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Article

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Development of a series of Kynurenine 3-Monooxygenase Inhibitors leading to a Clinical Candidate for the Treatment of Acute Pancreatitis

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ABSTRACT

Recently, we reported a novel role for KMO in the pathogenesis of acute pancreatitis (AP). A number of inhibitors of kynurenine 3-monooxygenase (KMO) have previously been described as potential treatments for neurodegenerative conditions and particularly for Huntington's disease. However the inhibitors reported to date have insufficient aqueous solubility relative to their cellular potency to be compatible with the intravenous (i.v.) dosing route required in AP. We have identified and optimised a novel series of high affinity KMO inhibitors with favourable physicochemical properties. The leading example is exquisitely selective, has low clearance in two species, prevents lung and kidney damage in a rat model of acute pancreatitis and is progressing into preclinical development.

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease of the pancreas that is usually triggered by gallstones or excessive alcohol consumption. In the majority of sufferers, symptoms resolve within a few days, but for approximately 20% of patients with AP, the disease progresses to multiple organ dysfunction syndrome (AP-MODS) requiring treatment in intensive care. The individual case fatality rate in AP-MODS is 21%.[1] Currently, there is no specific therapy for AP-MODS and treatment is solely supportive. Until now, no disease-modifying treatments for AP-MODS have been identified and there are no specific treatments in routine clinical practice. Therefore, it is clear that AP represents a significant unmet medical need. We have recently demonstrated a key role for KMO in the pathogenesis of AP-MODS.[2] In experimental models of AP, mice that were genetically engineered to have absent KMO activity, and rats treated with a small molecule KMO inhibitor tool compound, were protected from AP-MODS.[2]

AP patients are treated in an intensive care setting and therefore an intravenous dosing route is strongly preferred. Where a fast onset of treatment is desirable, as it is in AP, initial drug loading with a bolus dose is advantageous to achieve the target drug plasma concentration rapidly. This plasma concentration may then be maintained by infusion dosing for as long as required. The initial bolus dose volume is limited in practise, to 50 mL, which in turn requires that the drug have high cellular potency, a significant free fraction and good solubility in aqueous solution at a biologically acceptable pH in order to achieve a therapeutically relevant free drug plasma concentration in vivo.

For reasons of storage and dosing the stability of the compound in solution is also important, with a necessity for excellent solution stability at relevant pHs and to light exposure.

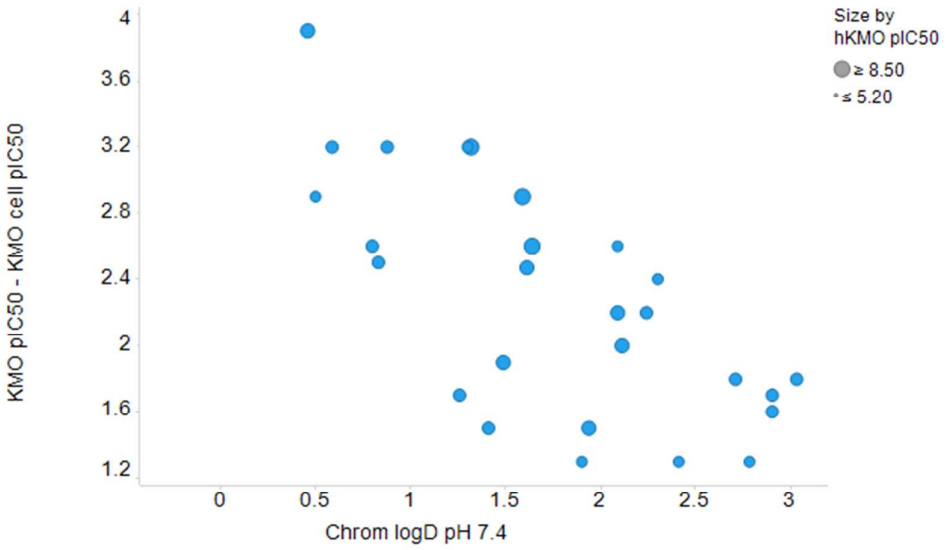
A number of series of KMO inhibitors have been described[3-10] including most recently those from CHDI.[3] The majority of the molecules described share a common pharmacophore containing both a carboxylic acid / acidic moiety and a mono or 1,2-dichloro substitution of the core phenyl ring. In our previous papers[2,11], we have shown that one such molecule, 3-(5,6-dichloro-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid **1** (GSK180[2]) protected animals from secondary organ dysfunction in a rat model of experimental AP.[2] However, the cellular potency of **1** was relatively weak so high exposures were required to inhibit KMO activity *in vivo* resulting in effects on the pathway that were not related to KMO inhibition. Moreover, while **1** has solubility in excess of 0.2mg/ml (Table 4), the compound showed significant instability precluding further development of this compound for an i.v. dosing modality.

We therefore sought to expand our existing series to discover better molecules that have optimised characteristics that will accelerate the translation of KMO inhibitors towards the clinic.

RESULTS AND DISCUSSION

We first prepared a number of other analogues from the chemical series represented by **1**; 3-(5-chloro-6-ethyl-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid **2** (GSK428[11]) retained potency in our KMO enzymatic activity assay[12], had improved stability compared with the 5,6-dichloro example and had excellent aqueous solubility as the TRIS salt (>120 mg/mL). Further expansion of the 6-methyl group to ethyl **3** or iso-butyl **4** was also well tolerated, as was replacement of the methyl by a small neutral alkoxy group **5** – **10**; however basic side chains, for example, compound **11** significantly reduced KMO inhibitory activity. During these changes, the activity in a cell based assay of KMO activity[2] remained at c.a. 1μM $IC_{50,}$ except for more polar side chains as in **10** where potency in the cellular assay declined. The artificial membrane permeability for the compounds was low, typically at around or below the lower level of quantification; this was therefore not a reliable discriminator of cellular behaviour. We also considered other predictors of permeability; the number of hydrogen bond donors and acceptors are largely unchanged for these molecules, as is the pKa and the molecular weights of the molecules are within a narrow distribution range. However, the lipophilicity (chromLogD[13]) did correlate reasonably with the relative cellular activity for these and other small halogenated, alkyl and alkoxy examples and also with the human serum albumin (HSA) binding[14]; suggesting both HSA binding and passage of the compounds into the cell were largely driven by lipophilicity (Figure 1). This is a potentially problematic scenario in an in vivo situation as any reduction in the target plasma free concentration due to increased cellular potency is likely be off-set by increased the protein binding so that the total plasma drug concentration required is not reduced.

a)



b)

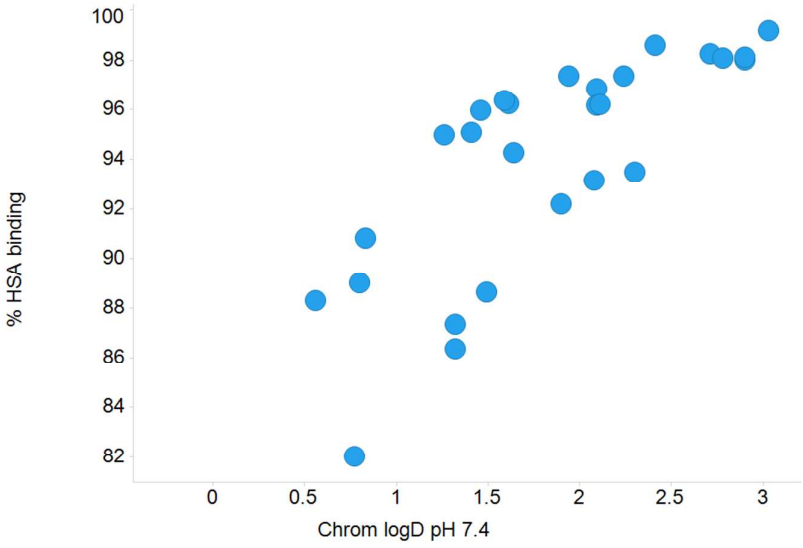
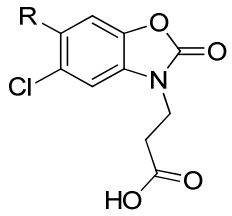


Figure 1 a) Drop-off from human KMO_{enzyme} to human KMO_{cell} pIC_{50} plotted against the Chrom LogD for examples of the series with a small 6-substituent including compounds **1** - **12**. b) % binding to HSA compared to Chrom LogD for the same compound set.

Addition of a benzyloxy group at the 6-position as in **12** followed previous trends with modest cell activity and high HSA binding (98.3%), however its 2-pyridylmethoxy analogue **13** showed increased KMO potency (pIC_{50} 8.5, which is close to the tight binding limit of the assay), but with a significantly lower level of plasma protein binding (HSA 87.4%). However, this increased potency did not translate into potency in the cellular assay with **13** having slightly weaker cellular activity than the more lipophilic **12**.

Surprisingly addition of a methyl group onto the benzylic position of **13** to give **14** resulted in a substantial (~30x) jump in cellular potency (pIC_{50} 6.9) and a higher free drug fraction than would be expected from the logD based on the previous trends. This substantial increase in cell potency was confirmed in primary human hepatocytes[2] (pIC_{50} 7.0).



Eg. No.	R	Salt	KMO mean pIC_{50} (n)	KMO LE /LLE	HEK mean cell pIC_{50} (n)	chro mlogD pH7.4	HSA (%)	hPPB (%)	AM permeability (nm / sec)
1	Cl		8.2 (6)	0.66 / 5.97	5.7 (4)	1.61	96.3	98.2	24
2	Me	Tris	7.9	0.64 /	6.2	1.26	95.0	97.6	<3 - <10

			(13)	5.76	(3)			(free acid)	
3	Et	Tris	8.0 (7)	0.61 / 5.33	6.5 (4)	1.94	97.4	98.7	<10-22
4	iBu	Tris	7.9 (5)	0.54 / 4.31	6.1 (3)	3.03	99.2	nd	<10
5	OMe	-	7.9 (8)	0.60 / 6.43	5.3 (2)	nd	89.0	nd	<3
6	OEt	Tris	8.3 (5)	0.60 / 6.30	6.4 (7)	1.49	88.7	95.8	<10
7	OcPr	Tris	8.5 (5)	0.58 / 6.45	5.9 (8)	1.64	94.3	96.9	15
8	OCH_2cPr	Tris	8.0 (5)	0.52 / 5.56	5.8 (11)	2.09	96.2	96.0	19
9	OCH_2cBu	Tris	7.6 (2)	0.47 / 4.60	5.9 (2)	2.90	98.1	98.1	12
10	$O(CH_2)_2OMe$	Tris	7.5 (5)	0.49 / 6.14	4.3 (5)	0.88	44.2	70.7	<3
11	$O(CH_2)_2$pyrrolidin-1-yl	Tris	6.1 (5)	0.35 / 6.13	nd	-0.26	15.1	nd	<3
12	OBn	Tris	7.6 (5)	0.43 / 4.37	5.8 (2)	2.71	98.3	nd	nd
13	OCH_2pyrid-2-yl	Tris	8.5 (7)	0.49 / 6.76	5.3 (11)	1.32	87.4	92.6	11
14	OCH(Me)pyrid-	Tris	8.5	0.47 /	6.9	1.86	87.1	81.7	<3

	2-yl		(7)	6.45	(9)				
15	(R)-OCH(Me)pyrid-2-yl	Tris	8.6 (12)	0.47 / 6.55	6.7 (15)	1.71	93.3	88.1	<3-20
16	(S)-OCH(Me)pyrid-2-yl	Tris	6.6 (7)	0.36 / 4.55	4.2 (3)	1.79	91.6	nd	<3
17	(R)-OCH(Me)oxazol-2-yl	Tris	8.5 (5)	0.49 / 7.28	5.1 (3)	1.11	77.3	nd	<3
18	(R)-OCH(Me)pyrimidin-2-yl	Tris	8.3 (5)	0.45 / 7.21	5.5 (3)	0.83	59.1	nd	<3
19	(R)-OCH(Me)pyridazin-3-yl	-	8.9 (5)	0.49 / 8.08	6.2 (3)	0.83	59.1	68.1	<3
20	(R)-OCH(Me)-4-methylpyridin-2-yl	Tris	8.4 (5)	0.44 / 5.85	4.5 (2)	2.15	94.4	nd	<3
21	(R)-OCH(Me)-5-methylpyridin-2-yl	Tris	8.5 (6)	0.45 / 5.95	8.2 (12)	2.02	nd	97.8	22
22	(R)-OCH(Me)-	Tris	8.5	0.45 /	7.0	2.65	97.9	99.0	60

	5-chloropyridin-2-yl		(6)	5.66	(3)				
23	(R)-OCH(Me)-5-fluoropyridin-2-yl	Tris	8.6 (3)	0.45 / 6.33	7.6 (9)	1.98	94.0	nd	31
24	(R)-OCH(Me)-6-methylpyridin-2-yl	Tris	8.3 (5)	0.44 / 5.75	6.5 (6)	2.12	93.2	nd	21

Table 1

The X-ray crystal structure of human KMO has not been reported. However KMO from the bacterium *Pseudomonas fluorescens* (Pf KMO) shows high levels of similarity to the human protein in the active site. All the residues directly lining the active site are conserved with human KMO, except His320 in Pf KMO which is a phenylalanine in human.

Modelling of these compounds based on the X-ray structure previously described for **1** in Pf KMO[2], gave a good fit of the smaller alkoxy groups into the available space around the 6-position, but the larger alkoxy and in particular the benzyl and pyrid-2-ylmethyl examples **12** and **13** could only be modelled into a pocket formed by Asn54, Glu195, Thr234 and Thr236 with displacement of the resident waters. This gave no explanation of the potency jump seen for **13** as the nitrogen of the pyridine appeared to make no additional contacts.

We were able to obtain a crystal structure of **14** in Pf KMO (Figure 2). The crystal structure showed only the R-enantiomer **15** bound into the active site, with the pyridyl moiety occupying an unexpected binding mode (Figure 2). In the X-ray structure of **14** / **15**, the bicyclic core and acid side chain are bound as we had seen previously for **1,** although as with

1 the exact interactions of the carboxylic acid vary between the A and B chains in the crystal structure (a direct hydrogen bond between carboxylate and Tyr98 hydroxyl in the A chain and a water mediated hydrogen bond in the B chain). However the tricyclic group of the catalytic FAD has rotated to accommodate **15,** allowing the (R)-1-(pyridine-2-yl)ethoxy to sit underneath forming a face to face π interaction between the pyridine of **15** and FAD. The pyridine nitrogen makes H-bonding interactions with a water network extending towards Pro318 and Val317, whilst the pyridine ring in addition to the face to face π interaction with FAD also forms two edge to face π interactions from the 5-position of the pyridine into Tyr193, and from Phe238 into the face of the pyridine ring.

A similar binding mode for the S-enantiomer **16** would result in obvious protein clashes, and indeed this compound is much less potent.

Whilst there is high homology between the Pf KMO and human KMO and the SAR from the human KMO is consistent with the Pf KMO crystal structures, these structures and the FAD movement must be interpreted with some caution**. 15** inhibited Pf KMO with a similar potency as the human enzyme (Pf KMO pIC_{50} 8.5, h KMO pIC_{50} 8.6), however the h KMO potency may be an underestimate as we are close to the tight binding limit of the assay.

Subsequent crystal structures on a small set of intermediately sized inhibitors **6**, **7** and **9**, showed **6** and **7** to bind in the same mode as **1** with the side-chain accommodated into a space in front of the FAD tricycle. **9** however shows significant differences in binding mode in the A and B chains of the protein crystal structure (Figures 3a / 3c). In the A chains, where the ligand carboxylic acid is directly H-bonded to Tyr98, the protein environment around the ligand alkyl groups is the same, including the position of the FAD tricycle. The different

sized alkyl groups are accommodated by small adjustments in the inhibitor bicycle position, with the largest, cyclobutyloxy, group adjacent to the FAD in a perpendicular position.

For the B chains, where the carboxylic acid is bonded to Tyr98 through a water molecule, the protein environment around the ligand alkyl groups is the same for the ethyl and cyclopropyl groups as in the three A chain structures, however the cyclobutyl group induces a change in the orientation of the FAD tricycle, and by changing its own conformation by approximately 90° it is able to adopt a conformation parallel to the FAD aromatic ring. This is the first example of the FAD tricycle adopting an intermediate conformation between the two extremes seen with **1** and **14**/**15** (Figure 3c, d). Although **9** is able to cause a change in FAD tricycle conformation, in this case it has a lower potency than **6** and **7**, suggesting that the conformational change results in overall less favourable interactions in the KMO active site. It appears that only when the larger and planar pyridyl group of **14**/**15** is present can the FAD tricycle achieve the full positional shift required to allow the favourable aromatic π-stacking and edge to face π interactions to be made.

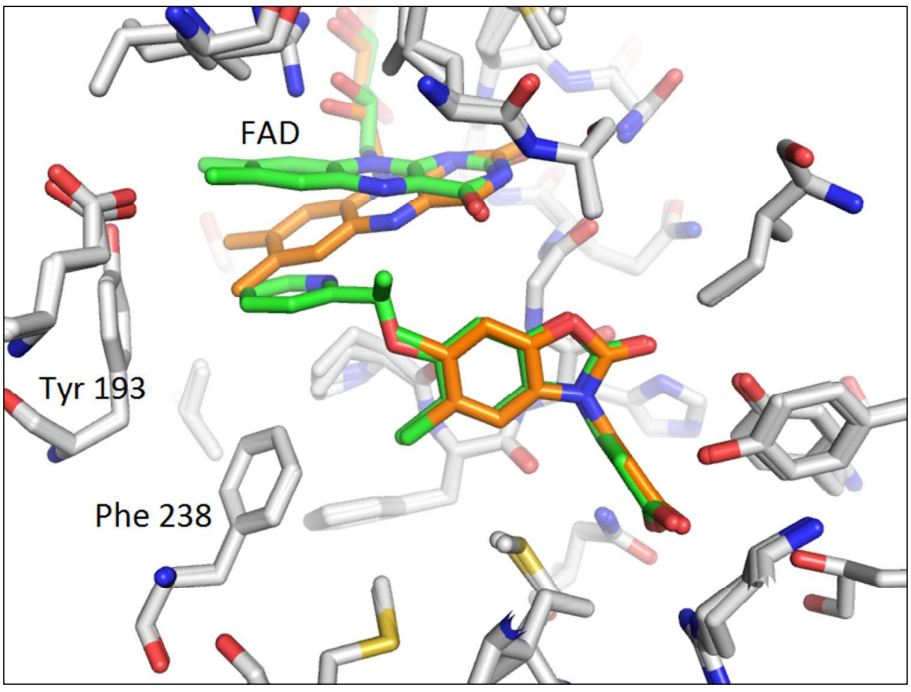


Figure 2 Crystal structures of **1** (orange) and **14/15** (green) bound to Pf KMO protein (A Chain). The tricycle of the catalytic FAD has rotated in the structure of **14** to accommodate the pyridine.

