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Synthesis and SAR of azolopyrimidines as potent and selective dipeptidyl peptidase-4 (DPP4) inhibitors for type 2 diabetes

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ABSTRACT

Several pyrazolo-, triazolo-, and imidazolopyrimidines were synthesized and evaluated as inhibitors of DPP4. Of these three classes of compounds, the imidazolopyrimidines displayed the greatest potency and demonstrated excellent selectivity over the other dipeptidyl peptidases. SAR evaluation for these scaffolds was described as they may represent potential treatments for type 2 diabetes.

Diabetes mellitus is a chronic disease and a major healthcare problem which is estimated to affect more than 171 million people worldwide.[1] It is further estimated that by the year 2030,[2] there will be over 366 million cases of diabetes, of which more than 90% will be type 2.[3] Recently, DPP4 inhibition has shown promise as a novel approach to treating type 2 diabetes.[4] DPP4 is a ubiquitous serine protease which belongs to a broad class of dipeptidyl peptidases (DPP's). Although the physiological functions of these other DPP's are not fully understood, the function of DPP4 has been well documented in the literature.[5] DPP4 specifically cleaves a proline or aniline at the penultimate position of the N-terminus of a variety of small peptides.[6] When food is ingested, the intestinal L-cells release a potent incretin hormone known as glucagon-like peptide-1 (GLP-1). This 30-mer peptide is responsible for increases in insulin secretion and β-cell function, and decreases in glucagon secretion.[7] These beneficial antidiabetic responses mediated by GLP-1 make it a highly attractive direct or indirect target for drug design. Upon its release, the active GLP-1 (7–36) is rapidly degraded by DPP4 ($t_{1/2}$ <1 min) to provide the inactive form of GLP-1 (9–36).[8] Inhibiting DPP4 has been shown in animals and clinically to effectively increase the half-life of active GLP-1 and regulate plasma glucose.[9]

To date, sitagliptin (**1**),[10] saxagliptin (**2**),[11] and vildagliptin (**3**, approved in Europe, Brazil, and Mexico)[12] have all obtained regulatory approval while alogliptin (**4**),[13] linagliptin (**5**),[14] and dutogliptin (**6**)[15] have successfully advanced to late stage clinical trials (Fig. 1). Compounds **2** and **3** belong to a potent class of α-amino acid derived DPP4 inhibitors which are dipeptide mimics, while **1** is a β-amino acid derived inhibitor.

Our continued interest in DPP4 inhibition as a therapy for type 2 diabetes prompted the investigation into other novel small molecule DPP4 inhibitor manifolds that were both potent and selective over the other DPP's. This investigation led to the discovery of the pyrazolopyrimidines, a class of compounds under investigation in

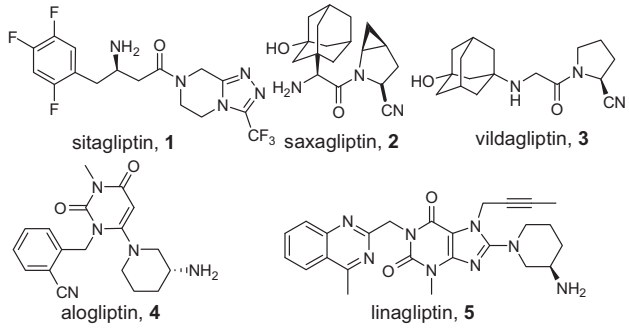

Figure 1. Structures of selected DPP4 inhibitors.

