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# Discovery of Second Generation Reversible

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# Covalent DPP1 Inhibitors

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# Leading to an Oxazepane

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# Amidoacetonitrile Based Clinical Candidate (AZD7986)

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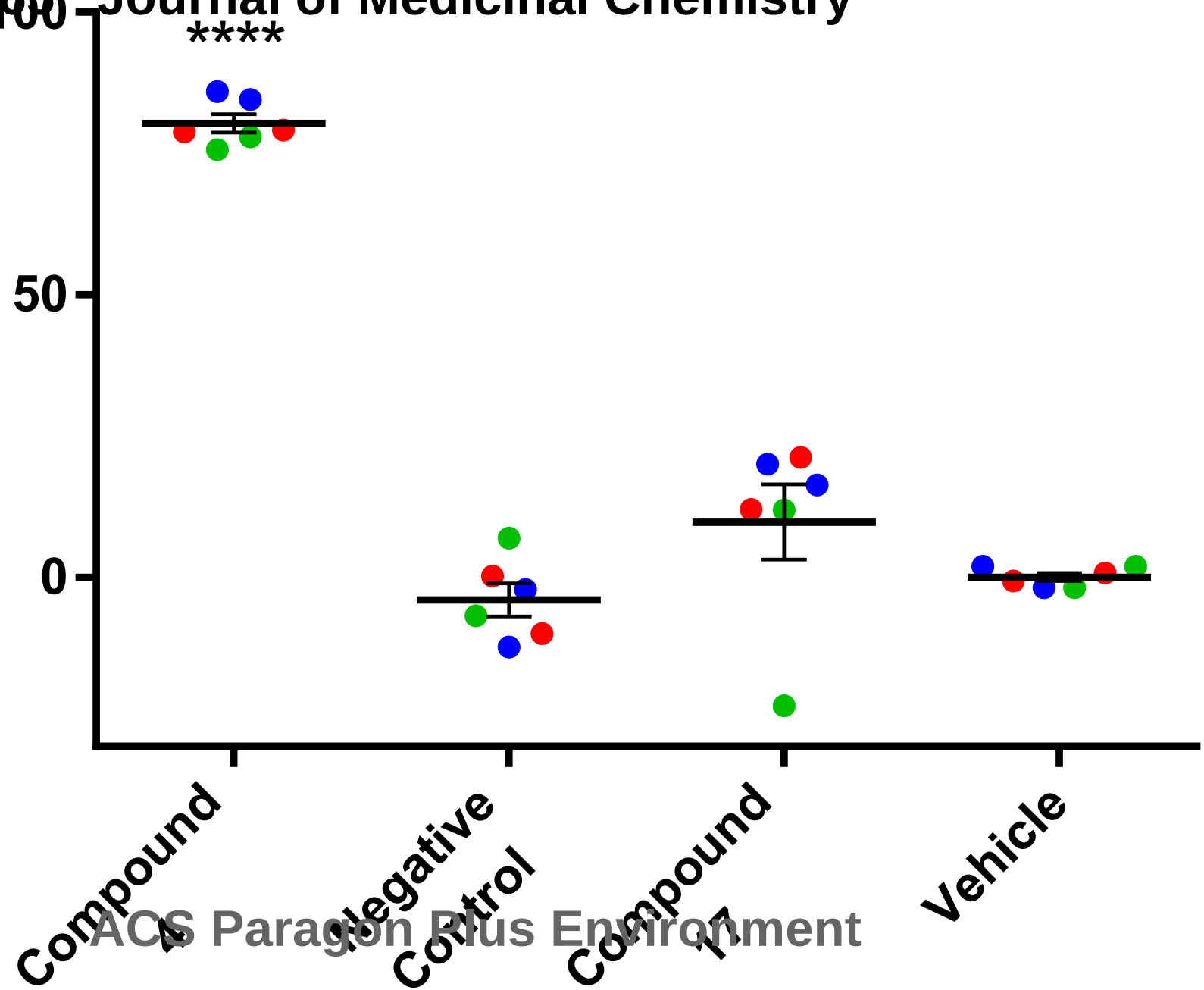

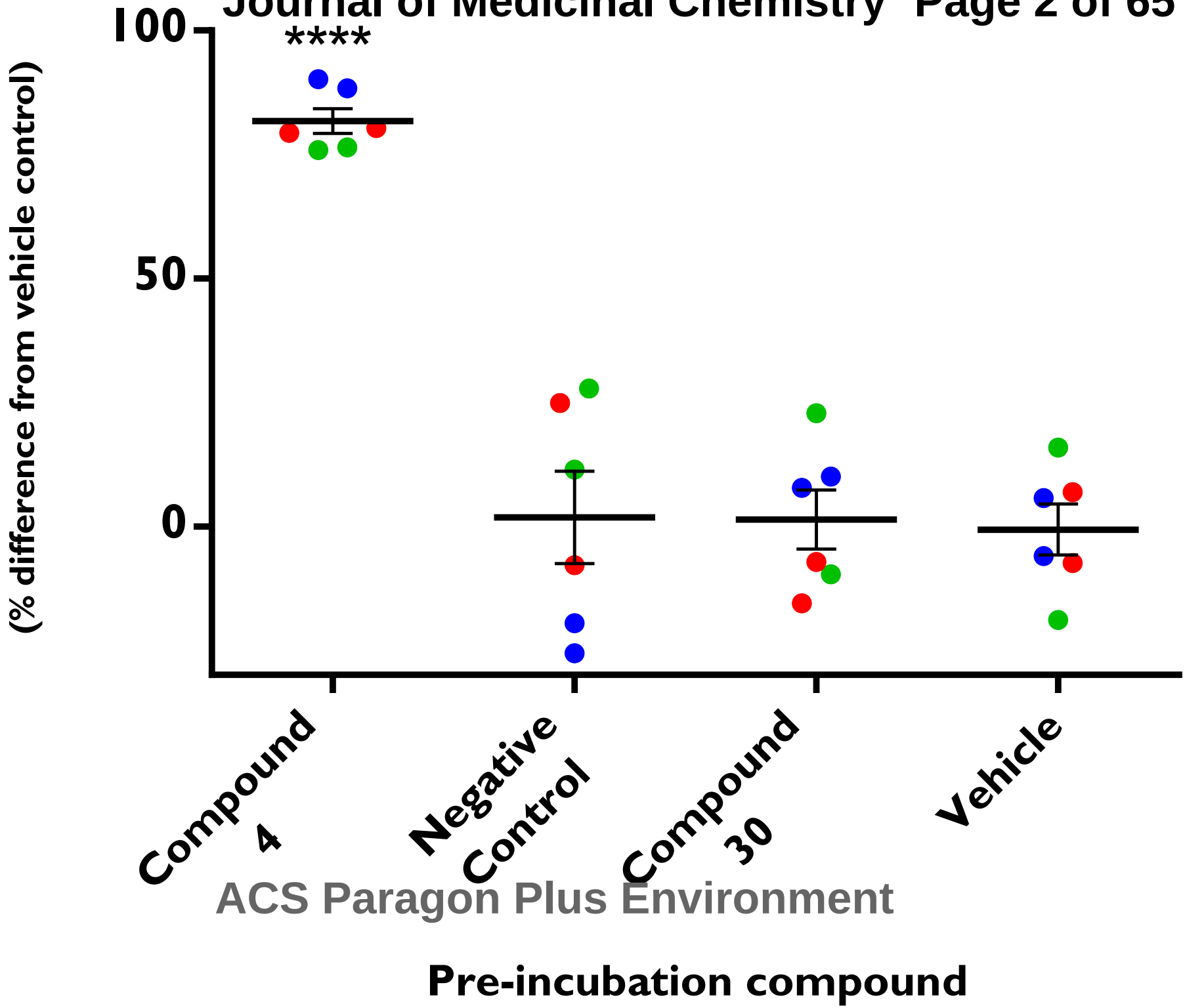
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Aortic Binding
(% difference from vehicle control)
Compound 4
Negative Control
Compound 30
Vehicle
ACS Paragon Plus Environment
Pre-incubation compound

# Discovery of Second Generation Reversible Covalent DPP1 Inhibitors Leading to an Oxazepane Amidoacetonitrile Based Clinical Candidate (AZD7986)

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**Abstract**

A novel series of second generation DPP1 inhibitors free from aorta binding liabilities found for earlier compound series was discovered. This work culminated in the identification of

compound **30** (AZD7986), as a highly potent, reversible and selective clinical candidate for COPD, with predicted human PK properties suitable for once daily human dosing.

## Introduction

DPP1 (dipeptidyl peptidase 1, or cathepsin C, CatC) is a lysosomal cysteine protease that plays a key role in the activation of the proinflammatory neutrophil serine proteases (NSPs) neutrophil elastase (NE), proteinase-3 (Pr3), and cathepsin G (CatG).[1-4] Inhibition of DPP1 has therefore been implicated as a therapeutic treatment of diseases that carry a high neutrophilic burden, such as COPD (chronic obstructive pulmonary disease)[2, 5, 6] which is projected to be the 3rd leading cause of death worldwide by 2030.[7] DPP1 activates NSPs by cleaving the *N*-terminal dipeptide during neutrophil maturation in the bone marrow. Inhibition of DPP1 in the bone marrow would therefore lead to neutrophils without stored active NE, Pr3, or CatG, and has the potential to reduce the high local release of active NSPs in the lung that cause inflammation and neutrophil driven lung damage[2, 3] (Figure 1).

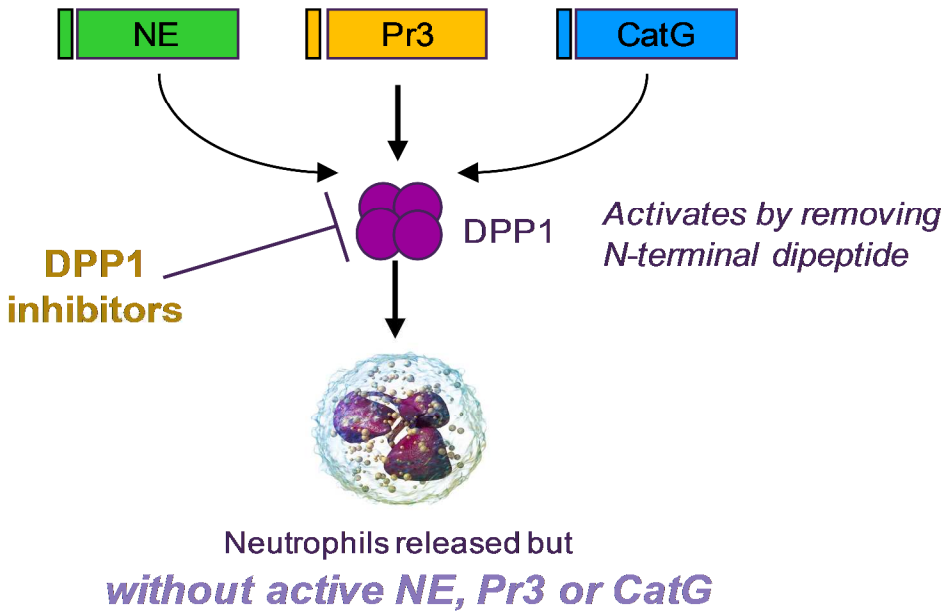



**Figure 1.** Inhibition of DPP1 in the bone marrow delivering neutrophils without stored active NE, Pr3 or CatG. Part of picture adapted from Blausen.com staff, Wikiversity Journal of Medicine 1 (2).[8]

Human DPP1 is a tetrameric enzyme containing four identical units.[9] Each of the identical subunits comprises three independent protein chains: the light chain, the heavy chain and the exclusion domain. The active site which harbours the catalytic cysteine residue (Cys234) is located at the interface of the three chains. DPP1 has an open S1 site positioned at the entrance of the active site, while the S2 pocket is large but enclosed within the protein. The exclusion domain, which is unique for DPP1, blocks access to substrates beyond the S2 site and explains the exopeptidase activity of DPP1. In addition, the side chain of Asp1 in the exclusion domain governs substrate recognition by interacting with the $\alpha$-amino group of the substrate peptide (Figure 2).

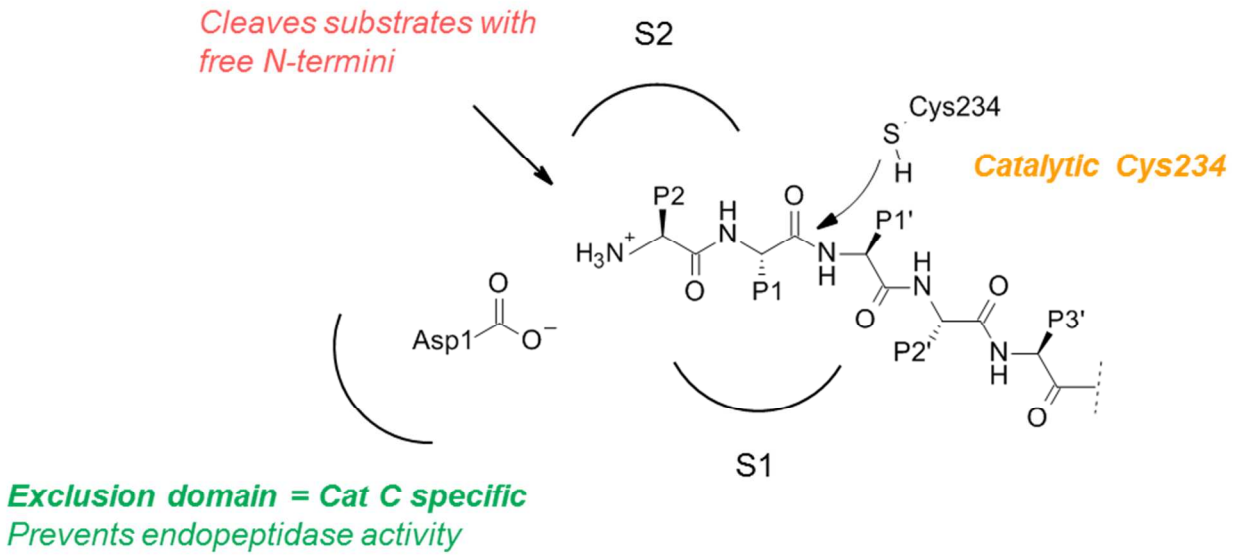



**Figure 2.** Schematic picture of the DPP1 active site, specifically showing the catalytic Cys234 and the Asp1 in the exclusion domain.

The medicinal chemistry of DPP1 has been reviewed and several programs towards developing oral inhibitors have been published.[2, 10-12] Most of the reported DPP1 inhibitors are dipeptidic structures based on preferred substrate motifs where the *C*-terminus has been modified to include an electrophilic warhead that reacts through covalent bond formation with the active site cysteine (Cys234). This covalent interaction can either be reversible or irreversible. The peptidic and electrophilic nature of DPP1 inhibitors is frequently associated with poor metabolic stability[2, 11] as well as presenting a potential safety concern because of high reactivity, potentially leading to poor selectivity and consequent off-target effects.[13] Overcoming such issues is a serious hurdle and a limited number of DPP1 inhibitors have reached clinical evaluation to date.

