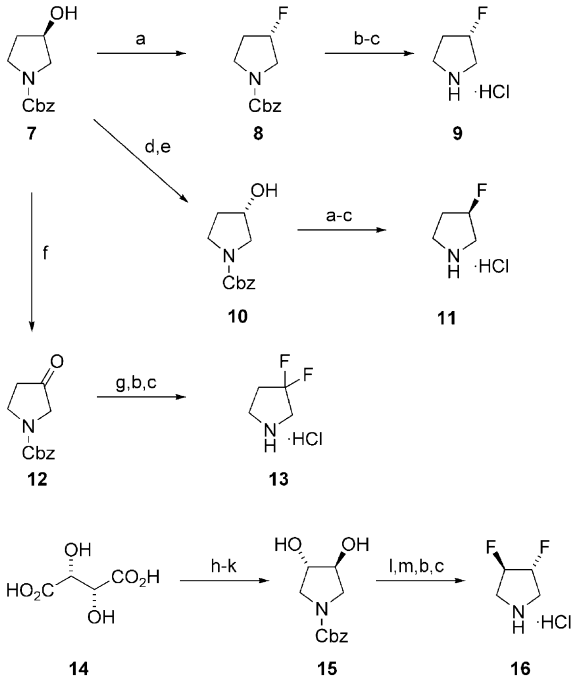
volunteers.[6] Introduction of the cyclohexylglycine subunit of compound **4** produced an analogue which is somewhat more potent than isoleucine pyrrolidide as a DP-IV inhibitor.[7]

An earlier report from our laboratories described 4-aminocyclohexylglycine derivatives (**5**) which are potent DP-IV inhibitors having good pharmacokinetic properties.[8] Augustyns has reported results for the (*S*)-3-fluoropyrrolidine derivative **6** and the corresponding mixture of epimeric 3-fluoropyrrolidides.[9] The work which we describe here is an examination of a series of DP-IV inhibitors resulting from the combination of fluorinated pyrrolidines with 4-substituted cyclohexylglycine subunits previously studied as their thiazolidine or pyrrolidine amides.[8]

Determinations of IC_{50} values for inhibition of DP-IV and quiescent cell proline dipeptidase (QPP, also known as DPP-II or DPP7) were carried out as described previously.[10] Compounds were also tested against prolyl endopeptidase, amino peptidase P, prolidase, and fibroblast activation protein α (FAP, also called seprase).[11] Since significant inhibition (IC_{50} < 10,000 nM) was not observed for these other enzymes, data is presented for DP-IV and QPP only. Measurements of binding to the hERG potassium ion channel were obtained on compounds of interest as a measure of general off-target activity.[12]

The syntheses of (*S*)-3-fluoropyrrolidine hydrochloride (**9**) and the corresponding (*R*)-isomer (**11**) were accomplished using modifications of the conditions described by Giardina and co-workers (see Scheme 1).[13] Fluorination of the (*R*)-3-hydroxypyrrolidine derivative **7** using DAST proceeded in good yield.[14] Following hydrogenolysis to remove the Cbz group and filtration to separate the catalyst, addition of HCl and evaporation of the solvent yielded the (*S*)-isomer **9**. Inversion of the stereochemistry of alcohol **7** was accomplished as described by Giardina, and the enantiomer **10** was then converted into (*R*)-3-fluoropyrrolidine hydrochloride (**11**). Swern oxidation of the 3-hydroxypyrrolidine intermediate **7** yielded ketone **12** which was fluorinated in an overnight reaction using [bis(2-methoxyethyl)amino]sulfur trifluoride (Deoxo-Fluor®) in dichoromethane, or in 3 h using neat DAST. Deprotection yielded 3,3-difluoropyrrolidine hydrochloride **13**.[13]

