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3,5-Dihydro-imidazo[4,5-*d*]pyridazin-4-ones: A class of potent DPP-4 inhibitors

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ABSTRACT

Systematic variations of the xanthine scaffold in close analogs of development compound **BI 1356** led to the class of 3,5-dihydro-imidazo[4,5-*d*]pyridazin-4-ones which provided, after substituent screening, a series of highly potent DPP-4 inhibitors.

Inhibition of the serine protease dipeptidyl peptidase 4 (DPP-4) has proven to be an effective treatment for improving glycemic control in type 2 diabetic patients.[1] Recovery of glucose homeostasis by DPP-4 inhibition results from the prolonged action of the incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) that are normally rapidly inactivated by DPP-4 through the cleavage of a dipeptide from the N-terminus of these oligopeptides.[2] GLP-1 and GIP are released from the gut in response to food intake, and exert a potent glucose-dependent insulinotropic action and thereby contribute to the maintenance of post-prandial glycemic control.[3] Further contributing factors are the inhibition of glucagon release from pancreatic α-cells, reduction of food intake and retardation of gastric emptying, which are mediated by GLP-1.[4] The observed beneficial effects on pancreatic β-cells have also been attributed to an extended action of GLP-1.[5] Consequently, inhibition of DPP-4 with small molecules has become an attractive approach to treat type 2 diabetes that has been pursued by several research groups.[6]

Recently, we reported the development of **BI 1356** (proposed trade name ONDERO), a highly potent and selective DPP-4 inhibitor that is currently undergoing clinical phase III trials (Fig. 1).[7] **BI 1356** is a xanthine-based molecule that was identified after broad exploration of the residue decoration around the xanthine core with regard to their effect on pharmacodynamic and pharmacokinetic properties as well as adverse side-effects. Although the xanthine scaffold proved to be an excellent platform leading to a very promising DPP-4 inhibitor, we decided to further explore variations within the xanthine framework with respect to the overall profile. Hence, we started evaluating different scaffolds based on the most favorable residue ensembles discovered for xanthine, with a particular emphasis on scaffolds that place the various residues in roughly the same spatial locations as that achieved by the xanthine core.

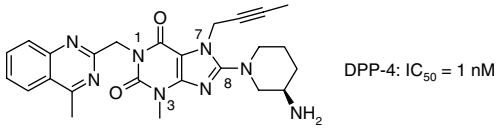

Figure 1. DPP-4 inhibitor **BI 1356**.

Our experience with substituents on the xanthine scaffold suggested an arrangement including 3-amino-piperidinyl at C-8 and but-2-ynyl at N-7 as particularly effective. We discovered that this arrangement is best complemented with methyl at N-3 and an arylmethyl group attached to N-1, among which inter alia naphth-1-ylmethyl and 3-methyl-isoquinolin-1-ylmethyl proved superior. An X-ray crystal structure of **BI 1356** complexed with human DPP-4 demonstrated the manner in which these residues provided a good fit: the primary amine of the piperidine moiety forms a highly attractive network of charge reinforced hydrogen bonds with the enzyme, while the butynyl group fits well into a hydrophobic pocket and the quinazoline interacts favorably by π-stacking.[7] A hydrogen bond between the C-6 carbonyl function of