GlaxoSmithKline was the first company to report clinical studies of a DPP1 inhibitor in healthy humans, completed in 2014 with their irreversible covalent $\alpha,\beta$-unsaturated amide based DPP1 inhibitor GSK2793660[14, 15] (**1**) (Chart 1) for bronchiectasis. However the development of this compound was recently reported to have been stopped, due to drug-related adverse events and lack of desired effect on biomarkers.[16]

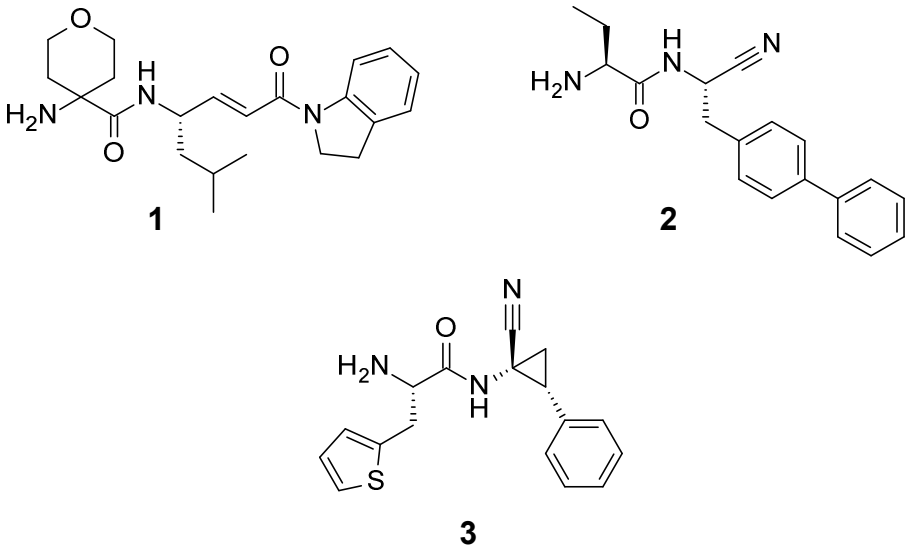
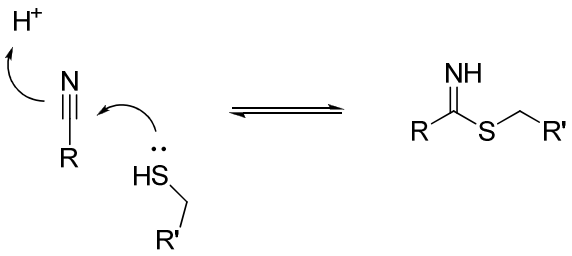



**Chart 1.** Examples of known DPP1 inhibitors.

Published reversible DPP1 inhibitors include dipeptide nitriles, and Combio and Merck have reported on such amidoacetonitrile based DPP1 inhibitors **2**[17, 18] and **3**[19] respectively (Chart 1). The nitrile function reacts with the active site cysteine in a Pinner type reaction to form a reversible thioimidate complex as illustrated in Scheme 1.[10] Thus, with a non-hydrolysable warhead, amidoacetonitriles are competitive DPP1 inhibitors but do not act as substrates.[2]

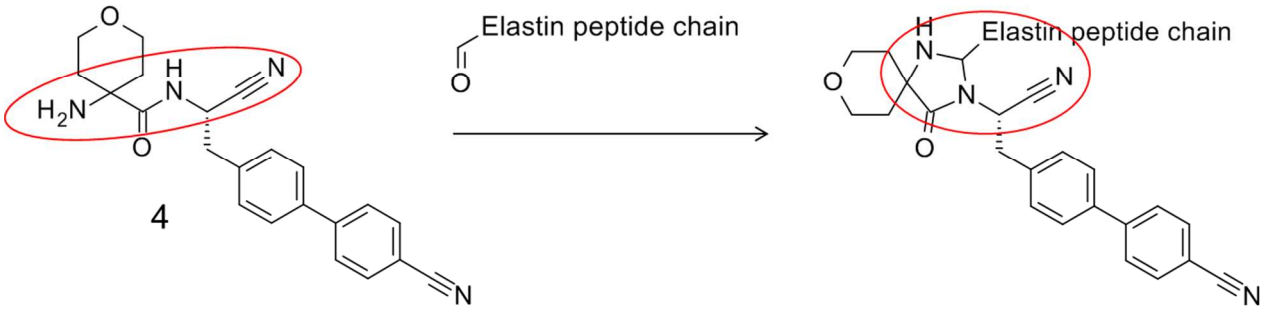
Amidoacetonitriles have been successfully used in other advanced development compounds such as the cathepsin K inhibitor Odanacatib[20] that reached late clinical development for osteoporosis, and in the marketed antidiabetic DPP4 inhibitors Saxagliptin and Vildagliptin, thus indicating the amidoacetonitrile function as a viable warhead.[10]




**Scheme 1.** Reaction of the nitrile function with the active site cysteine in a Pinner type reaction to form a reversible thioimidate complex.[10]

We previously reported[21] on our highly potent, selective, and metabolically stable first generation reversible DPP1 inhibitors that ultimately led to the oral clinical candidate **4**, (Scheme 2). Compound **4** showed aortic binding in a rat quantitative whole-body autoradiography (QWBA) study leading to its development being terminated prior to human dosing due to safety concerns.[22] A mechanistic hypothesis for this finding was established suggesting reactivity with the aldehyde in allysine side chains that are involved in the cross-linking of elastin, a major component of aortic tissue. Specifically, we proposed that the chemical reactivity of **4** with the aldehyde function in allysine occurs via formation of a stable 5-membered imidazolidin-4-one as depicted in Scheme 2, and formation of an imidazolidin-4-one through reaction with

propionaldehyde in buffer was subsequently demonstrated. A screening cascade to detect this reactivity *in vitro* was developed.[22] Compounds were tested in a medium throughput *in vitro* propionaldehyde reactivity assay that measures compound reactivity as half-life of the test compound. The half-life for compound **4** in this assay was less than 1 h (Table 1). As a second step selected compounds were also tested in a more biologically relevant but lower throughput *in vitro* covalent binding assay using aortic tissue homogenate. The utility of this cascade is demonstrated in the work herein reported towards the discovery of second generation DPP1 inhibitors.



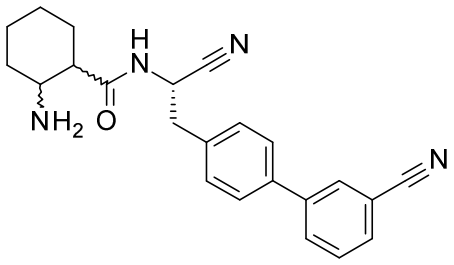

**Scheme 2.** Proposed mechanism for chemical reactivity of compound **4** with reactive aldehyde functions in allysine via formation of a stable 5-membered imidazolidin-4-one ring. Red encircled part of the molecules shows motifs involved in aldehyde reactivity.

## Results and discussion

A program to find new second generation DPP1 inhibitors, which retain the excellent profile and physicochemical properties of **4** but without the aorta binding liability, was initiated. One of

the key challenges was the fact that the pharmacophore required for DPP1 binding strongly overlaps with the motifs causing the aldehyde reactivity.

**Exocyclic amine *β*-amino acids.** As compound **4** was built around an *α*-amino acid scaffold, it was proposed to investigate compounds that do not contain this scaffolding motif. A re-analysis of the compounds prepared during the discovery program was undertaken, and a *β*-amino acid derivative **5** (Chart 2), with $pIC_{50}$ of 6.0, was identified as a reasonable starting point.



**5**

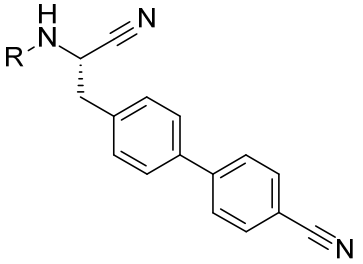
DPP1 enzyme $pIC_{50}$ 6.0

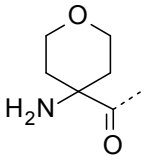
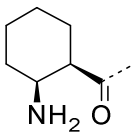
**Chart 2.** Structure of historical *β*-amino acid DPP1 inhibitor **5**.

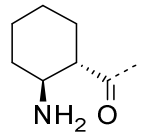
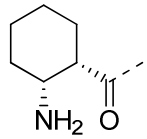
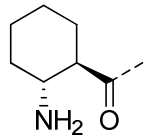
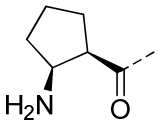
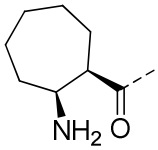
The *β*-amino acid derivative **5** had previously been prepared as a diastereomeric mixture, and initially work was undertaken to improve potency in this series. Pure enantiomers of the corresponding 4'-cyano-[1,1'-biphenyl]-4-yl substituted compounds **6** to **9** (Table 1) were synthesized to evaluate the effect on potency with the different isomers. Testing these compounds in the DPP1 enzyme and cellular assays, showed the most potent isomer **6** to have the 1*R*, 2*S*-configuration around the cyclohexane ring. Furthermore, all four isomers were shown to be stable in the propionaldehyde reactivity assay, with a half-life of over 50 h.

The cyclopentane and cycloheptane analogues of **6**, compounds **10** and **11** respectively, were subsequently prepared. Again, it was observed that both these compounds were stable in the propionaldehyde reactivity assay. It was noted that as the ring size increased, from 5 to either 6 or 7, there was an improvement in their enzyme and cellular potencies, probably in part due to their increased lipophilicity.

**Table 1.** Exocyclic *β*-amino acid examples.




| **Compound** | R | DPP1 enzyme $pIC_{50}$[a] | DPP1 cell $pIC_{50}$[b] | Aldehyde reactivity assay $t_{½}$ (h)[c] |
|---|---|---|---|---|
| **4** |  | 9.1 | 8.1 | <1 |
| **6** |  | 7.4 | 7.4 | >50 |

| | | | | |
|---|---|---|---|---|
| **7** |  | 6.3 | 6.4 | >50 |
| **8** |  | <5 | ND[d] | >50 |
| **9** |  | 6.5 | 6.8 | >50 |
| **10** |  | 6.0 | 5.9 | >50 |
| **11** |  | 7.4 | 7.5 | >50 |

[a]Human recombinant DPP1 isolated enzyme assay. Mean values from a minimum of two experiments. Average standard deviation for the assay was 0.17. [b]DPP1 cell assay using U937 cell line. Mean values from a minimum of two experiments. Average standard deviation for the assay was 0.16. [c]Reactivity with propionaldehyde measured as half-life of test compound, determined as described in reference 22. [d]ND = Not determined.

Compounds **6** and **11** were profiled further in a selection of *in vitro* assays (Table 2). Both compounds demonstrated moderate to good stability in human liver microsomes (HLM) and rat hepatocytes, as well as high kinetic solubility, and were highly permeable as determined by a Caco-2 assay. However, they also demonstrated affinity for the hERG channel, with $IC_{50}$ values

of 5.4 and 3.6 μM respectively. This was in part attributed to them being basic compounds with relatively high logDs of 1.6 and 2.0 respectively.[23, 24]

**Table 2.** Profiling data for exocyclic *β*-amino acid examples **6** and **11**.

| | **6** | **11** |
|---|---|---|
| HLM $CL_{int}$[a] | 9.3 | 13 |
| Rat Hepatocytes $CL_{int}$[b] | <3.5 | <3.5 |
| human/rat %PPB[c] | 93 / 88 | 97 / 92 |
| Kinetic solubility[d] (μM) | 175 | 175 |
| LogD[e] | 1.6 | 2.0 |
| Caco-2 $P_{app}$[f] A to B/B to A (Efflux ratio) | 11/36 (3.2) | 11/23 (2.0) |
| hERG $IC_{50}$ (μM) | 5.4 | 3.6 |
| Calculated pKa | 9 | 10 |

[a]HLM (μL/min/mg), [b]Hepatocytes (μL/min/cells x $10^6$), [c]PPB = plasma protein binding, [d]pH 7.4, [e]Octanol/water partitioning, pH 7.4, measured value, [f]$P_{app}$ = apparent permeability (cm/s x $10^{-6}$).

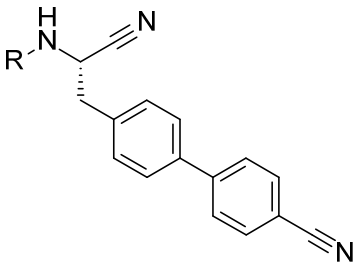
**Cyclic *β*-amino acids.** With a potential hERG liability noted, a series of alternative *β*-amino acids where the amino functionality is incorporated into the ring system were prepared (Table 3). To mitigate any potential hERG liabilities, it was proposed that the presence of an oxygen atom within the ring might help in lowering lipophilicity and assist in ameliorating any potential

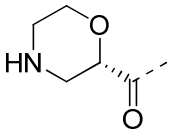
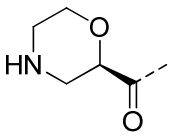
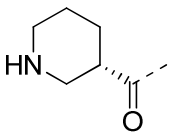
issues, although it was recognized that a ring-oxygen would also reduce the pKa, which would have a compensating increasing effect on logD. A series of 6- and 7-membered ring analogues, **12** to **19**, were prepared. The four 6-membered ring examples, **12** to **15**, were prepared as single diastereomers, whilst the two 7-membered analogues, **16** and **17**, were prepared as diastereomeric mixtures. It was observed that the presence of the oxygen atom lowered the logD for the 7-membered rings, with the oxazepane analogue **17** having a measured logD of 1.1 (corresponding calculated value 1.2) as compared to a logD of 1.6 (calculated value) for the azepane analogue **16**. In the 6-membered ring examples however, incorporation of the oxygen had a slight detrimental effect on logDs for the morpholine analogues **12** and **13**, compared to logDs for the equivalent piperidine analogues **14** and **15**. As discussed above, this is likely because the oxygen in the 6-membered ring lowers the pKa of the ring nitrogen to a greater extent than in the 7-membered oxazepane ring, leading to a logD increasing effect as seen in the 6-membered analogues **12** and **13**. Of the 6-membered analogues, only the morpholine (*S*)-diastereoisomer **12** demonstrated weak DPP1 inhibition, with $pIC_{50}$ 6.4, whilst all the other three 6-membered ring analogues were inactive. Of the 7-membered ring analogues, the azepane **16** demonstrated some DPP1 potency, as opposed to the equivalent piperidine analogues, **14** and **15**. The diastereomeric oxazepane **17** showed good DPP1 enzyme potency level with $pIC_{50}$ 7.2. Of the 6- and 7-membered ring analogues tested, all demonstrated stability in the propionaldehyde reactivity assay.

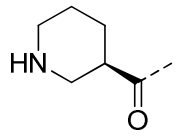
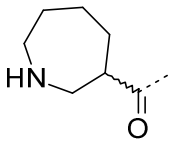
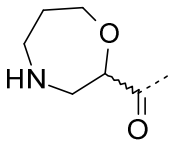
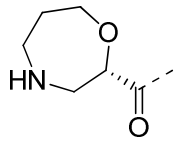
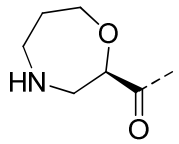
With the diastereomeric oxazepane **17** showing good DPP1 enzyme potency levels, preparation of the pure (*S*)- and (*R*)-oxazepane diasteroisomers **18** and **19** showed that, as in the case of **12**, potency resided within the diastereomer with the (*S*)-configuration for the ring

substituent, **18**. Diastereomer **18** was confirmed to have a good DPP1 enzyme potency level with $pIC_{50}$ 7.4, as well as retaining high potency in the DPP1 cellular assay.

**Table 3.** Data for 6- and 7-membered cyclic $\beta$-amino acids.



| **Compound** | R | DPP1 enzyme $pIC_{50}$[a] | DPP1 cell $pIC_{50}$[b] | Aldehyde reactivity assay t½ (h)[c] | LogD[d] | pKa |
|---|---|---|---|---|---|---|
| **12** |  | 6.4 | 6.3 | >50 | 1.4 | 7.4 |
| **13** |  | <5 | ND | >50 | 1.6 | ND[e] |
| **14** |  | <5 | ND | ND | 0.2 | 9.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **15** |  | <5 | ND | ND | 0.6 | ND |
| **16** |  | 5.9 | 6.3 | >50 | (1.6)[f] | ND |
| **17** |  | 7.2 | ND | >50 | 1.1 (1.2)[f] | ND |
| **18** |  | 7.4 | 7.5 | >50 | 1.1 (1.2)[f] | 8.0 |
| **19** |  | 5.2 | ND | >50 | 1.2 | ND |

[a]Human recombinant DPP1 isolated enzyme assay. Mean values from a minimum of two experiments. Average standard deviation for the assay was 0.17. [b]DPP1 cell assay using U937 cell line. Mean values from a minimum of two experiments. Average standard deviation for the assay was 0.16. [c]Reactivity with propionaldehyde measured as half-life of test compound, determined as described in reference 22. [d]Octanol/water partitioning, pH 7.4, measured value unless otherwise noted, [e]ND = Not determined, [f]Calculated value.

