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4-Amino cyclohexylglycine analogues as potent dipeptidyl peptidase IV inhibitors

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Abstract—Substituted 4-amino cyclohexylglycine analogues were evaluated for DP-IV inhibitory properties. Bis-sulfonamide **15e** was an extremely potent 2.6 nM inhibitor of the enzyme with excellent selectivity over all counterscreens. 2,4-Difluorobenzenesulfonamide **15b** and 1-naphthyl amide **16b**, however, combined an acceptable in vitro profile with good pharmacokinetic properties in the rat, and **15b** was orally efficacious at 3 mpk in an OGTT in lean mice.
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Inhibition of dipeptidyl peptidase IV (DP-IV), a proline specific serine dipeptidase, is a novel therapeutic approach to the treatment of type 2 diabetes.[1] DP-IV rapidly cleaves the active form of the incretin hormone glucagon-like peptide 1 (GLP-1) (GLP-1[7-36] amide) to its inactive form (GLP-1[9-36] amide) with a half-life of approximately one minute and is thought to be the primary enzyme responsible for this hydrolysis.[1c] GLP-1 plays an important role in glucose-dependent insulin biosynthesis and secretion in humans. Infusion of GLP-1 has been shown to normalize both postprandial and fasting glucose in diabetics.[2] Additionally, chronic administration of GLP-1 has resulted in significant decreases in both fasting plasma glucose and HbA1c.[3] Inhibition of DP-IV, therefore, is expected to significantly reduce inactivation of GLP-1[7-36] and should lead to an increase in circulating levels of the active form of the hormone. Supporting evidence for this comes from DP-IV deficient mice, which have elevated levels of GLP-1[7-36] amide.[4] Thus, DP-IV inhibitors act as indirect stimulators of insulin secretion by stabilization of GLP-1.

Importantly, in contrast to many current therapies for type 2 diabetes, this mechanism potentially carries with it only a low risk of hypoglycemia since GLP-1 stimulates insulin release solely in the presence of elevated plasma glucose levels. Other possible advantages of DP-IV inhibitors as a treatment for diabetes include the fact that no weight gain is anticipated and there are potential long-term beneficial effects on beta cell function.[5]

Several small molecule inhibitors of DP-IV have been reported in the literature and progressed into clinical trials with positive results.[6,7] Of these, compounds **1** and **2** are the most potent inhibitors of DP-IV ($IC_{50} = 22$ and 34 nM, respectively),[8] but both contain an electrophilic nitrile (Fig. 1).[9] Inhibitors lacking an electrophile, while more stable, are generally of only modest intrinsic potency. One such example is (*S*)-isoleucine thiazolidide **3**, which showed good effects in limited clinical trials despite moderate inhibitory properties (DP-IV $IC_{50} = 420$ nM).[7,8]

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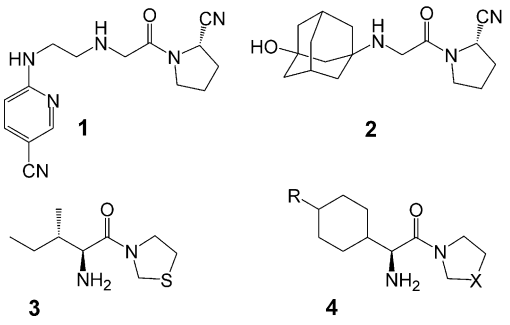


Figure 1. Reported DP-IV inhibitors.

