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1-Aminomethylisoquinoline-4-carboxylates as Novel Dipeptidylpeptidase IV Inhibitors

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Abstract—Structure–activity relationship within a series of 1-aminoalkylisoquinoline-4-carboxylates as inhibitors of DPP-IV is described. A primary aminomethyl group is required to maintain biological activity. Substitution of the isoquinoline at the 6- and 8-positions with methoxy groups increases potency to 53 times that of the lead compound SDZ 029-576.

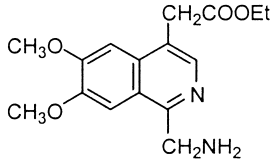
Diabetes mellitus is the sixth leading cause of death by disease in the United States. The overall incidence of noninsulin-dependent diabetes mellitus (Type 2 diabetes) is approximately 20% of the United States population over the age of 60, which translates to greater than 14 million people. Approximately 800,000 new cases are diagnosed each year.[1,2]

Type 2 diabetes is a progressive disease characterized by impaired insulin secretion by β-cells and glucagon secretion by the α-cells as well as insulin resistance in skeletal muscle and adipose tissue. This leads to increased fatty acid flux to the liver which stimulates lipoprotein production. The resulting dislipidemia leads to macrovascular complications such as atherosclerosis and stroke.

Recently, it has been demonstrated that injection of glucagon-like peptide-1 (GLP-1) in man shows promise as a therapeutic approach to Type 2 diabetes by its favorable actions on insulin and glucagon secretion, peripheral glucose disposal, and gastric emptying.[3–6] GLP-1 is a 30 amino acid peptide secreted by endocrine (L) cells of the distal intestinal mucosa in response to oral glucose loads or mixed meals. GLP-1 reaches peak plasma concentrations within 15–30 min; however, it has been shown that human plasma degrades the active GLP-1 (7–36) amide to the terminally truncated inactive form GLP-1 (9–36) amide with a half-life of about 1–2 min.[7–9]

The enzyme responsible for cleaving the N-terminal His-Ala fragment from GLP-1 has been identified as dipeptidylpeptidase IV (DPP-IV), a highly specialized aminopeptidase that removes dipeptides only from proteins containing an N-terminal penultimate proline or alanine.[10] Therefore, agents that inhibit DPP-IV should increase the circulation levels of endogenous GLP-1 and ultimately lower blood glucose levels.[11]

As part of our high capacity screening efforts, the isoquinoline derivative SDZ 029-576 (**1**) was identified as an inhibitor of DPP-IV. It is selective when compared to PPCE, APP, and Trypsin and possesses a mixed inhibition kinetic profile, which increases K_m and reduces V_{max}. A preliminary SAR study explored some replacements of the ester moiety and selected aryl substitutions but primarily focused on the generation of a library of 4-acetic acid amide derivatives of **1**.[12] Here we wished to further explore the effects of subtle variations to the functional groups at the 1- and 4-positions in addition to the oxy substitution of the benzene ring in an effort to increase potency of the lead structure.



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Chemistry

The chain-shortened analogues where the carboxylate is attached directly to the heterocycle were prepared by

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the acylation of **2** with phthaloylglycine followed by a Bischler–Napieralski cyclization to **3** ($n=1$). Aromatization with MnO_2 then hydrazinolysis of the phthaloyl group afforded the products (Scheme 1). The free amine was Boc-protected for purification purposes then the Boc group was removed to give pure **5**. This general method also allows for the preparation of the homologated amine derivative **5c** ($n=2$) simply by acylating **2** with 3-phthalimidopropionic acid.[13]