**Lead compound, 18.** At this point **18** was considered as a lead compound and was selected for further profiling (Table 4). It was profiled in a HLM stability assay as well as in a range of

hepatocyte stability assays (human, rat and dog), where it demonstrated good metabolic stability across all assays. The compound showed good kinetic solubility, and a weak inhibition of the hERG channel, $IC_{50}$ 17 μM. Progressing into rat and dog *in vivo* PK studies, the *in vitro* metabolic stability profile translated into low clearances with oral bioavailabilities of 25% and 71% respectively.

**Table 4.** *In vitro* and *in vivo* data for lead compound **18**.

| | |
|---|---|
| HLM $CL_{int}$[a] | 9.5 |
| Human/Rat/Dog Hepatocytes $CL_{int}$[b] | <3.5 / <3.5 / <4 |
| human/rat/dog %PPB[c] | 91 / 76 / 75 |
| Kinetic solubility (μM)[d] | 170 |
| LogD[e] | 1.1 |
| pKa | 8.0 |
| hERG $IC_{50}$ (μM) | 17 |
| Caco-2 $P_{app}$[f] A to B/B to A (Efflux ratio) | 19 / 45 (2.4) |
| Rat PK[g] (CL / $V_{ss}$ / $t_{½}$ / F%) | 3.8 / 2.0 / 6.2 / 25 |
| Dog PK[h] (CL / $V_{ss}$ / $t_{½}$ / F%) | 4.1 / 3.9 / 10.7 / 71 |

[a]HLM (μL/min/mg), [b]Hepatocytes (μL/min/cells x $10^6$), [c]PPB = plasma protein binding, [d]pH 7.4, [e]Octanol/water partitioning, pH 7.4, measured value, [f]$P_{app}$ = apparent permeability (cm/s x $10^{-6}$), [g]Rat PK (1 mg/kg *iv*, 3 mg/kg *po*), CL (ml/min/kg), $V_{ss}$ (L/kg), $t_{1/2}$ (h), [h]Dog PK (1 mg/kg *iv*, 2 mg/kg *po*).

As a second step in the screening cascade to support removal of aortic binding, after the propionaldehyde assay, we screened the diastereomeric oxazepane **17** in an *in vitro* competitive covalent binding assay based on competition for reactive sites within rat aortic tissue homogenate. This assay[22] detects irreversible aortic binding and uses [$^{14}$C]-radiolabelled compound **4**[25] as the competitive probe ligand. The results are shown in Figure 3, and confirmed that the diastereomeric oxazepane **17** did not bind to aortic tissue.

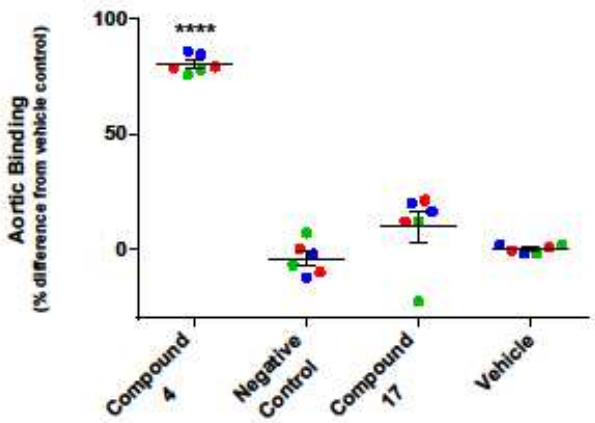
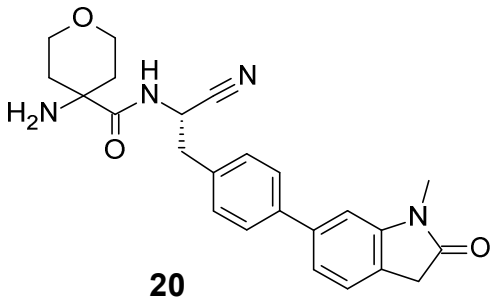



**Figure 3.** Combined datasets from several experimental sets showing relative aortic binding of compound **4**, a negative control AZD5672[26] and the diastereomeric oxazepane **17** of the lead oxazepane **18**. Compounds were screened in the *in vitro* competitive covalent binding assay based on competition for reactive sites within aortic tissue homogenate, and using [$^{14}$C]-radiolabelled compound **4**[25] as competitive probe ligand. Each experiment set is indicated by a different colour. Mean radioactivity of aortic homogenate samples pre-incubated with DMSO vehicle control for each experimental set was taken to be 100% binding, and results for samples pre-incubated with other compounds are expressed as % difference from experimental set-

matched vehicle controls. 1-way ANOVA and Bonferroni's multiple comparison tests were performed to calculate the significance of differences from experimental set-matched vehicle controls. Significance of difference from vehicle is calculated at $p < 0.0001$ (****, very highly significant), $p < 0.001$ (*** highly significant), $p < 0.01$ (**, very significant), and $p < 0.05$ (*, significant).

As a third step to confirm removal of aortic binding $^{14}$C-labelled **18** was prepared and profiled in a rat QWBA study. The results confirmed low levels of radiolabeled material in the aortic tissue after 24 h, and very little remaining radioactivity at all later time points, and thus supported our molecular hypothesis and developed screening cascade for removal of aortic binding.

**Optimization.** With the oxazepane motif demonstrated in the QWBA study of **18** to be free from aortic binding, focus was then turned towards improving the DPP1 inhibition potency for this series. The aim was to bring the series in-line with the potency observed for compound **4**, with a $pIC_{50} > 8$ for cellular activity being considered as acceptable. Re-analysis of the legacy data generated during the original discovery program highlighted a series of alternative right hand side chains, based around bicyclic ring systems on the distal phenyl ring, for example **20** (Chart 3), that had been shown to have excellent DPP1 enzyme and cellular potencies.



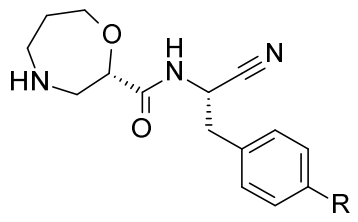
**20**

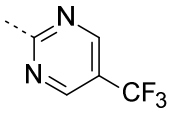
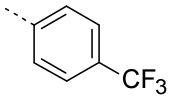
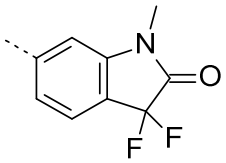
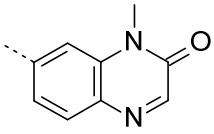
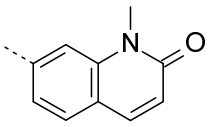
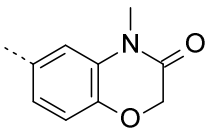
DPP1 enzyme $pIC_{50}$ 9.6
DPP1 cell $pIC_{50}$ 8.8

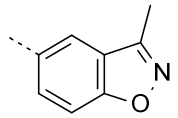
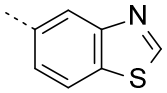
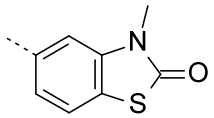
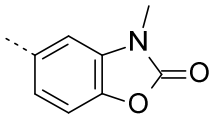
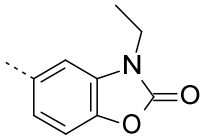
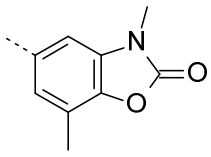
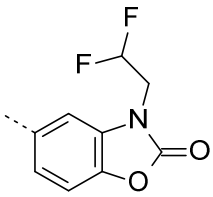
**Chart 3.** Historical right hand side chain example.

Based on this observation, a series of analogues containing ring systems built around the distal phenyl ring were prepared, and some selected examples, **21** to **33**, are shown in Table 5. Of these, the 2-oxo-1,3-benzothiazole example **29** and its equivalent 2-oxo-1,3-benzoxazole, **30**, gave DPP1 enzyme potencies with $pIC_{50}$ values 8.6 and 8.4 respectively, that also translated into good DPP1 cell potencies, with $pIC_{50}$ values of 8.5 and 8.4 respectively. Of these analogues, **29** had a higher logD of 1.5 compared to the lower logD of 0.8 for **30**, leading to **30** having a better ligand lipophilicity efficiency (LLE)[27, 28] of 7.6 compared to an LLE of 7.1 for **29**. Based on the better LLE for the 2-oxo-1,3-benzoxazole **30**, a series of close analogues were prepared, **31** to **33**, which all demonstrated good DPP1 enzyme and cellular potencies.

**Table 5.** Distal phenyl ring substituent examples.



| Compound | R | DPP1 enzyme $pIC_{50}$[a] | DPP1 cell $pIC_{50}$[b] | logD[c] | LLE[d] |
|---|---|---|---|---|---|
| **21** |  | 6.7 | 6.7 | 1.1 | 5.6 |
| **22** |  | 7.4 | ND | 2.8 | 4.6 |
| **23** |  | 7.8 | 7.8 | 1.4 | 6.4 |
| **24** |  | 7.6 | ND | 0.6 | 7.0 |
| **25** |  | 7.9 | 7.9 | 1.1 | 6.8 |
| **26** |  | 7.9 | 7.9 | 1.1 | 6.8 |

| | | | | | |
|---|---|---|---|---|---|
| **27** |  | 7.8 | 7.8 | 1.5 | 6.3 |
| **28** |  | 7.2 | ND[e] | 1.5 | 5.7 |
| **29** |  | 8.6 | 8.5 | 1.5 | 7.1 |
| **30** |  | 8.4 | 8.4 | 0.8 | 7.6 |
| **31** |  | 8.3 | 8.3 | 1.3 | 7.0 |
| **32** |  | 8.0 | 8.1 | 1.5 | 6.5 |
| **33** |  | 8.2 | 8.2 | 1.0 | 7.2 |

[a]Human recombinant DPP1 isolated enzyme assay. Mean values from a minimum of two experiments. Average standard deviation for the assay was 0.17. [b]DPP1 cell assay using U937 cell line. Mean values from a minimum of two experiments. Average standard deviation for the assay was 0.16. [c]Octanol/water partitioning, pH 7.4, [d]LLE (Ligand lipophilicity efficiency) = DPP1 enzyme $pIC_{50}$ - logD, [e]ND = Not determined.

Compounds **29** to **33** were profiled in microsomal and cross-species hepatocyte stability assays, where they all demonstrated excellent metabolic stability (Table 6). Profiling the compounds against the hERG channel showed them all to have noticeable safety windows, however compound **29** had the strongest hERG inhibitory effect with an $IC_{50}$ value of 23 μM. The compounds were then progressed into rat *in vivo* PK studies, where the excellent *in vitro* stabilities translated into low clearances and long half-lives, and with **30** and **31** having the highest bioavailabilities of 30% and 36% respectively. These two compounds were then taken into dog *in vivo* PK studies, again both demonstrated low clearances, and long half-lives, with bioavailabilities of 92% and 85% for **30** and **31** respectively. Based on its overall promising profile compound **30** was at this point selected for more extensive *in vitro* and *in vivo* profiling as a pre-clinical candidate drug.

**Table 6.** *In vitro* and *in vivo* data for advanced compounds **29** to **33**.

| | **29** | **30** | **31** | **32** | **33** |
|---|---|---|---|---|---|
| Aldehyde reactivity assay $t_{½}$ (h)[a] | >50 | >50 | >50 | >50 | >50 |
| pKa | 8.1 | 8.1 | 8.0 | 7.7 | ND[b] |
| HLM $CL_{int}$[c] | <15 | <14 (<3.0)[d] | <14 | <14 | <14 |
| Human/Rat/ Dog Hepatocytes $CL_{int}$[e] | <3.5/ <3.5/ <3.5 | <3.5/ <3.5/ <3.5 (<1/ 1.9/ <1)[d] | <3.5/ <3.5 / <3.5 | <3.5/ <3.5/ <3.5 | 5 /<3.5 / ND |
| human/rat/dog %PPB[f] | 93/ 95/ ND | 87/ 94/ 70 | 94/ 92/ 86 | 92/ 97/ 87 | 93 /94 /85 |
| Kinetic solubility | 170 | 180 | 185 | 175 | 190 |

| | | | | | |
|---|---|---|---|---|---|
| (μM)[g] | | | | | |
| Caco-2 $P_{app}$[h] A to B/B to A (efflux Ratio) | 11/ 51 (4.7) | 9.2/ 58 (6.3) (4.0/27 (6.9))[i] | 21/ 80 (3.9) | 13/ 76 (5.9) | 8.0/ 80 (10) |
| Rat PK[j] (CL / $V_{ss}$ / $t_{1/2}$ / F%) | 3/ 2/ 8h/ 26 | 1/ 1/ 7h/ 30 (7/ 2.5/ 5h/ 75)[k] | 4/ 2/ 8h/ 36 | 1/ 0.5/ 8h/ 24 | 4/ 2/ 7h/ 29 |
| Dog PK[l] (CL / $V_{ss}$ / $t_{1/2}$ / F%) | ND | 8/ 11/ 18h/ 92 | 6 /9 /16h /85 | ND | ND |
| hERG $IC_{50}$ (μM) | 23 | >33 | 31 | >33 | >33 |

[a]Reactivity with propionaldehyde measured as half-life of test compound, determined as described in reference 22. [b]ND = Not determined, [c]HLM (μL/min/mg), [d]Later stage data from assay version with lower detection limit, [e]Hepatocytes (μL/min/cells x $10^6$), [f]PPB = plasma protein binding, [g]pH 7.4, [h]$P_{app}$ = apparent permeability (cm/s x $10^{-6}$), [i]Data from later stage assay version, [j]Rat PK (Sprague Dawley unless otherwise noted), at 1 mg/kg *iv*, 3 mg/kg *po* in pH3 buffer, CL (ml/min/kg), $V_{ss}$ (L/kg), $t_{1/2}$ (h), [k](data gererated in Han Wistar rats), [l]dog PK at 1 mg/kg *iv*, 2 mg/kg *po* in pH4 buffer.

**Profiling of compound 30.** Compound **30** was stable in the propionaldehyde reactivity assay, with a half-life over 50 h (Table 6), and was shown to be free from binding in the *in vitro* aortic tissue homogenate assay, as shown in Figure 4.

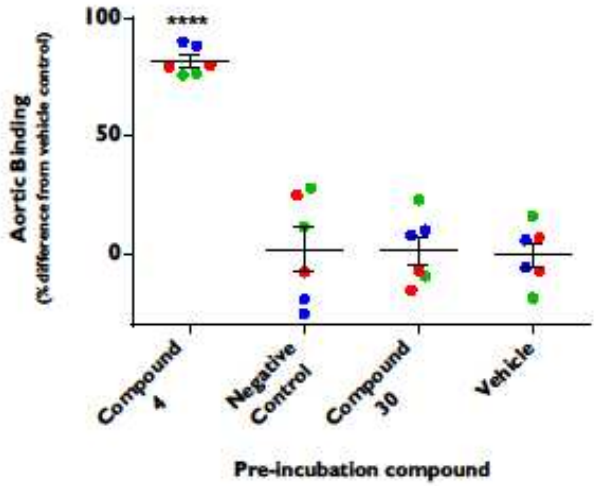



**Figure 4.** Combined datasets from several experimental sets showing relative aortic binding of compound **4**, a negative control AZD5672[26] and pre-clinical candidate drug **30**. Compounds were screened in the *in vitro* competitive covalent binding assay based on competition for reactive sites within aortic tissue homogenate, and using [$^{14}$C]-radiolabelled compound **4**[25] as competitive probe ligand. Each experiment set is indicated by a different colour. Mean radioactivity of aortic homogenate samples pre-incubated with DMSO vehicle control for each experimental set was taken to be 100% binding, and results for samples pre-incubated with other compounds are expressed as % difference from experimental set-matched vehicle controls. 1-way ANOVA and Bonferroni's multiple comparison tests were performed to calculate the significance of differences from experimental set-matched vehicle controls. Significance of

difference from vehicle is calculated at p <0.0001 (****, very highly significant), p<0.001 (*** highly significant), p <0.01 (**, very significant), and p <0.05 (*, significant).

As a third step to confirm no aortic binding $^{14}$C-labelled **30** was also prepared and profiled in a rat QWBA study, confirming no specific aorta binding after 24 h or at later time points.