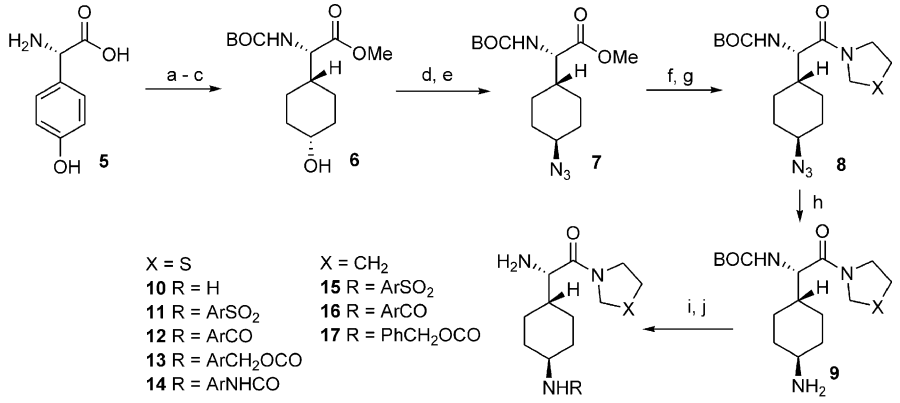
Related to compound **3**, (*S*)-cyclohexylglycine pyrrolidide **4** (R = H, X = CH_2, DP-IV IC_{50} = 320 nM)[8,10] has been reported as an inhibitor of DP-IV and we selected this as a scaffold for further modification in an effort to prepare DP-IV inhibitors of higher intrinsic potency but lacking an electrophile. We decided to install an amino substituent (R = NH_2) in the 4-position of the cyclohexyl ring of compound **4** and investigate the effect of a variety of aromatic acyl and sulfonyl capping groups on DP-IV inhibitory properties. Initially a thiazolidine ring was employed as the proline mimic (X = S). Inhibitors were also assessed for their selectivity profiles against a variety of DP-IV homologues and proline-specific enzymes [including prolyl endopeptidase, amino peptidase P, prolidase, quiescent prolyl peptidase (QPP/DPP-II), and seprase (FAP)].[11] Significant off-target activity (< 10,000 nM), however, only presented with QPP and hence only these data will be discussed. QPP is an enzyme reported to have DP-IV-like activity, although its exact physiological function is not currently well understood.[12] The QPP inhibitory properties of compounds **1**–**4** showed that nitrile containing analogues exhibited excellent selectivity for DP-IV (**1** and **2** at least 300-fold), while analogues **3** and **4** were 5–10-fold less selective.

Inhibitors were synthesized from alcohol **6**, prepared from (*S*)-4-hydroxyphenylglycine by esterification and protection of the amine as its *tert*-butylcarbamate followed by hydrogenation of the aromatic ring using catalytic platinum oxide in acetic acid at 45 psi (Scheme 1).

The *cis* isomer **6** was obtained in a 36% yield from **5** accompanied by the epimeric alcohol and fully reduced cyclohexyl compound. Alcohol **6** was converted to its methanesulfonate, followed by installation of the amine masked as its azide, which was carried through the subsequent saponification and amide coupling to afford amide **8**. The amino functionality was then released from the azide by treatment with triphenylphosphine in aqueous tetrahydrofuran, followed by acylation and deprotection to give compounds **11**–**17**.[13] Deprotection of the *tert*-butylcarbamate group in **9** gives the parent amine **10**.

Table 1 summarizes the inhibitory properties of amino containing thiazolidides. As can be seen from analogue **10**, a primary amino moiety at the 4-position was not well tolerated. Attenuation of the basicity by acylation of the nitrogen, however, provided a series of potent thiazolidide containing DP-IV inhibitors (IC_{50} < 60 nM). Sulfonamide derivatives **11a**–**e** were equipotent 20 nM inhibitors of the enzyme. Differences in selectivity over QPP were however evident, as the window for DP-IV inhibition ranged from 50-fold for 4-trifluoromethoxyphenyl compound **11b** to 9-fold for 2-naphthyl derivative **11e**. Incorporating a urea substituent onto the benzenesulfonamide as in analogue **11f**[14] increased potency (DP-IV IC_{50} = 7.5 nM) as well as selectivity (> 250-fold window over inhibition of QPP). Amide derivatives **12** showed a similar range of activities against DP-IV, with 3-quinolyl analogue **12g** being the most potent (DP-IV IC_{50} = 9 nM), while the 1-naphthyl derivative **12b** exhibited both excellent potency and a 60-fold window over inhibition of QPP. While the carbamate and urea analogues **13** and **14** were also potent inhibitors of DP-IV, benzyl carbamate **13a** was the only analogue to exhibit significant selectivity over QPP (36-fold). The epimeric *cis* analogues of **11a**,**b**,**e**, **12c**, and **13a** were prepared from the *trans* isomer of alcohol **6** in an identical manner to that described above. They were all found to be significantly less potent inhibitors of DP-IV (IC_{50}'s 100–500 nM) and no selectivity over QPP was observed (data not shown).