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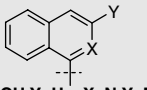
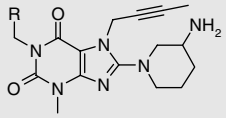
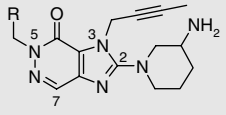
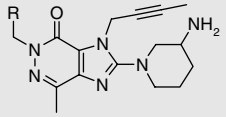
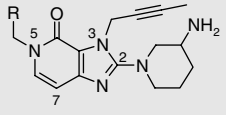
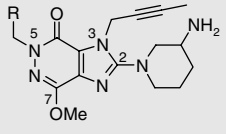
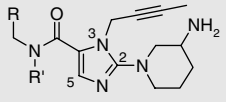
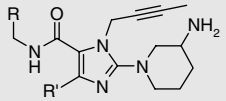
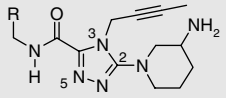
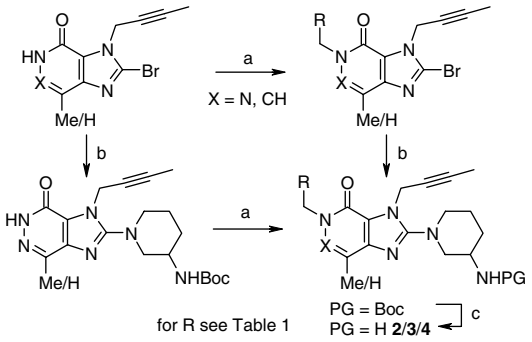
the xanthine and the enzyme completes the attractive interactions. Accordingly, we designed and synthesized a series of compounds with different scaffolds satisfying the basic requirements of orienting the residues comparably to xanthine and bearing one of the two residue ensembles mentioned, including the C-6 carbonyl group of xanthine.

The initial structural alterations varied the uracil substructure of xanthine but retained the integrity of the cyclic nature and the attachment site of the N-1 residue such as in pyridazone **2**, methylpyridazone **3**, pyridone **4**, and methoxypyridazone **5** (Table 1). The syntheses of these aromatics have been described before and are sketched in Schemes 1 and 2.[8,9]

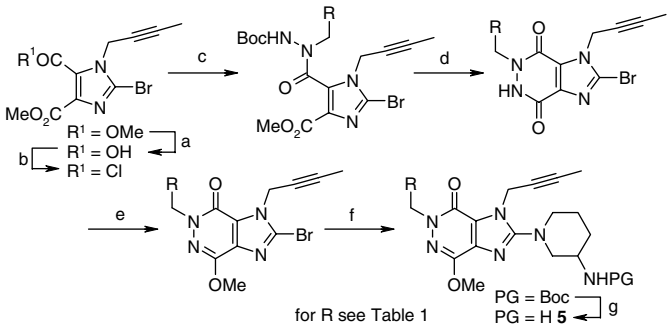
More substantial alterations of the uracil moiety included disbanding the ring structure leading to monocyclic imidazoles **6–9**. The synthetic access to all these imidazoles via the advanced intermediate **I-1** could not be accomplished as originally planned since the replacement of the 5-NH_2 group with H, Br, and CN after diazotization worked only well for H (Scheme 3)[10]; the latter two imidazoles had to be synthesized using alternative routes. Moreover, we prepared the triazole analog **10**[11] of imidazole **6** to investigate the effect on substituent conformation and, in turn, on activity of a C–H → N exchange at the 5-position of imidazole.

Table 1
DPP-4 inhibitory activity of xanthine related core structures

Compound	Structure		DPP-4 IC_{50} (nM)	
		R =		
			X=CH,Y=H	X=N,Y=Me
1			15	2
2			13	5
3			–	7
4			89	21
5			52	19
6		R′ = H	107	99
7		R′ = Me	3059	–
8		R′ = CN	582	–
9		R′ = Br	559	–
10			–	24

