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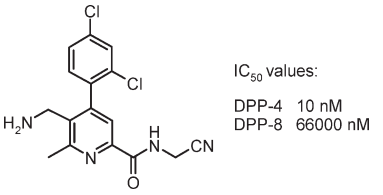
Design, Synthesis, and in Vitro Evaluation of Novel Aminomethyl-pyridines as DPP-4 Inhibitors

Katarzyna Kaczanowska, Karl-Heinz Wiesmüller, and Arnaud-Pierre Schaffner*

EMC microcollections GmbH, Sindelfinger Str. 3, D-72070 Tübingen, Germany

ABSTRACT A collection of novel aminomethyl-pyridines was designed, synthesized, and investigated as potential inhibitors of DPP-4. Optimization of the screening hit afforded a number of 5-aminomethyl-pyridines with inhibitory activity in the nanomolar range. Selected DPP-4 inhibitors were further evaluated for their selectivity over the closely related peptidase DPP-8. 5-Aminomethyl-4-(2,4-dichlorophenyl)-6-methyl-pyridine-2-carboxylic acid cyanomethyl-amide showed high potency and excellent DPP-4 selectivity [IC_{50}: 10 (DPP-4) and 6600 nM (DPP-8)] and no toxicity in mammalian cell culture.

KEYWORDS Aminomethyl-pyridines, DPP-4, DPP-8, incretins, diabetes, structure−activity relationship

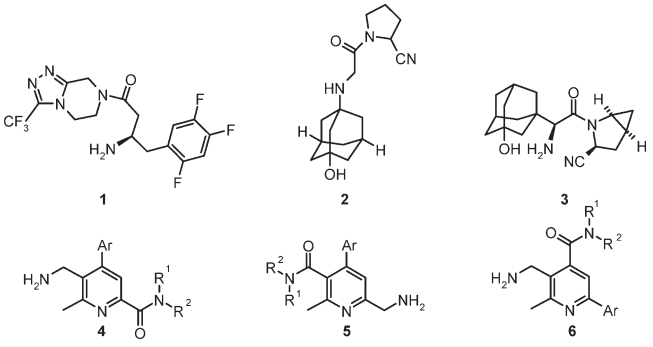


Dipeptidyl peptidase IV (DPP-4) is a serine protease, which specifically cleaves dipeptides from proteins and oligopeptides after a penultimate N-terminal proline or alanine.[1−3] It is responsible for the rapid deactivation of incretin hormones, glucose-dependent insulinotropic peptide (GIP), and glucagon-like peptide 1 (GLP-1), which stimulates the secretion of insulin, inhibits glucagon release, and slows gastric emptying, which benefits in the control of glucose homeostasis in patients with type 2 diabetes. Because of the impressive antidiabetic actions of GLP-1, an effective inhibition of DPP-4 became an original pathway for the treatment of diabetes and, in particular, the noninsulin-dependent diabetes and related diseases.[4−8] Extensive research efforts from both the academia and the pharmaceutical industry have led to the launch of Sitagliptin **1** (Merck & Co. Inc., 2006),[9] Vildagliptin **2** (Novartis AG, 2008),[10] and Saxagliptin **3** (Brystol-Myers Squibb Co., 2009) for the treatment of type 2 diabetes (Scheme 1).[11,12]

In recent years, a number of studies regarding a large variety of scaffolds for DPP-4 inhibition show a continuing search for small molecules as potent, selective, orally available inhibitors.[13] Particular efforts were made to prolong the stability and the long-acting potential of conventional DPP-4 inhibitors. Many DPP-4 inhibitors were developed, but the lack of selectivity and the inhibition of other members of the DPP family resulted in unforeseen side effects and low tolerance.[14−17] The high degree of sequence homology between, for example, dipeptidyl peptidase 8 (DPP-8) and DPP-4, makes the design of selective inhibitors a challenging task.[14]