Selected derivatives were also prepared in the pyrrolidide series, and their inhibitory properties are summarized in



Scheme 1. Synthesis of 4-amino cyclohexylglycine analogues: (a) HCl_g, MeOH, 45 °C; (b) BOC_2O, DIEA, EtOAc; (c) PtO_2, AcOH, H_2, 45 psi, 16 h; (d) MsCl, DIEA, CH_2Cl_2; (e) NaN_3, DMF, 60 °C; (f) aq LiOH, THF; (g) thiazolidine or pyrrolidine, EDC, HOBt, DIEA, DMF; (h) PPh_3, aq THF; (i) acylating agent, Et_3N, CH_2Cl_2; (j) TFA, CH_2Cl_2.

Table 1. Inhibitory properties of selected DP-IV inhibitors[8]

Entry	Ar	DP-IV IC_{50}, nM	QPP IC_{50}, nM
1	—	22	6600
2	—	34	>100,000
3	—	420	15,000
4	(R = H, X = CH_2)	320	19,000
10	—	2700	—
11a	4-IPh	23	280
11b	4-CF_3OPh	22	1100
11c	3-CNPh	24	820
11d	2,3,4-triFPh	24	960
11e	2-Naphthyl	18	170
11f	4-Me_2NCONHPh	7.5	2000
12a	4-IPh	33	340
12b	4-CF_3OPh	33	360
12c	3,4-diFPh	53	690
12d	3,4-diClPh	38	230
12e	1-Naphthyl	15	900
12f	2-Naphthyl	21	230
12g	3-Quinolinyl	9	230
13a	Ph	25	900
13b	3,4-diClPh	29	170
13c	1-Naphthyl	17	170
13d	2-Naphthyl	20	200
14a	4-IPh	10	210
14b	4-CF_3OPh	55	350
14c	3,4-diClPh	17	170
14d	3,4-diFPh	35	380

Table 2. Inhibitory properties of selected DP-IV inhibitors[8]

Entry	Ar	DP-IV IC_{50}, nM	QPP IC_{50}, nM
15a	4-CF_3OPh	89	6400
15b	2,4-diFPh	88	8800
15c	2,3,4-triFPh	98	8200
15d	4-Me_2NCONHPh	26	12,000
15e	4-$CF_3CH_2SO_2NHPh$	2.6	15,000
16a	3,4-diFPh	190	5000
16b	1-Naphthyl	46	8600
16c	3-Quinolinyl	64	4500
17	Ph	94	4100

Table 3. hERG binding and rat pharmacokinetic properties (1/2 mpk iv/po) of selected DP-IV inhibitors

Entry	hERG binding K_i (nM)	Clp (mL/min/kg)	$t_{1/2}$ (h)	F (%)
11b	2800	21	4.7	85
12c	5900	30	2	81
12e	22,000	22	1.2	51
14a	1900	74	2.3	12
15a	5700	31	3.6	38
15b	35,000	53	1.8	36
15d	>100,000	21	2.4	1
15e	>100,000	95	0.1	0
16b	17,000	13	2.3	30
17	40,000	28	4.0	52