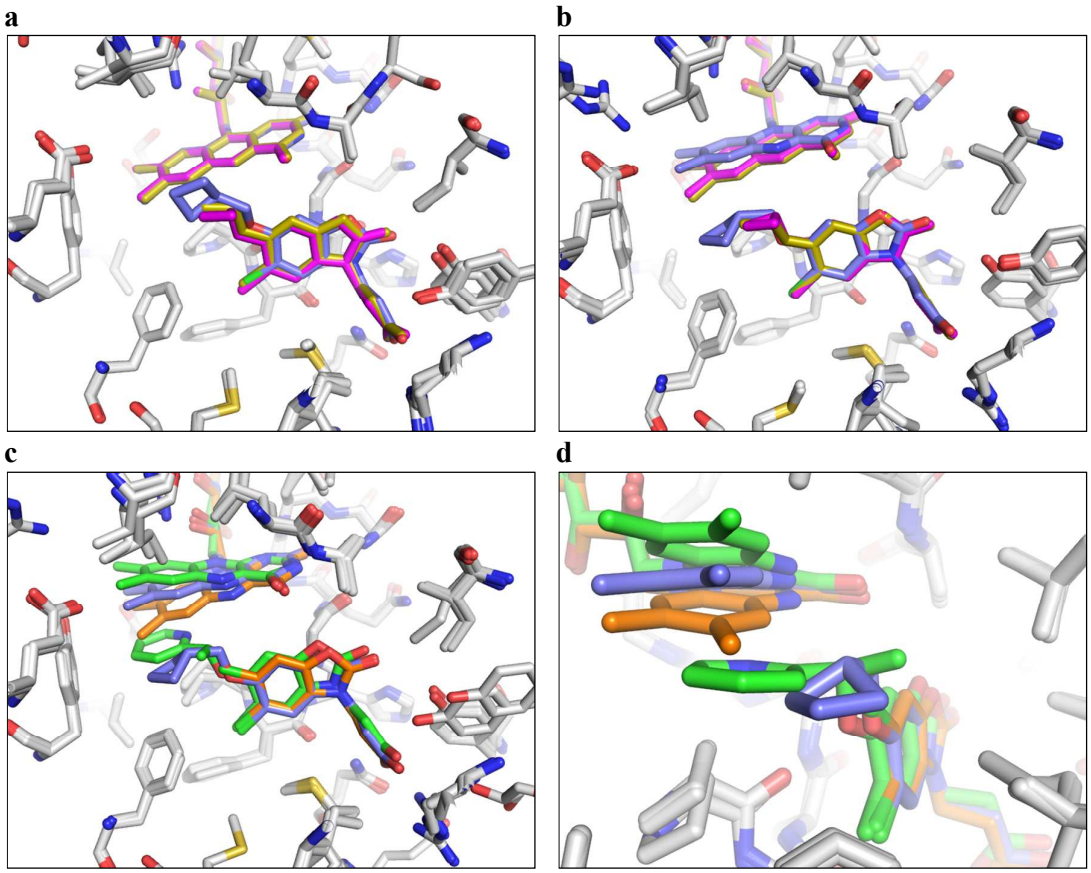


Figure 3 **a**, **b** Comparison of binding modes of **9** (blue) with **6** (gold) and **7** (magenta) bound to Pf KMO in the A chain (**a**) and in the B chain (**b**). In the A chain, the largest, cyclobutyl, group shifts the inhibitor slightly towards Tyr98. In contrast, in the B chain, whilst the FAD is twisted upon binding of the cyclobutyl substitutent, there is very little difference in the actual positions of the inhibitor bicycles. **c**, **d** Comparison of binding modes of **1** (orange), **9** (blue) and **14**/**15** (green) bound to Pf KMO in the B chain exhibiting the three different observed FAD conformations. (**c**) front view (**d**) side view.

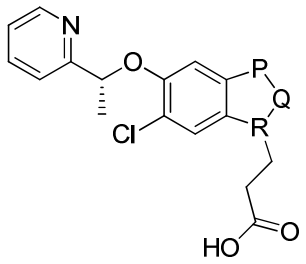
Three approaches were taken to further improve on **15** based on knowledge from the crystal structure: to modify the 1-(pyridine-2-ylethoxy) side chain, to substitute the pyridine ring and to explore alternative bicyclic cores.

Analogues where the pyridine was replaced by an alternative heteroaromatic (**17** – **19**) retained potent enzyme inhibition but had weaker cell potency, possibly due to a potentially negative impact of the additional polarity on cell permeability, although this was not confirmed. As expected the increased polarity correlated with lower protein binding.

From the crystal structure of **15** bound to Pf KMO, it was hypothesised that substitution of the pyridine would be tolerated at any of the 4-, 5- and 6-positions, but that a 5-position substituent would have the potential to form an interaction with the π cloud of Tyr193. Substitution at the 3-position was excluded as being likely to clash with the α-methyl group in the exemplified binding mode. In the KMO primary assay, substitution of the pyridine appeared to be tolerated at all of these positions, but only substitution at the 5-position was beneficial in the cell with **21**, **22** and **23**, all having enhanced cell potency compared with **15**.

The 1-(pyridin-2-ylethoxy) was introduced onto a number of 6/5 and 6/6 cores, including some which we had previously examined[11] and in others chosen based on the crystal structures we had generated (Table 2). All examples demonstrated KMO pIC_{50}s at around the theoretical tight binding limit of the KMO enzyme assay. The cellular potency was generally higher than for **15** with the exception of the indazole **27**. The benzisoxazole **28** was of particular interest with an approximately 60-fold increase in cell potency over **15**, although with a ~5-fold reduced free fraction (2.2% vs 11.9%). This cellular activity was confirmed in

primary human hepatocytes[1] (pIC_{50} 8.0). The thioheteroaryl **29** was also very potent with a HEK cell pIC_{50} of 7.5.

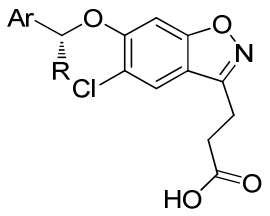


Eg. No.	PQR	Salt	KMO mean pIC50 (n)	HEK mean cell pIC50 (n)	chromlog D pH 7.4	hPPB (%)
15	OC(=O)-N	Tris	8.6 (12)	6.7 (15)	1.71	88.1
25	OCH_2-C(=O)-N	Tris	8.3 (6)	7.2 (11)	1.56	92.5
26	CH=CH-N	-	8.3 (5)	7.6 (2)	2.17	nd
27	CH=N-N	Tris	8.5 (5)	7.1 (5)	1.70	90.1
28	O-N=C	Tris	8.3 (7)	8.5 (12)	1.99	97.8
29	S-C(=O)-N	-	8.7 (5)	7.5 (1)	1.81	nd

Table 2

Translation of the substituted pyridines onto the more potent benzisoxazole core gave some extremely cell potent examples (**30**, **31**, **32**), although with lower free fractions than the less lipophilic benzoxazolones (Table 3). The (6-methylpyridazin-3-yl)ethoxy example **33** was also prepared on the benzisoxazole core, aiming to combine the large free fraction of **19** with the more lipophilic core and the additional methyl substituent (as in **30)** to achieve a logD consistent with good cellular potency. This combination, as desired, gave enhanced cellular

activity (pIC_{50} 8.5) and a higher plasma free fraction than the equivalent pyridine **30** (7.1% vs 0.7%).



Eg. No.	Ar	R	Salt	KMO mean pIC_{50} (n)	HEK mean cell pIC_{50} (n)	chromlog D pH 7.4	HSA (%)	hPPB (%)
30	5-methylpyridin-2-yl	Me	Tris	8.5 (6) (**30a** HCl salt)	8.8 (5)	2.42	98.4	99.7
31	5-chloropyridin-2-yl	Me	Tris	8.5 (5)	8.4 (4)	2.91	99.0	99.8
32	5-fluoropyridin-2-yl	Me	-	8.5 (9)	7.5 (6)	2.47	98.3	nd
33	6-methylpyridazin-3-yl	Me	-	8.7 (4)	8.5 (19)	2.97	89.2	92.9

Table 3

The enhanced cellular activity of **14** and later analogues compared with the smaller alkyl examples and **12** /**13** and movement of the FAD in Pf KMO led us to examine the kinetics of interaction between these molecules and hKMO in an attempt to understand the increase in potency. We had previously shown that **2** had a fast dissociation rate. These kinetics of interaction of a range of inhibitors with human KMO were studied and are reported in detail elsewhere.[15] In "recovery of activity" experiments **13** was found to have a dissociation $T_{1/2}$ in the region of 30min and a K_i in the order of 1nM, whereas the $T_{1/2}$ of **15** was ~2h and its K_i is approximately 100pM. For **28** the association kinetics were measured in onset of inhibition time-courses using the mass spectrometry assay. Observed rate constants increased linearly with [**28**], indicative of second order kinetics, with k_{on} = 6.2 x 10^5 M^{-1} s^{-1}. Due to the high potency of **28**, the recovery of activity approach could not be used to measure k_{off}. Instead the time-course was analysed by numerical fitting to a model of competitive inhibition with k_{on} fixed at 6.2 x 10^5 $M^{-1}s^{-1}$, giving a value of k_{off} = 1.94 x 10^{-5} s^{-1}. The calculated K_i of 28 is thus 50pM and the $T_{1/2}$ was around 10h.

A number of the small alkoxy examples **1**, **2**, **6**, **7**, **8**, chiral pyridyls **15, 28, 30** and the chiral pyridazinyl **33** were profiled in i.v. rat DMPK[16] (Table 4) and for stability to light and pH under oxidative and non-oxidative conditions and for solubility in saline. The examples with a small alkyl or alkoxy substituent had a small volume of distribution, low to moderate clearance and, except for **1** and **2**, a short half-life. By contrast **15** had a long half-life and a much higher volume of distribution. The pyridyl benzisoxazole examples, **28**, **30** and **31** had low volumes and clearance with moderate to long half lives, whereas pyridazinyl example **33** had a slightly higher volume, moderate clearance and moderate $T_{1/2}$.

Eg. No.	rPPB	Rat i.v.dmpk (~1 mg/kg)	Stability	Saline

	(%)						solubility
		$T_{1/2}$ (h)	Clb (mL/min/kg)	Vdss (l/kg)	pH6 oxidative ($T_{1/2}$)	Light (% remaining at 12h)	24 h (ug/mL)
1	91.7	4.3	4.3	0.28	334 h	41	270
2	95.5	3.7	4.7	0.52	>1000 h	100	>127000
6	81.6	0.43	16.0	0.36	>1000 h	99.5	>1880
7	90.3	0.39	9.4	0.29	>1000 h	100	>2400
8	90.2	0.68	4.1	0.26	>1000 h	104.9	>2060
15	78.5	5.6	16.8	2.96	>1000 h	99.2	>9500
28	97.8	6.6	0.8	0.42	>1000 h	97.7	>7440
30	99.1	3.6	1.1	0.31	>1000 h	96.5	>860
31	99.7	8.7	0.35	0.26	>1000 h	99.5	>620
33	93.3	1.5	12.0	1.10	>1000 h	85.2	230

Table 4

All examples showed excellent stability to both light and relevant pH and all had good levels of solubility in saline, particularly as the TRIS salts. We had identified the TRIS salt as a means of significantly improving the aqueous solubility of the smaller alkyl / alkoxy substituted molecules including **2** and for these the TRIS salts were well behaved solids. However, for molecules containing a chiral pyridine or chiral heteroaryl side chain, the TRIS salts were hygroscopic gums. We therefore returned to preparing the free acid for later examples and compound batches.

Following consideration of all available data compound **28** was chosen for further profiling. This compound showed no inhibitory effects on the other enzymes in the tryptophan metabolism pathway (IDO, TDO, KATI / II, kynureninase) and had remarkable selectivity in GSK's liability panel (57 assays) with a window of >20,000 fold over all the targets examined.

Compound **28** was evaluated in a rat PK/PD study using a loading i.v. bolus followed by a 3h i.v. infusion formulated as the ethanolamine salt formed in-situ in the dosing vehicle. The potency of **28** against rat KMO was lower than observed with human enzyme. When rat KMO was expressed in HEK cells the apparent cellular K_i was calculated as (45nM) and so three dose levels were tested that delivered steady free drug levels ranging from 10x above to 10x below this level (Table 5). **28** had no impact on circulating TRP levels, but showed a dose dependent effect on the concentration of 3-hydroxykynurenine (the product of KMO) and a corresponding increase in kynurenine (the substrate of KMO), with a half maximal effect at concentrations around the rat cell K_i (Figure 6). As expected, dose dependent increases were also observed in the diversionary products of kynurenine metabolism – kynurenic acid and anthranilic acid – consistent with the effects previously reported.[2]

Dose of 28 (free acid)	**Free plasma concentration**	**Concentration relative to cellular Ki**
0.025mg/kg & 0.0025mg/kg/hr	3nM	0.07x
0.25mg/kg & 0.025mg/kg/hr	50nM	1.1x
2.5mg/kg & 0.25mg/kg/hr	405nM	9.0x

Table 5

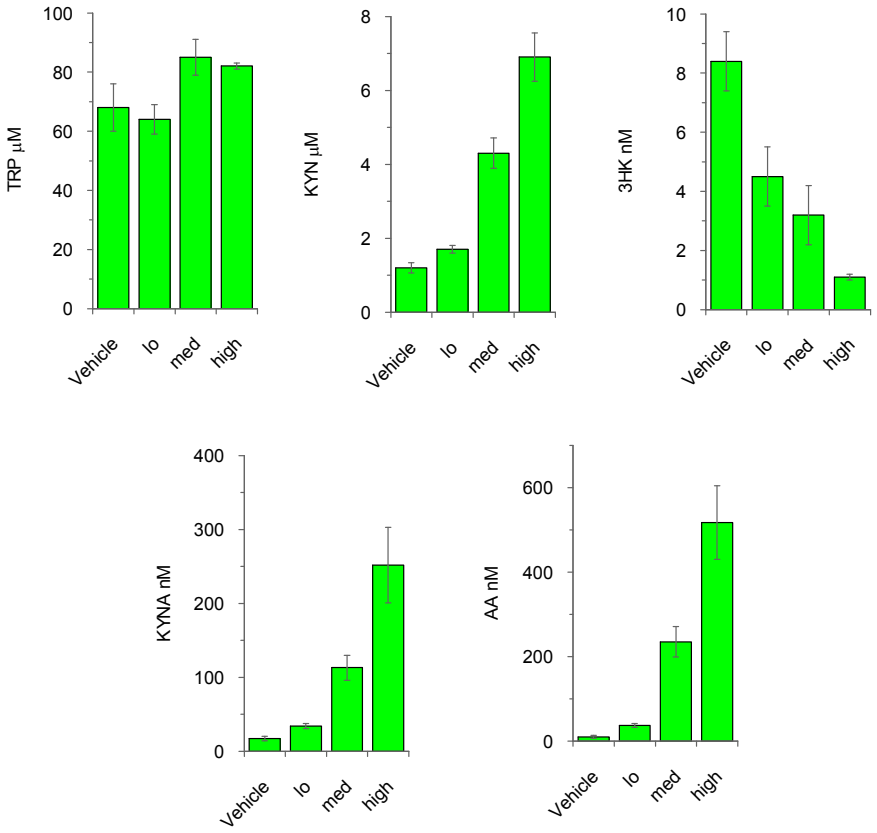
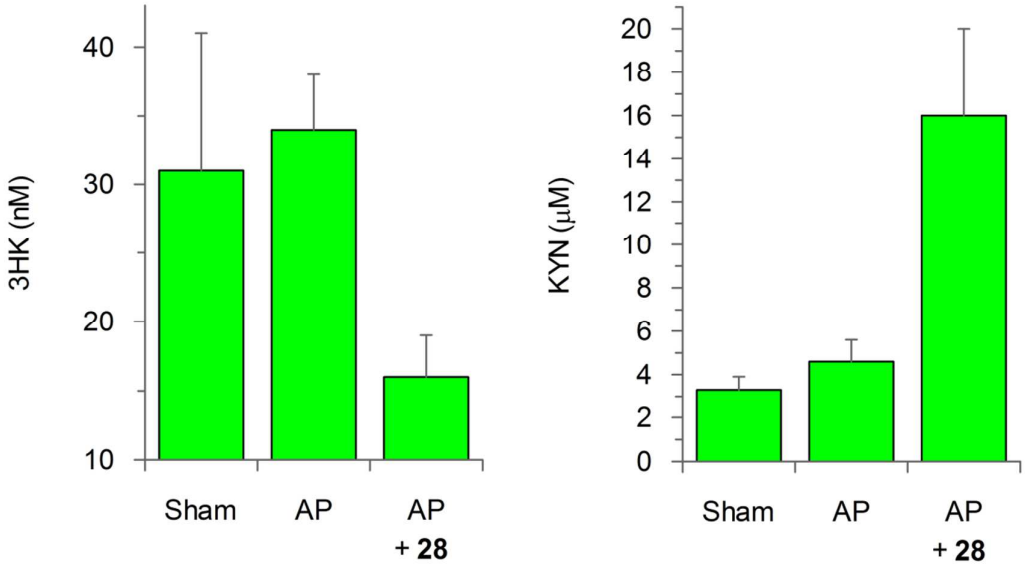


Figure 6 a) Effect of **28** on components of the kynurenine pathway measured at 3hrs after initiation of dosing with doses of 2.5mg/kg bolus & 0.25mg/kg/hr infusion (high), 0.25mg/kg bolus & 0.025mg/kg/hr infusion (med) and 0.025mg/kg bolus & 0.0025mg/kg/hr infusion (lo) doses (Table 5).