The aim of this study was to investigate novel aminomethyl-pyridines **4**−**6** (Scheme 1). The compounds are based on the phenyl-pyridine skeleton and include structural elements that are important for DPP-4 molecular recognition.[18−21] We anticipated that the presence and the position of the primary amine and the amide groups on the pyridine ring might be critical for the inhibitory activity. We describe the synthesis and in vitro evaluation of collections of the aminomethyl-pyridines **4**−**6** as potential DPP-4 inhibitors (Table 1). A novel, potent series of DPP-4 inhibitors was discovered, and lead optimization gave clear structure−activity relationships (SARs) and resulted in the identification of compounds **4e-2** and **4e-7** with IC_{50} values of 11 and 10 nM, respectively. The most active inhibitors **4** were further investigated for the DPP-8 activity, and a highly selective DPP-4 inhibitor **4e-7** [IC_{50}: 10 (DPP-4) and 6600 nM (DPP-8)] was identified.[22]

Scheme 1



To obtain the regioisomers **4**−**6** (Scheme 1), we modified 4-aryl-5-cyano-6-methyl-pyridine-2-carboxylic acids **7** (Schemes 2 and 3) and 6-aryl-3-cyano-2-methyl-isonicotinic acids **16** (Scheme 4), which are accessible by a straightforward protocol that we have reported recently.[23] The two different approaches to target the 3-aminomethyl-pyridines **4** are outlined in Scheme 2.

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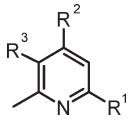
Table 1. Screening Results of the Aminomethyl-pyridines **4**−**6** for DPP-4 Inhibition



compd	R^1	R^2	R^3	IC_{50} (μM)[a]
8a	carboxyl	4-fluoro-phenyl	aminomethyl	<50
8b	carboxyl	2,4-difluoro-phenyl	aminomethyl	<50
11e	carboxylic acid amide	2,4-dichloro-phenyl	cyano	>50
11f	carboxylic acid methylamide	2,4-dichloro-phenyl	cyano	>50
4a-1	carboxylic acid amide	2-fluoro-phenyl	aminomethyl	<50
4a-2	carboxylic acid cyclopropylamide	2-fluoro-phenyl	aminomethyl	<50
4a-3	pyrrolidin-1-yl-methanone	2-fluoro-phenyl	aminomethyl	<50
4b-1	carboxylic acid cyclopropylamide	4-fluoro-phenyl	aminomethyl	0.080
4b-2	pyrrolidin-1-yl-methanone	4-fluoro-phenyl	aminomethyl	0.686
4b-3	morpholin-4-yl-methanone	4-fluoro-phenyl	aminomethyl	<50
4b-4	carbonyl-1-piperidine-4-carboxylic acid ethyl ester	4-fluoro-phenyl	aminomethyl	0.937
4c-1	carboxylic acid cyclopropylamide	2,4-difluoro-phenyl	aminomethyl	0.674
4c-2	carbonyl-amino-acetic acid methyl ester	2,4-difluoro-phenyl	aminomethyl	<50
4c-3	pyrrolidin-1-yl-methanone	2,4-difluoro-phenyl	aminomethyl	<50
4c-4	morpholin-4-yl-methanone	2,4-difluoro-phenyl	aminomethyl	<50
4c-5	carboxylic acid (5-methyl-isoxazol-3-yl)-amide	2,4-difluoro-phenyl	aminomethyl	0.674
4d	carboxylic acid methylamide	4-chloro-phenyl	aminomethyl	<50
4e-1	carboxylic acid amide	2,4-dichloro-phenyl	aminomethyl	0.016
4e-2	carboxylic acid methylamide	2,4-dichloro-phenyl	aminomethyl	0.011
4e-3	carboxylic acid cyclopropylamide	2,4-dichloro-phenyl	aminomethyl	<50
4e-4	pyrrolidin-1-yl-methanone	2,4-dichloro-phenyl	aminomethyl	0.044
4e-5	morpholin-4-yl-methanone	2,4-dichloro-phenyl	aminomethyl	<50
4e-6	carboxylic acid carbamoylmethyl-amide	2,4-dichloro-phenyl	aminomethyl	<50
4e-7	carboxylic acid cyanomethyl-amide	2,4-dichloro-phenyl	aminomethyl	0.010
4f-1	carboxylic acid cyclopropylamide	4-methoxy-phenyl	aminomethyl	>50
4f-2	pyrrolidin-1-yl-methanone	4-methoxy-phenyl	aminomethyl	>50
4f-3	morpholin-4-yl-methanone	4-methoxy-phenyl	aminomethyl	>50
4f-4	carboxylic acid 3,4-dichloro-benzylamide	4-methoxy-phenyl	aminomethyl	>50
4f-5	carboxylic acid (5-methyl-isoxazol-3-yl)-amide	4-methoxy-phenyl	aminomethyl	>50
4f-6	((r)-3-amino-pyrrolidin-1-yl)-methanone	4-methoxy-phenyl	aminomethyl	>50
4f-7	(4-amino-piperidin-1-yl)-methanone	4-methoxy-phenyl	aminomethyl	>50
4f-8	carboxylic acid (2-piperazin-1-yl-ethyl)-amide	4-methoxy-phenyl	aminomethyl	>50
5a	aminomethyl	2-fluoro-phenyl	carboxylic acid amide	>50
5b	aminomethyl	4-chloro-phenyl	carboxylic acid amide	>50
5c	aminomethyl	2,4-dichloro-phenyl	carboxylic acid amide	>50
5d	aminomethyl	4-methoxy-phenyl	carboxylic acid amide	>50
6a	4-fluoro-phenyl	pyrrolidin-1-yl-methanone	aminomethyl	>50
6b-1	4-chloro-phenyl	pyrrolidin-1-yl-methanone	aminomethyl	>50
6b-2	4-chloro-phenyl	morpholin-4-yl-methanone	aminomethyl	>50
6b-3	4-chloro-phenyl	carbonyl-1-piperidine-4-carboxylic acid ethyl ester	aminomethyl	>50
6c-1	2,4-dichloro-phenyl	carboxylic acid cyclopropylamide	aminomethyl	>50
6c-2	2,4-dichloro-phenyl	pyrrolidin-1-yl-methanone	aminomethyl	>50
6c-3	2,4-dichloro-phenyl	morpholin-4-yl-methanone	aminomethyl	>50
6d-1	4-methoxy-phenyl	carboxylic acid cyclopropylamide	aminomethyl	>50
6d-2	4-methoxy-phenyl	pyrrolidin-1-yl-methanone	aminomethyl	>50
6d-3	4-methoxy-phenyl	morpholin-4-yl-methanone	aminomethyl	>50