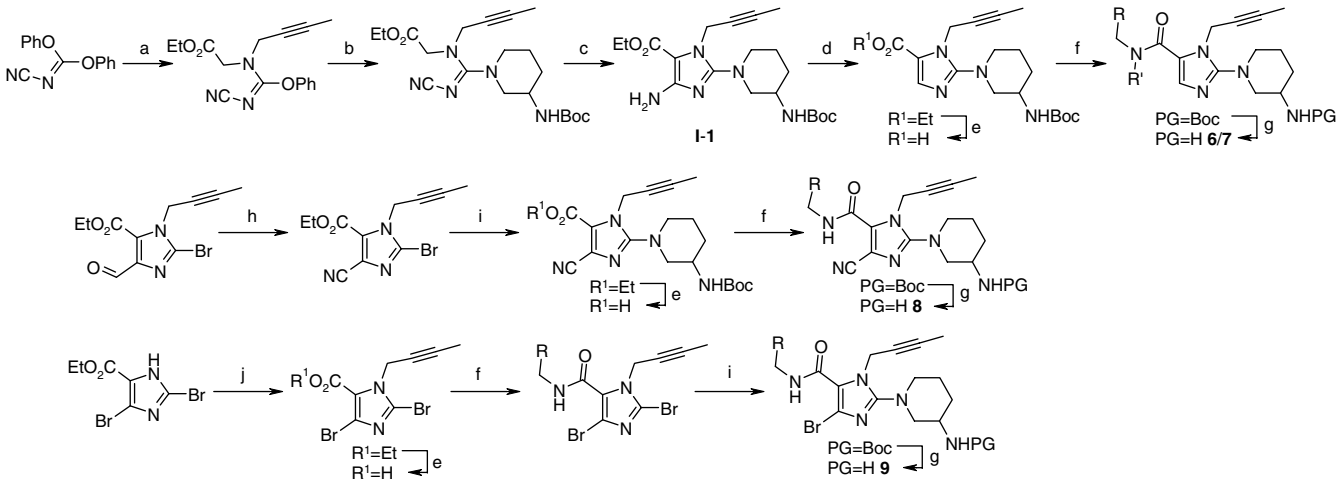


Scheme 1. Reagents: (a) RCH_2Cl/Br, K_2CO_3, DMF; (b) 3-BocHN-piperidine, Na_2CO_3, DMSO; (c) TFA, DCM.



Scheme 2. Reagents: (a) NaOH, H_2O/THF/MeOH; (b) $SOCl_2$, DCM; (c) RCH_2NHNH-Boc, NEt_3, DMF; (d) TFA, DCM; (e) MeI, K_2CO_3, DMF; (f) 3-BocHN-piperidine, Na_2CO_3, DMSO; (g) TFA, DCM.

The DPP-4 inhibitory activity was determined using human DPP-4,[12] and the results obtained for the various scaffolds substituted with the ensemble comprising either 1-naphthyl or 3-methyl-isoquinolin-1-yl are compiled in Table 1, including the data for the analogous xanthine compounds. Replacement of the uracil moiety of the original xanthine with a pyridazone (→ **2** and **3**), either with or without an additional methyl group, had no considerable effect on the inhibitory activity, while a methoxy substituent on pyridazone (→ **5**) or switching to a pyridone (→ **4**) led to a significant loss of potency. More pronounced scaffold alterations resulted in compounds of even lower activity. Although compound **6** still exhibited respectable DPP-4 inhibition, the C-5 derivatized imidazoles **8** and **9** were more than 30-fold less potent than xanthine **1**. Repulsion of the amide group and the residue at C-5 of the imidazole causing a different orientation of the arylmethyl group and the amide carbonyl would explain the considerably inferior potency of the monocyclic aromatics. Support for this rationalization is seen with imidazole **7** which has a bulkier secondary amide, most likely resulting in an even more distorted residue and amide group positioning and thus a further drop in potency. Additional evidence is provided by triazole **10** which should be capable of forming an intramolecular hydrogen bond between the amide N–H and the 5-N of the triazole by which the initial cyclic form would be reestablished: triazole **10** is significantly more active than its imidazole congener **6** exhibiting activity comparable to pyridone **4** and pyridazone **5**. These observations, combined

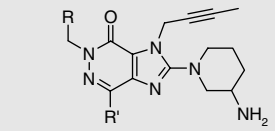
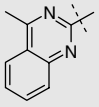
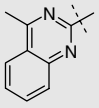
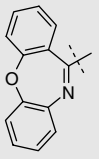
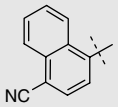
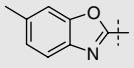