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another in-house program. This class of compounds possessed a basic amine functionality that was bound to a rigid system, a motif sharing commonalities with some other non-amino acid derived DPP4 inhibitors such as linagliptin and dutogliptin.[16]

Our SAR started from pyrazolopyrimidine containing compounds. Various substituents on the pyrazole ring which include alkyl, aryl, substituted aryl and carboxylic ester groups were used to explore the substitution pattern in the region. The synthesis of pyrazolopyrimidines **13–24** was achieved by condensing the appropriate 3-aminopyrazole **9** (obtained commercially or prepared according to the literature procedures[17]) with 2,4-dichlorobenzaldehyde **7** and methyl acetoacetate **8** in a three component, one pot reaction to give the desired dihydropyrimidine intermediate **10** (Scheme 1). Formation of **10** for compound **16** required a two-step process in which *t*-butyl acetoacetate was condensed with **7** to give the desired Knoevenagel product, which was then condensed with 4-carboethoxy-2-aminopyrrazole **9**. Compound **10** was then oxidized to the desired pyrazolopyrimidine core **11** by DDQ. The carboalkoxy group of **11** was reduced to the corresponding hydroxymethyl **12** using DIBAL directly or by a three-step process in which the ester was hydrolyzed to the corresponding acid with LiOH (TFA for compound **16**), converted to a mixed anhydride using ethyl chloroformate, and reduced to the desired alcohol with $NaBH_4$. The resultant alcohol was then converted to the corresponding azide via a chloride intermediate, and reduced by PPh_3 to the desired pyrazolopyrimidine **13–24**.

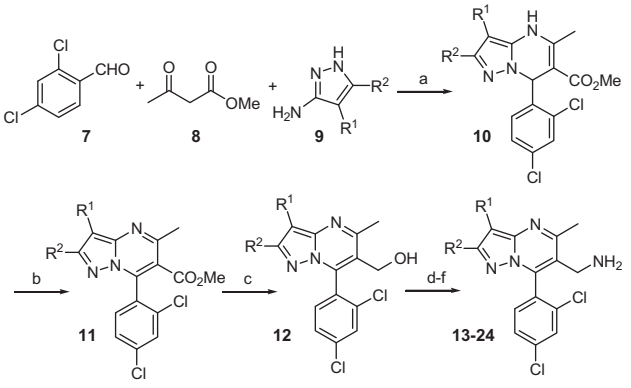
All inhibitors were tested in vitro against purified human DPP4 using H-Gly-Pro-*p*NA as a substrate and measuring the level of *p*-nitroaniline production at 405 nm over 15 min.[18] Inhibition of the related proteases DPP8 and DPP9 was also assessed (Table 1). This class of compounds possessed three desirable characteristics: favorable DPP4 activity, selectivity over DPP8 and DPP9, and ample opportunity for further optimization around the pyrazole ring. The majority of the compounds in this series exhibited good potency against DPP4, though only weak DPP8/DPP9 inhibition (most K_i's >30 μM) was observed. Additionally, all of these inhibitors occupied a narrow potency range, indicating tolerability for a wide variety of substituents around the pyrazole ring. Initially, compounds **13–16** were synthesized to determine the more favorable substitution pattern about the pyrazole ring (R^1 vs R^2). The SAR from this small group of compounds clearly indicated that substituents at R^2 showed >6-fold greater activity than those at R^1 (**14** = 141 nM and **15** = 22 nM). Once this was established, the remaining analogs (**17–18** and **20–24**) focused to probe the electronics and their substitution pattern on a phenyl group at the R^2 position of the pyrazole ring. It was observed that *meta*-substitution was somewhat less favored than *para*-substitution on the R^2 phenyl (comparing **20–24** and **22–23**).