Compound **30** showed good stability in plasma, with a half-life >10 h, and a comparison of *in vitro* and *in vivo* clearances in rat and dog, presented in Table 6, indicated a good *in vitro-in vivo* correlation. These data in combination with the low turnover seen in human hepatocytes (Table 6) predicted the compound to be suitable for once daily human dosing.

Good species crossover was seen for compound **30**, with the DPP1 enzyme $pIC_{50}$ values being determined to for mouse 7.6, rat 7.7, dog 7.8, and rabbit 7.8.

The compound showed high selectivity over other cathepsins, with $IC_{50}$ values all over 20 μM, when tested on human recombinant enzyme cathepsins S, L, B, K, D, E, Z, H, and G. In a panel of >200 *in vitro* radioligand binding and enzyme assays covering a diverse set of enzymes, receptors, ion channels, and transporters, compound **30** showed excellent selectivity.

The binding kinetics for compound **30** were characterized using a Surface Plasmon Resonance direct binding assay (SPR DBA), which confirmed that the compound is a reversible inhibitor of DPP1 (sensorgram available in supplementary materials). The interaction could be described with a simple 1:1 interaction model resulting in an on-rate ($k_{on}$) of 1.5E+6 (1/Ms), and an off-rate ($k_{off}$) of 4.0E-3 (1/s), giving a $pK_d$ value of 8.6. The residence time at the DPP1 enzyme, as determined by half-life, was 3 min.

The effect of DPP1 inhibition from compound **30** on the activation of NSPs was studied *in vitro* using human primary bone marrow-derived CD34+ neutrophil progenitor cells. After differentiation in the presence of compound **30** (38 pM to 10 μM) concentration-dependent decreases in cell lysate enzyme activity was observed for DPP1, as well as for all of the three NSPs, NE, Pr3, and CatG respectively (Figure 5). In conclusion compound **30** inhibited activation of all three NSPs in a concentration dependent manner, with $pIC_{50}$ values of around 7 for all three NSPs (Figure 5 and Table 7). The reduction of the activities was almost complete, with NE, Pr3 and CatG activities reduced to 4-10% of control at 10μM of compound **30** (Figure 5).

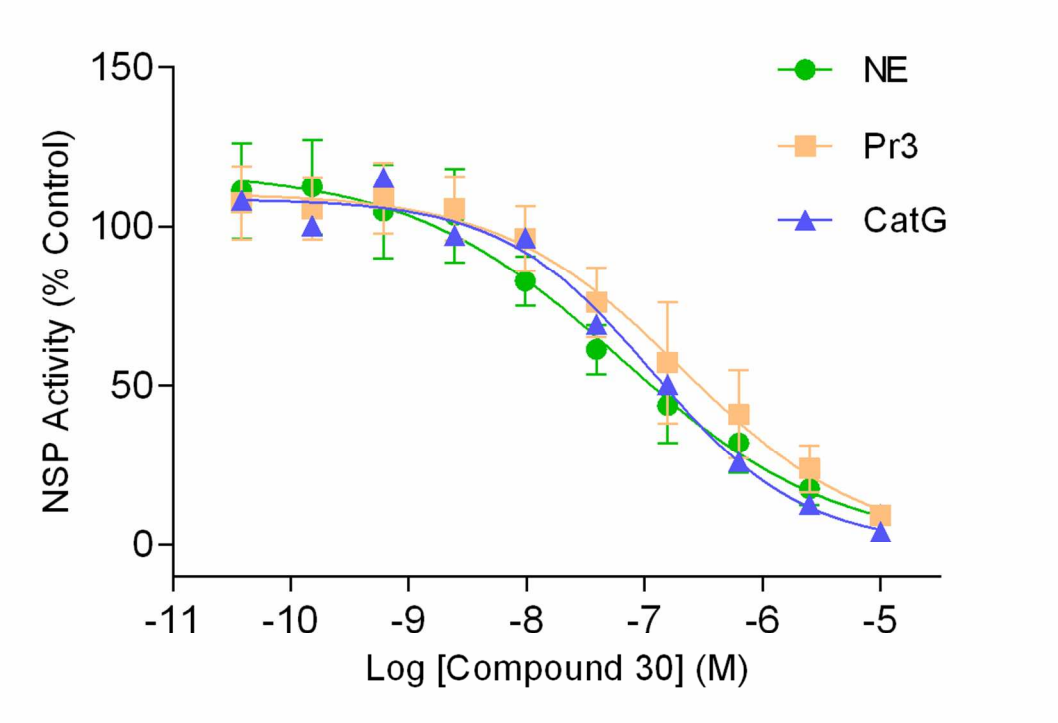



**Figure 5.** Compound **30** inhibited activation of NSPs NE, Pr3, and CatG in human primary bone marrow-derived CD34+ neutrophil progenitor cells, in a concentration dependent manner, with $pIC_{50}$ values around 7 (Table 7). The reduction of the activities was almost complete, with NE,

Pr3 and CatG activities all reduced to 4-10% of control at 10μM compound **30** (Figure 5). Data are mean ± SEM, n=4.

**Table 7**. $pIC_{50}$ values of compound **30** for inhibition of DPP1 and inhibition of activation of NSPs NE, Pr3, and CatG *in vitro* using differentiating human primary bone marrow-derived CD34+ neutrophil progenitor cells (n = 4). Data are mean ± SEM, n=4.

| | **DPP1** | **NE** | **Pr3** | **CatG** |
|---|---|---|---|---|
| $pIC_{50}$ | 6.85 ± 0.33 | 7.21 ± 0.14 | 6.68 ± 0.07 | 6.94 ± 0.12 |

The ability of compound **30** to inhibit activation of NSPs *in vivo* was assessed by treatment of naïve rats with the compound twice daily (0.2, 2 and 20 mg/kg/day) for eight days. Compound **30** inhibited activation of NE and Pr3, but not CatG, in bone marrow cell lysates in a dose dependent manner *in vivo* (Figure 6). Variability in the CatG assay was due to the colourmetric substrate used, as opposed to flourometric substrates used in the NE and Pr3 assays.

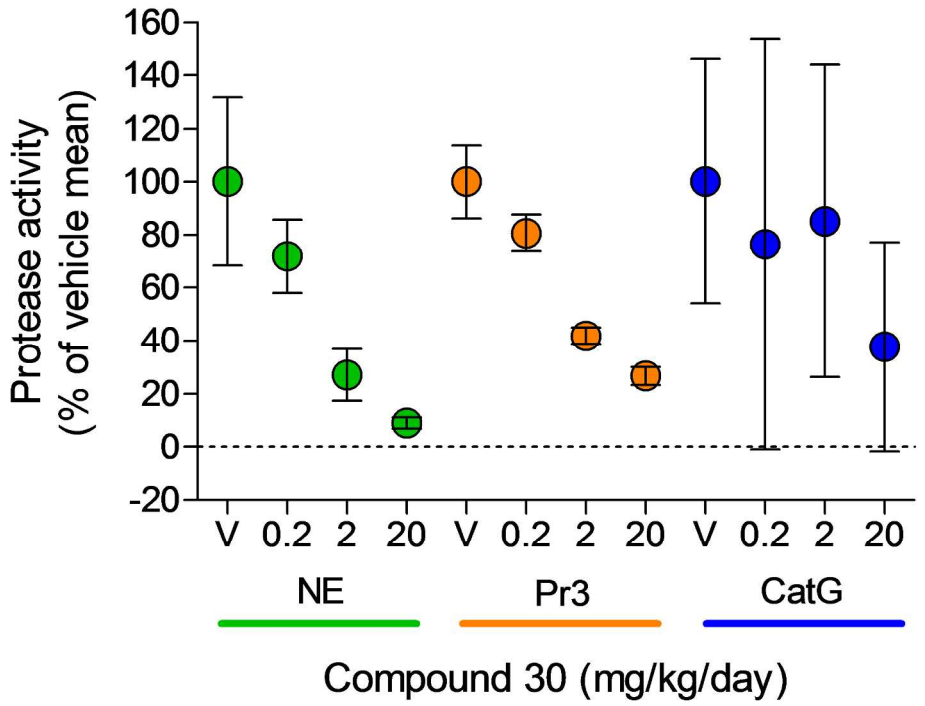



**Figure 6.** Compound **30** inhibited the activation of NE and Pr3, but not CatG, in a dose dependent manner *in vivo*, when administered twice daily to naïve rats (0.2, 2 and 20 mg/kg/day) for eight days. The vehicle mean was set to 100% activity and the compound **30** treated groups are presented as percent activity of vehicle mean. The graph shows mean % NSP activities with 95% CI in bone marrow cell lysates.

On the basis of the overall attractive profile described above compound **30** was selected as clinical candidate drug for COPD.

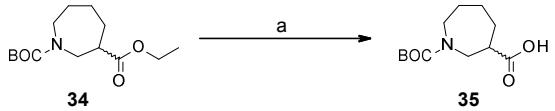
## Chemistry

The preparation of the cyclic *β*-amino acid series illustrated in Tables 1 and 3 was achieved using one of two coupling conditions between, when available, commercially available BOC

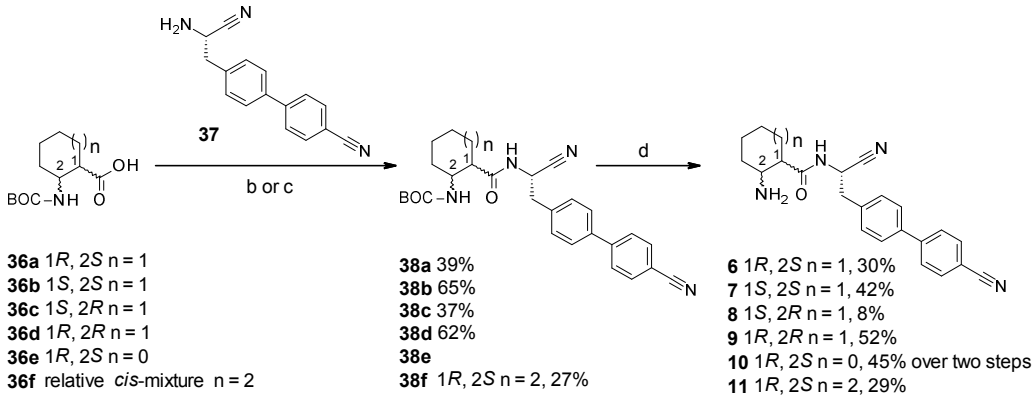
protected amino acids and (*S*)-4'-(2-amino-2-cyanoethyl)-[1,1'-biphenyl]-4-carbonitrile **37**[25] (Scheme 3). Compounds **6**, **8**, **9**, **11-13**, **18** and **19** were coupled using EDC·HCl and HOPO. The remaining compounds, compounds **7**, **10**, **14-17**, were coupled via T3P coupling conditions. Both methods afforded the BOC protected materials in good yield. Compound **11** was prepared from the relative *cis*-mixture of 2-((*tert*-butoxycarbonyl)amino)cycloheptanecarboxylic acid. The two diastereoisomers were separated by silica gel column chromatography after the amide coupling step and the absolute stereochemistry assigned arbitrarily by data comparison with analogous *cis* $\beta$-amino acids previously synthesized. The $\beta$-amino acid for compound **16** was prepared by hydrolysis of 1-*tert*-butyl 3-ethyl azepane-1,3-dicarboxylate **34** which was in turn synthesized according to the route described in the literature.[29]

**Scheme 3.** Synthetic Scheme for the Preparation of Compounds **6** – **19**[a]

Non-commercial $\beta$ - amino acid synthesis:



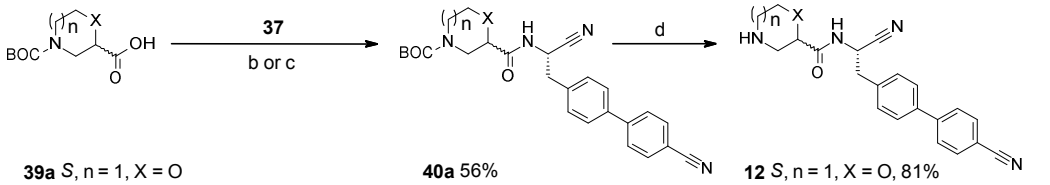

Exo-cyclic and cyclic $\beta$ -amino acid target compounds:




**36a** 1*R*, 2*S* n = 1
**36b** 1*S*, 2*S* n = 1
**36c** 1*S*, 2*R* n = 1
**36d** 1*R*, 2*R* n = 1
**36e** 1*R*, 2*S* n = 0
**36f** relative *cis*-mixture n = 2

**38a** 39%
**38b** 65%
**38c** 37%
**38d** 62%
**38e**
**38f** 1*R*, 2*S* n = 2, 27%

**6** 1*R*, 2*S* n = 1, 30%
**7** 1*S*, 2*S* n = 1, 42%
**8** 1*S*, 2*R* n = 1, 8%
**9** 1*R*, 2*R* n = 1, 52%
**10** 1*R*, 2*S* n = 0, 45% over two steps
**11** 1*R*, 2*S* n = 2, 29%




**39a** *S*, n = 1, X = O
**39b** *R*, n = 1, X = O
**39c** *S*, n = 1, X = $CH_2$
**39d** *R*, n = 1, X = $CH_2$
**39e** racemate, n = 2, X = $CH_2$
**39f** racemate, n = 2, X = O
**39g** *S*, n = 2, X = O
**39h** *R*, n = 2, X = O

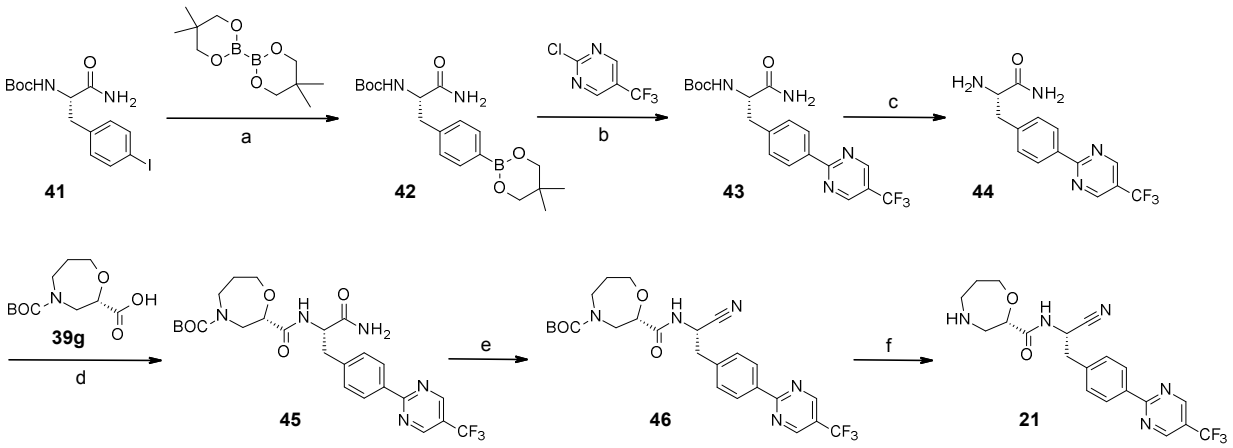
**40a** 56%
**40b** 96%
**40c**
**40d**
**40e** 49%
**40f**
**40g** 54%
**40h** 89%

**12** *S*, n = 1, X = O, 81%
**13** *R*, n = 1, X = O, 43%
**14** *S*, n = 1, X = $CH_2$, 46% over two steps
**15** *R*, n = 1, X = $CH_2$, 46% over two steps
**16** racemate, n = 2, X = $CH_2$, 41%
**17** racemate, n = 2, X = O, 50% over two steps
**18** *S*, n = 2, X = O, 77%
**19** *R*, n = 2, X = O, 44%

[a]Reagents and conditions: (a) LiOH, MeOH, $H_2O$, rt, 2 h, quantitative yield; (b) **37**, EDC·HCl, HOPO, DCM, rt, 18 h; (c) **37**, T3P, $Et_3N$, DMF, rt, 18 h; (d) $HCO_2H$, 50 °C, 10 min.