Scheme 3. Reagents: (a) i—$EtO_2CCH_2NH_2$, K_2CO_3, acetone; ii—$MeCCCH_2Br$, K_2CO_3, DMF; (b) 3-BocHN-piperidine, K_2CO_3, DMF; (c) NaOEt, EtOH; (d) $NaNO_2$, aq HCl; H_3PO_2; (e) aq KOH, THF; (f) RCH_2NHR' or RCH_2NH_2, TBTU, Et*i*-Pr_2N, DMF; (g) TFA, DCM; (h) H_2NOSO_3H, pyridine, EtOH; (i) 3-BocHN-piperidine, Na_2CO_3, DMSO; (j) $MeCCCH_2$ Br, K_2CO_3, DMF; for R and R' see Table 1.

with the X-ray data of **BI 1356**, allow for the assumption that a coplanar relationship of the imidazole ring and the amide group, with the carbonyl oxygen positioned next to the butynyl group, is a prerequisite for optimal arylmethyl and carbonyl group alignment.

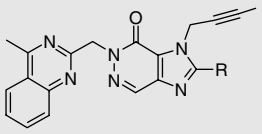
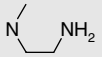
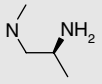
Of the different scaffolds prepared, only the promising data of the pyridazones warranted further studies.[13] In addition to in vitro DPP-4 inhibitory activity we included activity on the muscarinic receptor M_1 and inhibition of DPP-4 in rats[14] into our pro-

Table 2
Activities of 5-derivatized 3,5-dihydro-imidazo[4,5-*d*]pyridazin-4-ones

Compound[a]			DPP-4 IC_{50} (nM)	M_1 IC_{50} (nM)	DPP-4 inhib. in rat[b] (%)
	R	R′			
11-(*R*)		H	1	1190	80
12-(*R*)		Me	1	93	76
13		H	14	5607	73
13-(*S*)			25	—	9
13-(*R*)			3	>5000	63
14	PhCO	H	26	—	8
15		H	4	862	84
16		H	4	—	64

[a] (*R*) or (*S*) is appended to the compound number when the pure enantiomer was used.
[b] Determined ex vivo after 7 h post-compound administration (po, 10 mg/kg) versus control group.

Table 3
Activities with variations at C-2 of a 3,5-dihydro-imidazo[4,5-*d*]pyridazin-4-one

Compound[a]		DPP-4 IC_{50} (nM)	M_1 IC_{50} (nM)	DPP-4 inhib. in rat[b] (%)
11-(*R*)		1	1190	80
17		5	2804	45
18		3	954	20
19		1	>5000	29
20		2	>5000	50

[a] (*R*) is appended to the compound number when the pure enantiomer was used.
[b] Determined ex vivo after 7 h post-compound administration (po, 10 mg/kg) versus control group.

filing scheme for the subsequent optimization process; significant M_1 receptor inhibition was a characteristic of some early xanthines. We set out to confirm the substituent SAR established for the xanthines with the 3,5-dihydro-imidazo[4,5-*d*]pyridazin-4-ones. A set of residues known to be well suited from the xanthine series were attached to C-2 and N-5 of the imidazopyridazone core; substitution of the butynyl group was not considered as it proved to be an important feature of xanthines showing no unwanted side-effects, such as hERG or receptor M_1 inhibition. Attaching 4-methyl-quinazolin-2-ylmethyl, the residue of **BI 1356**, to the two pyridazones [→ **11**-(*R*) and **12**-(*R*)] led to very potent DPP-4 inhibitors in vitro as well as in vivo (Table 2). Unfortunately, methyl derivatized imidazopyridazone **12**-(*R*) inhibited the M_1 receptor to a considerable extent forcing us to discontinue the examination of this scaffold. Conversely, the methyl free congener **11**-(*R*) showed only poor affinity for the M_1 receptor. Additional N-5 derivatives confirmed the close relationship to the xanthines, giving similarly potent DPP-4 inhibitors. Compounds **13**-(*R*) and **13**-(*S*) exemplify this further by the former enantiomer being about 8-fold more potent than the latter, a trend generally seen within the xanthine series.