Table 1
Binding affinities for pyrazolopyrimidines **13–24**

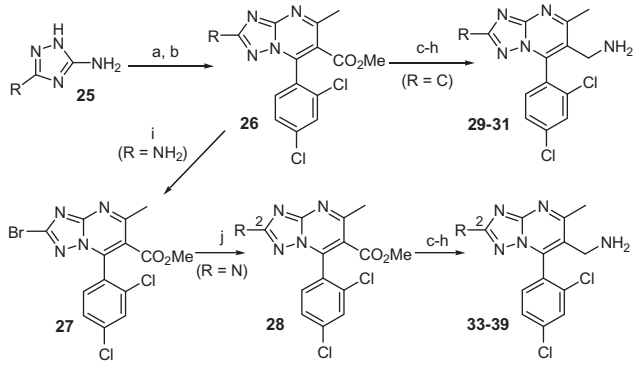
Compd	R^1	R^2	DPP4 K_i (nM)	DPP8 K_i (μM)	DPP9 K_i (μM)
13	H	Me	28 (±2.5)[a]	>30	>30
14	Ph	H	141 (±24)[a]	>30	>30
15	H	Ph	22	>30	>20
16	CO_2Et	H	73	>30	>30
17	H	2-Me-Ph	50	7.0	5.8
18	H	2-Cl-Ph	20	6.8	4.5
19	H	2-Furanyl	43	>30	>30
20	H	4-Cl-Ph	33	6.3	>30
21	H	4-MeO-Ph	30	5.5	3.1
22	H	4-Me-Ph	28	>30	>10
23	H	3-Me-Ph	75	12.2	7.8
24	H	3-Cl-Ph	163	14.6	5.3

[a] Values represent the mean of multiple determinations, standard deviation is shown in parentheses.

Expanded investigation into other fused heterobicyclic systems led us to examine triazolopyrimidines as DPP4 inhibitors. Except for compounds **32** and **40**, triazolopyrimidines were prepared by one of two similar methods. The synthesis of 2-alkyl or 2-aryl substituted triazolopyrimidines **29–31** began with a same three component, one pot reaction in which the appropriate 3-substituted 5-amino-1*H*-[1,2,4]-triazole **25** was condensed with **7** and **8**, followed with DDQ oxidation, to give triazolopyrimidine **26** (Scheme 2). Compound **26** (R = C) was then converted to the desired 5-aminomethyl triazolopyrimidine using the same methods employed in the pyrazolopyrimidine series. For 2-amino substituted triazolopyrimidines **33–39**, a common intermediate **27** provided a means for rapid analog generation and SAR evaluation, beginning with 3,5-diamino-1*H*-[1,2,4]-triazole **25**. To this end, **26** (R = NH_2) was converted to the corresponding 2-bromotriazolopyrimidine **27** by a Sandmeyer reaction, of which the bromide could be readily replaced with appropriate amines by nucleophilic substitution to form amine **28**. Compound **28** was further converted to the desired final products **33–39** using the same methodology as previously described. Compound **32** was generated by first Boc protecting amine **31** with di-*tert*-butyl dicarbonate and Et_3N in THF, followed by hydrolysis of the ester using aqueous LiOH. The final product **32** was then obtained by standard EDAC/HOAT coupling of the resultant carboxylic acid with aniline to give the corresponding amide, which was then deprotected with TFA. Compound **40** was generated by deprotection of compound **39** with TFA in CH_2Cl_2.



Scheme 1. Reagents and conditions: (a) piperidine, heptane, THF, 70 °C; (b) DDQ, CH_2Cl_2, 50–100% over two steps; (c) DIBAL, THF or LiOH, THF, H_2O, 50 °C; $ClCO_2Et$, Et_3N, THF; $NaBH_4$, THF, 38–100%; (d) MsCl, CH_2Cl_2, Et_3N; (e) NaN_3, DMF, 50 °C; (f) PPh_3, THF, H_2O, 50 °C, 26–65% over three steps.



Scheme 2. Reagents and conditions: (a) **7**, **8**, piperidine, THF, 70 °C; (b) DDQ, CH_2Cl_2, 50–61% over two steps; (c) LiOH, THF, H_2O, 50 °C; (d) $ClCO_2Et$, Et_3N, THF; (e) $NaBH_4$, THF, 30–60% over three steps; (f) MsCl, CH_2Cl_2, Et_3N; (g) NaN_3, DMF, 50 °C; (h) PPh_3, THF, H_2O, 50 °C, 39–50% over three steps; (i) *t*-BuONO, $CuBr_2$, CH_3CN, 65 °C, 75–85%; (j) amine, dioxane, 75 °C, 51–95%.