The preparation of the oxazepane series of compounds **21-33** in Table 5 were prepared by one of four routes. Compound **21** was prepared from (*S*)-*tert*-butyl (1-amino-3-(4-iodophenyl)-1-oxopropan-2-yl)carbamate (**41**)[30] through palladium catalyzed conversion of the iodide to the boronate ester **42** and subsequent reaction with 2-chloro-5-(trifluoromethyl)pyrimidine to afford **43**. This was deprotected with TFA and coupled using EDC·HCl coupling conditions. The primary amide **45** was dehydrated with Burgess reagent and deprotected using conditions previously discussed to afford compound **21** (Scheme 4).

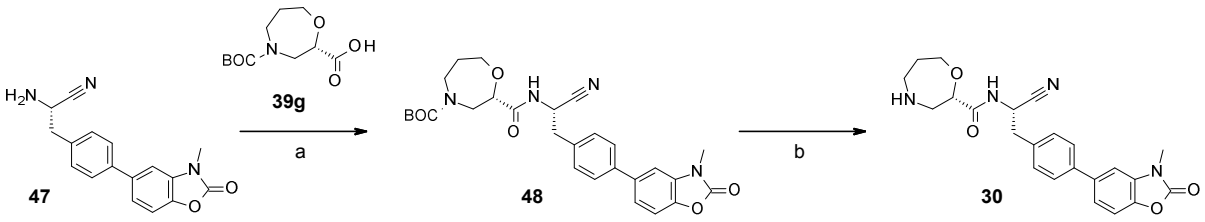
**Scheme 4.** Synthetic Scheme for the Preparation of Compound **21**[a]




[a]Reagents and conditions: (a) bis(neopentyl glycolato)diboron, Pd(dppf)$Cl_2$·$CH_2Cl_2$·KOAc, DMSO, 85 °C, 4 h, 57%; (b) Pd(dppf)$Cl_2$·$CH_2Cl_2$. $K_2CO_3$, MeCN, $H_2O$, 90 °C, 3 h, 22%; (c) TFA, DCM, rt, 1.5 h; (d) **39g**, EDC·HCl, HOPO, DIPEA, DCM, rt; (e) $Et_3N^+SO_2N^-CO_2Me$, DCM, rt, 18 h, 56%; (f) $HCO_2H$, 50 °C, 10 min, 58%.

Compound **30** was prepared in good yield by coupling of (*S*)-2-amino-3-(4-(3-methyl-2-oxo-2,3-dihydrobenzo[*d*]oxazol-5-yl)phenyl)propanenitrile **47**[25] and commercially available (*S*)-4-(tert-butoxycarbonyl)-1,4-oxazepane-2-carboxylic acid **39g**, followed by protecting group deprotection (Scheme 5).

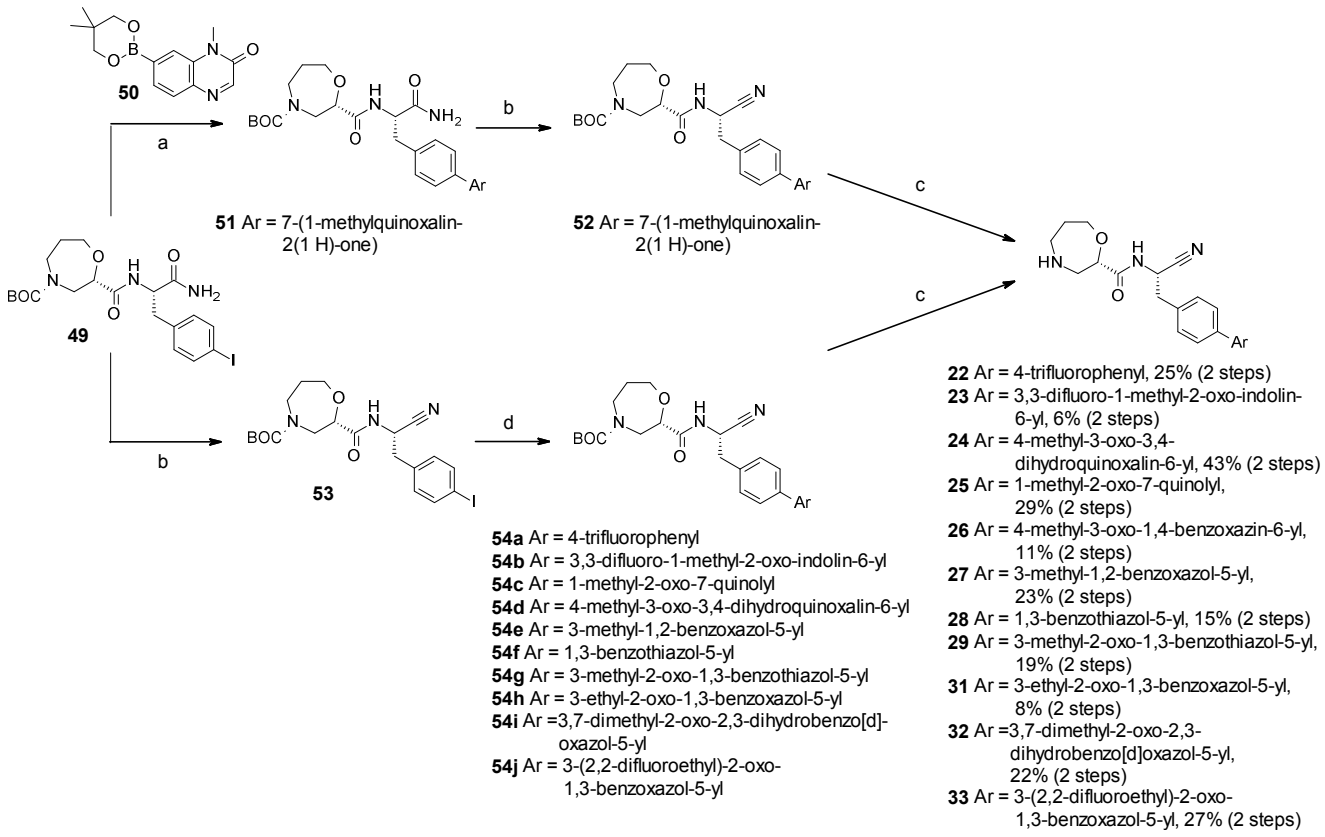
**Scheme 5.** Synthetic Scheme for the Preparation of Compound **30**[a]




[a]Reagents and conditions: (a) EDC·HCl, HOPO, DIPEA, DCM, rt, 18 h, 44%; (b) $HCO_2H$, 50 °C, 10 min, 64%.

The majority of the compounds in Table 5 were prepared from common intermediate **49**.[25] The primary amide was either dehydrated and the resultant aryl iodide **53**[25] was coupled with the appropriate boronate ester, or the Suzuki coupling was performed first followed by the dehydration step (Scheme 6). In all cases the BOC deprotection was the final step. Eight boronate esters were not commercially available and therefore required synthesis in order to prepare compounds **23**, **25-26**, **29**, **31-33**. The synthesis of these boronate esters was achieved according to the procedures as previously described.[25]

**Scheme 6.** Synthetic Scheme for the Preparation of Compounds **22-29** and **31-33**[a]




[a]Reagents and conditions: (a) $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, $K_2CO_3$, MeCN, $H_2O$, 80 °C, 1 h, 100%; (b) $Et_3N^+SO_2N^-CO_2Me$, DCM, rt, 18 h, 54%; c) $HCO_2H$, 50 °C, 10 min; (d) Aryl boronic acid or ester, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, $K_2CO_3$, MeCN, $H_2O$, 80 °C, 1 h.

## Conclusion

We herein describe the discovery of a second generation series of DPP1 inhibitors, which are free from the aorta binding liabilities found for our first generation clinical candidate. This work culminated in the identification of compound **30**, as a reversible, highly potent and selective, clinical candidate with predicted human PK properties suitable for once daily human dosing. Compound **30** is currently in clinical development for COPD. Human phase 1 studies were started in Q4 2014 and further progress will be reported in due course.

## EXPERIMENTAL SECTION

**General Procedures.** All chemicals purchased from commercial suppliers where used as received. Flash chromatography was carried out with prepacked $SiO_2$ SNAP cartridges (KP-SIL) from Biotage using a Biotage Isolera Four system using gradient elution. Analytical thin-layer chromatography (TLC) was performed on silica using Polygram®SIL G/UV254 with fluorescent indicator (200 μm thickness), and visualized under UV light. $^1$H NMR spectra were recorded on a Bruker AV 400 ($^1$H = 400.13MHz) instrument spectrometer and referenced in $CDCl_3$ to tetramethylsilane (0.00 ppm) and in DMSO-$d_6$ referenced to DMSO-$d_6$ (2.50 ppm). The following abbreviations are used: s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, q = quartet, m = multiplet. $^{13}$C NMR spectra were recorded on a Bruker AV 500 ($^{13}$C = 126 MHz) instrument spectrometer and referenced to DMSO-$d_6$ (39.5 ppm). Preparative HPLC was performed on a Waters Sunfire column eluting with a gradient of acetonitrile in aqueous sodium bicarbonate or trifluoroacetic acid solution. All final compounds were purified to >95% chemical purity as assayed by HPLC/MS. HRMS experiments were performed on a Waters Acquity, Waters 2777, or Waters 2700 UV-HPLC system and a Waters Xevo G2 TOF, Waters LCT Premiere, Waters LCT, or Waters QTOFmicro mass spectrometer. Compounds were named with the aid of the Cambridgesoft Chemistry Cartridge (v. 9.0.0.182) software. All reactions involving air or moisture-sensitive reagents were performed under a nitrogen atmosphere using dried solvents and glassware. Calculated pKa values were obtained using ACD software, version 12.0. Measured pKa values were obtained using a Sirius GLpKa instrument equipped with a Dip Probe Absorption Spectroscopy (DPAS) attachement. hERG $IC_{50}$ values were determined using electrophysiology IonWorks technology.[31]

**Amide coupling Method A:**

**_tert_-Butyl((1*S*,2*R*)-2-(((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)carbamoyl) cyclohexyl)carbamate (38a).**

*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (321 mg, 1.68 mmol) and 2-pyridinol 1-oxide (186 mg, 1.68 mmol) were added to a solution of (1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexanecarboxylic acid (334 mg, 1.37 mmol) in DCM (10 mL). The reaction was stirred at rt for 30 min before the addition of (*S*)-4'-(2-amino-2-cyanoethyl)-[1,1'-biphenyl]-4-carbonitrile **37** (339 mg, 1.37 mmol, prepared as described,[25] and *N*,*N*-diisopropylethylamine (0.38 mL, 2.10 mmol). The reaction was stirred at rt for 18 h before transferring to a separating funnel. The mixture was washed with 2 M hydrochloric acid, saturated sodium hydrogen carbonate solution and brine. The organic extract was dried (phase separator) and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 0-30% ethyl acetate in *iso*-hexane to afford the subtitled compound as a yellow oil (254 mg, 39%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.74 (d, *J*=8.5 Hz, 2H), 7.68 (d, *J*=8.4 Hz, 2H), 7.60 (d, *J*=8.3 Hz, 2H), 7.44 (d, *J*=8.2 Hz, 2H), 6.38 (s, 1H), 5.19 - 5.11 (m, 1H), 5.08 - 5.02 (m, 1H), 4.01 (s, 1H), 3.19 - 3.05 (m, 2H), 2.56 - 2.50 (m, 1H), 1.93 - 1.82 (m, 2H), 1.67 - 1.50 (m, 4H), 1.45 (s, 9H), 1.41 - 1.33 (2H, m).

**BOC deprotection conditions Method B:**

**(1*R*,2*S*)-2-Amino-*N*-((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)cyclohexane carboxamide (6).**

*tert*-Butyl((1*S*,2*R*)-2-(((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)carbamoyl)cyclohexyl) carbamate (**38a**) (249 mg, 0.53 mmol) was dissolved in formic acid (3 mL) and heated at 50 °C for 10 min on a pre-heated stirrer hotplate. After this time the reaction was concentrated under

reduced pressure, dissolved in DCM and washed with saturated sodium hydrogen carbonate solution. The organic extract was dried (phase separator) and concentrated under reduced pressure. The solid was purified by silica gel column chromatography eluting with 0-3% methanolic ammonia (7N) in DCM to afford the subtitled compound as a white solid (60 mg, 30%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 10.16 (d, *J*=8.0 Hz, 1H), 7.73 (d, *J*=8.4 Hz, 2H), 7.67 (d, *J*=8.3 Hz, 2H), 7.57 (d, *J*=8.2 Hz, 2H), 7.41 (d, *J*=8.0 Hz, 2H), 5.25 (q, *J*=7.5 Hz, 1H), 3.26 - 3.20 (m, 1H), 3.16 (d, *J*=7.0 Hz, 2H), 2.43 - 2.37 (m, 1H), 1.90 - 1.82 (m, 1H), 1.44 - 1.37 (m, 7H) (two exchangeable protons not observed). HRMS $C_{23}H_{24}N_4O$ $(M+H)^+$ calculated mass: 373.2028, found: 373.2023.

The following compounds were prepared in the analogous fashion starting from the commercially available *β*-amino acids:

**(1*S*,2*R*)-2-Amino-*N*-((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl) cyclohexanecarboxamide (8).**

Prepared from (1*S*,2*R*)-2-((*tert*-butoxycarbonyl)amino)cyclohexanecarboxylic acid following methods A and B to afford the subtitled compound as a white solid (5 mg, 3% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 10.17 (d, *J*=7.9 Hz, 1H), 7.73 (d, *J*=8.4 Hz, 2H), 7.68 (d, *J*=8.5 Hz, 2H), 7.57 (d, *J*=8.3 Hz, 2H), 7.41 (d, *J*=8.2 Hz, 2H), 5.19 (dt, *J*=6.5, 7.8 Hz, 1H), 3.23 - 3.09 (m, 3H), 2.42 - 2.36 (m, 1H), 1.95 - 1.87 (m, 1H), 1.58 - 1.38 (m, 7H) (two exchangeable protons not observed).

**(1*R*,2*R*)-2-Amino-*N*-((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)cyclohexanecarboxamide (9).**

Prepared from (1*R*,2*R*)-2-((*tert*-butoxycarbonyl)amino)cyclohexanecarboxylic acid following methods A and B to afford the subtitled compound as a white solid (47 mg, 32% over two steps).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.86 (d, *J*=8.2 Hz, 1H), 7.73 (d, *J*=8.4 Hz, 2H), 7.67 (d, *J*=8.3 Hz, 2H), 7.58 (d, *J*=8.2 Hz, 2H), 7.42 (d, *J*=8.2 Hz, 2H), 5.20 (q, *J*=7.4 Hz, 1H), 3.20 - 3.09 (m, 2H), 2.67 (dt, *J*=4.6, 10.5 Hz, 1H), 2.20 - 2.12 (m, 1H), 1.92 - 1.69 (m, 4H), 1.35 - 1.08 (m, 4H) (two exchangeable protons not observed). HRMS $C_{23}H_{24}N_4O$ $(M+H)^+$ calculated mass: 373.2028, found: 373.2018.

**(1*R*,2*S*)-2-Amino-*N*-((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)cycloheptane carboxamide (11).**

**i) *tert*-Butyl ((1*S*,2*R*)-2-(((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)carbamoyl) cycloheptyl)carbamate (38f).**

Prepared from (±)-*cis*-2-((*tert*-butoxycarbonyl)amino) cycloheptanecarboxylic acid following method A. The diastereoisomers were separated by silica gel column chromatography eluting with 30% ethyl acetate in *iso-hexane*. The first eluting compound corresponded to the subtitled compound, isolated as a white solid (129 mg, 27%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.74 (d, *J*=8.5 Hz, 2H), 7.68 (d, *J*=8.5 Hz, 2H), 7.60 (d, *J*=8.3 Hz, 2H), 7.43 (d, *J*=8.3 Hz, 2H), 6.55 (s, 1H), 5.17 - 5.11 (m, 1H), 4.89 - 4.86 (m, 1H), 4.07 - 4.04 (m, 1H), 3.19 - 3.05 (m, 2H), 2.73 - 2.67 (m, 1H), 2.01 - 1.85 (m, 2H), 1.79 - 1.60 (m, 5H), 1.52 - 1.46 (m, 3H), 1.44 (s, 9H).