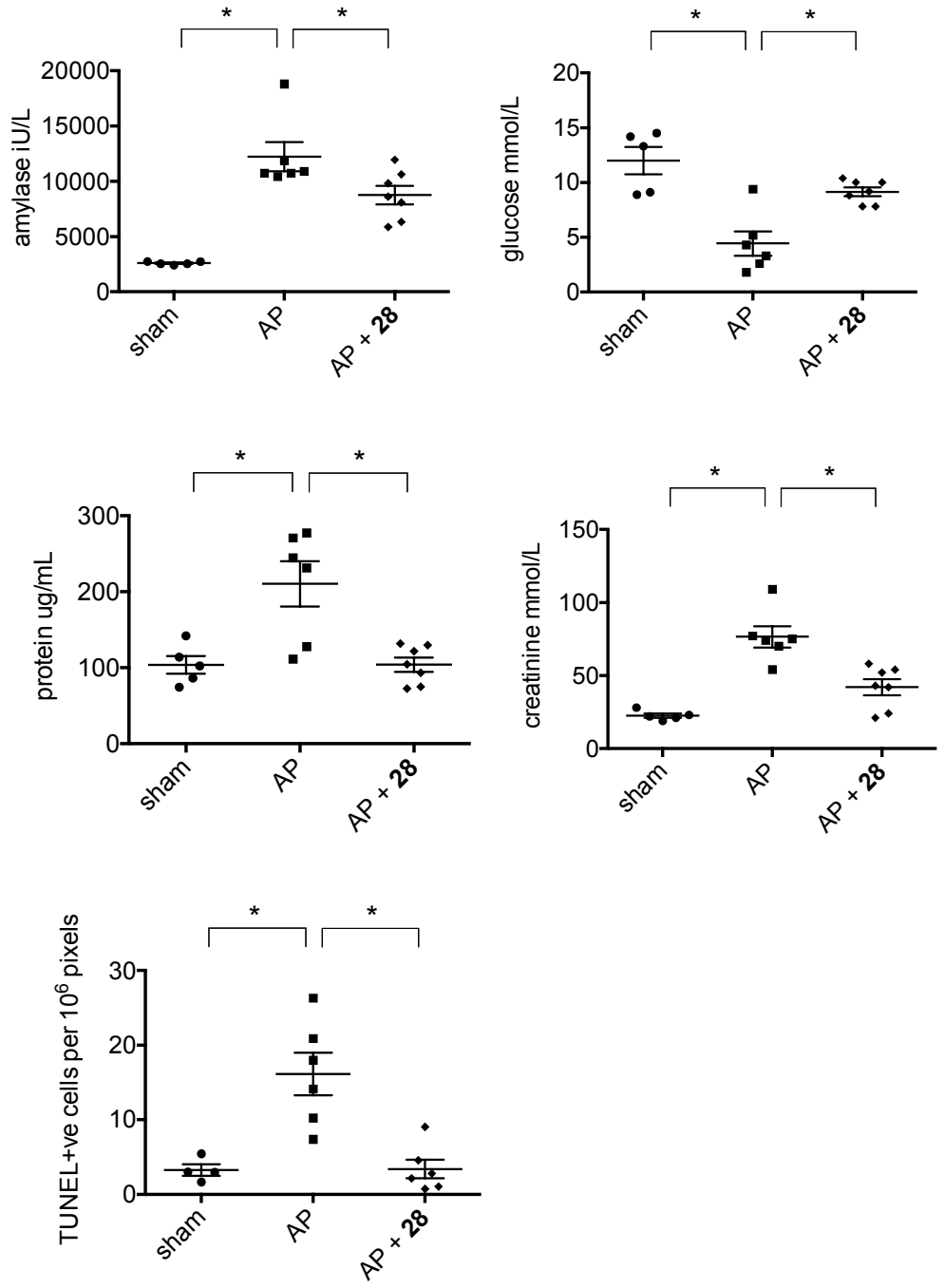
Compound **28** was assessed in an experimental model of AP in rats[17] dosing as an initial 20 mg/kg bolus followed by a 5h, 2.2 mg/kg/hr infusion therapeutically, commencing 1 hour after the induction of AP. This regime delivered free concentration in plasma of 50x the pIC_{50} for rat KMO, and resulted in a decrease in 3-hydroxykynurenine and an increase in

kynurenine as expected (Figure 7). The extent of pancreatic injury in this model (measured by serum amylase levels) was only marginally impacted by treatment with **28.** The stress response during experimental AP-MODS caused a decrease in plasma glucose concentration, which was less pronounced in rats with AP treated with **28**. Importantly, **28** showed significant protective effects against the secondary organ injury to lung as assessed by histology and measured by analysing protein leak into the alveolar space (in bronchoalveolar lavage fluid). Furthermore, kidney injury was reduced with **28** treatment, measured by a reduction in AP-induced plasma creatinine rise and a reduction in apoptotic cell count in the outer medullary stripe of the kidney assessed by TUNEL staining (Figure 7).

a)



b)


amylase iU/L
20000
15000
10000
5000
0
sham
AP
AP + 28
*
*
glucose mmol/L
20
15
10
5
0
protein ug/mL
300
200
100
creatinine mmol/L
150
100
50
TUNEL+ve cells per 10^6 pixels
30
20
10

c)

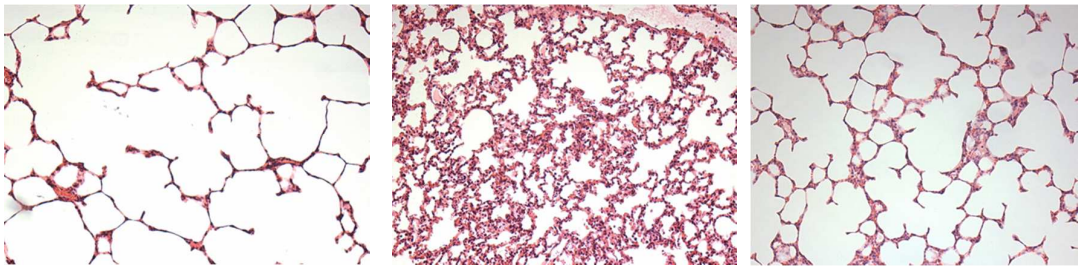


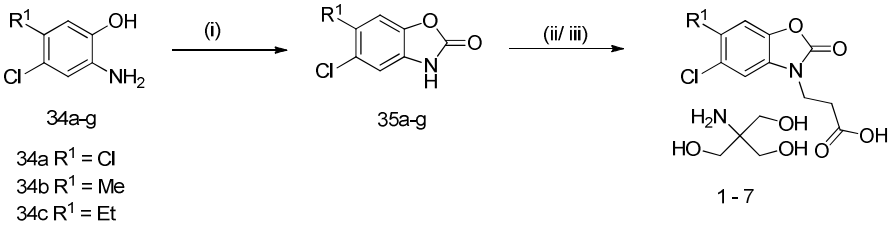
Figure 7 a) Reduced plasma levels of 3-HK and increased levels of kynurenine compared with both sham operated and AP control animals at 5 hours after start of administration of **28**. b) Therapeutic administration of **28** 1 hour after the induction of experimental AP in rats protects against secondary organ damage in lung and kidney. Each point represents data from an individual rat, with group sizes as follows: sham n=5, AP n=6, AP + **28** n=7. For the lung TUNEL figure panel, one extreme outlier data point was omitted from the sham group and one extreme outlier from the AP + **28** group after careful consideration. Both excluded data points were at least 7x standard deviations from the mean of the remainder of the corresponding group and were felt to be due to technical error. Statistical comparison between groups was by one-way ANOVA with post-hoc Student-Neuman-Keuls, *P < 0.05. c) Lung histology representative photomicrographs. Haematoxylin and eosin staining of formalin-fixed sections of lung tissue. Alveolar-epithelial thickening and inflammatory infiltrates are visible on images in the AP group, which is less marked in the AP + **28** group.

Dog i.v. infusion PK[18] of the free acid of **28** dosed at 1 mg/kg showed a similar profile to rat i.v. PK with long half-life and low volume and clearance (Dog: $T_{1/2}$ 5.8h, Vd 0.36, Clb 0.93). Based on scaling of PK parameters from rat and dog, combined with the high cellular potency and moderated free fraction, we estimate that a daily dose of less than 50mg should provide substantial suppression of 3-hydroxykynurenine levels in man.

SYNTHESIS

The synthesis of **1** and **2** have been described previously[2/19] and examples **3** to **7** were prepared using the same synthetic sequence (Scheme 1). The benzo[*d*]oxazol-2(*3H*)-ones

35a – g were prepared by cyclisation of the relevant 2-aminophenol (**34a-g**) using either CDI or triphosgene. The bicyclic product **35a-g** was then alkylated with 3-bromopropanoic acid and the target molecule was then optionally converted to the TRIS salt in aqueous methanol or ethanol.



34a-g

35a-g

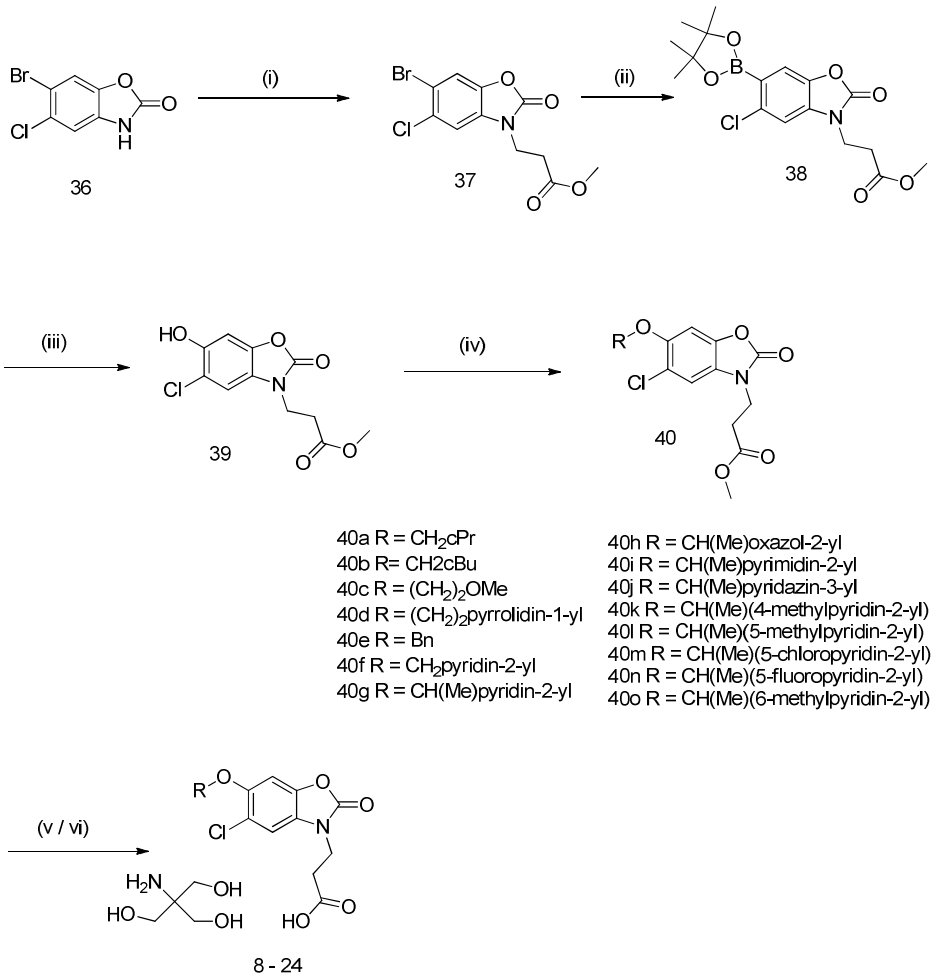
1 - 7

34a R^1 = Cl
34b R^1 = Me
34c R^1 = Et
34d R^1 = iBu
34e R^1 = OMe
34f R^1 = OEt
34g R^1 = OcPr

Scheme 1

(i) CDI, THF, 60 - 80 °C or Triphosgene, Et_3N, 0°C – rt (ii) 3-bromopropanonic acid, K_2CO_3, MeCN, 60 - 80 °C (iii) TRIS, EtOH or MeOH, H_2O, 80 °C.

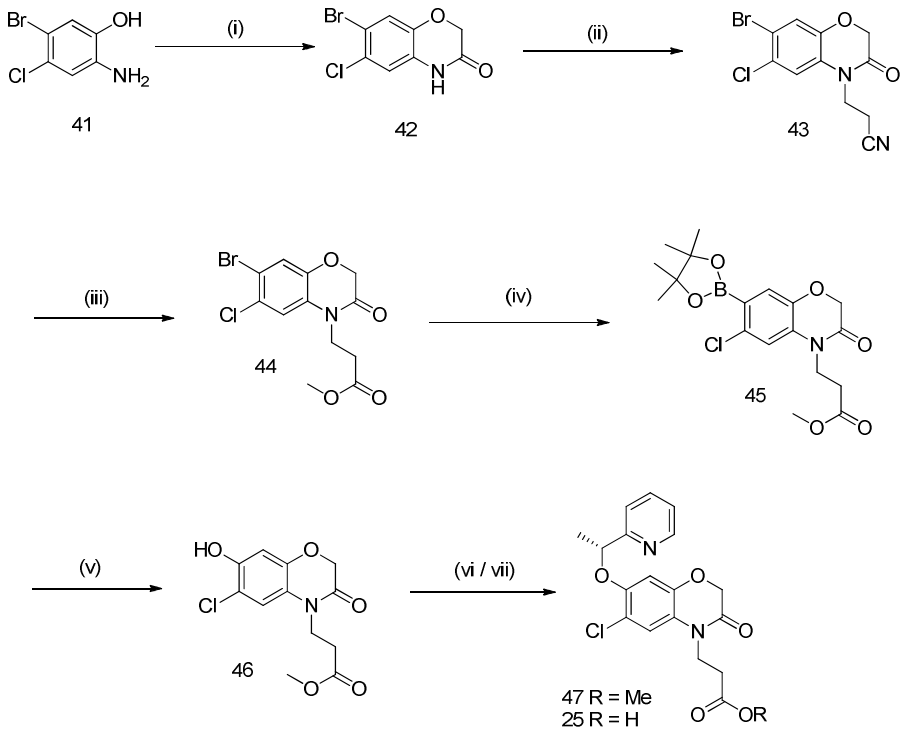
The remaining alkoxy substituted examples **8** to **24** were prepared as shown in Scheme 2 from the commercially available 6-bromo-5-chlorobenzo[d]oxazol-2(3H)-one **36**. This was alkylated with methyl 3-bromopropanoate to give **37**. Treatment of **37** with bis(pinacolato)diboron and palladium catalysis give the pinnacolborane **38** which was converted to the phenol **39** with hydrogen peroxide. The 6-alkoxy group was installed either under Mitsonubu conditions with the alcohol or by alkylation using either the appropriate bromide or an activated alcohol. Acid mediated hydrolysis of the esters **40a-o** was generally preferred to avoid ring opening, which gave the target compounds **8** to **24**.



Scheme 2

(i) methyl 3-bromopropanoate, K_2CO_3, MeCN, 70 °C (ii) bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc, dioxane, 100 °C (iii) 30% H_2O_2, AcOH, THF, rt (iv) ROH, DEAD, PPH_3 or RX / ROMs, K_2CO_3 (v) 0.5 N HCl, dioxane, 80 °C or 6N HCl 70-100 °C or LiOH, dioxane, 0 °C - rt (vi) TRIS, MeOH, rt.

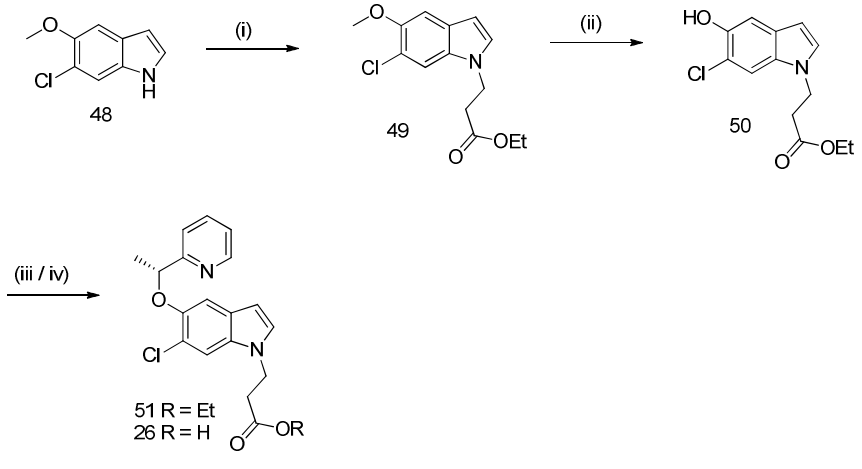
Compound **25** was synthesised in a similar manner to the equivalent benzoxazolone from 7-bromo-6-chloro-2H-benzo[b][1,4]oxazin-3(4H)-one **42,** but using acrylonitrile in place of methyl 3-bromopropionate for the alkylation which installs the side chain (Scheme 3). The nitrile **43** was converted to the ester **44** by hydrolysis with hydrochloric acid in methanol, before conversion of the 7-bromo to the 7-phenol **46** via the pinnacol borane **45**. A subsequent Mitsonubu coupling and ester hydrolysis gave **25**.



Scheme 3

(i) 2-chloroacetyl chloride, KOAc, DMF, rt (ii) acrylonitrile, K_2CO_3, MeCN, 70 °C (iii) c. HCl, MeOH, 90 °C (iv) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, dioxane 100 °C, Ar (v) H_2O_2, AcOH, THF, rt (vi) (S)-1-(pyrid-2-yl)ethanol, PPh_3, DEAD, toluene, rt (vii) 0.5N HCl, dioxane, 80 °C.

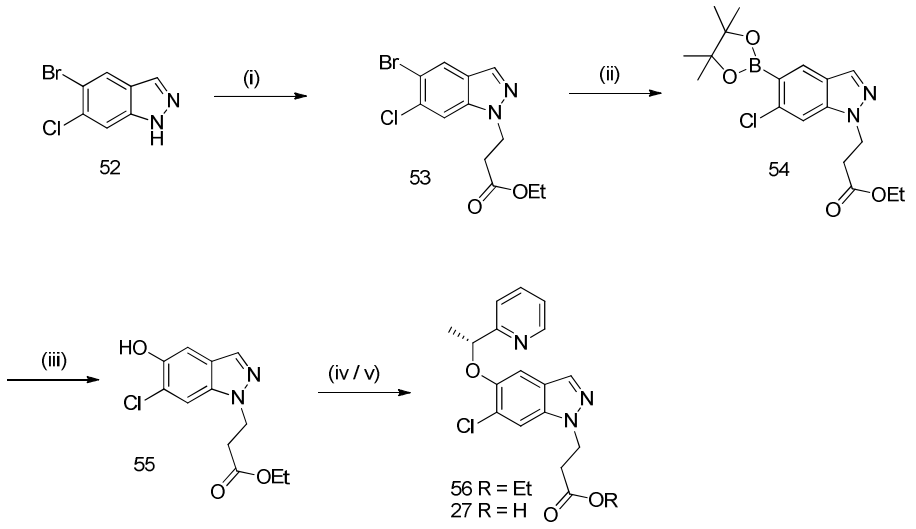
The choice of alkylation conditions was found to be highly sensitive to the core bicyclic, for 6-chloro-5-methoxy-1H-indole **48**, ethyl acrylate was preferred as the alkylating group (Scheme 4). Conversion of the 5-methoxy indole **49** to the 5-phenol **50** was followed by a Mitsonubu coupling and ester hydrolysis to give **26**.



Scheme 4

(i) ethyl acrylate, K_3PO_4, MeCN, rt (ii) $AlCl_3$, toluene, 100 °C (iii) (S)-1-(pyrid-2-yl)ethanol, PPh_3, DEAD, toluene, rt (iv) LiOH, THF, H_2O, rt.

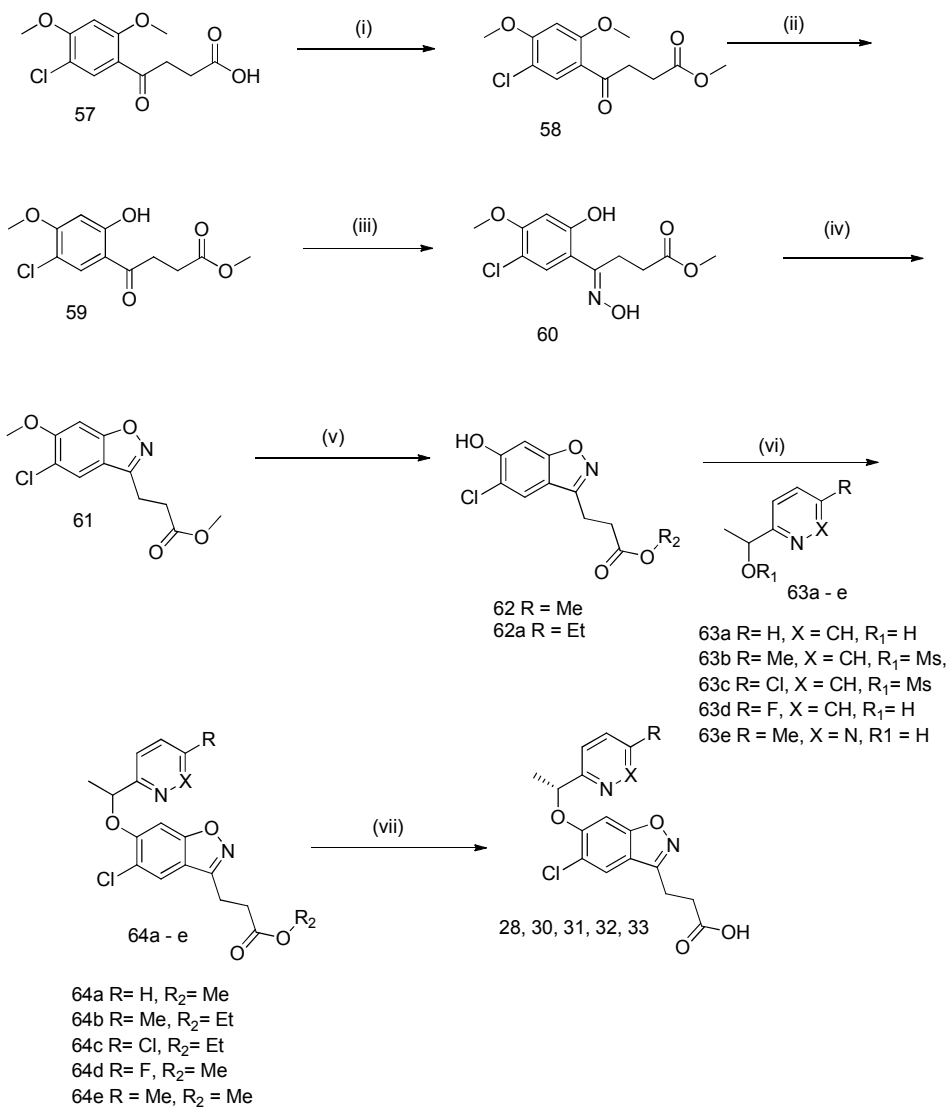
Commercially available 5-bromo-6-chloroindazole **52** was also alkylated with ethyl acrylate to give ester **53**. This was then converted from the 5-bromo compound in the same manner as the analogous benzoxazolone to give compound **27**.