Table 2
Binding affinities for triazolopyrimidines **29**–**40**

Compd	R	DPP4 K_i (nM)	DPP8 K_i (μM)	DPP9 K_i (μM)
29	Me	64	>30	>30
30	Ph	67	>30	>30
31	COOMe	137	>30	>30
32	CONHPh	93	13.3	>10
33	Et_2N	73	>30	>30
34	Me(Et)N	50	>30	>30
35	$MeOCH_2CH_2(Me)N$	18	>30	>30
36	Pyrrolidine	50	>10	>30
37	Morpholine	31	nd	>30
38	Thiomorpholine	29	>30	>30
39	*N*-Boc-piperazine	442	>30	>30
40	Piperazine	106	>30	>30

nd, not determined.

Table 3
Binding affinities for imidazolopyrimidines **44**–**52**

Compd	R	DPP4 K_i (nM)	DPP8 K_i (μM)	DPP9 K_i (μM)
44	H	12 (±3)[a]	>30	>30
45	Ph	6	>30	>30
46	2-Cl-Ph	18	5.3	9.8
47	3-Cl-Ph	24	7.2	5.9
48	4-Cl-Ph	9	4.7	4.8
49	4-Me-Ph	22	2.7	5.6
50	3-MeO-Ph	38	3.4	5.5
51	4-MeO-Ph	5.6	1.3	2.1
52	2-MeO-Ph	13 (±12)[a]	7.8	>10

[a] Values represent the mean of multiple determinations, standard deviation is shown in parentheses.

Table 4
Comparison of properties of azolopyrimidines **15**, **30**, and **45**

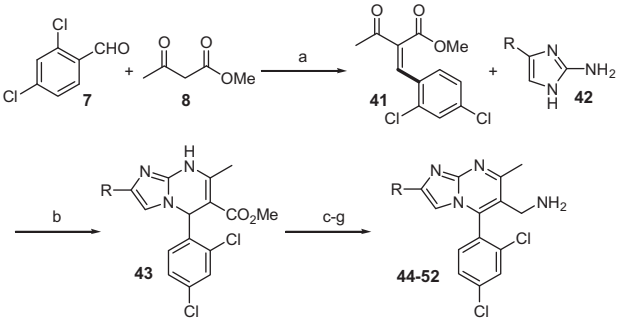
Compd	**15**	**30**	**45**
DPP4 K_i (nM)	22	67	6
DPP8 K_i (μM)	>30	>30	>30
DPP9 K_i (μM)	>30	>30	>30
hERG (% @ 10 μM)	95	85	74
Na^+ (% @ 10 μM)	95	67	58
L-type Ca^{++} (% @ 10 μM)	35	17	26
CYP3A4 IC_{50} (μM)	1	nd	1
CYP2C9 IC_{50} (μM)	12	nd	>40
CYP2C19 IC_{50} (μM)	3	nd	13
CYP1A2 IC_{50} (μM)	6	nd	19

nd, not determined.

These compounds, like the pyrazolopyrimidines, tolerated significant substituent diversity on the fused five-membered heterocycle, and retained DPP4 activity and DPP8/DPP9 selectivity (Table 2). The degree of DPP4 inhibition in this series was dependent on the nature of the substituent, with the most active compounds bearing cyclic or straight-chain secondary amines that contain either an oxygen or a sulfur (**35** = 18 nM, **37** = 31 nM, and **38** = 29 nM). Other substitution of triazole displayed slightly weaker activity as observed for **29**, **30**, **33**, **34**, and **36** (50–73 nM). DPP4 activity appeared to diminish when the substituent was changed to a carboalkoxy group or a secondary cyclic amine containing a second nitrogen (**31** = 137 nM, **39** = 442 nM, and **40** = 106 nM). Additionally, a direct comparison of DPP4 inhibition between compounds **13** (K_i = 28 nM) and **29** (K_i = 64 nM), and compounds **15** (K_i = 22 nM) and **30** (K_i = 67 nM) indicated that comparable compounds in the pyrazolopyrimidine series were 2–3-fold more active than those in the triazolopyrimidine series.