**ii) (1*R*,2*S*)-2-Amino-*N*-((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)cycloheptane carboxamide (11).**

Prepared from *tert*-butyl ((1*S*,2*R*)-2-(((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)carbamoyl) cycloheptyl)carbamate according to the procedure in method B to afford the subtitled compound as a white solid (43 mg, 29%). The relative stereochemistry was inferred by data comparison to other compounds prepared. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.54 (d, *J*=8.3 Hz, 1H), 7.73 (d, *J*=8.5 Hz, 2H), 7.67 (d, *J*=8.5 Hz, 2H), 7.57 (d, *J*=8.4 Hz, 2H), 7.42 (d, *J*=8.3 Hz,

2H), 5.25 - 5.18 (m, 1H), 3.36 - 3.31 (m, 1H), 3.15 (d, *J*=7.2 Hz, 2H), 2.48 - 2.44 (m, 1H), 1.94 - 1.85 (m, 1H), 1.70 - 1.43 (9H, m) (two exchangeable protons not observed). HRMS $C_{24}H_{26}N_4O$ $(M+H)^+$ calculated mass: 387.2185, found: 387.2172.

**(*S*)-*N*-((*S*)-1-Cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)morpholine-2-carboxamide (12).** Prepared from (*S*)-4-(*tert*-butoxycarbonyl)morpholine-2-carboxylic acid following methods A and B to afford the subtitled compound as a white solid (79 mg, 45% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.74 (d, *J*=8.4 Hz, 2H), 7.68 (d, *J*=8.5 Hz, 2H), 7.60 (d, *J*=8.3 Hz, 2H), 7.40 (d, *J*=8.3 Hz, 2H), 6.95 (d, *J*=8.9 Hz, 1H), 5.21 - 5.14 (m, 1H), 3.99 (dd, *J*=2.9, 10.4 Hz, 1H), 3.93 - 3.88 (m, 1H), 3.63 (ddd, *J*=4.4, 9.6, 11.3 Hz, 1H), 3.30 (dd, *J*=2.8, 12.4 Hz, 1H), 3.19 - 3.11 (m, 2H), 2.87 - 2.79 (m, 2H), 2.58 (dd, *J*=10.5, 12.4 Hz, 1H) (one exchangeable proton not observed). HRMS $C_{21}H_{20}N_4O_2$ $(M+H)^+$ calculated mass: 361.1664, found: 361.1666.

**(*R*)-*N*-((*S*)-1-Cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)morpholine-2-carboxamide (13).** Prepared from (*R*)-4-(*tert*-butoxycarbonyl)morpholine-2-carboxylic acid following methods A and B to afford the subtitled compound as a white solid (74 mg, 41% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.74 (d, *J*=8.5 Hz, 2H), 7.68 (d, *J*=8.4 Hz, 2H), 7.60 (d, *J*=8.3 Hz, 2H), 7.41 (d, *J*=8.4 Hz, 2H), 6.97 (d, *J*=8.9 Hz, 1H), 5.22 - 5.14 (m, 1H), 4.02 - 3.88 (m, 2H), 3.69 - 3.61 (m, 1H), 3.34 - 3.28 (m, 1H), 3.22 - 3.08 (m, 2H), 2.85 (dd, J=2.6, 8.0 Hz, 2H), 2.69 (dd, *J*=10.4, 12.4 Hz, 1H) (one exchangeable proton not observed). HRMS $C_{21}H_{20}N_4O_2$ $(M+H)^+$ calculated mass: 361.1664, found: 361.1662.

**(*S*)-*N*-((*S*)-1-Cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)-1,4-oxazepane-2-carboxamide (18).**

Prepared from (*S*)-4-(*tert*-butoxycarbonyl)-1,4-oxazepane-2-carboxylic acid following methods A and B to afford the subtitled compound as a white solid (439 mg, 41% over two steps). $^1$H

NMR (400 MHz, $CDCl_3$) $\delta$ 7.74 (d, *J*=8.4 Hz, 2H), 7.68 (d, *J*=8.4 Hz, 2H), 7.59 (d, *J*=8.2 Hz, 2H), 7.44 (d, *J*=8.2 Hz, 2H), 7.20 (d, *J*=8.9 Hz, 1H), 5.24 - 5.17 (m, 1H), 4.09 (dd, *J*=4.0, 6.5 Hz, 1H), 4.03 - 3.95 (m, 1H), 3.79 - 3.71 (m, 1H), 3.30 (dd, *J*=4.0, 14.4 Hz, 1H), 3.15 (d, *J*=6.7 Hz, 2H), 3.08 - 2.83 (m, 3H), 1.89 - 1.80 (m, 2H) (one exchangeable proton not observed). HRMS $C_{22}H_{22}N_4O_2$ $(M+H)^+$ calculated mass: 375.1821, found: 375.1856.

**(*R*)-*N*-((*S*)-1-Cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)-1,4-oxazepane-2-carboxamide (19).**

Prepared from (*R*)-4-(*tert*-butoxycarbonyl)-1,4-oxazepane-2-carboxylic acid following methods A and B to afford the subtitled compound as a white solid (220 mg, 39% over 2 steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.74 (d, *J*=8.5 Hz, 2H), 7.68 (d, *J*=8.5 Hz, 2H), 7.60 (d, *J*=8.3 Hz, 2H), 7.41 (d, *J*=8.2 Hz, 2H), 7.21 (d, *J*=8.9 Hz, 1H), 5.18 - 5.11 (m, 1H), 4.09 (dd, *J*=3.8, 7.3 Hz, 1H), 4.05 - 3.98 (m, 1H), 3.81 - 3.74 (m, 1H), 3.36 (dd, *J*=3.8, 14.4 Hz, 1H), 3.23 - 3.02 (m, 3H), 2.95 - 2.89 (m, 2H), 1.90 - 1.78 (m, 2H) (one exchangeable proton not observed). HRMS $C_{22}H_{22}N_4O_2$ $(M+H)^+$ calculated mass: 375.1821, found: 375.1840.

**Amide coupling Method C:**

**i) *tert*-Butyl 2-(((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)carbamoyl)-1,4-oxazepane-4-carboxylate (40f).**

*Rac*-4-(*tert*-butoxycarbonyl)-1,4-oxazepane-2-carboxylic acid (248 mg, 1.01 mmol) and (*S*)-4'-(2-amino-2-cyanoethyl)-[1,1'-biphenyl]-4-carbonitrile **37** (1200 mg, 0.81 mmol), prepared as previously described[25] were added to 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide solution (T3P, 700 mg, 50% solution in DMF) in DMF (2 mL). Triethylamine (640 µL, 4.54 mmol) was added and the reaction stirred at rt for 18 h. After this time the reaction mixture

was concentrated under reduced pressure. The resultant oil was dissolved in ethyl acetate and washed successively with 2 M aqueous hydrochloric acid, saturated aqueous solution of sodium hydrogen carbonate and sodium chloride. The organic extracts were dried (magnesium sulfate), filtered and concentrated under reduced pressure to afford the subtitled compound as a yellow oil which was used without further purification in the next step.

**ii) *N*-((*S*)-1-Cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)-1,4-oxazepane-2-carboxamide (17).**

Prepared according to procedure in Methods C and B using *tert*-butyl 2-(((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)carbamoyl)-1,4-oxazepane-4-carboxylate to afford the subtitled compound as a white solid (150 mg, 50% over two steps). The isolated compound was a mixture of two diastereomers, which were not separated. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.75 - 7.72 (m, 2H), 7.70 - 7.65 (m, 2H), 7.62 - 7.57 (m, 2H), 7.46 - 7.39 (m, 2H), 7.28 - 7.22 (m, 1H), 5.24 - 5.11 (m, 1H), 4.12 - 4.06 (m, 1H), 4.05 - 3.96 (m, 1H), 3.81 - 3.71 (m, 1H), 3.39 - 3.27 (m, 1H), 3.24 - 3.10 (m, 2H), 3.08 - 3.01 (m, 1H), 2.99 - 2.82 (m, 2H), 1.90 - 1.78 (m, 2H) (one exchangeable proton not observed). HRMS $C_{22}H_{22}N_4O_2$ $(M+H)^+$ calculated mass: 375.1821, found: 375.1824.

The following compounds were prepared in the analogous fashion staring from the commercially available $\beta$-amino acids:

**(1*S*,2*S*)-2-Amino-*N*-((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)cyclohexanecarboxamide (7).**

Prepared from (1*S*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclohexanecarboxylic acid following methods C and B to afford the subtitled compound as a white solid (40 mg, 27% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.98 (d, *J*=8.0 Hz, 1H), 7.74 (d, *J*=8.4 Hz, 2H), 7.68 (d, *J*=8.4 Hz,

2H), 7.58 (d, *J*=8.2 Hz, 2H), 7.41 (d, *J*=8.2 Hz, 2H), 5.18 (q, *J*=7.2 Hz, 1H), 3.20 - 3.08 (m, 2H), 2.79 - 2.71 (m, 1H), 2.12 (dd, *J*=2.8, 13.9 Hz, 1H), 1.94 - 1.70 (m, 4H), 1.38 - 1.09 (m, 4H) (two exchangeable protons not observed). HRMS $C_{23}H_{24}N_4O$ $(M+H)^+$ calculated mass: 373.2028, found: 373.2023.

**(1*R*,2*S*)-2-Amino-*N*-((*S*)-1-cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)cyclopentanecarboxamide (10).**

Prepared from (1*R*,2*S*)-2-((*tert*-butoxycarbonyl)amino)cyclopentanecarboxylic acid following methods C and B to afford the subtitled compound as a white solid (65 mg, 45% over two steps). $^1$H NMR (400 MHz, DMSO-$d_6$ @ 125 °C) $\delta$ 7.86 - 7.82 (m, 4H), 7.71 - 7.66 (m, 2H), 7.49 - 7.43 (m, 2H), 5.07 - 5.01 (m, 1H), 3.45 - 3.38 (m, 1H), 3.26 - 3.16 (m, 2H), 2.61 - 2.55 (m, 1H), 1.96 - 1.89 (m, 1H), 1.78 - 1.69 (m, 3H), 1.52 - 1.37 (m, 2H) (three exchangeable protons not observed). HRMS $C_{22}H_{22}N_4O$ $(M+H)^+$ calculated mass: 359.1872, found: 359.1877.

**(*S*)-*N*-((*S*)-1-Cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)piperidine-3-carboxamide (14).**

Prepared from (*S*)-1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid following methods C and B to afford the subtitled compound as a white solid (66 mg, 46% over 2 steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.33 (s, 1H), 7.74 (d, *J*=8.4 Hz, 2H), 7.68 (d, *J*=8.4 Hz, 2H), 7.58 (d, *J*=8.2 Hz, 2H), 7.41 (d, *J*=8.2 Hz, 2H), 5.25 - 5.18 (m, 1H), 3.22 - 3.11 (m, 2H), 3.08 - 2.95 (m, 2H), 2.84 - 2.78 (m, 1H), 2.67 - 2.63 (m, 1H), 2.46 - 2.43 (m, 1H), 2.00 - 1.95 (m, 1H), 1.70 - 1.47 (m, 4H). HRMS $C_{22}H_{22}N_4O$ $(M+H)^+$ calculated mass: 359.1872, found: 359.1880.

**(*R*)-*N*-((*S*)-1-Cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)piperidine-3-carboxamide (15).**

Prepared from (*R*)-1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid following method C and B to afford the subtitled compound as a white solid (66 mg, 46% over 2 steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.24 (s, 1H), 7.74 (d, *J*=8.4 Hz, 2H), 7.67 (d, *J*=8.4 Hz, 2H), 7.58 (d, *J*=8.2 Hz,

2H), 7.44 (d, *J*=8.3 Hz, 2H), 5.28 - 5.21 (m, 1H), 3.22 - 3.18 (m, 2H), 3.18 - 3.10 (m, 1H), 2.96 - 2.89 (m, 1H), 2.82 - 2.75 (m, 1H), 2.68 - 2.59 (m, 1H), 2.49 - 2.45 (m, 1H), 1.93 - 1.85 (m, 1H), 1.71 - 1.58 (m, 2H), 1.44 - 1.33 (m, 2H). HRMS $C_{22}H_{22}N_4O$ $(M+H)^+$ calculated mass: 359.1872, found: 359.1858.

**1-(*tert*-Butoxycarbonyl)azepane-3-carboxylic acid (35).**

Lithium hydroxide (233 mg, 5.55 mmol) was added to a solution of 1-*tert*-butyl 3-ethyl azepane-1,3-dicarboxylate **34**[29] (0.5 g, 1.84 mmol) in methanol (25 mL) and water (5 mL) at rt with stirring. After 2 h the reaction mixture was concentrated under reduced pressure. The resultant residue was dissolved in water and ethyl acetate and acidified with 2M hydrochloric acid. The layers were separated and the organic extracts washed with brine, dried (magnesium sulfate), filtered and concentrated under reduced pressure to afford a brown oil (0.46 g, >100%). Used without further purification in the next step.

***N*-((*S*)-1-Cyano-2-(4'-cyano-[1,1'-biphenyl]-4-yl)ethyl)azepane-3-carboxamide (16).**

Prepared *via* Method C and B starting from 1-(*tert*-butoxycarbonyl)azepane-3-carboxylic acid **35** to afford the subtitled compound as a white solid (30 mg, 20% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.07 - 8.96 (m, 1H), 7.74 (d, *J*=8.2 Hz, 2H), 7.67 (dd, *J*=2.6, 8.6 Hz, 2H), 7.58 (d, *J*=7.3 Hz, 2H), 7.42 (dd, *J*=8.3, 8.3 Hz, 2H), 5.25 - 5.16 (m, 1H), 3.25 - 2.96 (m, 4H), 2.76 (dd, *J*=3.5, 14.0 Hz, 1H), 2.68 - 2.58 (m, 1H), 2.54 - 2.45 (m, 1H), 1.98 - 1.89 (m, 1H), 1.77 - 1.47 (m, 5H) (one exchangeable proton not observed).

**(*S*)-*tert*-Butyl (1-amino-3-(4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)-1-oxopropan-2-yl)carbamate (42).**

Bis(neopentyl glycolato)diboron (1.51 g, 6.7 mmol) and potassium acetate (1.51 g, 15.42 mmol) were added to a solution of *tert*-butyl (*S*)-(1-amino-3-(4-iodophenyl)-1-oxopropan-2-

yl)carbamate **41**[30] (2.00 g, 5.14 mmol) in DMSO (10 mL). The reaction mixture was degassed under nitrogen for 15 min before [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (210 mg, 0.26 mmol, complex with DCM) was added. The reaction mixture was heated at 85 °C for 4 h. After this time the reaction mixture was cooled to rt and poured onto water (60 mL). The resulting mixture was extracted with ethyl acetate (3 × 60 mL). The combined extracts were washed with brine (60 mL), and separated. The organic extracts were dried (phase separator) and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 0 - 80% ethyl acetate in *iso*-hexane to afford the subtitled compound as a colorless oil (1.12 g, 57%).

***tert*-Butyl (*S*)-(1-amino-1-oxo-3-(4-(5-(trifluoromethyl)pyrimidin-2-yl)phenyl)propan-2-yl)carbamate (43).**

(*S*)-*tert*-Butyl (1-amino-3-(4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)-1-oxopropan-2-yl)carbamate **42** (1.12 g, 2.90 mmol) and 2-chloro-5-trifluoromethyl pyrimidine (0.53 g, 2.90 mmol) were dissolved in acetonitrile (25 mL) and water (2.5 mL). The reaction mixture was degassed under nitrogen for 10 min before potassium carbonate (600 mg, 4.35 mmol) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (235 mg, 0.29 mmol, complex with DCM) were added. The reaction mixture was heated at 90 °C for 3 h. After this time the reaction was concentrated under reduced pressure. The residue was triturated with DCM. The filtrate was purified by silica gel column chromatography eluting with a gradient of 0 - 100% ethyl acetate in *iso*-hexane to afford the subtitled compound as a yellow solid (260 mg, 21%). Used without further purification in the next step.