Scheme 5

(i) ethyl acrylate, DBU, MeCN, rt (ii) bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc, dioxane 100 °C, Ar (iii) H_2O_2, THF, AcOH, rt (iv) (S)-1-(pyrid-2-yl)ethanol, PPh_3, DEAD, toluene, rt (v) LiOH, THF, H_2O, rt.

The benzisoxazole core can be synthesised by several routes[20], the shorter improved route is shown in Scheme 6. Acid **57** was converted to the ester with thionyl chloride and methanol and then the methoxyl adjacent to the ketone was selectively demethylated with aluminium trichloride and sodium iodide to give phenol **59**. The ketone was converted to the hydroxylamine with ammonium hydroxide and the isoxazole closed by heating in a mixture of acetic anhydride and pyridine. Demethylation of **61** using aluminium chloride gave phenol **62.** This was then converted to ether **64a-d** by addition of the chiral pyridine alcohol **63a** under Mitsonubu conditions or by alkylation with the relevant mesylate **63b-d**. Subsequent lithium hydroxide hydrolysis of the esters gave compounds **28**, **30**, **31**, **32** and **33**.

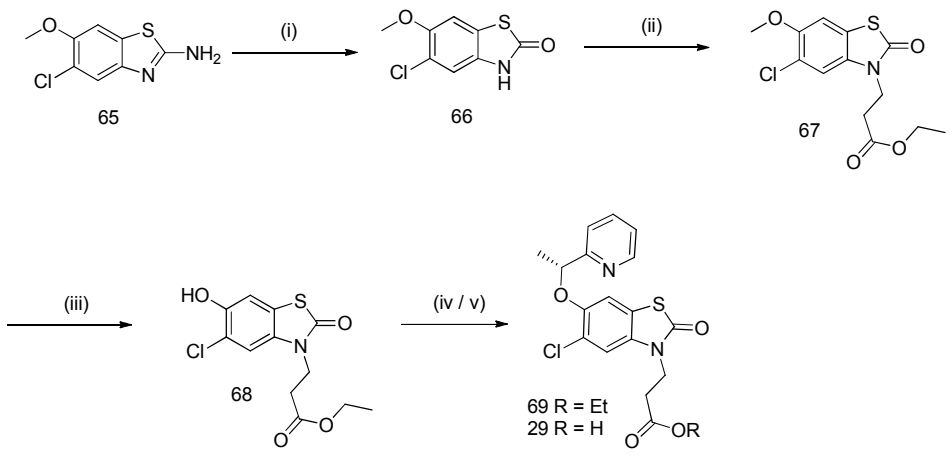


Scheme 6

(i) $SOCl_2$, MeOH, 0 °C - rt (ii) NaI, $AlCl_3$, MeCN, 80 °c (iii) pyridine, MeOH, $NH_2OH.HCl$, 100 °C (iv) pyridine, Ac_2O, 110 °C to 120 °C, (v) $AlCl_3$, DCM, rt (vi) 63a: ROH, PPh_3, DEAD, THF or 63b-d: ROMs, K_2CO_3, DMF or MeCN, 70 - 80 °C (vii) LiOH, THF, H_2O, MeOH or EtOH, 0 °C - rt

The 5-chloro-6-methoxybenzo[d]thiazol-2(3H)-one **66** was prepared from the 5-chloro-6-methoxybenzo[d]thiazol-2-amine **65** by diazotization and treatment with acetic acid (Scheme 7). Alkylation with ethyl acrylate and demethylation with boron tribromide gave phenol **68**.

Treatment of **68** with the mesylate of the chiral pyridine under alkylation conditions and subsequent acid mediated hydrolysis of the ester **69** gave **29**.



Scheme 7

(i) HCO_2H, AcOH, c. HCl, $NaNO_2$, H_2O, -10 - 100 °C (ii) ethylacrylate, $Si(OEt)_4$, CsF, toluene, 110 °C (iii) BBr_3, DCM, rt (iv) (S)-1-(pyrid-2-yl)ethanol, PPh_3, DEAD, toluene, rt (v) 0.5N HCl, dioxane, 90 °C.

CONCLUSION

We report here the development and optimisation of a series of KMO inhibitors arising from a Discovery Partnership with Academia collaboration between the University of Edinburgh and GSK. Using novel Pf KMO crystal structures, we have rationalised some unexpected SAR on the human KMO enzyme. This has enabled further optimisation using the principles of structure-driven design to generate molecules with very high KMO inhibitory potential. In the process we have also converted the series from compounds with a rapid off-rate (**2**) to examples with very slow dissociation kinetics (**15**, **28**). The resulting molecules have potent cellular activity, excellent aqueous solubility and a DMPK profile suitable for i.v. dosing. The effect on plasma concentrations of kynurenine pathway metabolites after administration of **28** recapitulates the previously observed effect using tool KMO inhibitor compounds and

Kmo knockout mice.[2] The effect on the kynurenine pathway *in vivo* and at pharmacologically active doses is devoid of the non-KMO mediated effects (reduction in TRP, large increase in KYNA) previously observed with **1**.[2] Compound **28** showed protection against extrapancreatic tissue injury to kidney and lung during experimental AP in rats. Compound **28** will now be progressed towards clinical evaluation.

EXPERIMENTAL SECTION

General Experimental Details. All commercial reagents and solvents were obtained from commercial sources and used without further purification. LC/MS was conducted on a number of systems for which details are provided in the supplementary data.

^{1}H NMR spectra, chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard. Purity of the final compounds was of >95% using interpretation of a combination of LCMS and NMR data unless stated otherwise.

3-(5,6-Dichloro-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid (**1**) was prepared as described in reference 2.

2-Amino-2-(hydroxymethyl)propane-1,3-diol 3-(5-chloro-6-methyl-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid (**2**) was prepared as described in reference 19.

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-(5-chloro-6-ethyl-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid (**3**). 5-Chloro-6-ethyl-2,3-dihydro-1,3-benzoxazol-2-one **36c** (475 mg, 2.41 mmol), 2-bromopropanoic acid (443 mg, 2.89 mmol) and potassium carbonate (665 mg, 4.82 mmol) were mixed in acetonitrile (10 mL) and stirred at 75 °C for

14 h. The cooled mixture was treated with hydrochloric acid (0.5N) until the mixture achieved a pH of between 2 and 4. The solvent was evaporated and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient (45 – 50%) containing TFA (0.1%) to give 3-(5-chloro-6-ethyl-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid (180 mg, 20%). A portion of this material (150 mg, 0.56 mmol) and 2-amino-2-(hydroxymethyl)propane-1,3-diol (68 mg, 0.56 mmol) were mixed in ethanol (9 mL) and water (3 mL) and the mixture stirred at room temperature for 1 h. The solvents were removed under reduced pressure to give the title compound as an off-white solid (218 mg, 100 %). ^{1}H NMR (CD_3OD) 7.37 (s, 1H), 7.17 (s, 1H), 4.06 (t, J = 7.2Hz, 2H), 3.65 (s, 6H), 2.77 (q, J = 7.4, 2H), 2.60 (t, J = 7.1, 2H), 1.21 (t, J = 7.5, 2H). LCMS: MH+ 268 / 270, retention time 1.55 min (method B).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-[5-chloro-6-(2-methylpropyl)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid (**4**). 5-Chloro-6-(2-methylpropyl)-2,3-dihydro-1,3-benzoxazol-2-one **36d** (200 mg, 0.88 mmol), 2-bromopropanoic acid (153 mg, 1.06 mmol) and potassium carbonate (138 mg, 0.88 mmol) were mixed in acetonitrile (10 mL) and stirred at 80 °C for 2 h. Water was added and the pH of the mixture adjusted to pH 7 by addition of ammonium chloride. The mixture was extracted with ethyl acetate, the organics dried over sodium sulphate and the solvent evaporated in vacuo. The residue was purified by preparative HPLC eluting with acetonitrile / water (55: 45) containing 0.1% TFA to give 3-[5-chloro-6-(2-methylpropyl)-2-oxo-2,3- dihydro-1,3-benzoxazol-3-yl]propanoic acid (45 mg, 17%). This material and 2-amino-2-(hydroxymethyl)propane-1,3-diol (18 mg, 0.15 mmol) were mixed in methanol (3 mL) and water (3 mL) and the mixture stirred at room temperature for 1 h. The solvents were removed under reduced pressure to give the title compound as a yellow oil (63 mg, 100 %). ^{1}H NMR (CD_3OD) 7.39 (s, 1H), 7.12 (s, 1H),

4.06 (t, J = 7.2Hz, 2H), 3.65 (s, 6H), 2.62 (m, 4H), 1.95 (m, 1H), 0.93 (d, J = 6.8, 6H). LCMS: MH+ 296, retention time 1.66 min (method B).

3-(5-Chloro-6-methoxy-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid (**5**). 5-Chloro-6-methoxy-2,3-dihydro-1,3-benzoxazol-2-one **36e** (150 mg, 0.75 mmol) in acetonitrile (10 mL) were added 2-bromopropanoic acid (115 mg, 0.75 mmol) and potassium carbonate (138 mg, 1.5 mmol) and the mixture stirred at 60 °C for 2 h. The solvent was evaporated and the residue adjusted to pH 4 by addition of hydrochloric acid (1 N). The mixture was extracted with ethyl acetate (3x 15 mL) and the combined organics dried over sodium sulphate. The residue was purified by chiral SFC (CO_2 / methanol containing 0.1 % TFA) to give the title compound as a white solid (60 mg, 29 %). ^{1}H NMR (CD_3OD) 7.39 (s, 1H), 7.16 (s, 1H), 4.08 (t, J = 6.7Hz, 2H), 3.89 (s, 3H), 2.76 (t, J = 6.2Hz, 2H). LCMS: MH+ 272, retention time 1.37 min (method C).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-(5-chloro-6-ethoxy-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid (**6**). A mixture of 5-chloro-6-ethoxy-2,3-dihydro-1,3-benzoxazol-2-one **36f** (150 mg, 0.70 mmol), 2-bromopropanoic acid (107 mg, 0.70 mmol) and potassium carbonate (97 mg, 0.7 mmol) in acetonitrile (10 mL) was heated at 80 °C for 16 h. The reaction mixture was diluted with water and acidified with hydrochloric acid. The mixture was extracted with ethyl acetate, the organic phase washed with water (3x 30 mL) and dried over sodium sulphate. The solvent was evaporated in vacuo and the residue purified by preparative HPLC (acetonitrile / water 1: 1, including 0.1% TFA) to give 3-(5-chloro-6-ethoxy-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid (38 mg, 19%). This material was treated with 2-mino-2-(hydroxymethyl)propane-1,3-diol (16 mg, 0.133 mmol) in methanol (5 mL) and

water (6 mL) and the mixture stirred for 1 h. The solvents were evaporated in vacuo to give the title compound as a yellow solid (54 mg, 100%). ^{1}H NMR (CD_3OD) 7.37 (s, 1H), 7.05 (s, 1H), 4.02 (m, 4H), 3.57 (s, 6H), 2.54 (m, 2H), 1.38 (3H, m). LCMS: MH+ 284, retention time 1.43 min (method B).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-(5-chloro-6-cyclopropoxy-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid (**7**). A mixture of 5-chloro-6-cyclopropoxy-2,3-dihydro-1,3-benzoxazol-2-one **36g** (183 mg, 0.81 mmol), 2-bromopropanoic acid (149 mg, 0.98 mmol) and potassium carbonate (224 mg, 1.62 mmol) in acetonitrile (5 mL) was heated at 73 °C for 14 h. The reaction was cooled to room temperature and acidified to between pH2 and pH4 with hydrochloric acid (0.5 N). The solvent was removed and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (35-45 % acetonitrile) to give 3-(5-chloro-6-cyclopropoxy-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)propanoic acid as a white solid (70 mg, 29 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (28.5 mg, 0.24 mmol) in ethanol (3 mL) and water (1 mL) at room temperature for 0.5 h. The solvent was evaporated to give the title compound as a white solid (48.5 mg, 100 %). ^{1}H NMR (CD_3OD) 7.38 (s, 1H), 7.37 (s, 1H), 4.04 (t, J = 7.2Hz, 2H), 3.86 (m, 1H), 3.64 (s, 6H), 2.59 (t, J = 7.1, 2H), 0.86 – 0.73 (m, 4H). LCMS: MH+ 286 / 288, retention time 1.51 min (method B).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-[5-chloro-6-(cyclopropylmethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid (**8**). Methyl 3-[5-chloro-6-(cyclopropylmethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate **40a** (38 mg, 0.12 mmol) in dioxane (5 mL) was added hydrochloric acid (0.6 N, 5 mL) and the mixture heated at 80 °C for 3 h. The mixture was poured into water (10 mL), extracted with ethyl acetate (2x

10 mL) and the combined organics dried (sodium sulphate). The residue was purified by preparative HPLC, eluting with an acetonitrile / water gradient containing 0.1 % TFA (40 - 70 % acetonitrile) to give 3-[5-chloro-6-(cyclopropylmethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid as a white solid (12 mg, 54 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (19 mg, 0.16 mmol) in methanol (5 mL) and the mixture stirred at room temperature for 0.5 h. The solvent was evaporated to give the title compound as a white solid (30 mg). ^{1}H NMR (CD_3OD) 7.39 (s, 1H), 7.09 (s, 1H), 4.06 (t, J = 6.9Hz, 2H), 3.88 (d, J = 6.6Hz, 2H), 3.66 (s, 6H), 1.29 (m, 1H), 0.63 (m, 2H), 0.40 (m, 2H). LCMS: MH+ 312, retention time 1.51 min (method B).

2-(Hydroxymethyl)propane-1,3-diol; 3-[5-chloro-6-(cyclobutylmethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid (**9**). To methyl 3-[5-chloro-6-(cyclobutylmethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate **40b** (80 mg, 0.24 mmol) in dioxane (2 mL) was added hydrochloric acid (0.5 N, 2 mL) and the reaction stirred at 85 °C for 3h. Water (5 mL) was added and the mixture extracted with ethyl acetate (3x 10 mL). The combined organic phases were dried (sodium sulphate) and purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (60-70 % acetonitrile) to give a white solid (30 mg). This material was dissolved in methanol (5 mL) and treated with 2-(hydroxymethyl)propane-1,3-diol (10 mg, 0.08mmol). The mixture was stirred at ambient temperature for 30 mi and concentrated to give the title compound as a white solid (40 mg, 38 %). ^{1}H NMR (CD_3OD) 7.40 (s, 1H), 7.11 (s, 1H), 4.05 (t, J = 7.0Hz, 2H), 3.98 (d, J = 6.4, 2H), 3.66 (s, 6H), 2.80 (m, 1H), 2.64 (t, J = 7.0Hz, 2H), 2.14 (m, 2H), 1.99 (m, 4H). LCMS: MH+ 326, retention time 1.57 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-[5-chloro-6-(2-methoxyethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid (**10**). Methyl 3-[5-chloro-6-(2-methoxyethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate **40c** (49 mg, 0.15 mmol) was treated with hydrochloric acid (0.5 N, 2 mL) in dioxane (4 mL) at 80 °C for 2 h. The solvent was evaporated and the residue purified by column chromatography (silica gel 200-300 mesh, petroleum ether / ethyl acetate 2:1, 200 mL). Evaporation of the solvents gave 3-[5-chloro-6-(2-methoxyethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid as a white solid (20 mg, 43 %). A sample of this material (10 mg, 0.03 mmol) was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (3.8 mg, 0.03 mmol) in ethanol (3 mL) and water (1 mL) at ambient temperature for 1 h. Removal of the solvents gave the title compound as an off-white solid (13.8 mg, 100 %). ^{1}H NMR (CD_3OD) 7.41 (s, 1H), 7.13 (s, 1H), 4.12 (m, 2H), 4.04 (t, J = 7.2Hz, 2H), 3.76 (m, 2H), 3.59 (s, 6H), 3.44 (s, 3H), 2.57 (t, J = 7.2Hz, 2H). LCMS: MH+ 316/318, retention time 1.40 min (method B).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-2-oxo-6-[2-(pyrrolidin-1-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**11**). Methyl 3-{5-chloro-2-oxo-6-[2-(pyrrolidin-1-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate **40d** (0.1 g, 0.27 mmol) was treated with hydrochloric acid (0.5 N, 2 mL) in dioxane (2 mL) and the mixture stirred at 80 °C for 3 h. The mixture was concentrated and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (10 – 15 % acetonitrile), to give 3-{5-chloro-2-oxo-6-[2-(pyrrolidin-1-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as a yellow oil (20 mg, 21 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (10 mg) in methanol (2 mL) and stirred at ambient temperature for 0.5 h. The mixture was concentrated to give the title compound as a yellow oil (3 mg, 20 %). ^{1}H NMR (CD_3OD) 7.45 (s, 1H), 7.17 (s, 1H), 4.27 (t, J = 5.1Hz,

2H), 4.05 (t, J = 6.9Hz, 2H), 3.64 (s, 6H), 3.38 (t, J = 5.1Hz, 2H), 3.20 (m, 4H), 2.58 (t, J = 7.0Hz, 2H), 2.00 (m, 4H). LCMS: MH+ 355, retention time 1.11 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-[6-(benzyloxy)-5-chloro-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid (**12**). Methyl 3-[6-(benzyloxy)-5-chloro-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate **40e** (80 mg, 0.22 mmol) was treated with hydrochloric acid (0.5 N, 3 mL) in dioxane (5 mL) and the mixture stirred at 80 °C for 2 h. The cooled mixture was diluted with water (10 mL) and extracted with ethyl acetate (3x 10 mL). The combined organic phases were dried over sodium sulphate and the solvent removed. The residue was purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (40 – 70 % acetonitrile), to give 3-[6-(benzyloxy)-5-chloro-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid as a white solid (17 mg, 22 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (6 mg, 0.05 mmol) in ethanol (3 mL) and water (1 mL) and stirred at ambient temperature for 0.5 h. The mixture was concentrated to give the title compound as a white solid (23 mg, 100 %). ^{1}H NMR (CD_3OD) 7.48 – 7.30 (m, 6H0, 7.15 (s, 1H), 5.14 (s, 2H), 3.55 (s, 6H), 4.04 (t, J = 7.1Hz, 2H), 2.57 (t, J = 7.2Hz, 2H). LCMS: MH+ 348, retention time 1.55 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-[5-chloro-2-oxo-6-(pyridin-2-ylmethoxy)-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic acid (**13**). Methyl 3-[5-chloro-2-oxo-6-(pyridin-2-ylmethoxy)-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate **40f** (135 mg, 0.37 mmol) was treated with hydrochloric acid (0.5 N, 4 mL) in dioxane (8 mL) and the mixture stirred at 80 °C for 2 h. The mixture was concentrated and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (20 – 60 % acetonitrile), to give 3-[5-chloro-2-oxo-6-(pyridin-2-ylmethoxy)-2,3-dihydro-1,3-benzoxazol-3-yl]propanoic

acid as a white solid (35 mg, 27 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (13 mg, 0.1 mmol) in ethanol (3 mL) and water (1 mL) and stirred at ambient temperature for 1 h. The mixture was concentrated to give the title compound as a white solid (50 mg, 100 %). ^{1}H NMR (CD_3OD) 8.65 (d, J = 4.5Hz, 1H), 8.00 (m, 1H), 7.90 (d, J = 7.9Hz, 1H), 7.53 (s, 1H), 7.49 (m, 1H), 7.33 (s, 1H), 5.32 (s, 2H), 4.17 (t, J = 6.7Hz, 2H), 3.76 (s, 6H), 2.86 (t, J = 6.7Hz, 2H). LCMS: MH+ 349/351, retention time 1.34 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-2-oxo-6-[1-(pyridin-2-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**14**) was prepared as described in reference 19.