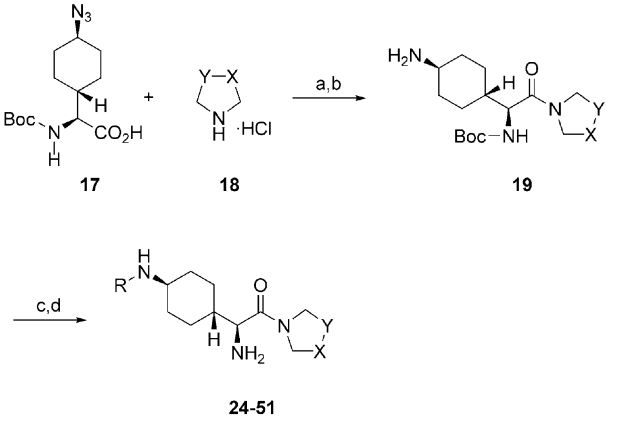


Scheme 1. Reagents: (a) DAST, CH_2Cl_2, −78 °C to rt; (b) H_2, 10% Pd/C, EtOH; (c) HCl; (d) AcOH, DEAD, Ph_3P, $PhCH_3$; (e) KOH, EtOH, H_2O; (f) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (g) Deoxo-Fluor®, CH_2Cl_2, rt, 15 h, or DAST (neat), rt, 3 h; (h) $BnNH_2$, xylenes, reflux; (i) LAH, THF; (j) H_2, 20% $Pd(OH)_2$/C, EtOH; (k) BnOC(O)Cl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2; (l) Tf_2O, pyridine, CH_2Cl_2; (m) $(n\text{-}Bu)_4NF$, THF.

L-Tartaric acid (**14**) reacted with benzylamine to give the *N*-benzyl imide derivative which was reduced using LAH to give (3*S*,4*S*)-1-benzyl-3,4-dihydroxypyrrolidine.[15] After hydrogenolysis to remove the benzyl group, the pyrrolidine nitrogen was protected to give the Cbz derivative **15**. The vicinal hydroxyl groups were then activated by formation of the corresponding ditriflate, and displacement was accomplished by treatment with tetrabutylammonium fluoride.[16] Hydrogenolysis to remove the Cbz group proceeded as for the previous examples to give (3*R*,4*R*)-3,4-difluoropyrrolidine hydrochloride (**16**). The enantiomeric (3*S*,4*S*)-isomer was prepared from D-tartaric acid using the same synthetic sequence.

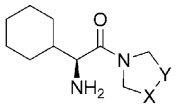
The cyclohexylglycine amides **21**–**23** (Table 1) were prepared by EDC-mediated coupling of the fluorinated pyrrolidines with Boc-L-cyclohexylglycine, with subsequent removal of the protecting group. The synthesis of the 4-amino derivatives shown in Table 2 utilized the 4-azido intermediate **17** described earlier (see Scheme 2).[9] Coupling of the pyrrolidines with **17** was followed by hydrogenation to give amine **19**. After acylation or sulfonylation, removal of the Boc protecting group provided the 4-substituted cyclohexylglycine derivatives **24**–**51**.

The data presented in Table 1 compare the activities of three fluorinated pyrrolidine derivatives of cyclohexyl-



Scheme 2. Reagents: (a) EDC, HOBt, Et_3N, DMF; (b) H_2, 10% Pd/C, EtOH; (c) RCl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2; (d) HCl, CH_3OH.

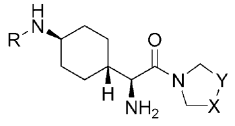
Table 1. Cyclohexylglycine amides



Compd	X–Y	DP-IV IC_{50} (nM)	QPP IC_{50} (nM)	hERG K_i (nM)
20	S-CH_2	89	2800	28,000
4	CH_2-CH_2	320	19,000	—
21	CHF-CH_2 (*S*)–	170	20,000	>90,000
22	CHF-CH_2 (*R*)–	340	38,000	>90,000
23	CF_2-CH_2	73	1900	>90,000

glycine to the parent compound as well as to the thiazolidide. Pyrrolidide **4** is approximately 4-fold less potent than thiazolidide **20** in the DP-IV assay. The (*R*)-3-fluoro analogue **22** shows no improvement relative to pyrrolidide **4** in DP-IV potency, although it does show some increase in selectivity versus QPP. The (*S*)-3-fluoro derivative **21** shows improved DP-IV potency, while retaining 100-fold selectivity versus QPP. The 3,3-difluoro analogue **23** is approximately equipotent to the thiazolidide **20** as a DP-IV inhibitor, although it has lost the increased selectivity versus QPP seen for the monofluoropyrrolidides **21** and **22**.