**(*S*)-2-Amino-3-(4-(5-(trifluoromethyl)pyrimidin-2-yl)phenyl)propanamide trifluoroacetic acid salt (44).**

A solution of 30% TFA in DCM (13 mL) was added to *tert*-butyl (*S*)-(1-amino-1-oxo-3-(4-(5-(trifluoromethyl)pyrimidin-2-yl)phenyl)propan-2-yl)carbamate **43** (260 mg, 0.63 mmol) and the reaction stirred at room temperature for 1.5 h. The solvents were removed under reduced pressure and the resultant salt co-evaporated with toluene (2 × 20 mL) to yield the subtitled compound as a tan solid. Used without further purification in the next step.

**(*S*)-*tert*-Butyl 2-(((*S*)-1-amino-1-oxo-3-(4-(5-(trifluoromethyl)pyrimidin-2-yl)phenyl)propan-2-yl)carbamoyl)-1,4-oxazepane-4-carboxylate (45).**

*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (147 mg, 0.77 mmol) and 2-pyridinol 1-oxide (86 mg, 0.77 mmol) were added to a solution of (*S*)-4-(*tert*-butoxycarbonyl)-1,4-oxazepane-2-carboxylic acid (154 mg, 0.63 mmol) in DCM (5 mL) and DMF (1 mL). *N*,*N*-di*iso*propylethylamine (0.17 mL, 0.97 mmol) and (*S*)-2-amino-3-(4-(5-(trifluoromethyl)pyrimidin-2-yl)phenyl)propanamide trifluoroacetic acid salt **44** (0.63 mmol) were added and the reaction was stirred at rt for 18 h before transferring to a separating funnel. The mixture was diluted with ethyl acetate (50 mL) then washed with 2 M hydrochloric acid (20 mL) and saturated sodium hydrogen carbonate solution (20 mL). The organic extract was dried (phase separator) and concentrated under reduced pressure. The crude material was used without further purification in the next step.

***tert*-Butyl (*S*)-2-(((*S*)-1-cyano-2-(4-(5-(trifluoromethyl)pyrimidin-2-yl)phenyl)ethyl)carbamoyl)-1,4-oxazepane-4-carboxylate (46).**

Burgess reagent (167 mg, 0.70 mmol) was added to a solution of (*S*)-*tert*-butyl 2-(((*S*)-1-amino-1-oxo-3-(4-(5-(trifluoromethyl)pyrimidin-2-yl)phenyl)propan-2-yl)carbamoyl)-1,4-oxazepane-4-carboxylate **45** (280 mg, 0.52 mmol) in DCM (10 mL) at rt with stirring. After 18 h the reaction was diluted with water. The layers were separated and the aqueous phase extracted with DCM.

The combined organic extracts were dried (phase separator) and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography eluting with 0-40% ethyl acetate in *iso*-hexane to afford the subtitled compound as a colorless oil (150 mg, 56%). NMR broad used in the next step without further purification.

**(*S*)-*N*-((*S*)-1-Cyano-2-(4-(5-(trifluoromethyl)pyrimidin-2-yl)phenyl)ethyl)-1,4-oxazepane-2-carboxamide (21).**

*tert*-Butyl (*S*)-2-(((*S*)-1-cyano-2-(4-(5-(trifluoromethyl) pyrimidin-2-yl)phenyl)ethyl) carbamoyl)-1,4-oxazepane-4-carboxylate **46** (150 mg, 0.29 mmol) was dissolved in formic acid (3 mL) and heated at 50 °C for 10 min on a pre-heated stirrer hotplate. After this time the reaction was concentrated under reduced pressure, dissolved in DCM and washed with saturated sodium hydrogen carbonate solution. The organic extract was run through a hydrophobic frit and concentrated under reduced pressure. The resultant foam was purified by silica gel column chromatography eluting with 0 - 5% methanolic ammonia (7N) in DCM to afford the title compound as a white solid (70 mg, 58%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.03 (s, 2H), 8.51 (d, *J*=8.4 Hz, 2H), 7.48 (d, *J*=8.4 Hz, 2H), 7.18 (d, *J*=9.0 Hz, 1H), 5.27 - 5.20 (m, 1H), 4.09 (dd, *J*=3.9, 6.5 Hz, 1H), 4.02 - 3.95 (m, 1H), 3.78 - 3.70 (m, 1H), 3.29 (dd, *J*=4.0, 14.4 Hz, 1H), 3.19 (d, *J*=6.5 Hz, 2H), 3.06 - 2.92 (m, 2H), 2.90 - 2.82 (m, 1H), 1.88 - 1.78 (m, 2H) (one exchangeable proton not observed). HRMS $C_{20}H_{20}F_3N_5O_2$ $(M+H)^+$ calculated mass: 420.1647, found: 420.1641.

**(*S*)-*tert*-Butyl 2-(((*S*)-1-amino-3-(4-(4-methyl-3-oxo-3,4-dihydroquinoxalin-6-yl)phenyl)-1-oxopropan-2-yl)carbamoyl)-1,4-oxazepane-4-carboxylate (51).**

7-(5,5-Dimethyl-1,3,2-dioxaborinan-2-yl)-1-methylquinoxalin-2(1*H*)-one **50**[25] (100 mg, 0.37 mmol) and (*S*)-*tert*-butyl 2-(((*S*)-1-amino-3-(4-iodophenyl)-1-oxopropan-2-yl)carbamoyl)-1,4-

oxazepane-4-carboxylate **49**[25] (182 mg, 0.35 mmol) were dissolved in acetonitrile (9 mL) and water (0.4 mL). The reaction mixture was degassed under nitrogen for 30 min before the addition of potassium carbonate (73 mg, 0.53 mmol) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (29 mg, 0.035 mmol, complex with DCM). The reaction mixture was heated at 80 °C for 1 h. After this time the reaction was concentrated under reduced pressure, purified by silica gel column chromatography eluting with 8% methanol in ethyl acetate to afford the subtitled compound as a brown oil (192 mg, 100%). Used without further purification in the next step.

**(*S*)-*tert*-Butyl 2-(((*S*)-1-cyano-2-(4-(4-methyl-3-oxo-3,4-dihydroquinoxalin-6-yl)phenyl)ethyl)carbamoyl)-1,4-oxazepane-4-carboxylate (52).**

1-Methoxy-*N*-triethylammoniosulfonyl-methanimidate (Burgess reagent, 167 mg, 0.70 mmol) was added to a solution of (*S*)-*tert*-butyl 2-(((*S*)-1-amino-3-(4-(4-methyl-3-oxo-3,4-dihydroquinoxalin-6-yl)phenyl)-1-oxopropan-2-yl)carbamoyl)-1,4-oxazepane-4-carboxylate **51** (192 mg, 0.35 mmol) in DCM (15 mL). The reaction mixture was stirred at rt for 24 h, after which the reaction was transferred to a separating funnel and washed with water. The organic extracts were dried (phase separator cartridge) and concentrated under reduced pressure. The resultant solid was purified by silica gel column chromatography eluting with 65% ethyl acetate in *iso*-hexane to afford a yellow oil. Trituration with diethyl ether afforded the subtitled compound as an oil (101 mg, 54%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.32 (s, 1H), 7.95 (d, *J*=8.4 Hz, 1H), 7.66 (d, *J*=8.0 Hz, 2H), 7.57 (dd, *J*=1.6, 8.3 Hz, 1H), 7.49 (d, *J*=1.8 Hz, 1H), 7.43 (d, *J*=8.2 Hz, 2H), 7.13 - 7.12 (m, 1H), 5.26 - 5.13 (m, 1H), 4.23 - 3.99 (m, 3H), 3.77 (s, 3H), 3.57 - 3.33 (m, 3H), 3.27 - 3.09 (m, 3H), 2.02 - 1.82 (m, 2H), 1.47 (s, 9H).

**(*S*)-*N*-((*S*)-1-Cyano-2-(4-(4-methyl-3-oxo-3,4-dihydroquinoxalin-6-yl)phenyl)ethyl)-1,4-oxazepane-2-carboxamide (24).**

Prepared according to method B starting from (*S*)-*tert*-butyl 2-(((*S*)-1-cyano-2-(4-(4-methyl-3-oxo-3,4-dihydroquinoxalin-6-yl)phenyl)ethyl)carbamoyl)-1,4-oxazepane-4-carboxylate **52** (101 mg, 0.19 mmol) to afford the title compound as a yellow solid (65 mg, 80%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.32 (s, 1H), 7.95 (d, *J*=8.3 Hz, 1H), 7.65 (d, *J*=8.3 Hz, 2H), 7.57 (dd, *J*=1.8, 8.3 Hz, 1H), 7.49 - 7.45 (m, 3H), 7.21 (d, *J*=9.1 Hz, 1H), 5.25 - 5.19 (m, 1H), 4.10 (dd, *J*=3.8, 6.6 Hz, 1H), 4.03 - 3.97 (m, 1H), 3.80 - 3.72 (m, 4H), 3.32 (dd, *J*=3.9, 14.3 Hz, 1H), 3.19 - 3.15 (m, 2H), 3.06 (dd, *J*=6.6, 14.4 Hz, 1H), 3.00 - 2.84 (m, 2H), 1.89 - 1.80 (m, 2H), (one exchangeable proton not observed). HRMS $C_{24}H_{25}N_5O_3$ $(M+H)^+$ calculated mass: 432.2036, found: 432.2035.

**Suzuki coupling Method D:**

**(*S*)-*tert*-Butyl 2-(((S)-1-cyano-2-(4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)ethyl)carbamoyl)-1,4-oxazepane-4-carboxylate (54a).**

4-Trifluoromethyl phenyl boronic acid (167 mg, 0.88 mmol) and (*S*)-tert-butyl 2-(((*S*)-1-cyano-2-(4-iodophenyl)ethyl)carbamoyl)-1,4-oxazepane-4-carboxylate **53**[25] (399 mg, 0.80 mmol) were dissolved in acetonitrile (20 mL) and water (0.8 mL). The reaction mixture was degassed under nitrogen for 15 min before the addition of potassium carbonate (166 mg, 1.20 mmol) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (65 mg, 0.08 mmol, complex with DCM). The reaction mixture was heated at 80 °C for 1.5 h. After this time the reaction was cooled to rt and concentrated under reduced pressure. The residue was dissolved in DCM and washed with water. The organic extracts were dried (hydrophobic frit) and reduced under reduced pressure. The resulting brown oil was purified by silica gel column chromatography

eluting with 25-40% ethyl acetate in *iso*-hexane to afford compound the title compound as a light brown solid (342 mg, 88%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.72 - 7.68 (m, 4H), 7.61 (d, *J*=8.0 Hz, 1H), 7.40 (d, *J*=7.9 Hz, 1H), 7.10 - 7.00 (m, 2H), 5.16 - 5.16 (m, 1H), 4.20 - 4.03 (m, 3H), 3.75 (s, 1H), 3.57 - 3.47 (m, 2H), 3.30 (d, *J*=8.8 Hz, 1H), 3.22 - 3.05 (m, 2H), 1.94 - 1.94 (m, 2H), 1.47 (s, 9H), (one exchangeable proton not observed).

**(*S*)-*N*-((*S*)-1-cyano-2-(4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)ethyl)-1,4-oxazepane-2-carboxamide (22).**

Prepared according to procedure in Method B to afford the subtitled compound as a white solid (57 mg, 25%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.73 - 7.66 (m, 4H), 7.60 (d, *J*=8.3 Hz, 2H), 7.43 (d, *J*=8.3 Hz, 2H), 7.20 (d, *J*=8.8 Hz, 1H), 5.24 - 5.18 (m, 1H), 4.09 (dd, *J*=3.9, 6.4 Hz, 1H), 4.02 - 3.95 (m, 1H), 3.78 - 3.71 (m, 1H), 3.30 (dd, *J*=4.0, 14.4 Hz, 1H), 3.15 (d, *J*=6.6 Hz, 2H), 3.04 (dd, *J*=6.2, 14.2 Hz, 1H), 2.99 - 2.83 (m, 2H), 1.88 - 1.79 (m, 2H), (one exchangeable proton not observed). HRMS $C_{22}H_{22}F_3N_3O_2$ $(M+H)^+$ calculated mass: 418.1742, found: 418.1732.

The following compounds were prepared in an analogous fashion using commercially available boronic acids or esters.

**(2*S*)-*N*-[(1*S*)-1-Cyano-2-[4-(3-methyl-1,2-benzoxazol-5-yl)phenyl]ethyl]-1,4-oxazepane-2-carboxamide (27).**

Prepared from 3-methyl benzoisoxazole-5-boronic acid pinacol ester according to methods D and B to afford the subtitled compound as a white solid (91 mg, 23% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.78 - 7.75 (m, 2H), 7.63 - 7.59 (m, 3H), 7.43 (d, *J*=8.2 Hz, 2H), 7.20 (d, *J*=9.0 Hz, 1H), 5.24 - 5.18 (m, 1H), 4.10 (dd, *J*=3.9, 6.5 Hz, 1H), 4.03 - 3.96 (m, 1H), 3.79 - 3.72 (m, 1H), 3.32 (dd, *J*=4.0, 14.4 Hz, 1H), 3.17 - 3.14 (m, 2H), 3.05 (dd, *J*=6.7, 14.5 Hz, 1H), 3.00 -

2.84 (m, 2H), 2.63 (s, 3H), 1.89 - 1.80 (m, 2H), (one exchangeable proton not observed). HRMS $C_{23}H_{24}N_4O_3$ $(M+H)^+$ calculated mass: 405.1927, found: 405.1915.

**(2*S*)-*N*-[(1*S*)-2-[4-(1,3-Benzothiazol-5-yl)phenyl]-1-cyano-ethyl]-1,4-oxazepane-2-carboxamide (28).**

Prepared from 5-benzothiazole boronic acid pinacol ester according to methods D and B to afford the subtitled compound as an off-white solid (50 mg, 15% over two steps). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.44 (s, 1H), 8.63 (d, *J*=8.5 Hz, 1H), 8.35 (d, *J*=1.5 Hz, 1H), 8.25 (d, *J*=8.4 Hz, 1H), 7.82 - 7.74 (m, 3H), 7.43 (d, *J*=8.2 Hz, 2H), 5.09 - 5.01 (m, 1H), 4.00 (dd, *J*=3.8, 7.8 Hz, 1H), 3.85 (ddd, *J*=4.4, 6.1, 12.4 Hz, 1H), 3.77 - 3.69 (m, 1H), 3.29 - 3.20 (m, 2H), 3.03 (dd, *J*=3.8, 14.3 Hz, 1H), 2.80 - 2.71 (m, 1H), 2.64 - 2.53 (m, 3H), 1.80 - 1.67 (m, 2H). HRMS $C_{22}H_{22}N_4O_2S$ $(M+H)^+$ calculated mass: 407.1541, found: 407.1518.

**(2*S*)-*N*-[(1*S*)-1-Cyano-2-[4-(3,3-difluoro-1-methyl-2-oxo-indolin-6-yl)phenyl]ethyl]-1,4-oxazepane-2-carboxamide (23).**

Prepared from 6-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-3,3-difluoro-1-methylindolin-2-one[25] according to methods D and B to afford the subtitled compound as a pale orange solid (10 mg, 6% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.63 - 7.56 (m, 2H), 7.44 (d, *J*=8.3 Hz, 2H), 7.37 (d, *J*=7.5 Hz, 1H), 7.20 (d, *J*=9.2 Hz, 1H), 7.06 (s, 1H), 5.24 - 5.17 (m, 1H), 4.10 (dd, *J*=3.9, 6.5 Hz, 1H), 4.03 - 3.97 (m, 1H), 3.79 - 3.72 (m, 1H), 3.34 - 3.29 (m, 4H), 3.18 - 3.15 (m, 2H), 3.10 - 2.84 (m, 3H), 1.88 - 1.81 (m, 2H), (two exchangeable protons not observed). HRMS $C_{24}H_{24}F_2N_4O_3$ $(M+H)^+$ calculated mass: 455.1895, found: 455.1913.