2-Amino-2-(hydroxymethyl)propane-1,3-diol; (R)-3-(5-chloro-2-oxo-6-(1-(pyridin-2-yl)ethoxy)benzo[d]oxazol-3(2H)-yl)propanoate (**15**) was prepared as described in reference 19.

2-Amino-2-(hydroxymethyl)propane-1,3-diol; (S)-3-(5-chloro-2-oxo-6-(1-(pyridin-2-yl)ethoxy)benzo[d]oxazol-3(2H)-yl)propanoate (**16**) was prepared as described in reference 19.

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(1,3-oxazol-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**17**). A stirred solution of (R)-ethyl 3-(5-chloro-6-(1-(oxazol-2-yl)ethoxy)-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **40h** (50 mg, 0.13 mmol) in THF (5 mL) and water (1 mL) was cooled to 0 °C and lithium hydroxide monohydrate (6 mg, 0.13 mmol) added slowly. The reaction was maintained at 0

°C for 30 min and then allowed to warm to ambient temperature and maintained at this temperature for 2 h. The THF was evaporated in vacuo and cool water (50 mL) added to the residue. The aqueous phase pH was adjusted to pH 4 by addition of 10 % citric acid solution. The mixture was extracted with ethyl acetate (3x 30 mL), the combined organic layers dried (sodium sulphate) and concentrated under reduced pressure. The residue was purified by washing with 20% ether in n-pentane (3x 5 mL) to give 3-{5-chloro-6-[(1R)-1-(1,3-oxazol-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as a white solid (30 mg, 65 %). This material, dissolved in methanol (5 mL) at 20 °C was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (10 mg, 0.085 mmol) and the reaction stirred at this temperature for 10 min. The reaction was allowed to warm to room temperature, then warmed slowly to 60 °C and maintained at 60 °C for 2 h. The reaction was cooled to ambient temperature, the solvent evaporated in vacuo and the residue purified by washing with 30% ether in n-pentane (3x 5 mL) to give the title compound as an off-white solid (40 mg, 98 %). 1H NMR (D_6-DMSO) includes 8.34 (s, 1H), 8.12 (s, 1H), 7.49 (s, 1H), 7.39 (s, 1H), 5.49 (q, J = 6.5Hz, 1H), 3.92 (t, J = 7.0, 2H), 3.34 (s, 6H), ~2.50 (m, partially obscured by DMSO), 1.60 (d, J = 6.4Hz, 3H). LCMS: $[M-H]^-$ 351, retention time 1.81 min (method D).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-2-oxo-6-[(1R)-1-(pyrimidin-2-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**18**). A stirred solution of (R)-ethyl 3-(5-chloro-2-oxo-6-(1-(pyrimidin-2-yl)ethoxy)benzo[d]oxazol-3(2H)-yl)propanoate **40i** (200 mg, 0.51 mmol) in THF (5 mL) and water (1 mL) was cooled to 0 °C and lithium hydroxide monohydrate (42 mg, 1.02 mmol) added slowly. The reaction was maintained at 0 °C for 30 min and then allowed to warm to ambient temperature and maintained at this temperature for 2 h. The THF was evaporated and water (50 mL) added to the residue. The aqueous phase pH was adjusted to pH 4 by addition of 10 % citric acid solution. The mixture

was extracted with ethyl acetate (3x 30 mL), the combined organic layers dried (sodium sulphate) and concentrated under reduced pressure. The residue was purified by preparative HPLC (column: Sunfire 250x 30 mm, 10 μ, eluent: 0.1% formic acid in aqueous acetonitrile, flow rate: 30 mL / min) to give 3-{5-chloro-2-oxo-6-[(1R)-1-(pyrimidin-2-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as an off white solid (90 mg, 48 %). This material, dissolved in methanol (10 mL) at 20 °C was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (30 mg, 0.247 mmol) and the reaction stirred at 20-25 °C for 10 min. The reaction was allowed to warm to room temperature and then maintained at 60 °C for 2 h. The solvent was evaporated in vacuo and the residue purified by washing with n-pentane (3x 5 mL) to give the title compound as an off-white solid (80 mg, 70 %). 1H NMR (D_6-DMSO) 8.83 (d, J = 5.0Hz, 2H), 7.49 (s, 1H), 7.45 (t, J = 4.9Hz, 1H), 7.08 (s, 1H), 3.54 (q, J = 6.4, 1H), 3.91 (t, J = 7.0Hz, 2H), 3.33 (s, 6H), 1.68 (d, 3H). LCMS: MH+ 364, retention time 255 min (method E).

3-{5-Chloro-2-oxo-6-[(1R)-1-(pyridazin-3-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**19**). Methyl 3-{5-chloro-2-oxo-6-[1-(pyridazin-3-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate **40j** (380 mg, ~60% purity) was treated with hydrochloric acid (0.5 N, 10 mL) in dioxane (10 mL) and the mixture stirred at 90 °C for 5 h. The mixture was concentrated and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (20 – 50 % acetonitrile, Gemini-C18 column (150*21.2mm, 5μm)), to give 3-{5-chloro-2-oxo-6-[1-(pyridazin-3-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as a white solid (120 mg). The racemic mixture was separated by chiral chromatography (chiral column-IC, mobile phase: 30% Hexane:70% EtOH + 0.2%DEA-FA, flow rate-0.8 mL/min) to give 3-{5-chloro-2-oxo-6-[(1S)-1-(pyridazin-3-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (54 mg, 25 %,

chiral retention time 27.44 min) and the title compound as a white solid (58 mg, 26 %, chiral retention time 13.72 min). ^{1}H NMR (CD_3OD) 9.12 (d, J = 4.4Hz, 1H), 7.95 (d, J = 8.5Hz, 1H), 7.76 (dd, J = 8.5Hz, J = 5.6Hz, 1H), 7.39 (s, 1H), 7.11 (s, 1H), 5.77 (q, J = 5.9, 1H), 4.05 (t, J = 6.2Hz, 2H), 2.76 (t, J = 5.9Hz, 3H), 1.77 (d, J = 6.5Hz, 3H). LCMS: MH+ 364/366, retention time 1.37 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(4-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**20**). Methyl 3-{5-chloro-6-[(1R)-1-(4-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate **40k** (130 mg, 0.33 mmol) in hydrochloric acid (6 N, 5 mL) was heated at 100 °C for 2 h. The mixture was concentrated and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (30 – 60 % acetonitrile, Gemini-C18 column (150*21.2mm, 5µm)), to give 3-{5-chloro-6-[(1R)-1-(4-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as a yellow solid (25 mg, 20 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (8 mg, 0.06 mmol) in methanol (3 mL) and stirred at ambient temperature for 1 h. The mixture was concentrated to give the title compound as a yellow solid (33 mg, 100 %). ^{1}H NMR (D_6-DMSO) 8.38 (d, J = 4.8Hz, 1H), 7.49 (s, 1H), 7.33 (s, 1H), 7.17 (s, 1H), 7.12 (d, J = 4.6Hz, 1H), 5.48 (q, J = 6.4Hz, 1H), 3.90 (t, J= 6.3Hz, 2H), 3.43 (s, 6H), 2.46 (m, partially obscured by DMSO), 2.30 (s, 3H), 1.58 (d, J = 6.3Hz, 3H). LCMS: MH+ 377, retention time 1.33 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(5-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**21**). Methyl 3-{5-chloro-6-[(1R)-1-(5-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate

40l (125 mg) in hydrochloric acid (6 N, 10 mL) was heated at 100 °C for 4 h. The mixture was concentrated and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (30 – 60 % acetonitrile, Gemini-C18 column (150*21.2mm, 5µm)), to give 3-{5-chloro-6-[(1R)-1-(5-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as a yellow oil (27 mg, 23 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (9 mg, 0.07 mmol) in methanol (5 mL) and stirred at ambient temperature for 1 h. The mixture was concentrated to give the title compound as a yellow solid (37 mg, 100 %). ^{1}H NMR (CD_3OD) 8.46 (br s, 1H), 8.35 (m, 1H), 7.65 (dd, J= 8.1Hz, J = 1.8Hz, 1H), 7.45 (d, J = 7.9Hz, 1H), 7.39 (s, 1H), 6.90 (s, 1H), 5.40 (q, J = 6.5Hz, 1H), 4.03 (t, J = 6.6Hz, 2H), 3.67 (s, 6H), 2.65 (t, J = 6.6Hz, 2H), 2.34 (s, 3H), 1.67 (d, J = 6.3Hz, 3H). LCMS: MH+ 377, retention time 1.49 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(5-chloropyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**22**). Methyl 3-{5-chloro-6-[1-(5-chloropyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate **40m** (210 mg, 0.5 mmol) in hydrochloric acid (6 N, 8 mL) was heated at 100 °C for 2 h. The mixture was concentrated and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (30 – 70 % acetonitrile, Gemini-C18 column (150*21.2mm, 5µm)). The resulting yellow oil (130 mg) was further purified by chiral HPLC (column: AD-H) to give 3-{5-chloro-6-[(1R)-1-(5-chloropyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as a yellow oil (54 mg, 27 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (16 mg, 0.13 mmol) in methanol (5 mL) and stirred at ambient temperature for 1 h. The mixture was concentrated to give the title compound as a yellow oil (70 mg, 100 %). ^{1}H NMR (CD_3OD) 8.47 (d, J =

2.3Hz, 1H), 7.80 (dd, J = 8.6Hz, J = 2.3Hz, 1H), 7.52 (d, J = 8.3Hz, 1H), 7.37 (s, 1H), 6.91 (s, 1H), 5.41 (q, J = 6.5Hz, 1H), 3.98 (t, J = 7.1Hz, 2H), 3.62 (s, 6H), 2.53 (t, J = 7.1Hz, 2H), 1.63 (d, J= 6.3Hz, 3H). LCMS: MH+ 397, retention time 1.54 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(5-fluoropyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**23**). Methyl 3-{5-chloro-6-[(1R)-1-(5-fluoropyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate **40n** (120 mg, 0.30 mmol) in hydrochloric acid (6 N, 10 mL) was heated at 70 °C for 4 h. The volatiles were evaporated in vacuo and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (30 – 60 % acetonitrile, Gemini-C18 column (150*21.2mm, 5µm)), then further purified by preparative chiral HPLC (Chiralpak AD-H colmn (250*20 mm, 5µm), mobile phase was: EtOH (Formic Acid):Hexane = 50:50, flow rate = 0.8 mL/min, retention time = 7.979min) to give 3-{5-chloro-6-[(1R)-1-(5-fluoropyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as a yellow oil (37 mg, 32 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (12 mg, 0.09 mmol) in methanol (5 mL) and stirred at ambient temperature for 2 h. The mixture was concentrated to give the title compound as a yellow solid (49 mg, 100 %). ^{1}H NMR (CD_3OD) 8.42 (s, 1H), 7.61 (d, J = 1.7Hz, 1H), 7.59 (m, 1H), 7.41 (s, 1H), 6.95 (s, 1H), 5.46 (q, J = 6.6Hz, 1H), 4.02 (t, J = 7.3Hz, 2H), 3.65 (s, 6H), 2.57 (t, J = 7.1Hz, 2H), 1.67 (d, J = 6.6Hz, 3H). LCMS: MH+ 381, retention time 1.45 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(6-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid (**24**). Methyl 3-{5-chloro-6-[(1R)-1-(6-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate

40o (110 mg, 0.28 mmol) in hydrochloric acid (6 N, 10 mL) was heated at 100 °C for 2 h. The solvent was evaporated in vacuo and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (40 – 60 % acetonitrile, Gemini-C18 column (150*21.2mm, 5µm)), then further purified by preparative chiral HPLC (AD-H, formic acid) to give 3-{5-chloro-6-[(1R)-1-(6-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoic acid as a yellow solid (14 mg, 13 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (4 mg, 0.03 mmol) in methanol (5 mL) and stirred at ambient temperature for 1 h. The mixture was concentrated to give the title compound as a white solid (17 mg, 100 %). ^{1}H NMR (CD_3OD) 8.64 (br. S, 1H), 7.68 (t, J = 7.6Hz, 1H), 7.39 (s, 1H), 7.34 (d, J = 7.9Hz, 1H), 7.18 (d, J = 7.6Hz, 1H), 6.89 (s, 1H), 5.38 (q, J = 6.4Hz, 1H), 4.02 (t, J = 6.8Hz, 2H), 3.67 (s, 6H), 2.65 (t, J = 6.8Hz, 2H), 2.55 (s, 3H), 1.67 (d, J = 6.6Hz, 3H). LCMS: MH+ 377, retention time 1.35 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{6-chloro-3-oxo-7-[(1R)-1-(pyridin-2-yl)ethoxy]-3,4-dihydro-2H-1,4-benzoxazin-4-yl}propanoic acid (**25**). To methyl 3-{6-chloro-3-oxo-7-[(1R)-1-(pyridin-2-yl)ethoxy]-3,4-dihydro-2H-1,4-benzoxazin-4-yl}propanoate **47** (0.28 g 0.41 mmol) in dioxane (5 mL) was added hydrochloric acid (0.5 N, 5 mL) and the mixture stirred at 80 °C for 16 h. The solvent was removed and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (20 – 30 % acetonitrile). Further purification was attempted by SFC (c. hexane/ethanol 1:1, 0.2% formic acid, and diethylamine on chiralpak-AD). The material was further purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (20 – 40 % acetonitrile) to give 3-{6-chloro-3-oxo-7-[(1R)-1-(pyridin-2-yl)ethoxy]-3,4-dihydro-2H-1,4-benzoxazin-4-yl}propanoic acid as a white solid (50 mg, 32 %). This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (15 mg, 0.03 mmol) in methanol (2

mL) and stirred at ambient temperature for 0.5 h. The mixture was concentrated to give the title compound as a white solid (65 mg, 100 %). ^{1}H NMR (CD_3OD) 8.51 (m, 1H), 7.83 (m, 1H), 7.54 (d, J = 7.9Hz, 1H), 7.33 (ddd, J= 7.6Hz, J = 5.0Hz, J = 1.3Hz, 1H), 7.28 (s, 1H), 6.57 (s, 1H0, 5.41 (q, J = 6.6Hz, 1H), 4.51 (d, J = 2.3Hz, 2H), 4.15 (t, J = 7.6Hz, 2H), 3.65 (s, 6H), 2.59 (t, J = 7.6Hz, 2H), 1.67 (d, J= 6.3Hz, 3H). LCMS: MH+ 377, retention time 1.37 min (method A).

3-{6-Chloro-5-[(1R)-1-(pyridin-2-yl)ethoxy]-1H-indol-1-yl}propanoic acid (**26**). 3-(6-chloro-5-hydroxy-1H-indol-1-yl)propanoate **50** (112 mg, 0.42 mmol) and (1S)-1-(pyridin-2-yl)ethan-1-ol (57 mg, 0.46 mmol), triphenyl phosphine (132 mg, 0.50 mmol) and DEAD (88 mg, 0.50 mmol) were mixed in toluene (5 mL) and the reaction stirred at ambient temperature for 16 h. The solvent was removed by evaporation and the residue ethyl 3-{6-chloro-5-[(1R)-1-(pyridin-2-yl)ethoxy]-1H-indol-1-yl}propanoate **51** (390 mg, crude) used directly in the next reaction. To this material was added aqueous lithium hydroxide (0.5 N, 10mL) and THF (8mL) and the mixture stirred at ambient temperature for 2h. Water (20 mL) was added and the mixture washed with ethyl acetate (3x 10 mL). The aqueous was acidified (pH 4 -5) with hydrochloric acid (0.5N) and extracted with ethyl acetate (4x 10 mL). The combined organics were reduced to dryness and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (30 – 70 % acetonitrile, Gemini-C18 column (150*21.2mm, 5μm)) to give the title compound as a white solid (26 mg, 18%). ^{1}H NMR (CD_3OD) 8.49 (m, 1H), 7.80 (td, J = 7.6Hz, J = 7.6Hz, J = 1.8Hz, 1H), 7.60 (d, J = 7.9Hz, 1H), 7.49 (s, 1H), 7.29 (ddd, J = 7.6Hz, J = 5.0Hz, J = 1.2Hz, 1H), 7.15 (d, J = 3.2Hz, 1H), 6.96 (s, 1H), 6.22 (dd, J = 3.1Hz, J = 0.7Hz, 1H), 5.41 (q, J = 6.3Hz, 1H), 4.36 (t, J = 6.6Hz, 2H), 2.74 (t, J = 6.6Hz, 2H), 1.68 (d, J = 6.5Hz, 3H). (LCMS: MH+ 345 / 347, retention time 1.42 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{6-chloro-5-[(1R)-1-(pyridin-2-yl)ethoxy]-1H-indazol-1-yl}propanoic acid (**27**). Ethyl 3-{6-chloro-5-[(1R)-1-(pyridin-2-yl)ethoxy]-1H-indazol-1-yl}propanoate **56** (0.1 g, 0.27 mmol) in THF (5 mL) was added lithium hydroxide (1 N, 0.5 mL) and the reaction stirred at ambient temperature for 16 h. The solvents were evaporated and the residue diluted with water (5 mL). The mixture was acidified (< pH 7) with hydrochloric acid (1 N) and the mixture extracted with ethyl acetate (2x 10 mL). The combined organic extracts were dried (sodium sulphate) and purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (20 – 50 % acetonitrile, Gemini-C18 column (150*21.2mm, 5µm)) to give 3-{6-chloro-5-[(1R)-1-(pyridin-2-yl)ethoxy]-1H-indazol-1-yl}propanoic acid (45 mg, 43 %) as a yellow oil. This material was treated with 2-amino-2-(hydroxymethyl)propane-1,3-diol (16 mg, 0.13 mmol) in methanol (2 mL) and the mixture stirred at ambient temperature for 30 min. The mixture was concentrated to give the title compound as a white solid (60 mg, 100%). ^{1}H NMR (CD_3OD) 8.52 (m, 1H), 7.80 (m, 3H), 7.57 (d, J=7.9Hz, 1H), 7.32 (ddd, J = 7.6Hz, J = 5.0Hz, J = 1.0Hz, 1H), 7.07 (s, 1H), 5.47 (q, J = 6.3Hz, 1H), 4.56 (t, J = 7.1Hz, 2H), 3.66 (s, 6H), 2.72 (t, J = 7.1Hz, 2H), 1.71 (d, J = 6.6Hz, 3H). LCMS: MH+ 346, retention time 1.44 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(pyridin-2-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid (**28**) was prepared as described in reference 20.