Table 2. 4-Aminocyclohexylglycine derivatives



Compd	R	X–Y	DP-IV IC_{50} (nM)	QPP IC_{50} (nM)	hERG K_i (nM)
24	CO(3,4-$F_2C_6H_3$)	S-CH_2	53	690	5900
25	CO(3,4-$F_2C_6H_3$)	CH_2-CH_2	190	5000	29,000
26	CO(3,4-$F_2C_6H_3$)	CHF-CH_2 (*S*)–	54	5000	19,000
27	CO(3,4-$F_2C_6H_3$)	CHF-CH_2 (*R*)–	110	4500	73,000
28	CO(3,4-$F_2C_6H_3$)	CF_2-CH_2	55	660	16,000
29	CO(2-(CF_3)C_6H_4)	S-CH_2	67	2000	39,000
30	CO(2-(CF_3)C_6H_4)	CHF-CH_2 (*S*)–	210	25,000	—
31	CO(2-(CF_3)C_6H_4)	CHF-CH_2 (*R*)–	250	21,000	—
32	CO(2-(CF_3)C_6H_4)	CF_2-CH_2	70	1100	—
33	Cbz	S-CH_2	25	900	—
34	Cbz	CH_2-CH_2	94	4100	40,000
35	Cbz	CHF-CH_2 (*S*)–	56	5200	37,000
36	Cbz	CHF-CH_2 (*R*)–	88	3500	43,000
37	Cbz	CF_2-CH_2	27	410	42,000
38	Cbz	CHF-CHF (*R,R*)–	360	3200	—
39	Cbz	CHF-CHF (*S,S*)–	830	3000	—
40	SO_2(4-(CF_3O)C_6H_4)	S-CH_2	22	1100	2800
41	SO_2(4-(CF_3O)C_6H_4)	CH_2-CH_2	89	6400	5700
42	SO_2(4-(CF_3O)C_6H_4)	CHF-CH_2 (*S*)–	35	8300	5500
43	SO_2(4-(CF_3O)C_6H_4)	CHF-CH_2 (*R*)–	75	12,000	6000
44	SO_2(4-(CF_3O)C_6H_4)	CF_2-CH_2	23	600	5300
45	SO_2(4-(CF_3O)C_6H_4)	CHF-CHF (*R,R*)–	270	6100	—
46	SO_2(4-(CF_3O)C_6H_4)	CHF-CHF (*S,S*)–	870	7500	—
47	SO_2(2,4-$F_2C_6H_3$)	CH_2-CH_2	88	8800	35,000
48	SO_2(2,4-$F_2C_6H_3$)	CHF-CH_2 (*S*)–	48	12,000	49,000
49	SO_2(2,4-$F_2C_6H_3$)	CHF-CH_2 (*R*)–	58	9400	52,000
50	SO_2(2,4-$F_2C_6H_3$)	CF_2-CH_2	21	810	—
51	SO_2(2,4-$F_2C_6H_3$)	CHF-CHF (*R,R*)–	250	8100	—

In the 3,4-difluorobenzoyl series of compounds (**24–28**) shown in Table 2, the (*S*)-3-fluoro analogue **26** and the 3,3-difluoro analogue **28** are equipotent with the thiazolidide **24** as DP-IV inhibitors, while the (*R*)-3-fluoro derivative **26** shows a slight drop in potency. Both **26** and **27** have improved selectivity versus QPP.

For the 2-(trifluoromethyl)benzoyl series of derivatives, the (*S*)- and (*R*)-3-fluoropyrrolidides **30** and **31** showed approximately 100-fold selectivity for DP-IV versus QPP, although their IC_{50} values for DP-IV had increased to >200 nM. The 3,3-difluoropyrrolidine derivative **32** was more potent as a DP-IV inhibitor, although selectivity over QPP was relatively low. A similar trend was observed for the Cbz derivatives **33–37**, with 3,3-difluoropyrrolidide **37** showing the greatest potency versus DP-IV, while having only 15-fold selectivity over QPP. Both the (3*R*,4*R*)- and (3*S*,4*S*)-3,4-difluoropyrrolidine derivatives **38** and **39** had decreased activity as DP-IV inhibitors.