[a] Measured in three independent experiments.

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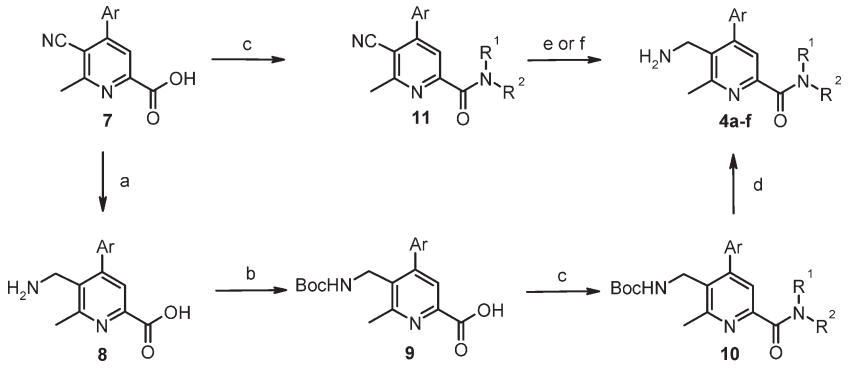
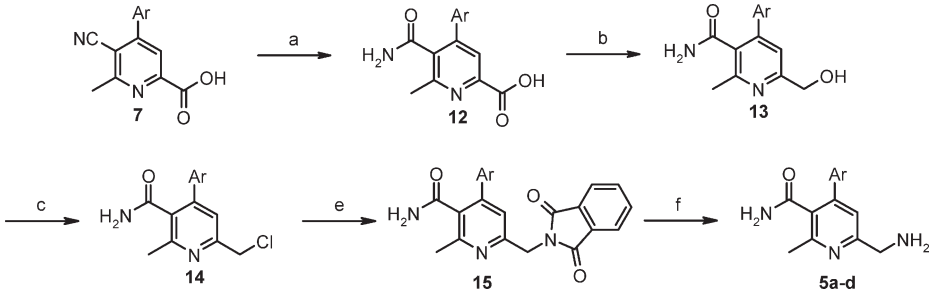
Scheme 2[a]