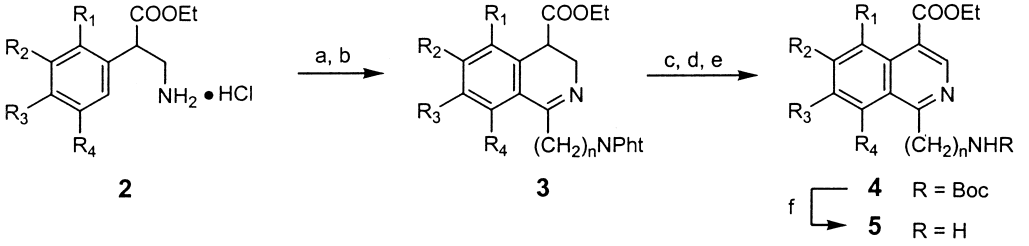
The chain-lengthened analogue **11** was readily available from **4b** using Wittig methodology (Scheme 2). The requisite aldehyde **7** was prepared by reduction of the ester function of **4b** to alcohol **6** followed by oxidation with MnO_2. A Horner–Emmons olefination of **7** furnished α,β-unsaturated ester **8**, which was then hydrogenated to propionate **9**. Removal of the Boc group furnished the desired product **11**.

N-methylated analogues **14** and **15** were prepared from the 1-chloromethylisoquinoline **13** according to previously published procedures[13] (Scheme 3).

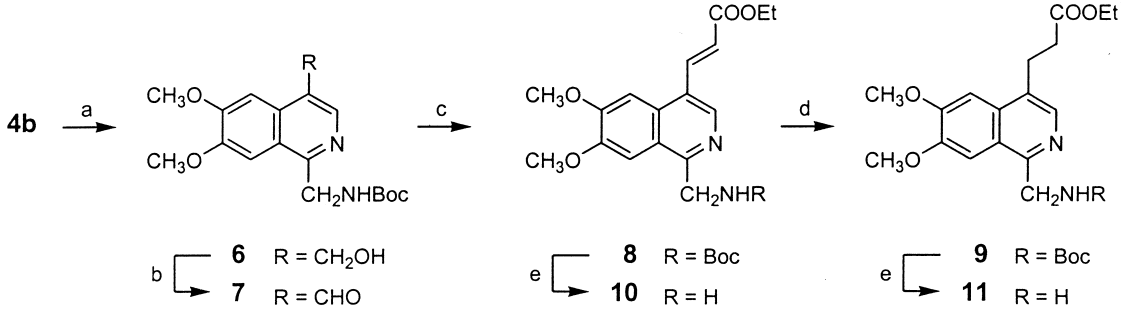
Amides **21** and **22** were prepared from the versatile acid intermediate **16**. Activation of **16** as a *p*-nitrophenyl ester produced the yellow crystalline **17** in good yield. Reaction of **17** with ammonium hydroxide or propylamine provided amides **18** and **19** in high yield. Removal of the Boc group afforded the desired products (Scheme 4). Curtius rearrangement of **16** in the presence of ethanol or *t*-butanol afforded carbamates **23** and **24**. Removal of the Boc group from **23** gave amine **25**, which can be considered an analogue of **1** where the CH_2 of the acetic acid is replaced with NH.

Biology

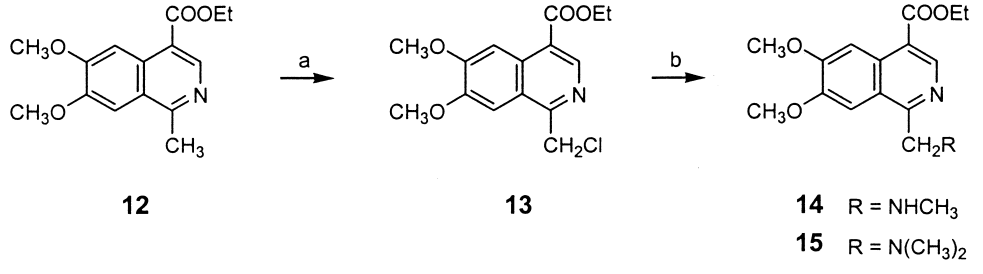
Compound effects on DPP-IV activity in extracts of human colonic carcinoma cells were assessed as described by Hughes.[14] In brief, the human colonic carcinoma cell line Caco-2 was obtained from the American Type Culture Collection (ATCC HTB 37). Differentiation of the cells to induce DPP-IV expression was accomplished as described by Reisher.[15] Cell extract is prepared from cells solubilized in 10 mM Tris–HCl, 0.15 M NaCl, 0.04 t.i.u. aprotinin, 0.5% nonidet-P40, pH 8.0, which was centrifuged at 35,000 *g* for 30 min at 4 °C to remove cell debris. The assay was conducted by adding 20 μg solubilized Caco-2 protein, diluted to a final volume of 125