Similarly, imidazolopyrimidines were prepared and evaluated as DPP4 inhibitors. The chemistry utilized to prepare these analogs was similar to that employed in the pyrazolopyrimidine and triazolopyrimidine syntheses (Scheme 3). Formation of dihydropyrimidine **43**, however, required two steps. Compound **8** was first condensed with **7** to give the desired Knoevenagel product **41**, which in turn, was condensed with the appropriate 2-aminoimidazole **42** obtained commercially or prepared according to the literature.[19] The dihydropyrimidine **43** was then converted to the desired 5-aminomethyl imidazolopyrimidines (**44–52**) using the same methods as described earlier.

Several compounds in this series were quite potent against DPP4 and most had no significant DPP8/DPP9 inhibition (Table 3). Compound **45** was synthesized to allow direct comparison of DPP4 activity and DPP8/DPP9 selectivity among the three respective azolopyrimidine series. Compounds **46–52** were generated to assess the SAR associated with the aryl substitution pattern. All of these compounds were active against DPP4 and displayed wide DPP8/DPP9 (200- to >2000-fold) selectivity. The degree of DPP4 activity, however, exhibited more of a regiochemical dependence (steric) than type of substitution (electronic), in that *ortho*- and *para*-substituted aryls (**46**, **48**, **51**, and **52**) were preferred over *meta*-substituted aryls (**47** and **50**).



Scheme 3. Reagents and conditions: (a) piperidine, IPA, AcOH, 70%; (b) DMF, 70 °C; (c) DDQ, CH_2Cl_2, 26–49% over two steps; (d) DIBAL, THF, 38–53%; (e) MsCl, CH_2Cl_2, Et_3N, 87–100%; (f) NaN_3, DMF, 50 °C, 79–100%; (g) PPh_3, THF, H_2O, 50 °C, 56–72%.

Based on its overall profile, the in vivo activity of compound **45** was assessed using an oral glucose tolerance test (oGTT) in *ob*/*ob* mice, dosing at 3 and 10 μmol/kg 1 h prior to a glucose challenge.[20] Blood samples were taken and analyzed for insulin 15 min post oGTT. Relative to the vehicle treated mice, the animals receiving the 3 μmol/kg dose had a mean increase in plasma insulin of 51% and those receiving the 10 μmol/kg dose had a mean increase in plasma insulin of 74%. The pursuit of potent small molecule DPP4 inhibitors that are selective over DPP8 and DPP9 led to the discovery of pyrazolo-, triazolo-, and imidazolopyrimidine-based inhibitors. These novel leads represent potential scaffolds for the design of clinically useful DPP4 inhibitors. With potency and selectivity already achieved, further exploration of SAR around the periphery of the imidazolopyrimidine core was targeted in order to further minimize CYP, hERG, and ion channel liabilities of the current compounds in this class. The results of these explorations will be reported in due course.