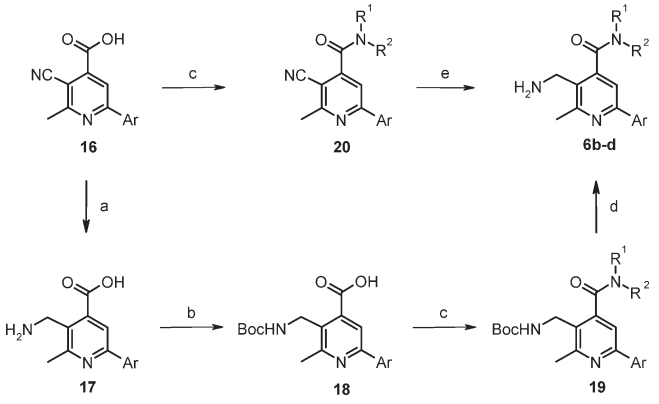
[a] Reagents and conditions: (a) H_2, 10% Pd/C, AcOH, 60 °C, 24 h, 52−72%. (b) Boc_2O, dioxane/H_2O, 60 °C, 16 h, 62−79%. (c) Amine, PyBOP, NEt_3, DCM_{abs}, 3−16 h, 46−92%. (d) 20% TFA/DCM, 1 h, 68−99%. (e) Raney Ni, AcOH, 16 h, 34−70%. (f) Raney Ni, AcOH, 50 °C, 48 h, 60%; $POCl_3$, 70 °C, 2 h, 70%.

Scheme 3[a]



[a] Reagents and conditions: (a) *t*-BuOK, H_2O, 100 °C, 3 h, 65−99%. (b) Ethyl chloroformate, NEt_3, THF, room temperature, 30 min; $NaBH_4$, H_2O, 30 min, 0 °C; 61−99%. (c) $SOCl_2$, toluene, 60 °C, 16 h, 75−85%. (e) Phthalimide, *t*-BuOK, DMF, 60 °C, 4 h, 65−90%. (f) 30% KOH, 110 °C, 4 h, 79−91%.

Scheme 4[a]



[a] Reagents and conditions: (a) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, 24 h, 64% or H_2, 10% Pd/C, AcOH, 60 °C, 24 h, 76%. (b) Boc_2O, dioxane, 60 °C, 16 h, 38−43%. (c) Amine, PyBOP, NEt_3, DCM_{abs}, 3−16 h, 54−92%. (d) 20% TFA/DCM, 1 h, 57−83%. (e) 50% NH_2NH_2/H_2O, Raney Ni, THF, 20 min; TFA, 46−53%.

In both pathways, the critical steps were the conversion of the cyano group to the corresponding aminomethyl functionality. The hydrogenation of **7** using palladium on charcoal was found to be most reliable for the synthesis of **8**.[24]

Compounds **8** were protected to **9** and amidated using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) as a coupling reagent to afford **10**. Deprotection of **10** with TFA afforded the 3-aminomethyl-pyridines **4** in high overall yields. Alternatively, the carboxylic acids **7** were first amidated to afford **11** that was then selectively hydrogenated with Raney Nickel[25] to give the aminomethyl-pyridines **4** in good yields. To obtain the aminomethyl-pyridine **4e-6** ($-NR^1R^2 = -NHCH_2CN$), the pyridine carboxylic acid **7** was reacted with 2-amino acetamide to afford the amide **11g** ($-NR^1R^2 = -NHCH_2CONH_2$). The cyano group of **11f** was then reduced to the corresponding methylamine **4e-6** with Raney Nickel as a catalyst. Finally, the dehydration of primary amide functionality using phosphorus oxychloride gave the product **4e-7** in good yield.

The basic hydrolysis of the cyano group of **7** led to the corresponding primary amides **12** in good to excellent yields. Reduction of the carboxylic acids moiety to the alcohols **13**[25] followed by treatment with thionyl chloride afforded the corresponding chlorides **14** (Scheme 3). Displacement of the chloride using phthalimide as nucleophile led to **15** that was then directly hydrolyzed with a 30% potassium hydroxide solution to afford **5** in good yields.