**(2*S*)-*N*-[(1*S*)-1-Cyano-2-[4-(1-methyl-2-oxo-7-quinolyl)phenyl]ethyl]-1,4-oxazepane-2-carboxamide (25).**

Prepared from 7-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-1-methylquinolin-2(1*H*)-one[25] according to methods D and B to afford the subtitled compound as a white solid (100 mg, 29% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.71 (d, *J*=9.4 Hz, 1H), 7.64 (dd, *J*=8.7, 8.7 Hz, 3H), 7.53 - 7.51 (m, 1H), 7.47 - 7.43 (m, 3H), 7.21 (d, *J*=9.2 Hz, 1H), 6.73 (d, *J*=9.4 Hz, 1H), 5.25 - 5.19 (m, 1H), 4.10 (dd, *J*=4.0, 6.5 Hz, 1H), 4.03 - 3.96 (m, 1H), 3.79 (s, 3H), 3.78 - 3.72 (m, 1H), 3.32 (dd, *J*=4.0, 14.4 Hz, 1H), 3.19 - 3.15 (m, 2H), 3.05 (dd, *J*=6.6, 14.4 Hz, 1H), 3.00 - 2.93 (m, 1H), 2.91 - 2.84 (m, 1H), 1.89 - 1.80 (m, 2H), (one exchangeable proton not observed). HRMS $C_{25}H_{26}N_4O_3$ $(M+H)^+$ calculated mass: 431.2083, found: 431.2091.

**(2*S*)-*N*-[(1*S*)-1-Cyano-2-[4-(4-methyl-3-oxo-1,4-benzoxazin-6-yl)phenyl]ethyl]-1,4-oxazepane-2-carboxamide (26).**

Prepared from 6-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-4-methyl-2H-benzo[b][1,4]oxazin-3(4*H*)-one[25] according to methods D and B to afford the subtitled compound as a white solid (20 mg, 11% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.54 (d, *J*=8.3 Hz, 2H), 7.40 (d, *J*=8.2 Hz, 2H), 7.23 - 7.17 (m, 2H), 7.15 (d, *J*=2.0 Hz, 1H), 7.06 (d, *J*=8.3 Hz, 1H), 5.23 - 5.16 (m, 1H), 4.66 (s, 2H), 4.10 (dd, *J*=4.0, 6.6 Hz, 1H), 4.02 - 3.95 (m, 1H), 3.79 - 3.71 (m, 1H), 3.43 (s, 3H), 3.31 (dd, *J*=3.9, 14.4 Hz, 1H), 3.16 - 3.12 (m, 2H), 3.04 (dd, *J*=6.7, 14.5 Hz, 1H), 2.99 - 2.91 (m, 1H), 2.91 - 2.83 (m, 1H), 1.88 - 1.80 (m, 2H), (one exchangeable proton not observed).

**(2*S*)-*N*-[(1*S*)-1-Cyano-2-[4-(3-methyl-2-oxo-1,3-benzothiazol-5-yl)phenyl]ethyl]-1,4-oxazepane-2-carboxamide (29).**

Prepared from 5-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-3-methylbenzo[d]thiazol-2(3*H*)-one[25] according to methods D and B to afford the subtitled compound as a white solid (50 mg, 19% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.59 (d, *J*=8.3 Hz, 2H), 7.50 (d, *J*=8.0 Hz, 1H), 7.44 - 7.37 (m, 3H), 7.23 - 7.18 (m, 2H), 5.24 - 5.18 (m, 1H), 4.10 (dd, *J*=4.0, 6.5 Hz, 1H), 4.03 -

3.96 (m, 1H), 3.79 - 3.72 (m, 1H), 3.52 (s, 3H), 3.31 (dd, *J*=3.9, 14.4 Hz, 1H), 3.17 - 3.14 (m, 2H), 3.05 (dd, *J*=6.7, 14.5 Hz, 1H), 3.00 - 2.83 (m, 2H), 1.89 - 1.80 (m, 2H), (one exchangeable proton not observed). HRMS $C_{23}H_{24}N_4O_3S$ $(M+H)^+$ calculated mass: 437.1647, found: 437.1639.

**(2*S*)-*N*-[(1*S*)-1-Cyano-2-[4-(3-ethyl-2-oxo-1,3-benzoxazol-5-yl)phenyl]ethyl]-1,4-oxazepane-2-carboxamide (31).**

Prepared from 5-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-3-ethylbenzo[d]oxazol-2(3*H*)-one[25] according to methods D and B to afford the subtitled compound as a white solid (20 mg, 8% over 2 steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.55 (d, *J*=8.0 Hz, 2H), 7.41 (d, *J*=8.0 Hz, 2H), 7.29 (dd, *J*=7.8, 16.7 Hz, 2H), 7.21 (d, *J*=8.9 Hz, 1H), 7.15 (s, 1H), 5.24 - 5.16 (m, 1H), 4.10 (dd, *J*=3.9, 6.4 Hz, 1H), 4.02 - 3.90 (m, 3H), 3.80 - 3.69 (m, 1H), 3.31 (dd, *J*=3.8, 14.4 Hz, 1H), 3.17 - 3.12 (m, 2H), 3.08 - 2.83 (m, 3H), 1.90 - 1.78 (m, 2H), 1.42 (t, *J*=7.2 Hz, 3H), (one exchangeable proton not observed). HRMS $C_{24}H_{26}N_4O_4$ $(M+H)^+$ calculated mass: 435.2032, found: 435.2026.

**(*S*)-*N*-((*S*)-1-Cyano-2-(4-(3,7-dimethyl-2-oxo-2,3-dihydrobenzo[d]oxazol-5-yl)phenyl)ethyl)-1,4-oxazepane-2-carboxamide (32).**

Prepared from 5-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-3,7-dimethylbenzo[d]oxazol-2(3*H*)-one[25] according to methods D and B to afford the subtitled compound as a white solid (54 mg, 22% over two steps). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.62 (d, *J*=8.5 Hz, 1H), 7.65 (d, *J*=8.3 Hz, 2H), 7.38 (d, *J*=8.2 Hz, 3H), 7.28 (s, 1H), 5.06 - 4.99 (m, 1H), 4.00 (dd, *J*=3.6, 7.9 Hz, 1H), 3.89 - 3.82 (m, 1H), 3.77 - 3.69 (m, 1H), 3.39 (s, 3H), 3.26 - 3.15 (m, 2H), 3.04 (dd, *J*=3.6, 14.3 Hz, 1H), 2.81 - 2.74 (m, 1H), 2.68 - 2.53 (m, 2H), 2.38 (s, 3H), 1.78 - 1.68 (m, 2H), (one exchangeable proton not observed). HRMS $C_{24}H_{26}N_4O_4$ $(M+H)^+$ calculated mass: 435.2032, found: 435.2048.

**(2*S*)-*N*-[(1*S*)-1-Cyano-2-[4-[3-(2,2-difluoroethyl)-2-oxo-1,3-benzoxazol-5-yl]phenyl]ethyl]-1,4-oxazepane-2-carboxamide (33).**

Prepared from 3-(2,2-difluoroethyl)-5-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzo[d]oxazol-2(3H)-one[25] according to methods D and B to afford the subtitled compound as a colorless glassy solid (50 mg, 27% over two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.55 (d, *J*=8.2 Hz, 2H), 7.41 (d, *J*=8.2 Hz, 2H), 7.36 (dd, *J*=1.7, 8.3 Hz, 1H), 7.30 (d, *J*=8.3 Hz, 1H), 7.26 (d, *J*=9.0 Hz, 1H), 7.19 (d, *J*=9.0 Hz, 1H), 6.12 (tt, *J*=4.0, 55.2 Hz, 1H), 5.24 - 5.17 (m, 1H), 4.28 - 4.18 (m, 2H), 4.10 (dd, *J*=3.9, 6.5 Hz, 1H), 4.03 - 3.96 (m, 1H), 3.79 - 3.72 (m, 1H), 3.31 (dd, *J*=4.0, 14.4 Hz, 1H), 3.15 (d, *J*=6.5 Hz, 2H), 3.04 (dd, *J*=6.7, 14.8 Hz, 1H), 2.99 - 2.83 (m, 2H), 1.88 - 1.80 (m, 2H), (one exchangeable proton not observed). HRMS $C_{24}H_{24}F_2N_4O_4$ $(M+H)^+$ calculated mass: 471.1844, found: 471.1831.

**(*S*)-*tert*-Butyl 2-(((*S*)-1-cyano-2-(4-(3-methyl-2-oxo-2,3-dihydrobenzo[*d*]oxazol-5-yl)phenyl)ethyl)carbamoyl)-1,4-oxazepane-4-carboxylate (48).**

Prepared according to method A using (*S*)-4-(*tert*-butoxycarbonyl)-1,4-oxazepane-2-carboxylic acid **39g** (490 mg, 2.0 mmol) and (*S*)-2-amino-3-(4-(3-methyl-2-oxo-2,3-dihydrobenzo[d]oxazol-5-yl)phenyl)propanenitrile **47**[25] (586 mg, 2.0 mmol) to afford the subtitled compound as a foaming oil (457 mg, 44%). Used without further purification in the next step.

**(*S*)-*N*-((*S*)-1-cyano-2-(4-(3-methyl-2-oxo-2,3-dihydrobenzo[d]oxazol-5-yl)phenyl)ethyl)-1,4-oxazepane-2-carboxamide (30).**

Prepared according to method B to afford the title compound as a white solid (230 mg, 64%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.56 (d, *J*=8.2 Hz, 2H), 7.41 (d, *J*=8.3 Hz, 2H), 7.32 (dd, *J*=1.8, 8.3 Hz, 1H), 7.29 - 7.25 (m, 1H), 7.20 (d, *J*=9.0 Hz, 1H), 7.14 (d, *J*=1.5 Hz, 1H), 5.23 - 5.17 (m, 1H), 4.11 (dd, *J*=3.9, 6.5 Hz, 1H), 4.03 - 3.96 (m, 1H), 3.79 - 3.72 (m, 1H), 3.46 (s, 3H), 3.33

(dd, *J*=3.9, 14.4 Hz, 1H), 3.17 - 3.13 (m, 2H), 3.05 (dd, *J*=6.6, 14.4 Hz, 1H), 3.00 - 2.85 (m, 2H), 1.90 - 1.81 (m, 2H), (one exchangeable proton not observed). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 171.10, 154.19, 141.54, 138.49, 136.06, 134.93, 132.42, 129.97, 126.68, 120.50, 119.19, 109.69, 107.38, 80.98, 67.07, 52.12, 47.44, 40.74, 36.63, 33.04, 28.11. HRMS $C_{23}H_{24}N_4O_4$ $(M+H)^+$ calculated mass: 421.1876, found: 421.1877.

**SPR DBA:** The SPR DBA experiments were run on a Biacore T200 instrument (GE Healthcare) at 20 degrees centigrade. The buffer used (running buffer) was 25 mM NaAc pH 5.0, 50 mM NaCl, 0.005% Triton X-100 and 5 mM DTT. A CM7 sensor chip (GE Healthcare) was docked into the instrument and the surface was activated using a 10 min injection of NHS/EDC followed by a 5 min injection of 120 µg/ml DPP1 diluted in running buffer. All surfaces were then deactivated using 1M ethanolamine, pH 8.5. Compound interaction experiments were performed in running buffer supplemented with 1% DMSO. Compound was diluted from a 10 mM DMSO stock solution to 1 µM and then serially diluted to 250 nM, 63 nM, 16 nM and 4 nM. All five compound concentrations were injected in quick succession after one another ("single-cycle kinetics") and the resulting sensorgram was reference subtracted, blank subtracted and solvent corrected. Data fitting to corrected sensorgrams were conducted using the Biacore T200 evaluation software (GE Healthcare) and the 1:1 binding model.

**DPP1 isolated enzyme assays:** Inhibition of isolated human recombinant DPP1 enzyme (synthesized as previously described[21]) activity was assessed using a fluorometric 384-well plate-based assay with a piperazine-based buffer. Compounds were pre-incubated with enzyme for 30 min at rt prior to addition of a fluorescent dipeptide substrate Gly-Arg-AMC (MP Biomedicals). In a separate study to assess species potency translation, mouse, rat, dog and human DPP1 enzyme (generated as previously described[21], and rabbit DPP1 enzyme transiently expressed in CHO cells) were studied using the same assay conditions. Potency values were obtained by fitting data using SmartFit via Screener software (Genedata AG, Switzerland).

**DPP1 cell assay:** Cellular potency was studied using the DPP1-expressing monocytic U937 cell line (European Collection of Cell Cultures) and based on the methods previously described,[32] and Gly-Phe-AFC substrate (MP Biomedicals). Briefly, cells grown in RPMI were

plated on 384-well polypropylene v-bottom plates at a density of 5 x 105 cells/mL per well, added to this was 10 µl of DPP1 inhibitor at 37 ºC for 60 min, followed by 350 µM Gly-Phe-AFC (MP Biomedicals). The well fluorescence was read using a Perkin Elmer EnVision Multilabel Plate Reader. Data were analyzed using in-house software (Excel add-in) to calculate $pIC_{50}$ values.

**Downstream effect of DPP1 inhibition on NSP activation:** Activation of NSPs was assessed *in vitro* using primary bone marrow-derived CD34+ neutrophil progenitor cells (Lonza). Cells were cultured in StemlineII media (Sigma) supplemented with rhSCF (Immunotools) and rhIL-3 (Immunotools) for seven days, and then for a further seven days in the presence of G-CSF (Immunotools) and different concentrations of compound **30** (38 pM to 10 µM). After harvesting and lysis with 10% Triton X-100 buffer cell lysates were kept at -20 °C until NSP activity analyses.

For analysis of the NSP activities, cell lysates were added to 384-well plates together with DMSO control (to measure activity within the sample) or protease inhibitors (to confirm that the measured activity was due to NE, Pr3 and CatG, respectively). The inhibitors used were compound **4**[21] (AstraZeneca) for DPP1, AZD9668[33] (AstraZeneca) for NE, sivelestat[34] for Pr3 and Cathepsin G inhibitor I (Merck Millipore) for CatG. Synthetic peptide substrates (Methoxysuccinyl-Ala-Ala-Pro-Val-AMC (Sigma) for NE, Aminobenzoyl-Val-Ala-Asp-Cys-Ala-Asp-Gln-ethylenediamine 2,4-dinitrophenyl (Peptide Synthetics) for Pr3 and N-Succinyl-Ala-Ala-Pro-Phe-pNA (Sigma) for CatG) were added before plates were read using a Tecan Safire plate reader (Tecan Group Ltd, Switzerland).

Data were fitted using GraphPad Prism software (GraphPad Software Inc, CA, USA) and was plotted as log [compound] versus % NSP activity compared to control. Curves were fitted using

all data points (cloud fit) and non-linear, 4 parameter, logistical regression analysis, with arithmetic means of $pIC_{50}$ and Emax values reported.

**Rat *in vivo* study:** The study was performed as previously described.[4] Briefly, naïve rats were dosed orally twice daily with compound **30** at 0.2, 2 and 20 mg/kg/day for eight days. At termination, bone marrow was taken by femural aspiration for NSP activity analysis using commercial synthetic peptide substrates.

## ASSOCIATED CONTENT

**Supporting Information**.

Compound sensorgram from characterization of binding kinetics for compound **30** using a Surface Plasmon Resonance direct binding assay (SPR DBA). Experimental information on assays used to generate profiling data in Tables 2, 4, and 6. This material is available free of charge via the Internet at http://pubs.acs.org.

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All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

CatC, cathepsin C; CatG, cathepsin G; $CL_{int}$, intrinsic clearance; COPD, chronic obstructive pulmonary disease; DIPEA, *N*,*N*-diisopropylethylamine; DPP1, dipeptidyl peptidase 1; DPP4, dipeptidyl peptidase 4; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HLM, human liver microsomes; HOPO, 2-pyridinol 1-oxide; LLE, ligand lipophilicity efficiency; NE, neutrophil elastase; NSPs, neutrophil serine proteases; $P_{app}$, apparent permeability; $Pd(dppf)Cl_2$, [1,1´-bis(diphenylphosphino)ferrocene]dichloropalladium(II); Pr3, proteinase-3; QWBA, quantitative whole-body autoradiography; SPR DBA, surface plasmon resonance direct binding assay; T3P, 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide; $V_{ss}$, apparent stady-state volume of distribution.

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