Based on the structure of compound **11**-(*R*), the impact of different amino groups at C-2 on the inhibitory potency was studied (Table 3). Compounds **11**-(*R*) and **17**–**20**, bearing the most potent amino residues discovered in the xanthine project, showed potent inhibition of DPP-4 throughout and low affinity for the M_1 receptor. The DPP-4 inhibition results in rats confirmed the superiority of 3-amino-piperidinyl over the other amino substituents: the residual inhibition of DPP-4 7 h after oral administration with all compounds but **11**-(*R*) had declined considerably from earlier time points.

Examination of the residues of the imidazopyridazones confirmed **BI 1356**'s highly favorable arrangement for closely related scaffolds yielding compound **11**-(*R*) as one of the most promising ones. **11**-(*R*) was submitted to advanced testing including preliminary experiments on pharmacokinetic parameters in rats (Table 4). Additionally, compound **11**-(*R*) was investigated in various in vitro receptor binding and enzyme assays that indicated no known liabilities at test concentrations of 3 μM.[15] Among the data listed,

Table 4
Preliminary pharmacological and kinetic data of **11**-(*R*)

hERG, current remaining at 1 μM	88%
Cyp_{450}, 3A4/2D6/2C9/2C19/1A2	>50 μM
Human liver cytosol $t_{1/2}$	>90 min
Human liver microsome $t_{1/2}$	>90 min
CL (rat)	56 mL/min/kg
V_{ss} (rat)	40 L/kg
MRT (rat)	8.8 h
F_{oral} (rat)	15%
DPP-4 inhibition in rats, ex vivo	71% after 24 h (10 mg/kg po)

the long-lasting strong DPP-4 inhibition (>70% after 24 h after compound administration) is particularly noteworthy. These findings support a more detailed study of the 3,5-dihydro-imidazo[4,5-*d*]pyridazin-4-one class and of compound **11**-(*R*) in particular, as potential candidates for development in the treatment of type 2 diabetes.

In conclusion, we have shown that variations of the xanthine scaffold used in the clinical development compound **BI 1356**, which retain the spatial substituent alignment, lead to alternative, potent DPP-4 inhibitor classes such as the 3,5-dihydro-imidazo[4,5-*d*]pyridazin-4-ones. One representative of the latter class, compound **11**-(*R*), has been further advanced and shows a very promising activity profile, demonstrating that this series is an appealing gateway to new, highly potent DPP-4 inhibitors.

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10. Eckhardt, M.; Himmelsbach, F.; Langkopf, E.; Thomas, L.; Tadayyon, M. WO 2006000354, 2006.
11. For synthesis of compound **10** see Ref. 10.
12. For experimental DPP-4 and M_1 activity determination see supporting information of Ref. 7a.
13. Imidazopyridazones with deviating structure have been reported as DPP-4 inhibitors in: (a) Yoshikawa, S.; Emori, E.; Matsuura, F.; Richard, C.; Ikuta, H.; Kira, K.; Yasuda, N.; Nagakura, T.; Yamazaki, K. WO 2003104229, 2003; (b) Kurukulasuriya, R.; Rohde, J. J.; Szczepankiewicz, B. G.; Basha, F.; Lai, C.; Jae, H.-S.; Winn, M.; Stewart, K. D.; Longenecker, K. L.; Lubben, T. W.; Ballaron, S. J.; Sham, H. L.; von Geldern, T. W. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6226.
14. In vitro inhibition data of rat DPP-4 were comparable to the results obtained for the human enzyme.
15. Enzyme set *Diversity profile* and receptor set *ExpresSProfile* offered by Cerep (Poitiers), France.