3-Aminomethyl-2-methyl-6-aryl-isonicotinamides **6** were synthesized using two similar approaches as for the

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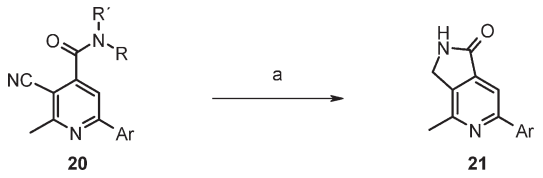
aminomethyl-pyridines **4** (Scheme 4). Compounds **18** were synthesized from 3-cyano-pyridines **16** via a hydrogenation/*tert*-butyloxycarbonyl (Boc) protection sequence.[26] Amidations of **18** were accomplished using PyBOP as a coupling reagent followed by deprotection of the aminomethyl group with TFA to yield **6**. In the second pathway, the pyridine carboxylic acids **16** were first amidated with a collection of amines to give compounds **20** that were then hydrogenated to afford aminomethyl-pyridines **6**. During the catalytic hydrogenation step with Raney Nickel as a catalyst, the use of hydrazine hydrate as hydrogen source was more efficient than the conditions applied for the synthesis of **4**. Interestingly, under basic conditions, the expected products **6** tend to cyclize spontaneously to form the pyrrolopyridines **21** if the reaction mixture was not acidified (Scheme 5).

The purified regioisomers **4**−**6** and different isolated intermediates were then evaluated for inhibition of human DPP-4 in an in vitro assay using Ile-thiazolidide as positive control. The inhibition was measured by following the increase of absorbance at 405 nm upon cleavage of the chromogenic substrate Gly-Pro-pNA.[28] The initial SAR breakthrough was the discovery that the aminomethyl-pyridines **4** exhibited high inhibitory activity (Table 1, entries **4a**−**f**) contrary to the analogues **5** (Table 1, entries **5a**−**d**) and **6** (Table 1, entries **6a**−**d**). The substitution pattern appeared to be crucial for the inhibitory activity. The pyridines **4a**−**e** bearing an aminomethyl moiety in the β-position to the ring nitrogen showed an activity with IC_{50} values below 50 μM, in comparison to the regioisomers **5a**−**d** (the aminomethyl group in the α-position) exhibiting IC_{50} values higher than 50 μM. Similarly, the distance between the primary amino group and the amide functionality was proven to be of great importance. The change in the position of the amide group from α (5-aminomethyl-pyridines **4**) to β (3-aminomethyl-pyridines **6**) results in a loss of the inhibitory activity.

Scheme 5[a]

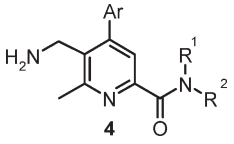


[a] Reagents and conditions: (a) 50% NH_2NH_2/H_2O, Raney Ni, MeOH, 3 h, 66−80%.

The screening results of aminomethyl-pyridines **4** and intermediates allowed prioritization of initial analogue synthesis. As expected, the pyridine amides **11** (Table 1, entries **11e-f**) without aminomethyl group were not active. The essential role of hydrogen bonding between the amine groups in ligands and the defined amino acids residues in DPP-4 have been previously reported.[18] Additional hydrogen bonds were observed due to polar side chains in DPP-4 that interact with the amido groups of the inhibitors. Interestingly, the free carboxylic acids **8a** and **8c** presented a good inhibitory activity (IC_{50} < 50 μM). The rigidity of DPP-4 binding pocket appears to be highly specific to proline residues, but inhibitors with substituted aromatic ring were reported to fit in the pocket as well. Further affinity gains might be achieved by aromatic−aromatic interactions between the biaryl system of the inhibitors and the phenyl rings of some aromatic residues in DPP-4 that are exposed to the ligand binding site.[18−21] The comparison of the analogues in regard to the variations of the substitution on the aryl ring of compounds **4** indicated the importance of the halogen substitution of the aromatic ring; for example, **4a-e** (IC_{50} < 50 μM) was more potent as compared to **4f** (IC_{50} > 50 μM).