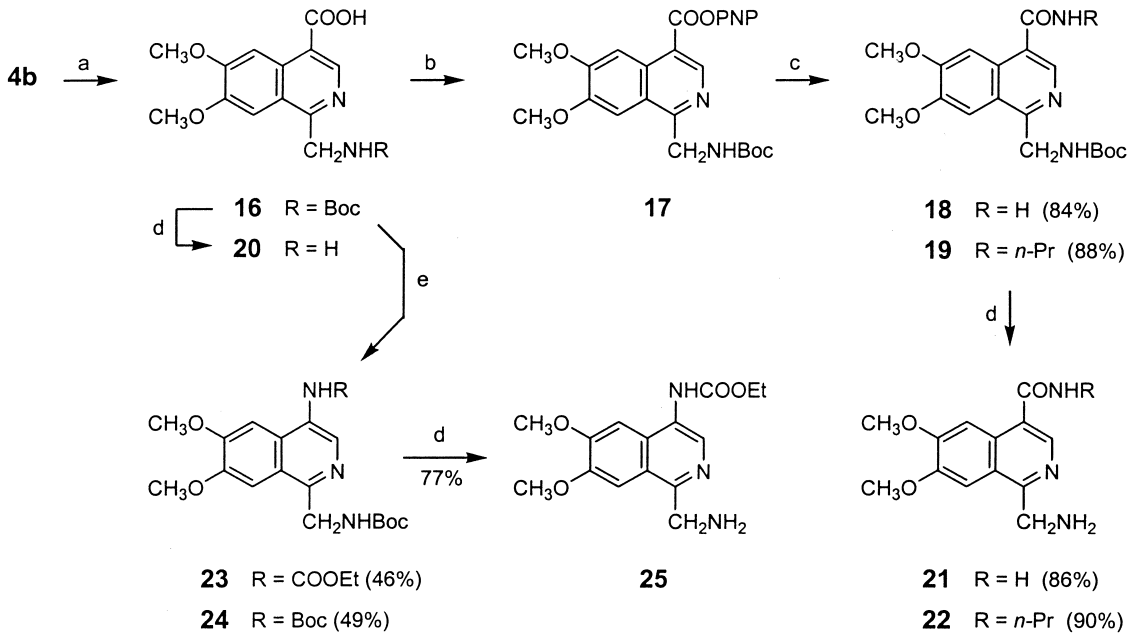
Scheme 1. (a) *N*-Phthaloylglycine, Et_3N, EDC, HOAt, DMF; (b) $POCl_3$, DMF 80 °C; (c) MnO_2, benzene reflux; (d) N_2H_4, CH_2Cl_2:EtOH (10:1); (e) BoC_2O, Et_3N, CH_2Cl_2; (f) HCl (g), EtOH:CH_2Cl_2 (8:2).



Scheme 2. (a) $[(Et)_2AlH_2]Na$, ether, 0–5 °C (98%); (b) MnO_2, CH_2Cl_2 (95%); (c) triethyl phosphonoacetate, NaH THF (47%); 9d) H_2, 5% Pd/C, EtOH (1 atm) (89%); (e) CF_3COOH, CH_2Cl_2.