Among the 4-(trifluoromethoxy)benzenesulfonamides in Table 2, the (*S*)-3-fluoro analogue **42** and the 3,3-difluoro analogue **44** had potency versus DP-IV similar to the thiazolidide **40**. The 3,3-difluoropyrrolidine derivative once again showed relatively low selectivity for inhibition of DP-IV over QPP. The (*R*)-3-fluoro compound **43** had a moderate decrease in DP-IV potency relative to thiazolidide **40**, while the (3*R*,4*R*)- and (3*S*,4*S*)-3,4-difluoropyrrolidine derivatives (**45** and **46**) both had ≥10-fold reductions in activity versus DP-IV.

In the 2,4-difluorobenzenesulfonamide series of compounds (**47–51**), the (3*R*,4*R*)-3,4-difluoropyrrolidine derivative **51** was the least active DP-IV inhibitor. The 3,3-difluoropyrrolidine analogue **50** showed the greatest potency versus DP-IV, but the selectivity of this compound over QPP was relatively low. The (*S*)- and (*R*)-3-fluoropyrrolidides **48** and **49** both showed good potency with IC_{50} values of 48 and 58 nM, respectively, for DP-IV inhibition. Potassium channel activity as measured by hERG binding was weak, with K_i values approximately 1000-fold higher than the IC_{50} for DP-IV inhibition. In addition, compound **48** showed 250-fold selectivity for inhibition of DP-IV over QPP.

Studies of the pharmacokinetic properties of (*S*)-3-fluoropyrrolidide **48** revealed that this compound was 53% orally bioavailable in rats (see Table 3), while studies in dogs showed 75% oral bioavailability. The compound was found to have a moderate clearance in dogs of 9.1 mL/min/kg and a half-life of 5.9 h. Peak

Table 3. Pharmacokinetic properties of compound **48**

		Rat	Dog
Dosage:	iv	1 mg/kg	1 mg/kg
	po	2 mg/kg	2 mg/kg
Cl_p		24 mL/min/kg	9.1 mL/min/kg
Vd_{ss}		2.5 L/kg	4.4 L/kg
$t_{1/2}$		1.6 h	5.9 h
C_{max} (po)		0.40 μM	1.0 μM
Oral bioavailability		53%	75%

concentrations following oral dosing at 2 mg/kg were 1.0 μM. Compound **48** was also examined in an oral glucose tolerance test. Dosing of the compound in lean C57BL6/N mice at 3 mg/kg resulted in a 42% reduction in the glucose excursion relative to vehicle-treated controls.

In summary, modification of the substituted 4-aminocyclohexylglycine pyrrolidide lead structure by introduction of fluorine substituents on the pyrrolidine ring has been examined. The (3*S*,4*S*)- and (3*R*,4*R*)-3,4-difluoropyrrolidine derivatives were less active inhibitors of DP-IV, but the 3,3-difluoropyrrolidine analogues showed good potency against the enzyme. Selectivity versus QPP for the 3,3-difluoropyrrolidine analogues was similar to that observed for the thiazolidine derivatives. Compounds derived from (*S*)-3-fluoropyrrolidine showed good potency versus DP-IV, with these compounds generally having slightly greater activity than the (*R*)-3-fluoropyrrolidides. The (*S*)-3-fluoro derivative **48** was found to have good pharmacokinetic properties and produced significant activity in an oral glucose tolerance test in lean mice.