Selected compounds possessing superior potency profiles were investigated for the effectiveness in inhibiting DPP-4 in dose-dependent experiments. The substitution of the primary amide **4e-1** with a methyl group as in **4e-2** revealed

Table 2. Selectivity of the Novel DPP-4 Inhibitors **4** over DPP-8 in Comparison to IPI



				IC_{50}/K_i (μM)[a]		
compd	Ar	R^1	R^2	DPP-4	DPP-8	LC_{50} (μM)
4e-1	2,4-dichloro-phenyl	H	H	0.016	33	>10
4e-2	2,4-dichloro-phenyl	Me	H	0.011 (0.006)	39	>10
4e-7	2,4-dichloro-phenyl	cyanomethyl	H	0.010	66	>10
4e-4	2,4-dichloro-phenyl	pyrrolidin-1-yl		0.044	25	>10
4b-1	4-fluoro-phenyl	cyclopropyl	H	0.080	106	>10
4b-2	4-fluoro-phenyl	pyrrolidin-1-yl		0.686	137	>10
4c-1	2,4-difluoro-phenyl	cyclopropyl	H	0.937	182	>10
4c-5	2,4-difluoro-phenyl	5-methyl-isoxazol-3-yl	H	0.674	179	>10
	IPI			3	39	

[a] Measured in three independent experiments.

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a significant improvement in the potency (Table 1). It was interesting to observe that the analogue **4e-7** with the cyano group had a similar IC_{50} value as **4e-2**. Several studies confirmed that the presence of the cyano group increases inhibitor activity, even up to 1000-fold, due to its interaction with the oxygen atom of the catalytic serine side chain in DPP-4.[18] The increase in the size of the amide group in **4e-4** lowered its potency in dropping the IC_{50} value down to 44 nM.

One of the most potent analogues, **4e-2** was then chosen for further investigation. Measurements of the inhibitory activity at various inhibitor and substrate concentrations allowed data plotting,[27] and a Lineweaver−Burk plot indicated a competitive, reversible type of inhibition, and the inhibitor affinity $K_i = 5.5 \pm 2$ nM was determinated.

For the investigation of the DPP-4 selectivity over DPP-8 in dose-dependent experiments, a group of representative inhibitors **4** was selected (Table 2). The in vitro assay was based on the same catalytic reaction as for DPP-4. The results from the biological screening in comparison to the experimental data for Ile-Pro-Ile (IPI) are summarized in Table 2. All of the tested compounds showed high DPP-4 selectivity (2500−6600-fold) over DPP-8. The secondary amide **4e-2** was slightly more selective in comparison to the primary amide **4e-1**. Interestingly, the aminomethyl-pyridines **4e-2** and **4e-7** with cyano groups were similarly potent against DPP-4, but **4e-7** (with cyano group on the side chain) was almost 2-fold less potent against DPP-8 than **4e-2**. The pyridine **4e-4** with a bulkier pyrrolidine ring showed lower potency and selectivity.

The toxicity of the aminomethyl-pyridines **4**−**6** was tested in an in vitro cellular assay. All derivatives showed no toxic effect on HeLa cells at a 10 μM concentration (Table 2, $LC_{50} >$ 10 μM).[29] The calculated log *P* values of the most active inhibitors **4e** were in the 2−3 range and, together with the structural elements and molecular masses (250−400 Da), are in accordance with Lipinski's rule of five.[30]

In summary, we have discovered a novel series of 5-aminomethyl-4-aryl-pyridines **4** that are potent and selective DPP-4 inhibitors. The substitution pattern proved to be a key discovery in increasing the potency and selectivity of this structural class of inhibitors. Further optimization afforded compounds **4** with high DPP-4 inhibitory potency exhibiting IC_{50} values in the nanomolar range. The IC_{50} levels of novel inhibitors are comparable to the values of drugs Vildagliptin and Sitagliptin.[28] Notably, 5-aminomethyl-pyridine **4e-7** showed excellent 6600-fold selectivity to DPP-4 over DPP-8.

SUPPORTING INFORMATION AVAILABLE

Procedures for the preparation of all compounds, analytical data, and in vitro assay conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *E-mail: emc@microcollections.de.

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ABBREVIATIONS

DPP-4, dipeptidyl peptidase IV; DPP-8, dipeptidyl peptidase 8; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; Boc, *tert*-butyloxycarbonyl; SAR, structure−activity relationship; IPI, Ile-Pro-Ile.

REFERENCES

(1) Lambeir, A. M.; Durinx, C.; Scharpé, S.; De Meester, I. Dipeptidyl-peptidase IV from bench to bedside: An update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit. Rev. Clin. Lab. Sci.* **2003,** *40*, 209–294.

(2) Durinx, C.; Lambeir, A. M.; Bosmans, E.; Falmagne, J. B.; Berghmans, R.; Haemers, A.; Scharpé, S.; De Meester, I. Molecular characterization of dipeptidyl peptidase activity in serum: Soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *Eur. J. Biochem.* **2000,** *267*, 5608–5613.