3-{5-Chloro-2-oxo-6-[(1R)-1-(pyridin-2-yl)ethoxy]-2,3-dihydro-1,3-benzothiazol-3-yl}propanoic acid (**29**). Ethyl 3-(5-chloro-6-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-3-yl)propanoate **68** (106 mg, 0.35 mmol), (1S)-1-(pyridin-2-yl)ethan-1-ol (48 mg, 0.39 mmol),

triphenyl phosphine (120 mg, 0.45 mmol) and DEAD (92 mg, 0.53 mmol) were mixed in toluene (5 mL) and the mixture stirred at ambient temperature for 16 h. The solvent was evaporated and the residue purified by column chromatography (silica gel, 200-300 mesh, 4 g) eluting with petroleum ether / ethyl acetate (4:1). To give ethyl 3-{5-chloro-2-oxo-6-[(1R)-1-(pyridin-2-yl)ethoxy]-2,3-dihydro-1,3-benzothiazol-3-yl}propanoate **69** as a yellow oil (212 mg, crude). This material was treated with hydrochloric acid (0.5 N, 3 mL) in dioxane (3 mL) at 90 °C for 3 h. The reaction was concentrated and the residue purified by preparative HPLC eluting with an acetonitrile / water gradient containing 0.1 % TFA (40 – 60 % acetonitrile, Gemini-C18 column (150*21.2mm, 5μm)) to give the title compound as an off-white solid (59 mg, 44 %). ^{1}H NMR (CD_3OD) 8.67 (d, J = 4.7Hz, 1H), 8.22 (t, J = 7.2Hz, 1H), 7.87 (d, J = 7.9Hz, 1H), 7.67 (m, 1H), 7.48 (s, 1H), 7.27 (s, 1H), 5.61 (q, J = 6.5Hz, 1H), 4.17 (t, J = 6.9Hz, 2H), 2.70 (t, J = 6.9Hz, 2H), 1.76 (d, J = 6.5Hz, 3H). LCMS: MH+ 379 / 381, retention time 1.46 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(5-methylpyridin-2-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid (**30**). To a stirred suspension of (R)-ethyl 3-(5-chloro-6-(1-(5-methylpyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate **64b** (120 mg, 0.31 mmol) in THF / water (1:1, 3 mL) at 0 °C, lithium hydroxide (25.8 mg, 0.62 mmol) was added portion-wise and the reaction stirred at ambient temperature for 2 h. The volatiles were evaporated and the residue diluted with water. The mixture was washed with diethyl ether, the aqueous phase cooled to 0 °C and acidified to pH 4 by addition of 2N hydrochloric acid. The mixture was extracted with 10 % methanol in DCM, the organic washed with water and dried (sodium sulphate). The solvent was evaporated to give 3-{5-chloro-6-[(1R)-1-(5-methylpyridin-2-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid as an off white solid (90 mg, 81 %). This material was suspended in methanol (5 mL), 2-amino-2-

(hydroxymethyl)propane-1,3-diol (27.3 mg) added to the mixture and the reaction heated at reflux for 3 h. The volatiles were evaporated in vacuo and the residue co-distilled with DCM. The white solid was washed with n-pentane and dried to give the title compound as an off-white solid (75mg, 63%). ^{1}H NMR (D_6-DMSO) 8.41 (s, 1H), 8.03 (s, 1H), 7.62 (d, J = 7.9Hz, 1H), 7.38 (d, J = 7.9Hz, 1H), 7.32 (s, 1H), 5.71 (q, J = 6.4Hz, 1H), 3.08 (t, J = 7.1Hz, 2H), 2.60 (t, J = 7.3Hz, 2H), 2.27 (s, 3H), 1.66 (d, J = 6.4Hz, 3H). LCMS: MH+ 361, retention time 2.08 min (method D).

3-{5-chloro-6-[(1R)-1-(5-methylpyridin-2-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid hydrochloride salt (**30a**). 3-{5-chloro-6-[(1R)-1-(5-methylpyridin-2-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid (39 g, 108 mmol) in hydrochloric acid (0.5 N, 500 mL) was stirred at ambient temperature for 16 h. The solid was isolated by filtration and dried. The solid was dissolved in DCM (600 mL) and methanol(100 mL) and n-hexane (2 L) was added dropwise. The resulting solid was isolated by filtration to give the title compound as a white solid (35 g, 18 %). ^{1}H NMR (CD_3OD) 8.75 (s, 1H), 8.51 (dd, J = 8.3, 1.4 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H), 7.99 (s, 1H), 7.39 (s, 1H), 6.01 (q, J = 6.5 Hz, 1H), 3.25 (q, J = 7.4 Hz, 2H), 2.86 (t, J = 7.2 Hz, 2H), 2.59 (s, 3H), 1.89 (d, J = 6.5 Hz, 3H) LCMS: MH+ 361, retention time 1.48 min (method A).

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 3-{5-chloro-6-[(1R)-1-(5-chloropyridin-2-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid (**31**). To a stirred suspension of (R)-ethyl 3-(5-chloro-6-(1-(5-chloropyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate **64c** (140 mg, 0.34 mmol) in THF (5 mL) and water (2 mL), lithium hydroxide.monohydrate (43 mg, 1.02 mmol) was added and the reaction stirred at ambient temperature for 3 h. The reaction was concentrated to removed the THF and the aqueous acidified to pH 2 with 2N hydrochloric

acid. The mixture was extracted with 10 % methanol in DCM, the organic dried (sodium sulphate) and the solvent was evaporated. The residue was triturated with pentane to give 3-{5-chloro-6-[(1R)-1-(5-chloropyridin-2-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid as an off white solid (105 mg, 80 %). 100mg of this material was dissolved in methanol (5 mL), 2-amino-2-(hydroxymethyl)propane-1,3-diol (30.2 mg, 0.25 mmol) added to the mixture and the reaction heated at 70 °C for 3 h. The volatiles were evaporated in vacuo and the residue triturated with pentane to give the title compound as an off-white solid (85mg, 65%). ^{1}H NMR (D_6-DMSO) 8.64 (d, J = 2.0Hz, 1H), 8.06 (s, 1H), 7.96 (dd, J = 8..4Hz, J = 2.5Hz, 1H), 7.56 (d, J = 8.3Hz, 1H), 7.40 (s, 1H), 5.80 (q, J = 6.4Hz, 1H), 3.31 (s, 6H), 3.10 (t, J = 7.3Hz, 2H), 2.63 (t, J = 7.3Hz, 2H), 1.67 (d, J = 6.4Hz, 3H). LCMS: MH+ 381, retention time 2.44 min (method D).

3-{5-Chloro-6-[(1R)-1-(5-fluoropyridin-2-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid (**32**). To a stirred suspension of (R)-methyl 3-(5-chloro-6-(1-(5-fluoropyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate **64d** (150 mg, 0.40 mmol) in THF (10 mL) was added lithium hydroxide solution (0.5 N, 3.2 mL) and the reaction stirred at ambient temperature for 2 h. The reaction was concentrated, the residue diluted with water (10 mL) and acidified to pH 3 - 4 with 0.5N hydrochloric acid. The mixture was extracted with ethyl acetate (4x 10 mL). The combined organic extracts were dried (sodium sulphate) and concentrated to give the title compound as an off-white solid (65mg, 45%). NMR (CD_3OD) 8.49 (s, 1H), 7.89 (s, 1H), 7.72 – 7.56 (m, 2H), 7.14 (s, 1H), 5.68 (q, J = 6.4Hz, 1H), 3.21 (t, J = 7.3Hz, 2H), 2.84 (t, J = 7.3Hz, 2H), 1.76 (d, J = 6.4Hz, 3H). LCMS: MH+ 365, retention time 1.50 min (method A).

3-{5-chloro-6-[(1R)-1-(6-methylpyridazin-3-yl)ethoxy]-1,2-benzoxazol-3-yl}propanoic acid (**33**) was prepared as described in reference 20.

Amino-4-chloro-5-isobutylphenol (**34d**). A mixture of 4-chloro-5-isobutyl-2-nitrophenol **77** (420 mg, 1.83 mmol), zinc (600 mg, 9.17 mmol) and acetic acid (1 mL) in ethanol (20 mL) was heated at 85 °C for 1 h. The mixture was filtered and the solvents removed in vacuo. The title compound as the residual yellow solid was used without further purification (420 mg). LCMS: MH+ 200, retention time 1.55 min (method A).

2-Amino-4-chloro-5-ethoxyphenol (**34f**). 4-Chloro-5-ethoxy-2-nitrophenol[21] (700 mg, 3.22 mmol) in ethanol (20 mL) and acetic acid (2 mL) was treated with zinc (2 g, 32.2 mmol) and stirred at 60 °C for 1 h. The reaction was filtered and the filtrate reduced to dryness in vacuo to give the crude title as a yellow solid, which was used without further purification (600 mg). LCMS: MH+ 188, retention time 1.21 min (method A).

2-Amino-4-chloro-5-cyclopropoxyphenol (**34g**). 5-Chloro-4-cyclopropoxy-2-methoxyaniline **81** (470 mg, 2.21 mmol) was dissolved in DCM (10 mL) at 0 °C. Boron tribromide (1.1 g, 4.42 mmol) was added slowly and the reaction stirred for 1 h at ambient temperature following completion of the addition. The solvent was evaporated in vacuo and water (30mL) added slowly to the residue. The pH was adjusted to ~pH 8 with saturated sodium hydrogen carbonate and the mixture extracted with ethyl acetate (4x 20 mL). The combined organic phases were dried (sodium sulphate) and reduced to dryness in vacuo to give the crude title compound as a black oil (360 mg, 82 %). LCMS: MH+ 200 / 202, retention time 1.20 min (method A).

5-Chloro-6-ethyl-2,3-dihydro-1,3-benzoxazol-2-one (**35c**). 2-Amino-4-chloro-5-ethylphenol **34c**[22] (360 mg, 2.1 mmol) in THF (15 mL) was treated with CDI (683 mg, 4.2 mmol) and the mixture stirred at 60 °C for 1 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 10 g) eluting with petroleum ether : ethyl acetate (4:1) to give the title compound as a brown solid (47 mg, 100 %). LCMS: MH+ 196 / 198, retention time 1.38 min (method B).

5-Chloro-6-(2-methylpropyl)-2,3-dihydro-1,3-benzoxazol-2-one (**35d**). 2-Amino-4-chloro-5-isobutylphenol **34d** (400 mg, 2.0 mmol) in THF (20 mL) was treated with CDI (648 mg, 4.0 mmol) and the mixture heated at 80 °C for 2 h. The solvent was evaporated in vacuo and the residue purified by chromatography (silica gel), eluting with petroleum ether / ethyl acetate (20:1) to give the title compound as a yellow oil (240 mg, 54 %). LCMS: MH+ 226, retention time 1.61 min (method A).

5-Chloro-6-methoxy-2,3-dihydro-1,3-benzoxazol-2-one (**35e**). 2-Amino-4-chloro-5-methoxyphenol[23] **34e** (435 mg, 2.57 mmol) in DCM (20 mL) were added triethylamine (635 mg, 6.28 mmol) and triphosgene (260 mg, 0.88 mmol) at 0 °C and the mixture stirred at 0 °C for 2 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 4 g) eluting with petroleum ether / ethyl acetate (4:1) to give the title compound as a yellow solid (0.3 g, 60 %). ^{1}H NMR (D_6-DMSO) 11.57 (br. s, 1H), 7.30 (s, 1H), 7.16 (s, 1H), 3.82 (s, 3H).

5-Chloro-6-ethoxy-2,3-dihydro-1,3-benzoxazol-2-one (**35f**). 2-Amino-4-chloro-5-ethoxyphenol **34f** (700 mg, 3.74 mmol) in DCM (20 mL) at ambient temperature was treated with triethylamine (1 mL) and then with triphosgene (1.1 g, 3.74 mmol). The reaction was

stirred for 4 h. The solvent was evaporated in vacuo and the residue purified by chromatography (silica gel) eluting with petroleum ether / ethyl acetate (9:1, 5:1 then 4:1) to give the title compound as a pink solid (380 mg, 44 %). ^{1}H NMR (CD_3OD) 7.09 (s, 1H), 7.08 (s, 1H), 4.08 (q, J = 6.9Hz, 2H), 1.42 (t, J = 6.9Hz, 3H).

5-Chloro-6-cyclopropoxy-2,3-dihydro-1,3-benzoxazol-2-one (**35g**). A mixture of 2-amino-4-chloro-5-cyclopropoxyphenol **34g** (300 mg, 1.89 mmol), CDI (587 mg, 3.62 mmol) in THF (20 mL) was stirred at 60 °C for 1 h. A second mixture of 4-chloro-5-cyclopropoxy-2-nitrophenol **34g** (22 mg, 0.11 mmol), CDI (25.3 mg, 0.22 mmol) in THF (5 mL) was stirred at 60 °C for 2 h. The solvents were removed from both reactions, the residues combined and purified by chromatography (silica gel, 200-300 mesh) eluting with petroleum ether / ethyl acetate (30:1) to give the title compound as a brown solid (220 mg, 51 %). LCMS: MH+ 224 / 226, retention time 1.47 min (method B).

Methyl 3-[5-chloro-6-(cyclopropylmethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate (**40a**). To methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate[19] **39** (70 mg, 0.26 mmol) in toluene (10 mL) were added cyclopropylmethanol (22 mg, 0.31 mmol), DEAD (45 mg, 0.26 mmol) and triphenylphosphine (68 mg, 0.26 mmol) and the mixture stirred at ambient temperature for 1.5 h. Water (10 mL) was added and the mixture extracted with ethyl acetate (2x 10 mL). The combined organics were dried (sodium sulphate) and purified by chromatography (silica gel, 200-300 mesh, 2 g), eluting with petroleum ether / ethyl acetate (10:1) to give the title compound as a purple solid (38 mg, 45 %). LCMS: MH+ 326, retention time 1.63 min (method A).

Methyl 3-[5-chloro-6-(cyclobutylmethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl] propanoate (**40b**). Methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (130 mg, 0.48 mmol) in DMF (5 mL) was treated with cyclobutylmethyl 4-methylbenzenesulfonate (140 mg, 0.57 mmol) and potassium carbonate (130 mg, 0.96 mmol) and the mixture stirred at 80 °C for 2 h. The mixture was poured into water (20 mL), extracted with ethyl acetate (3x 10 mL) and the combined organics dried (sodium sulphate). The residue was purified by chromatography (silica gel, 200-300 mesh, 10 g) eluting with petroleum ether / ethyl acetate (10:1 to 4:1) to give the title compound as a colourless oil (180 mg, 49 %). LCMS: MH+ 340, retention time 1.73 min (method A).

Methyl 3-[5-chloro-6-(2-methoxyethoxy)-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate (**40c**). Methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (100 mg, 0.37 mmol), 1-bromo-2-methoxyethane (62 mg, 0.44 mmol) and cesium carbonate (180 mg, 0.55 mmol) were mixed in acetone (5 mL) and stirred at 70 °C for 15 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 10 g) eluting with petroleum ether / ethyl acetate (3:1, 150 mL) to give the title compound (49 mg, 40 %). LCMS: MH+ 330/332, retention time 1.51 min (method A).

Methyl 3-{5-chloro-2-oxo-6-[2-(pyrrolidin-1-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate (**40d**). To methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (130 mg, 0.48 mmol) in toluene (10 mL) were added 2-(pyrrolidin-1-yl)ethanol (66 mg, 0.57 mmol), DEAD (84 mg, 0.48 mmol) and triphenylphosphine (126 mg, 0.48 mmol) and the mixture stirred at ambient temperature for 48 h. Water (40 mL) was added and the mixture extracted with ethyl acetate (2x 20 mL). The combined organics were dried (sodium sulphate) and purified by chromatography (silica gel, 200-300 mesh, 10 g),

eluting with petroleum ether / ethyl acetate (4:1) to give the title compound as a yellow oil (101 mg, 56 %). LCMS: MH+ 369, retention time 1.18 min (method A).

Methyl 3-[6-(benzyloxy)-5-chloro-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate (**40e**). Methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (100 mg, 0.37 mmol), benzyl bromide (76 mg, 0.44 mmol) and potassium carbonate (102 mg, 0.74 mmol) were mixed in acetone (5 mL) and stirred at 70 °C for 15 h. The solvent was evaporated in vacuo and the residue purified by chromatography (silica gel, 200-300 mesh) eluting with petroleum ether / ethyl acetate (5:1, 300 mL) to give the title compound (58 mg, 44 %). LCMS: MH+ 362/364, retention time 1.67 min (method A).

Methyl 3-[5-chloro-2-oxo-6-(pyridin-2-ylmethoxy)-2,3-dihydro-1,3-benzoxazol-3-yl]propanoate (**40f**). To methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (300 mg, 1.1 mmol) in toluene (10 mL) were added pyridin-2-ylmethanol (121 mg, 1.1 mmol), DEAD (192 mg, 1.1 mmol) and triphenylphosphine (290 mg, 1.1 mmol) and the mixture stirred at ambient temperature for 15 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 15 g), eluting with petroleum ether / ethyl acetate (1:1) to give the title compound as a yellow oil (135 mg, 34 %). LCMS: MH+ 363/365, retention time 1.51 min (method A).

(R)-ethyl 3-(5-chloro-6-(1-(oxazol-2-yl)ethoxy)-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate (**40h**). To a stirred solution of ethyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (700 mg, 2.45 mmol) in DMF (20 mL) were added potassium carbonate (676 mg, 4.9 mmol) and 1-(oxazol-2-yl)ethyl methanesulfonate **83** (468 mg, 2.45 mmol). The tube was sealed and heated at 60 °C for 3 h. The reaction was cooled, filtered through

Celite™. The filtrate was diluted with cool water (300 mL) and the mixture extracted with ethyl acetate (3x 100 mL). The combined organic phases were dried (sodium sulphate) and concentrated under reduced pressure. The residue was purified by chromatography (silica gel) eluting with ethyl acetate / hexane (1:1). The resulting racemic compound was further purified by chiral HPLC (column: OY-H, elutent: 0.5% diethylamine in methanol, flow rate 30 mL /min) to give the title compound (peak 1) as an off-white solid (50 mg, 5 %) and (S)-ethyl 3-(5-chloro-6-(1-(oxazol-2-yl)ethoxy)-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate (peak 2, 30 mg, 3%).

(R)-ethyl 3-(5-chloro-6-(1-(oxazol-2-yl)ethoxy)-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate LCMS: MH+ 381, retention time 2.30 min (method D). Chiral SFC: retention time 2.98 min, e.e. 99.0 % (method J).

(R)-ethyl 3-(5-chloro-6-(1-(oxazol-2-yl)ethoxy)-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate Chiral SFC: retention time 3.14 min, e.e. 93.5 % (method J).