Table 2. These analogues were at least 3-fold less potent than their thiazolidide congeners, but exhibited improved selectivity over QPP. Thus, sulfonamide **15a** was 4-fold less potent than **11b**, but was more than 70-fold selective over inhibition of QPP. Further improvements in selectivity were achieved. For example, 2,4-difluorobenzenesulfonamide **15b** (DP-IV IC_{50} = 88 nM) displayed a 100-fold window over QPP. Increasing the polarity of the phenyl substituents again resulted in dramatic improvements in the in vitro profile of these compounds, exemplifed with *N*,*N*-dimethylurea **15d**[14] (DP-IV IC_{50} = 26 nM, >450-fold selective over QPP). A more profound improvement in intrinsic activity was obtained, however, with bis-sulfonamide **15e**[14] which is a highly potent 2.6 nM inhibitor of DP-IV with >5000-fold selectivity over inhibition of QPP. This analogue represents the most potent DP-IV inhibitor reported to date lacking an electrophile on the pyrrolidide ring. In the amide series, the 1-naphthyl derivative **16b** was again the most potent and selective analogue (DP-IV IC_{50} = 46 nM, 180-fold selective over inhibition of QPP).

Representative analogues were selected for evaluation of pharmacokinetic properties in the rat and possible ion channel activity as a measure of general off-target activity. The latter is illustrated here with binding to the hERG potassium channel (Table 3).[15] In the sulfonamide series, trifluoromethoxyphenyl analogues (**11b** and **15a**) had demonstrable ion channel binding (hERG binding K_i = 2800 and 5700 nM, respectively). These compounds, however, had excellent pharmacokinetic properties with a moderate clearance and long half-life (4–5 h). The thiazolidide ring appeared to impart superior oral bioavailability in the rat [F = 85% (entry 1) vs 38% (entry 5)]. Selectivity over binding to the hERG potassium channel was significantly improved by replacement of the trifluoromethoxyphenyl group with a 2,4-difluorophenyl moiety in analogue **15b** which was now devoid of hERG binding up to concentrations of 35000 nM whilst maintaining good pharmacokinetic properties in the rat. Analogues **15d** and **15e**, which had displayed enhanced selectivity over QPP (vide supra), displayed negligible ion channel binding (hERG binding K_i >100,000 nM). Incorporation of the polar functionality was, however, detrimental to pharmacokinetic properties as evidenced by the extremely poor oral bioavailability of both these analogues.

Mimicking the simple sulfonamides, amides **12c**,**e**, and **16b** also possessed acceptable to good pharmacokinetic properites in the rat, with the naphthyl derivatives showing the best selectivity profiles over hERG binding (K_i >17,000 nM). Urea **14a** exhibited one of the poorest overall profiles, with significant affinity for the hERG channel, a high clearance and low oral bioavailability. Carbamate **17** had the best pharmacokinetic profile of the pyrrolidide analogues (F = 52%) and was devoid of ion channel activity up to concentrations of 40,000 nM.

In summary, we have discovered a novel series of potent and selective DP-IV inhibitors. These are among the most potent compounds reported to date lacking an electrophilic trap. Notable among these is bis-sulfonamide **15e**, which is a 2.6 nM DP-IV inhibitor with an exceptional selectivity profile over both QPP and ion channel binding. This compound, however, lacks oral bioavailability in the rat. Sulfonamide **15b** and amide **16b** both exhibit an acceptable 100-fold window over QPP, with excellent ion channel selectivity and good pharmacokinetic properties in the rat. Sulfonamide **15b** was selected for assessment in the dog where it had an

excellent pharmacokinetic profile with a moderate clearance (14 mL/min/kg), 6 h half-life, and oral bioavailability equal to 100%. This compound was evaluated in vivo for glucose lowering potential. Thus, a dose of 3 mpk given 1 h prior to an oral glucose tolerance test (OGTT) in lean C57BL6/N mice resulted in a 36% reduction in glucose excursion when compared to vehicle.

This important result confirmed the possible utility of substituted 4-aminocyclohexylglycine DP-IV inhibitors as orally active hypoglycemic agents for the treatment of type-2 diabetes. It was subsequently found that modifications to the pyrrolidide ring resulted in a further enhancement in both the selectivity and pharmacokinetic profile of these inhibitors. These alterations will be the subject of a future publication.

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