References and notes

1. Diabetes Facts and Figures. International Diabetes Foundation, October 6, 2009. http://www.idf.org/Facts_and_Figures.; (b) Wild, S.; Roglic, C.; Green, A.; Sicree, R.; King, H. *Diabetes* **2004**, *27*, 1047.
2. For latest information, please visit the website of World Health Organization at http://www.who.int/diabetes/facts/world_figures/en/.
3. Ross, S. A.; Gulve, E. A.; Wang, M. *Chem. Rev.* **2004**, *104*, 1255.
4. Rizzo, M.; Rizvi, A. A.; Spinas, G. A.; Rini, G. B.; Berneis, K. *Expert Opin. Invest. Drugs* **2009**, *18*, 1495.
5. (a) Holst, J. J.; Deacon, C. J. *Diabetes* **1998**, *47*, 1663; (b) Lankas, G. R.; Leiting, B.; Roy, R. S.; Eiermann, G. J.; Beconi, M. G.; Biftu, T.; Chan, C. C.; Edmonson, S.; Feeney, W. P.; He, H.; Ippolito, D. E.; Kim, D.; Lyons, K. A.; Ok, H. O.; Patel, R. A.; Petrov, A. N.; Pryor, K. A.; Qian, X.; Reigle, L.; Woods, A.; Wu, J. K.; Zaller, D.; Zhang, X.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes* **2005**, *54*, 2988.
6. Ahrén, B.; Hughes, T. E. *Endocrinology* **2005**, *146*, 2055.
7. (a) Zander, M.; Madsbad, S.; Madsen, J. L.; Holst, J. J. *Lancet* **2002**, *359*, 824; (b) Nauck, M. A.; Wollschlaeger, D.; Werner, J.; Holst, J. J.; Orskov, C.; Creutzfeldt, W.; Williams, B. *Diabetologia* **1996**, *39*, 1546.
8. Hansen, L.; Deacon, C. F.; Orskov, C.; Holst, J. J. *Endocrinology* **1999**, *140*, 5356.
9. Ahrén, B. *Expert Opin. Invest. Drugs* **2006**, *15*, 431.
10. Kim, D.; Wang, L.; Beconi, M.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
11. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S. P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2005**, *48*, 5025.
12. Villhauer, E.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. F.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774.
13. Feng, J.; Zhang, Z.; Wallace, M. B.; Stafford, J. A.; Kaldor, S. W.; Kassel, D. B.; Navre, M.; Shi, L.; Skene, R. J.; Asakawa, T.; Takeuchi, K.; Xu, R.; Webb, D. R.; Gwaltney, S. L. *J. Med. Chem.* **2007**, *50*, 2297.
14. Eckhardt, M.; Langkopf, E.; Mark, M.; Tadayyon, M.; Thomas, L.; Nar, H.; Pfrengle, W.; Guth, B.; Lotz, R.; Sieger, P.; Fuchs, H.; Himmelsbach, F. *J. Med. Chem.* **2007**, *50*, 6450.
15. Pattzi, H. M. R.; Pitale, S.; Alpizar, M.; Bennett, C.; O'Farrell, A. M.; Li, J.; Cherrington, J. M.; Guler, H.-P.PHX1149-PROT202 Study Group *Diabetes. Obes. Metab.* **2010**, *12*, 348.
16. For other small molecular DPP-IV inhibitors, see: Havale, S. H.; Pal, M. *Bioorg. Med. Chem.* **2009**, *17*, 1783. and references cited therein.
17. Settepani, J. A.; Stokes, J. B. *J. Org. Chem.* **1968**, *33*, 2606.
18. Magnin, D. R.; Robl, J. A.; Sulsky, R. B.; Augeri, D. J.; Huang, Y.; Simpkins, L. M.; Taunk, P. C.; Betebenner, D. A.; Robertson, J. G.; Abboa-Offei, B. E.; Wang, A.; Cap, M.; Xin, L.; Tao, L.; Sitkoff, D. F.; Malley, M. F.; Gougoutas, J. Z.; Khanna, A.; Huang, Q.; Han, S. P.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2004**, *47*, 2587.
19. Little, T. L.; Webber, S. E. *J. Org. Chem.* **1994**, *59*, 7299.
20. Oral glucose tolerance test in *ob*/*ob* mice. Male 13–14 weeks old *ob*/*ob* mice (Jackson Labs) were maintained under constant temperature and humidity conditions, a 12 h:12 h light–dark cycle, and had free access to a 10% fat rodent diet (D1245B Research Diets) and tap water. After an overnight fasting period, animals were dosed orally with vehicle (water) or DPP4 inhibitor (3 and 10 μmol/kg) at −60 min. Two blood samples were collected at −60 and 0 min by tail bleed for glucose and insulin determinations. Glucose (2 g/kg) was then administered orally (at 0 min). Additional blood samples were collected at 15, 30, 60, 90, and 120 min for glucose and insulin determinations. Blood samples were collected into EDTA containing tubes (Sarstedt). Plasma insulin was assayed using a mouse insulin ELISA kit (ALPCO Diagnostics). Data represent the mean of 12–24 mice/group. All procedures were performed according to BMS-IACUC guidelines.