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References and notes

1. (a) Bailey, C. J. *Trends in Pharmacol. Sci.* **2000**, *21*, 259. (b) Moller, D. E. *Nature* **2001**, *414*, 821. (c) Patel, M.; Rybczynski, P. J. *Expert Opin. Investig. Drugs* **2003**, *12*, 623.
2. (a) Holst, J. J. *Curr. Med. Chem.* **1999**, *6*, 1005. (b) Deacon, C. F.; Holst, J. J.; Carr, R. D. *Drugs of Today* **1999**, *35*, 159. (c) Livingston, J. N.; Schoen, W. R. *Ann. Rep. Med. Chem.* **1999**, *34*, 189.
3. Zander, M.; Madsbad, S.; Madsen, J. L.; Holst, J. J. *The Lancet* **2002**, *359*, 824.
4. (a) Augustyns, A.; Van der Veken, P.; Senten, K.; Haemers, A. *Exp. Opin. Ther. Patents* **2003**, *13*, 499. (b) Drucker, D. J. *Exp. Opin. Invest. Drugs* **2003**, *12*, 87. (c) Villhauer, E. B.; Coppola, G. M.; Hughes, T. E. *Ann. Rep. Med. Chem.* **2001**, *36*, 191. (d) Holst, J. J.; Deacon, C. F. *Diabetes* **1998**, *47*, 1663.
5. (a) Hughes, T. E.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Villhauer, E. B. *Biochemistry* **1999**, *38*, 11597. (b) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russel, M. E.; Welden, S. C.; Hughes, T. E. *J. Med. Chem.* **2002**, *45*, 2362. (c) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774.
6. (a) Schön, E.; Born, I.; Demuth, H.-U.; Faust, J.; Neubert, K.; Noll, F.; Stenmetzer, T.; Barth, A.; Ansorge, S. *Biol. Chem. Hoppe Seyler* **1991**, *372*, 305. (b) Pospisilik, J. A.; Stafford, S. G.; Demuth, H.-U.; Brownsey, R.; Parkhouse, W.; Finegood, D. T.; McIntosh, C. H. S.; Pederson, R. A. *Diabetes* **2002**, *51*, 943. (c) Sorbera, L. A.; Revel, L.; Castaner, J. *Drugs of the Future* **2001**, *26*, 859.
7. Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163.
8. Parmee, E. R.; He, J.; Mastracchio, A.; Edmondson, S. D.; Colwell, L. E.; Eiermann, G.; Feeney, W. P.; Habulihaz, B.; He, H.; Kilburn, R.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R.; Petrov, A.; di Salvo, J.; Wu, J. K.; Thornberry, N.; Weber, A. E. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 43.
9. Augustyns, K. J. L.; Lambeir, A. M.; Borloo, M.; De Meester, I.; Vedernikova, I.; Vanhoof, G.; Hendriks, D.; Scharpé, S.; Haemers, A. *Eur. J. Med. Chem.* **1997**, *32*, 301.
10. Leiting, B.; Pryor, K. D.; Wu, J. K.; Marsilio, F.; Patel, R. A.; Craik, C. S.; Ellman, J. A.; Cummings, R. T.; Thornberry, N. A. *Biochem. J.* **2003**, *371*, 525.
11. (a) Rosenblum, J. S.; Kozarich, J. W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 1. (b) Cunningham, D. F.; O'Connor, B. *Biochem. Biophys. Acta* **1997**, *1343*, 160.
12. (a) Friesen, R. W.; Ducharme, Y.; Ball, R. G.; Blouin, M.; Boulet, L.; Cote, B.; Frenette, R.; Girard, M.; Guay, D.; Huang, Z.; Jones, T. R.; Laliberte, F.; Lynch, J. J.; Mancini, J.; Martins, E.; Masson, P.; Muise, E.; Pon, D. J.; Siegel, P. K. S.; Styhler, A.; Tsou, N. N.; Turner, M. J.; Young, R. N.; Girard, Y. *J. Med. Chem.* **2003**, *46*, 2413 and references therein. (b) Pearlstein, R.; Vaz, R.; Rampe, D. *J. Med. Chem.* **2003**, *46*, 2017.
13. Giardina, G.; Dondio, G.; Grugni, M. *Synlett* **1995**, 55.
14. For compound **8**: $[\alpha]_D$ +23.2 ($c = 3.96$, CH_3OH). [Lit.[13] $[\alpha]_D$ +22.36 ($c = 4$, CH_3OH)].
15. Kang, Y. K.; Shin, K. J.; Yoo, K. H.; Seo, K. J.; Park, S. Y.; Kim, D. J.; Park, S. W. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2385.
16. Marson, C. M.; Melling, R. C. *Chem. Commun.* **1998**, 1223.