(R)-ethyl 3-(5-chloro-2-oxo-6-(1-(pyrimidin-2-yl)ethoxy)benzo[d]oxazol-3(2H)-yl)propanoate (**40i**). To a stirred solution of ethyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (800 mg, 2.80 mmol) in DMF (20 mL) were added potassium carbonate (773 mg, 5.60 mmol) and 1-(pyrimidin-2-yl)ethyl methanesulfonate[24] (566 mg, 2.80 mmol) in a single portion. The tube was sealed and heated at 60 °C for 3 h. The reaction was cooled, filtered through Celite™. The filtrate was diluted with aqueous ammonium chloride (20 %, 200 mL) and the mixture extracted with ethyl acetate (3x 50 mL). The combined organic phases were dried (sodium sulphate) and concentrated under reduced pressure. The residue was purified by chromatography (silica gel) eluting with ethyl acetate / hexane (1:1). The resulting racemic compound was further

purified by chiral HPLC (column: IC, elutent: 0.5% diethylamine in methanol) to give the title compound (peak 1) as an off-white solid (200 mg, 18 %) (S)-ethyl 3-(5-chloro-2-oxo-6-(1-(pyrimidin-2-yl)ethoxy)benzo[d]oxazol-3(2H)-yl)propanoate (peak 2, 200 mg, 18 %).

(R)-ethyl 3-(5-chloro-2-oxo-6-(1-(pyrimidin-2-yl)ethoxy)benzo[d]oxazol-3(2H)-yl)propanoate LCMS: MH+ 392, retention time 2.11 min (method D). Chiral SFC: retention time 3.52 min, e.e. 100 % (method K).

(S)-ethyl 3-(5-chloro-2-oxo-6-(1-(pyrimidin-2-yl)ethoxy)benzo[d]oxazol-3(2H)-yl)propanoate Chiral SFC: retention time 4.32 min, e.e. 89.4 % (method K).

Methyl 3-{5-chloro-2-oxo-6-[1-(pyridazin-3-yl)ethoxy]-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate (**40j**). Methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (236 mg, 0.87 mmol), 1-(pyridazin-3-yl)ethyl methanesulfonate **84** (160 mg, 0.79 mmol) and potassium carbonate (142 mg, 1.03 mmol) were mixed in acetonitrile (5 mL) and the mixture stirred at 72 °C for 16 h. The solvent was evaporated to give crude title compound as a yellow oil (380 mg, purity 60 %). LCMS: MH+ 378/380, retention time 1.87 min (method A).

3-{5-Chloro-6-[(1R)-1-(4-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate (**40k**). To methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (316 mg, 1.16 mmol) in THF (20 mL) were added (S)-1-(4-methylpyridin-2-yl)ethanol **85** (160 mg, 1.16 mmol), DEAD (201 mg, 1.16 mmol) and triphenylphosphine (304 mg, 1.16 mmol) and the mixture stirred at ambient temperature for 16 h. The solvent was evaporated in vacuo and the residue purified by chromatography (silica gel, 200-400 mesh, 20 g), eluting with petroleum ether / ethyl acetate (9:1) to give the title compound as a yellow oil (130 mg, 28 %). LCMS: MH+ 391, retention time 1.57 min (method A).

Methyl 3-{5-chloro-6-[(1R)-1-(5-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate (**40l**). A mixture of methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (190 mg, 0.73 mmol), (S)-1-(5-methylpyridin-2-yl)ethanol **86** (100 mg, 0.73 mmol), DEAD (127 mg, 0.73 mmol) and triphenylphosphine (191 mg, 0.73 mmol) in THF (10 mL) was heated at 45 °C for 14 h. The solvent was evaporated in vacuo and the residue purified by chromatography (silica gel), eluting with petroleum ether / ethyl acetate (9:1, 7:1, 5:1 and 4:1) to give the title compound as a yellow oil (125 mg, 45 %). LCMS: MH+ 391, retention time 1.63 min (method A).

Methyl 3-{5-chloro-6-[1-(5-chloropyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate (**40m**). Methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (140 mg, 0.51 mmol), 1-(5-chloropyridin-2-yl)ethyl methanesulfonate **87** (121 mg, 0.51 mmol) and potassium carbonate (70 mg, 0.51 mmol) were mixed in acetonitrile (10 mL) and the mixture stirred at 60 °C for 16 h. The reaction was cooled and filtered and the solvent was evaporated in vacuo to give crude title compound as a yellow oil (210 mg). LCMS: MH+ 411, retention time 1.66 min (method A).

Methyl 3-{5-chloro-6-[(1R)-1-(5-fluoropyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate (**40n**). A mixture of methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (153 mg, 0.56 mmol), (S)-1-(5-fluoropyridin-2-yl)ethanol (80 mg, 0.56 mmol), DEAD (98 mg, 0.56 mmol) and triphenylphosphine (146 mg, 0.56 mmol) in THF (10 mL) was heated at 50 °C for 16 h. The solvent was evaporated in vacuo and the residue purified by chromatography (silica gel), eluting with petroleum ether /

ethyl acetate (12:1, 9:1, 7:1, and 6:1) to give the title compound as a yellow oil (120 mg, 54 %). LCMS: MH+ 395, retention time 1.66 min (method A).

Methyl 3-{5-chloro-6-[(1R)-1-(6-methylpyridin-2-yl)ethoxy]-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl}propanoate (**40o**). A mixture of methyl 3-(5-chloro-6-hydroxy-2-oxobenzo[d]oxazol-3(2H)-yl)propanoate **39** (190 mg, 0.73 mmol), (S)-1-(6-methylpyridin-2-yl)ethanol (100 mg, 0.73 mmol), DEAD (127 mg, 0.73 mmol) and triphenylphosphine (191 mg, 0.73 mmol) in THF (20 mL) was stirred at ambient temperature for 16 h. The solvent was evaporated in vacuo and the residue purified by chromatography (silica gel), eluting with petroleum ether / ethyl acetate (10:1, 7:1, 5:1, and 4:1) to give the title compound as a yellow oil (110 mg, 41 %). LCMS: MH+ 391, retention time 1.58 min (method A).

7-Bromo-6-chloro-2H-benzo[b][1,4]oxazin-3(4H)-one (**42**). To 2-amino-5-bromo-4-chlorophenol **41** (2.0 g, 8.99 mmol) in DMF (80 mL) at 0 °C was added 2-chloroacetyl chloride (1.1 g, 9.89 mmol) and the mixture stirred at 0 °C for 1 h. Potassium acetate (2.5 g, 17.98 mmol) was added and the reaction stirred at ambient temperature for 16 h. Water (100 mL) was added and the resulting brown solid isolated by filtration to give the title compound as a brown solid (1.94 g, 82 %). LCMS: MH+ 261, retention time 1.59 min (method B).

3-(7-Bromo-6-chloro-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)propanenitrile (**43**). To 7-bromo-6-chloro-2H-benzo[b][1,4]oxazin-3(4H)-one **42** (1.94 g, 7.39 mmol) in acetonitrile (100 mL) were added acrylonitrile (0.78 g, 14.8 mmol) and potassium carbonate (2.04 g, 14.8 mmol) and the reaction stirred at 70 °C for 16 h. The solvent was evaporated and water (50 mL) added to the residue. The mixture was extracted with ethyl acetate (3x 50 mL), the combined organics dried (sodium sulphate) and concentrated to give the title compound as a

light brown solid (2.1g, 90 %). ^{1}H NMR ($CDCl_3$) 7.30 (s, 1H), 7.11 (s, 1H), 4.65 (s, 2H), 4.21 (t, J = 7.0Hz, 2H), 2.80 (t, J = 7.0Hz, 2H).

Methyl 3-(7-bromo-6-chloro-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)propanoate (**44**). To 3-(7-bromo-6-chloro-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)propanenitrile **43** (2.1 g, 6.66 mmol) in methanol (100 mL) was added concentrated hydrochloric acid (10 mL) and the reaction heated at 90 °C for 16 h. The solvent was evaporated and water (100 mL) added to the residue. The mixture was extracted with ethyl acetate (3x 80 mL), the combined organics dried (sodium sulphate) and purified by chromatography (silica gel, 200-300 mesh, 20 g), eluting with petroleum ether / ethyl acetate (20:1) to give the title compound as a yellow solid (1.2 g, 52 %). LCMS: MH+ 349, retention time 1.59 min (method A).

Methyl 3-(6-chloro-3-oxo-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2H-benzo[b][1,4]oxazin-4(3H)-yl)propanoate (**45**). To methyl 3-(7-bromo-6-chloro-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)propanoate **44** (1.2 g, 3.44 mmol) in dioxane (100 mL) were added 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (2.62 g, 10.3 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride (126 mg, 0.17 mmol) and potassium acetate (675 mg, 6.88 mmol) and the mixture stirred at 100 °C under an argon atmosphere for 16 h. The solvent was evaporated, water (100 mL) added to the residue and the mixture extracted with ethyl acetate (3x 80 mL). The combined extracts were dried (sodium sulphate) and purified by chromatography (silica gel, 200-300 mesh, 10 g), eluting with petroleum ether / ethyl acetate (10:1) to give the crude title compound as a yellow oil (1.61g). LCMS: MH+ 396, retention time 1.77 min (method A).

Methyl 3-(6-chloro-7-hydroxy-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)propanoate (**46**). To methyl 3-(6-chloro-3-oxo-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2H-benzo[b][1,4]oxazin-4(3H)-yl)propanoate **45** (1.6 g, 3.44 mmol) in THF (40 mL) were added acetic acid (4 mL) and hydrogen peroxide (30% solution, 5 mL) and the reaction stirred at ambient temperature for 3 h. The reaction was poured into water (20 mL) and extracted with ethyl acetate (3x 5 mL). The combined organics were dried (sodium sulphate) and purified by chromatography (silica gel, 200-300 mesh, 10 g), eluting with petroleum ether / ethyl acetate (4:1) to give the title compound as a white solid (0.49 g, 42 %). LCMS: MH+ 286, retention time 1.37 min (method A).

Methyl 3-{6-chloro-3-oxo-7-[(1R)-1-(pyridin-2-yl)ethoxy]-3,4-dihydro-2H-1,4-benzoxazin-4-yl}propanoate (**47**). To methyl 3-(6-chloro-7-hydroxy-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)propanoate **46** (116 mg, 0.41 mmol) in toluene (10 mL) were added (S)-1-(pyridin-2-yl)ethanol (50 mg, 0.41 mmol), triphenyl phosphine (161 mg, 0.62 mmol) and DEAD (107 mg, 0.62 mmol) and the mixture stirred at ambient temperature for 2 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 2 g), eluting with petroleum ether / ethyl acetate (4:1 to 3:1) to give the crude title compound as a white solid (0.27 g). LCMS: MH+ 391, retention time 1.54 min (method A).

Ethyl 3-(6-chloro-5-methoxy-1H-indol-1-yl)propanoate (**49**). 6-chloro-5-methoxy-1H-indole[25] **48** (2.23g, 12.3 mmol), ethyl acrylate (1.48 g, 14.8mmol) and potassium phosphate (653 mg, 3.0 mmol) were mixed in acetonitrile (50 mL) and the reaction stirred at ambient temperature for 18 h. The reaction was filtered and the filtrate reduced to dryness to give crude title compound as a yellow oil (2.52g). This was used directly in the next step without further purification. LCMS: MH+ 282/284, retention time 1.63 min (method A).

Ethyl 3-(6-chloro-5-hydroxy-1H-indol-1-yl)propanoate (**50**). Ethyl 3-(6-chloro-5-methoxy-1H-indol-1-yl)propanoate **49** (crude, 2.52 g) and aluminium chloride (4.0 g, 26.9 mmol) were dissolved in toluene (20 mL) and the mixture stirred at 100 °C for 15 min. The reaction was cooled to 0 °C and water (100 mL) added drop-wise. The mixture was extracted with ethyl acetate (5x 20 mL) and the combined organic phases dried (sodium sulphate). The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 12 g), eluting with petroleum ether / ethyl acetate (5:1) to give the title compound as a black oil (1.1 g, 33% over 2 steps). LCMS: MH+ 268/270, retention time 1.47 min (method A).

Ethyl 3-(5-bromo-6-chloro-1H-indazol-1-yl)propanoate (**53**). 5-bromo-6-chloro-1H-indazole **52** (2.0 g, 8.7 mmol), ethyl acrylate (955 mg, 9.54 mmol) and DBU (1.45 g, 9.54 mmol) were mixed in acetonitrile (20 mL) and the reaction stirred at ambient temperature for 5 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 4 g), eluting with petroleum ether / ethyl acetate (6:1, 200 mL) to give the title compound as a yellow oil (1.0 g, 35 %). ^{1}H NMR ($CDCl_3$) 8.00 (s, 1H), 7.94 (d, J = 0.9, 1H), 7.68 (s, 1H), 4.62 (t, J = 6.6Hz, 2H), 4.11 (q, J = 7.1Hz, 2H), 2.98 (t, J = 6.6Hz, 2H), 1.21 (t, J = 7.1Hz, 3H).

Ethyl 3-(6-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazol-1-yl)propanoate (**54**). To ethyl 3-(5-bromo-6-chloro-1H-indazol-1-yl)propanoate **53** (0.75 g, 2.36 mmol)) in dioxane (50 mL) were added 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.8 g, 7.09 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride (86 mg, 0.12 mmol) and potassium acetate (463 mg, 4.72 mmol) and the mixture stirred at 100 °C under an argon atmosphere for 16 h. The solvent was evaporated and the

residue purified by chromatography (silica gel, 200-300 mesh, 20 g), eluting with petroleum ether / ethyl acetate (10:1) to give the crude title compound as a yellow oil (1.5 g). LCMS: MH+ 379, retention time 1.81 min (method A).

Ethyl 3-(6-chloro-5-hydroxy-1H-indazol-1-yl)propanoate (**55**). To ethyl 3-(6-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazol-1-yl)propanoate **54** (1.5 g, 2.36 mmol) in acetic acid (6 mL) were added THF (6 mL) and hydrogen peroxide (30 % solution, 2 mL) and the reaction stirred at ambient temperature for 2 h. The solvent was evaporated and water (10 mL) added to the residue. The mixture was extracted with ethyl acetate (3x 10 mL) and the combined organics dried (sodium sulphate). The residue was purified by chromatography (silica gel, 200-300 mesh, 20 g), eluting with petroleum ether / ethyl acetate (10:1 to 1:1) to give the title compound as a yellow solid (0.5 g, 79 %). LCMS: MH+ 269, retention time 1.44 min (method A).

(R)-ethyl 3-(5-chloro-6-(1-(5-methylpyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate %). ^{1}H NMR (D_6-DMSO) 8.41 (d, J= 0.9Hz, 1H), 8.06 (s, 1H), 7.62 (dd, J = 7.9Hz, J = 1.5Hz, 1H), 7.39 (d, J = 7.7Hz, 1H), 7.34 (s, 1H), 5.72 (q, J = 6.7Hz, 1H), 4.40 (q, J = 7.2Hz, 2H), 3.16 (t, J = 7.1Hz, 2H), 2.81 (t, J = 7.3Hz, 2H), 2.27 (s, 3H), 1.66 (d, J = 6.4Hz, 3H), 1.14 (t, J = 7.1Hz, 3H). LCMS: MH+ 389, retention time 2.56 min (method D).

Ethyl 3-{6-chloro-5-[(1R)-1-(pyridin-2-yl)ethoxy]-1H-indazol-1-yl}propanoate (**56**). Ethyl 3-(6-chloro-5-hydroxy-1H-indazol-1-yl)propanoate **55** (90 mg, 0.33 mmol) in toluene (10 mL) were added (S)-1-(pyridin-2-yl)ethanol (42 mg, 0.33 mmol), triphenyl phosphine (130 mg, 0.50 mmol) and DEAD (87 mg, 0.50 mmol) and the mixture stirred at ambient temperature for 16 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 5 g), eluting with petroleum ether / ethyl acetate

(10:1 to 3:1) to give the crude title compound as a yellow oil (0.1 g). LCMS: MH+ 374, retention time 1.65 min (method A).

1-(5-Methylpyridin-2-yl)ethyl methanesulfonate (**63b**). To a stirred solution of 1-(5-methylpyridin-2-yl)ethanol (100 mg, 0.729 mmol) in DCM (5 mL) at 0 °C was added triethylamine (110 mg, 1.0 mmol) and the reaction stirred for 15 min at 0 °C. Mesyl chloride (91.4 mg, 0.802 mmol) was added drop-wise and stirring continued for 2 h. The reaction was washed with brine, the aqueous extracted with DCM. The combined organic phases were washed with brine, dried (sodium sulphate) and concentrated to give the crude title compound as a brown oil (150 mg). Used without further purification.

(R)-ethyl 3-(5-chloro-6-(1-(5-methylpyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate (**64b**). Potassium carbonate (192 mg, 1.39 mmol) was added to a stirred suspension of ethyl 3-(5-chloro-6-hydroxybenzo[d]isoxazol-3-yl)propanoate[20] **62a** (250 mg, 0.929 mmol) in DMF (5 mL) and the mixture stirred for 30 min. 1-(5-Methylpyridin-2-yl)ethyl methanesulfonate **63b** (220mg, 1.02 mmol) was dissolved in DMF (5 mL) and added to the mixture. The reaction was heated in a sealed tube at 80 °C overnight. The reaction was filtered through Celite™, the Celite washed with ethyl acetate (60 mL) and the filtrate diluted with saturated ammonium chloride solution. The mixture was extracted with ethyl acetate (3x 50 mL), the combined organic phases washed with brine, dried (sodium sulphate) and concentrated. The residue was purified by chromatography eluting with ethyl acetate / petroleum ether (3:17) to give the racemic compound as an off-white solid. The isomers were separated by chiral SFC (column: Chiralpak AD-H (260x 4.6 mm, 5µ, Solvents: CO2 / methanol (60:40), flow 4.0 g/min, uv 210 nm) to give the title compound (120 mg, 30 %) and

(S)-ethyl 3-(5-chloro-6-(1-(5-methylpyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate (130 mg).

(R)-ethyl 3-(5-chloro-6-(1-(5-methylpyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate ^{1}H NMR (D_6-DMSO) 8.40 (d, J = 0.9Hz, 1H), 8.05 (s, 1H), 7.61 (dd, J = 7.9Hz, J = 1.5Hz, 1H), 7.38 (d, J = 7.7Hz, 1H), 7.34 (s, 1H), 5.71 (q, J = 6.7Hz, 1H), 4.04 (q, J = 7.2Hz, 2H), 3.15 (t, J = 7.1Hz, 2H), 2.80 (t, J = 7.3Hz, 2H), 2.26 (s, 3H), 1.65 (d, J = 6.4Hz, 3H), 1.13 (t, J = 7.1Hz, 3H). LCMS: MH+ 389, retention time 2.56 min (method D). Chiral SFC: retention time 2.67 min, e.e. 99.9% (method I).

(S)-ethyl 3-(5-chloro-6-(1-(5-methylpyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate Chiral SFC: retention time 5.18 min, e.e. 99.1% (method I).

(R)-Ethyl 3-(5-chloro-6-(1-(5-chloropyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate (**64c**). A stirred solution of potassium carbonate (513 mg, 3.71 mmol) and ethyl 3-(5-chloro-6-hydroxybenzo[d]isoxazol-3-yl)propanoate **62a** (500 mg, 1.85 mmol) in acetonitrile (10 mL) was treated with 1-(5-chloropyridin-2-yl)ethyl methanesulfonate[26] **63c** (524mg, 2. 23 mmol) and the reaction was heated at 70 °C for 6 h. The reaction was filtered through Celite™, the Celite washed with ethyl acetate (100 mL) and the filtrate washed with water (50 mL) and brine. The organic phase was dried (sodium sulphate) and concentrated. The residue was triturated with pentane to give the racemic compound as an off-white solid (400mg). The isomers were separated by chiral SFC (column: Chiralpak AD-H (250x 4.6 mm, 5µ, Solvents: CO_2 / 0.5% diethylamine in methanol) to give the title compound (140mg, 18 %) and (S)-ethyl 3-(5-chloro-6-(1-(5-chloropyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate (160 mg).