Scheme 3. (a) NCS, AlBN, CCl_4; (b) methylamine or dimethylamine, THF.



Scheme 4. (a) NaOH, EtOH, rt, 18 h (86%); (b) 4-nitrophenyltrifluoroacetate, pyridine, rt, 18 h (62%); (c) NH_4OH or propylamine, dioxane, 3 h; (d) HCl (g), EtOH; (e) DPPA, Et_3N, EtOH or *t*-BuOH, 80 °C.

μL in assay buffer (25 mM Tris–HCl pH 7.4, 140 mM NaCl, 10 mM KCl, 1% bovine serum albumin) to microtiter plate wells. The reaction was initiated by adding 25 μL of 1 mM substrate (*H*-Alanine-Proline-pNA; pNA is *p*-nitroaniline). The reaction was run at room temperature for 10 min after which time a 19 μL volume of 25% glacial acetic acid was added to stop the reaction. Where indicated, compounds were added as 30 μL additions and the assay buffer volume was reduced to 95 μL. A standard curve of free *p*-nitroaniline was generated using 0–500 μM solutions of free pNA in assay buffer. The curve generated was linear and was used for interpolation of substrate consumption (catalytic activity in nmoles substrate cleaved/min). The endpoint is determined by measuring absorbance at 405 nm in a Molecular Devices UV Max microtiter plate reader. IC_{50} values were calculated using a four-parameter logistic equation (MicroCal Origin software package) in which the upper and lower asymptotes were fixed at 0 and 100, respectively.

Within the series of compounds in Table 1 it is evident that a primary amine is required for activity. Mono- and di-methylated analogues **14** and **15** are not active. Lengthening the carbon chain by one methylene (**5c**) also reduces potency relative to **1**. At the 4-position, chain length is also important. Lengthening the chain by one methylene unit (**11**) results in a 3-fold loss of activity whereas shortening the chain (**5b**) increases potency by a factor of five. Replacing the ester with an acid (**20**), aldehyde (**27**) or primary amide (**21**) results in significant loss of activity whereas the methanol derivative **26** is only about half as potent as **1**.

One can take advantage of the oxygenation pattern around the benzene ring of the heterocycle to further increase DPP-IV inhibitory activity in this series (Table 1). The optimum substitution is 6,8-dimethoxy which results in compound **5h** with an IC_{50} of 0.32 μM which is 53 times as potent as **1**. It was also found that replacing the methylene of the acetic acid of **1** with an NH (**25**) increases potency 7-fold.

Table 1. In vitro DPP-IV inhibitory activity of 1-aminoalkylisoquinoline-4-carboxylates

Compd	R_1	R_2	R_3	R_4	*n*	di-Salt	IC_{50} (μM)
5a	H	OCH_3	H	H	1	HCl	207
5b	H	OCH_3	OCH_3	H	1	HCl	3.4
5c	H	OCH_3	OCH_3	H	2	TFA	746
5d	H	OCH_3	OEt	H	1	HCl	6.7
5e	H	OEt	OCH_3	H	1	HCl	4.7
5f	H	OCH_3	OPr	H	1	HCl	8.4
5g	H	OCH_3	OH	H	1	HCl	14
5h	H	OCH_3	H	OCH_3	1	HCl	0.32
5i	OCH_3	H	H	OCH_3	1	HCl	2.4
5j	OCH_3	OCH_3	OCH_3	H	1	HCl	18
5k	H	OCH_3	OCH_3	OCH_3	1	HCl	1.1
10						TFA	18
11						TFA	52.6
14						HCl	731
15						HCl	948
20						HCl	103
21						HCl	170
22						HCl	51.4
25						HCl	2.3
26[a]						HCl	31.8
27[b]						HCl	277
1						HCl	17

[a]This compound was prepared from **6** by removal of the Boc group with HCl.
[b]This compound was prepared from **7** by removal of the Boc group with HCl.

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