(3) Christopherson, K. W.; Hangoc, G.; Broxmeyer, H. E. Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34+ progenitor cells. *J. Immunol.* **2002,** *169*, 7000–7008.

(4) Havale, S. H.; Pal, M. Medicinal chemistry approaches to the inhibition of dipeptidyl peptidase-4 for the treatment of type 2 diabetes. *Bioorg. Med. Chem.* **2009,** *17*, 1783–1802.

(5) Mohler, M. L.; He, Y.; Wu, Z.; Hwang, D. J.; Miller, D. D. Recent and Emerging Anti-DiabetesTargets. *Med. Res. Rev.* **2009,** *29*, 125–195.

(6) Chia, C. W.; Egan, J. M. Incretin-Based Therapies in Type 2 Diabetes Mellitus. *J. Clin. Endocrinol. Metab.* **2008,** *93*, 3703–3716.

(7) Drucker, D. J. Dipeptidyl Peptidase-4 Inhibition and the Treatment of Type 2 Diabetes. Preclinical biology and Mechanisms of Action. *Diabetes Care* **2007,** *30* (6), 1335–1343.

(8) Geelhoed-Duijvestijn, P. H. Incretins: A new treatment option for type 2 diabetes? *Neth. J. Med.* **2007,** *65*, 60–64.

(9) Thornberry, N. A.; Weber, A. E. Discovery of JANUVIA (Sitagliptin), a Selective Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type2 Diabetes. *Curr. Top. Med. Chem.* **2007,** *7*, 557–568.

(10) Pratley, R. E.; Jauffret-Kamel, S.; Galbreath, E.; Holmes, D. Twelve-week monotherapy with the DPP-4 inhibitor vildagliptin improves glycemic control in subjects with type 2 diabetes. *Horm. Metab. Res.* **2006,** *38*, 423–428.

(11) Richter, B.; Bandeira-Echtler, E.; Bergerhoff, K.; Lerch, C. L. Dipeptidyl peptidase-4 (DPP-4) inhibitors for type 2 diabetes mellitus. *Cochrane Database Syst. Rev.* **2008,** *16*, CD006739.

(12) Sebokova, E.; Christ, A. D.; Boehringer, M.; Mizrahi, J. Dipeptidyl Peptidase IV Inhibitors: The Next Generation of New Promising Therapies for the Management of Type 2 Diabetes. *Curr. Top. Med. Chem.* **2007,** *7*, 547–555.

(13) Mulakayala, N.; Reddy, Ch. U.; Pal, M. Synthesis of dipeptidyl peptidase-4 inhibitors. *Tetrahedron* **2010,** *66*, 4919–4938.

(14) Van der Veken, P.; Haemers, A.; Augustyns, K. Prolyl Peptidases Related to Dipeptidyl Peptidase IV: Potential of Specific Inhibitors in Drug Discovery. *Curr. Top. Med. Chem.* **2007,** *7*, 621–635.

(15) Connolly, B. A.; Sanford, D. G.; Chiluwal, A. K.; Healey, S. E.; Peters, D. E.; Dimare, M. T.; Wu, W.; Liu, Y.; Maw, H.; Zhou, Y.; Li, Y.; Jin, Z.; Sudmeier, J. L.; Lai, J. H.; Bachovchin, W. W. Dipeptide Boronic Acid Inhibitors of Dipeptidyl Peptidase IV:

DOI: 10.1021/ml100200c | *ACS Med. Chem. Lett.* **2010,** *1*, 530–535

Determinants of Potency and in Vivo Efficacy and Safety. *J. Med. Chem.* **2008,** *51*, 60056013.
(16) Kang, N. S.; Ahn, J. H.; Kim, S. S.; Chae, C. H.; Yoo, S. E. Docking-based 3D-QSAR study for selectivity of DPP4, DPP8, and DPP9 inhibitors. *Bioorg. Med. Chem. Lett.* **2007,** *17*, 3716–3721.
(17) Burkey, B. F.; Hoffmann, P. K.; Hassiepen, U.; Trappe, J.; Juedes, M.; Foley, J. E. Adverse effects of dipeptidyl peptidases 8 and 9 inhibition in rodents revisited. *Diabetes, Obes. Metab.* **2008,** *10*, 1057–1061.
(18) Kuhn, B.; Hennig, M.; Mattei, P. Molecular recognition of ligands in dipeptidyl peptidase IV. *Curr. Top. Med. Chem.* **2007,** *7*, 609–619.
(19) Lorey, S.; Stöckel-Maschek, A.; Faust, J.; Brandt, W.; Stiebitz, B.; Gorrell, M. D.; Kähne, T.; Mrestani-Klaus, C.; Wrenger, S.; Reinhold, D.; Ansorge, S.; Neubert, K. Different modes of dipeptidyl peptidase IV (CD26) inhibition by oligopeptides derived from the N-terminus of HIV-1 Tat indicate at least two inhibitor binding sites. *Eur. J. Biochem.* **2003,** *270*, 2147–2156.
(20) Lee, H. J.; Chen, Y. S.; Chou, C. Y.; Chien, C. H.; Lin, C. H.; Chang, G. G.; Chen, X. Investigation of the dimer interface and substrate specificity of prolyl dipeptidase DPP8. *J. Biol. Chem.* **2006,** *281*, 38653–38662.
(21) Liang, G. B.; Qian, X.; Biftu, T.; Singh, S.; Gao, Y. D.; Scapin, G.; Patel, S.; Leiting, B.; Patel, R.; Wu, J.; Zhang, X.; Thornberry, N. A.; Weber, A. E. Discovery of new binding elements in DPP-4 inhibition and their applications in novel DPP-4 inhibitor design. *Bioorg. Med. Chem. Lett.* **2008,** *13*, 3706–3710.
(22) Schaffner, A.-P.; Kaczanowska, K.; Baechle, D. Substituted pyridines as inhibitors of dipeptidyl peptidase IV and their application for treatment of diabetes and related diseases. EP 09012781.2.
(23) Kaczanowska, K.; Eickhoff, H.; Albert, K.; Wiesmüller, K.-H.; Schaffner, A.-P. A Simple, Diversity Oriented Synthesis of Highly Substituted Pyridines. *J. Heterocycl. Chem.* **2010**, in press.
(24) Peglion, J.-L.; Poitevin, C.; Mannoury La Cour, C.; Dupuis, D.; Millan, M. J. Modulations of the amide function of the preferential dopamine D3 agonist (R,R)-S32504: Improvements of affinity and selectivity for D3 versus D2 receptors. *Bioorg. Med. Chem. Lett.* **2009,** *19*, 2133–2138.
(25) Bemis, J. E.; Vu, C. B.; Xie, R.; Nunes, J. J.; Yee, Ng, P.; Disch, J. S.; Milne, J. C.; Carney, D. P.; Lynch, A. V.; Jin, L.; Smith, J. J.; Lavu, S.; Iffland, A.; Jirousek, M. R.; Perni, R. B. Discovery of oxazolo[4,5-b]pyridines and related heterocyclic analogs as novel SIRT1 activators. *Bioorg. Med. Chem. Lett.* **2009,** *19*, 2350–2353.
(26) Khurana, J. M.; Kukreja, G. Rapid reduction of nitriles to primary amines with nickel boride at ambient temperature. *Synth. Commun.* **2002,** *32*, 1265–1269.
(27) Dixon, M.; Webb, E. C. *Enzymes*, 2nd ed.; Longman: London, 1971.
(28) Van der Veken, P.; De Meester, I.; Dubois, V.; Soroka, A.; Van Goethem, S.; Maes, M.-B.; Brandt, I.; Lambeir, A.-M.; Chen, X.; Haemers, A.; Scharpé, S.; Augustyns, K. Inhibitors of dipeptidyl peptidase 8 and dipeptidyl peptidase 9. Part 1: Identification of dipeptide derived leads. *Bioorg. Med. Chem. Lett.* **2008,** *18*, 4154–4158.
(29) Kleymann, G.; Werling, H. O. A Generally Applicable, High-Throughput Screening—Compatible Assay to Identify, Evaluate, and Optimize Antimicrobial Agents for Drug Therapy. *J. Biomol. Screening* **2004,** *9*, 578–587.
(30) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997,** *23*, 3–25.

DOI: 10.1021/ml100200c | *ACS Med. Chem. Lett.* **2010,** *1*, 530–535