(R)-ethyl 3-(5-chloro-6-(1-(5-chloropyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate. ^{1}H NMR ($CDCl_3$) 8.56 (d, J = 2.2Hz, 1H), 7.66 (m, 2H), 7.44 (d, J= 8.6Hz, 1H), 6.94 (s, 1H),

5.52 (q, J = 6.6Hz, 1H), 4.17 (q, J = 7.2Hz, 2H), 3.21 (t, J = 7.5Hz, 2H), 2.86 (t, J = 7.5Hz, 2H), 1.78 (d, J = 6.6Hz, 3H), 1.26 (J = 7.1Hz, 3H). LCMS: MH+ 409, retention time 2.88 min (method D). Chiral SFC: retention time 2.50 min, e.e. 99.8% (method H).

(S)-ethyl 3-(5-chloro-6-(1-(5-chloropyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate Chiral SFC: retention time 1.73 min, e.e. 99.6% (method H).

(R)-methyl 3-(5-chloro-6-(1-(5-fluoropyridin-2-yl)ethoxy)benzo[d]isoxazol-3-yl)propanoate (**64d**). To a mixture of methyl 3-(5-chloro-6-hydroxybenzo[d]isoxazol-3-yl)propanoate[20] **62** (435 mg, 1.7 mmol) in THF (20 mL) were added (1R)-1-(5-fluoropyridin-2-yl)ethan-1-ol **63d** (200 mg, 1.42 mmol), triphenyl phosphine (559 mg, 2.13 mmol), DEAD (371 mg, 2.13 mmol) and the reaction stirred at room temperature for 2 h. The solvent was evaporated and the residue purified by chromatography (silica, 200-300 mesh, 10 g) eluting with petroleum ether / ethyl acetate (3 : 1) to give the title compound as a colourless oil (150 mg, 28%). LCMS: MH+ 379, retention time 1.69 min (method A).

5-Chloro-6-methoxybenzo[d]thiazol-2(3H)-one (**66**). 5-Chloro-6-methoxybenzo[d]thiazol-2-amine[27] **65** (8.0 g, 37.3 mmol) was dissolved in a mixture of formic acid (25 mL), acetic acid (10 mL), hydrochloric acid (36 %, 20 mL) at -10 °C. A solution of sodium nitrite (2.83 g, 41 mmol) in water (15 mL) was added drop-wise and the reaction stirred at -10 °C for 1 h before allowing the reaction to warm to ambient temperature. The reaction was then heated at 100 °C for 18 h. The cooled reaction was diluted with water (200 mL) and extracted with ethyl acetate (5x 50 mL). The organics were washed with brine (2x 100mL), dried, filtered and the solvent evaporated. The residue was purified by chromatography (silica gel, 200-300 mesh, 12 g), eluting with ethyl acetate / DCM (1:10, 500 mL) to give the title compound as a grey

solid (2.2 g, 25 %). ^{1}H NMR (D_6-DMSO) 11.81 (br. s, 1H), 7.50 (s, 1H), 7.12 (s, 1H), 3.34 (s, 3H). LCMS: MH+ 216/218, retention time 1.48 min (method A).

Ethyl 3-(5-chloro-6-methoxy-2-oxobenzo[d]thiazol-3(2H)-yl)propanoate (**67**). 5-Chloro-6-methoxybenzo[d]thiazol-2(3H)-one **66** (600 mg, 2.78 mmol), ethyl acrylate (333 mg, 3.33 mmol), tetraethyl orthosilicate (580 mg, 2.78 mmol) and cesium fluoride (51 mg, 0.33 mmol) were mixed in toluene (15 mL) and the reaction heated at 110 °C for 16 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 4 g), eluting with petroleum ether / ethyl acetate (2:1, 300 mL) to give the title compound as a yellow solid (740 mg, 84 %). LCMS: MH+ 316/318, retention time 1.64 min (method A).

Ethyl 3-(5-chloro-6-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-3-yl)propanoate (**68**). Boron tribromide (2.93 g, 11.7 mmol) was added drop-wise at ambient temperature to a mixture of ethyl 3-(5-chloro-6-methoxy-2-oxobenzo[d]thiazol-3(2H)-yl)propanoate **67** in DCM (15 mL) and the reaction stirred at ambient temperature for 15 h. The mixture was cooled to 0 °C and water (50 mL) added drop-wise. The mixture was extracted with ethyl acetate (3x 50 mL) and the combined organics dried (sodium sulphate). The solvent was evaporated to give the title compound as a yellow solid (580 g, 82 %). LCMS: MH+ 302/304, retention time 1.50 min (method A).

(4-Chloro-3-methoxyphenyl)(methyl)sulfane (**71**). To 4-chloro-3-methoxyaniline **70** (10 g, 63. 5 mmol) in hydrochloric acid (37 %, 13.2 g, 133 mmol) and water (50 mL) at 0 °C was added a solution of sodium nitrite (4.38 g, 63.5 mmol) in water (5 mL) and the reaction stirred at 0 °C for 30 min. Sodium methanethiolate (20 % aqueous, 22.25 g, 63.5 mmol) was added to the mixture and the reaction warmed to ambient temperature and stirred for 15 min.

The reaction was extracted with ethyl acetate (3x 100 mL), the combined organics dried (sodium sulphate) and the residue purified by chromatography (silica gel, 200-300 mesh, 50 g), eluting with petroleum ether / ethyl acetate (50:1) to give the title compound as a yellow oil (7.3 g, 61 %). ^{1}H NMR ($CDCl_3$) 7.28 (d, J = 8.1Hz, 1H), 6.84 (d, J = 2.0Hz, 1H), 6.79 (dd, J = 8.3Hz, J = 2.2Hz, 1H), 3.91 (s, 3H), 2.51 (s, 3H).

4-(5-Chloro-4-methoxy-2-(methylthio)phenyl)-4-oxobutanoic acid (**72a**). To (4-chloro-3-methoxyphenyl)(methyl)sulfane **71** (7.3 g, 38.7 mmol) in DCM (100 mL) were added dihydrofuran-2,5-dione (3.87g, 38.7 mmol) and aluminium chloride (5.68 g, 42.6 mmol) and the reaction stirred at ambient temperature for 16 h. Water (20 mL) was added, the solid isolated by filtration to give the title compound as a white solid (6.3 g, 56 %). LCMS: MH+ 289, retention time 1.56 min (method A).

Methyl 4-(5-chloro-4-methoxy-2-(methylthio)phenyl)-4-oxobutanoate (**72b**). To 4-(5-chloro-4-methoxy-2-(methylthio)phenyl)-4-oxobutanoic acid **72a** (6.3 g, 21.8 mmol) in methanol (100 mL) was added thionyl chloride (3.12g, 26.2 mmol) and the mixture stirred at ambient temperature for 16 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 20 g), eluting with DCM / methanol (100:1) to give the title compound as a white solid (5.2 g, 78 %). LCMS: MH+ 303, retention time 1.67 min (method A).

Methyl 4-(5-chloro-4-methoxy-2-(methylthio)phenyl)-4-(hydroxyimino)butanoate (**73**). To methyl 4-(5-chloro-4-methoxy-2-(methylthio)phenyl)-4-oxobutanoate **72b** (5.2 g, 17.2 mmol) in acetonitrile / ethanol (1:1, 100 mL) was added ammonium hydroxide hydrochloride (5.97 g, 85.9 mmol) and the reaction stirred at 110 °C for 16 h. The solvent was evaporated and

the residue purified by chromatography (silica gel, 200-300 mesh, 20 g), eluting with DCM / methanol (200:1) to give the title compound as a yellow oil (4.5 g, 82 %). LCMS: MH+ 318, retention time 1.63 min (method A).

Methyl 3-(5-chloro-6-methoxybenzo[d]isothiazol-3-yl)propanoate (**74**). Methyl 4-(5-chloro-4-methoxy-2-(methylthio)phenyl)-4-(hydroxyimino)butanoate **73** (4.5 g, 14.16 mmol) in pyridine / acetic anhydride (1:1, 50 mL) was heated at 120 °C for 16 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 20 g), eluting with DCM / methanol (200:1) to give the title compound as a yellow solid (1.57 g, 39 %). LCMS: MH+ 286, retention time 1.73 min (method A).

Methyl 3-(5-chloro-6-hydroxybenzo[d]isothiazol-3-yl)propanoate (**75b**). Methyl 3-(5-chloro-6-methoxybenzo[d]isothiazol-3-yl)propanoate **74** (1.57 g, 5.49 mmol) in hydrobromic acid (48%, 30 mL) was heated at 125 °C for 16 h. The reaction was cooled to room temperature, filtered and the solid dried to give crude 3-(5-chloro-6-hydroxybenzo[d]isothiazol-3-yl)propanoic acid **75a** (1.8g). LCMS: MH+ 258, retention time 1.28 min (method A).
To this material in methanol (50 mL) was added thionyl chloride (784 mg, 6.6mmol) and the reaction stirred at ambient temperature for 16 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 20 g), eluting with DCM / methanol (30:1) to give the title compound as a purple solid (1.1 g, 52 %). LCMS: MH+ 272, retention time 1.43 min (method A).

Methyl 3-{5-chloro-6-[(1R)-1-(pyridin-2-yl)ethoxy]-1,2-benzothiazol-3-yl}propanoate (**76**). Methyl 3-(5-chloro-6-hydroxybenzo[d]isothiazol-3-yl)propanoate **75b** (200 mg, 0.74 mmol) in toluene (20 mL) was treated with (1S)-1-(pyridin-2-yl)ethan-1-ol (91 mg, 0.74 mmol),

triphenyl phosphine (290 mg, 1.10 mmol) and DEAD (192 mg, 1.10 mmol) and the mixture stirred at 80 °C for 16 h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 10 g), eluting with DCM / methanol (100:1) to give the crude title compound as a white solid (0.4 g). LCMS: MH+ 377, retention time 1.54 min (method A).

4-Chloro-5-isobutyl-2-nitrophenol (**77**). 4-Chloro-3-isobutylphenol **78** (500 mg, 2.71 mmol) in acetic acid (10 mL) at room temperature was treated with nitric acid (70 %, 2.2 mL) and the reaction stirred at ambient temperature for 2 h. The reaction was diluted with water and extracted with ethyl acetate. The organic phase was washed with water (100 mL x3) and dried (magnesium sulphate) and the solvent evaporated in vacuo. The residue was purified by chromatography (silica gel, 200-400 mesh, 40 g) eluting with petroleum ether / ethyl acetate (200:1 then 100:1) which gave crude title compound as a yellow oil (420 mg, 68 %). ^{1}H NMR ($CDCl_3$) includes 10.30 (s, 1H), 7.98 (s, 1H), 6.88 (s, 1H), 2.51 (d, J = 7.3Hz, 2H), 1.90 (m, 1H), 0.85 (d, J = 6.8Hz, 6H).

4-Chloro-3-isobutylphenol (**78**). 1-Chloro-2-isobutyl-4-methoxybenzene **79** (1.69 g, 8.7 mmol) in DCM (20 mL) was treated with boron tribromide (2.63 g, 10.4 mmol) at room temperature and the mixture stirred for 4 h. Water was added and the mixture extracted with DCM. The organic phase was dried (magnesium sulphate) and the solvent evaporated in vacuo. The residue was purified by chromatography (silica gel, 200-400 mesh, 30 g) eluting with petroleum ether / ethyl acetate (20:1) to give the title compound as a yellow oil (1.1 g, 74 %). ^{1}H NMR ($CDCl_3$) 7.06 (d, J = 8.4Hz, 1H), 6.55 (d, J = 3.0Hz, 1H), 6.50 (dd, J = 8.4Hz, J = 3.0Hz, 1H), 2.42 (d, J = 7.1Hz, 2H), 1.85 (m, 1H), 0.82 (6H, J = 6.6Hz, d).

1-Chloro-2-isobutyl-4-methoxybenzene (**79**). 1-Chloro-4-methoxy-2-(2-methylprop-1-en-1-yl)benzene **80** (2.59 g, 12.7 mmol) and platinum dioxide (200 mg, 1.0 mmol) in toluene (30 mL) were stirred at ambient temperature under 1 Atm. of hydrogen for 16 h. The mixture was filtered and the solvent evaporated in vacuo. The residue was purified by chromatography (silica gel, 200-400 mesh, 30 g) eluting with petroleum ether / ethyl acetate (20:1) to give the title compound as a colourless oil (1.6 g, 64 %). ^{1}H NMR ($CDCl_3$) 7.25 (d, J = 8.6Hz, 1H), 6.74 (d, J = 2.9Hz, 1H), 6.71 (dd, J = 8.6Hz, J = 3.2Hz, 1H), 3.81 (s, 3H), 2.59 (d, J =7.3Hz, 2H), 2.00 (m, 1H), 0.96 (d, J = 6.8Hz, 6H).

1-Chloro-4-methoxy-2-(2-methylprop-1-en-1-yl)benzene (**80**). To isopropyltriphenylphosphonium iodide (10.93 g, 25.2 mmol) in THF (50 mL) at room temperature was added sodium hydride (60%, 1 g, 27.5 mmol) and the mixture stirred for 1 h. 2-Chloro-5-methoxybenzaldehyde (3.93 g, 22,9 mmol) was added to the reaction and stirring continued for 16 h. Water was added and the mixture extracted with ethyl acetate. The organic phase was dried (magnesium sulphate) and the solvent evaporated in vacuo. The residue was purified by chromatography (silica gel) eluting with petroleum ether / ethyl acetate (40:1) to give the title compound as a brown oil (2.5 g, 55 %). ^{1}H NMR ($CDCl_3$) 7.28 – 7.23 (m, 2H), 6.80 (d, J = 3.0Hz, 1H), 6.72 (dd, J = 8.8, J = 3.0, 1H), 6.27 (s, 1H), 3.80 (s, 3H), 1.95, (s, 3H), 1.81 (s, 3H).

5-Chloro-4-cyclopropoxy-2-methoxyaniline (**81**). Iron (421 mg, 7.53 mmol) was added portion-wise to a solution of 1-chloro-2-cyclopropoxy-4-methoxy-5-nitrobenzene **82** (609 mg, 2.51 mmol) in a mixture of water (10 mL) and acetic acid (10 mL) at 80 °C and the mixture stirred at 80 °C for a further 1 h after completion of the addition. The reaction was cooled to room temperature, filtered and the filtrate extracted with ethyl acetate (4x 50 mL).

The combined organics were reduced to dryness in vacuo. The residue was redissolved in ethyl acetate (50mL), washed with brine (2x 100mL) dried (sodium sulphate) and the solvent evaporated in vacuo to give the title compound as a black oil (470 mg, 88%). LCMS: MH+ 214 / 216, retention time 1.50 min (method A).

1-Chloro-2-cyclopropoxy-4-methoxy-5-nitrobenzene (**82**). Cyclopropanol (314 mg, 5.4 mmol) was dissolved in DMF (10 mL) at 0 °C and treated with sodium hydride (60%, 360 mg, 9.0 mmol) added portion-wise. The mixture was stirred at 0 °C for 30 min. 1,2-dichloro-4-methoxy-5-nitrobenzene (1.0 g, 4.5 mmol) was added in a single portion and the mixture stirred at 40 °C for 45 min, before cooling to ambient temperature. Water (30 mL) was added slowly and the resulting mixture extracted with ethyl acetate (4x 20 mL). The combined organic phases were concentrated in vacuo and the residue purified by chromatography (silica gel, 12 g) eluting with petroleum ether / ethyl acetate (30:1, 250 mL) to give the title compound as a pale yellow solid (610 mg, 56%). LCMS: MH+ 244 / 246, retention time 1.69 min (method A).

1-(Oxazol-2-yl)ethyl methanesulfonate (**83**). To a stirred solution of 1-(oxazol-2-yl)ethanol (650 mg, 5.75 mmol) in DCM (10 mL) at 0 °C was added triethylamine (1.25 mL, 8.62 mmol) and the mixture stirred for 10 min. To this was added mesyl chloride (0.53 mL, 6.90 mmol) and the reaction stirred for 3 h at between 5 and 20 °C. The reaction was quenched by addition of brine (200 mL) and the mixture extracted with DCM (3x 15 mL). The combined organic phases were dried (sodium sulphate) and concentrated under reduced pressure to give crude title compound as a pale yellow oil (700 mg). This material was used without further purification or analysis due to instability.

1-(Pyridazin-3-yl)ethyl methanesulfonate (**84**). 1-(Pyridazin-3-yl)ethanol[28] (200 mg, 1.61 mmol) and triethylamine (212 mg, 2.09 mmol) were mixed in DCM (8 mL) at 0 °C. To this was added drop-wise a solution of mesyl chloride (203 mg, 1.77 mL) dissolved in DCM (2 mL) and the reaction was stirred at 0 °C for 1h. The solvent was evaporated and the residue purified by chromatography (silica gel, 200-300 mesh, 4 g) eluting with petroleum ether / ethyl acetate (1:2, 150 mL) to give the title compound as a pale yellow oil (160 mg, 49 %). LCMS: MH+ 203, retention time 1.03 min (method A).

(S)-1-(4-Methylpyridin-2-yl)ethanol (**85**). To R-CBS (3.7 mL, 3.7 mmol) in THF (20 mL) at 0 °C was added borane.dimethyl sulphide solution (2M, 1.85 mL, 3.7 mmol) and the mixture stirred at 0 °C for 1 h. 1-(4-Methylpyridin-2-yl)ethanone (400 mg, 2.96 mmol) was added and the reaction stirred at ambient temperature for 2 h. Methanol (15 mL) was added and the solvent evaporated in vacuo. The residue was purified by chromatography (silica gel, 200-400 mesh, 20 g) eluting with DCM / methanol (20:1 then 10:1) to give the title compound as a yellow oil (160 mg, 40 %). LCMS: MH+ 138, retention time 0.42 min (method A).

(S)-1-(5-Methylpyridin-2-yl)ethanol (**86**). To R-CBS (3.7 mL, 3.7 mmol) in THF (10 mL) at 0 °C was added borane.dimethyl sulphide solution (2M, 1.85 mL, 3.7 mmol) and the mixture stirred at 0 °C for 1 h. 1-(5-Methylpyridin-2-yl)ethanone (400 mg, 2.96 mmol) was added and the reaction stirred at ambient temperature for 16 h. Water (50 mL) was added and the mixture extracted with ethyl acetate (2x 50 mL). The combined organic phases were dried (magnesium sulphate) and the solvent evaporated in vacuo. The residue was purified by chromatography (silica gel) eluting with petroleum ether / ethyl acetate (9:1, 7:1, 5:1) to give the title compound as a colourless oil (110 mg, 28 %). LCMS: MH+ 138, retention time 1.34 min (method A).

1-(5-Chloropyridin-2-yl)ethyl methanesulfonate (**87**). A mixture of 1-(5-chloropyridin-2-yl)ethanol (390 mg, 1.2 mmol), mesyl chloride (206 mg, 1.8 mmol) and triethylamine (180 mg, 1.8 mmol) in DCM was stirred at ambient temperature for 6 h. the solvent was evaporated in vacuo and the residue purified by chromatography (silica gel, 200-400 mesh, 20 g), eluting with petroleum ether / ethyl acetate (7:1 then 4:1) to give the title compound as a yellow oil (121 mg, 21 %). LCMS: MH+ 236, retention time 1.41 min (method A).

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All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

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The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

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ACCESSION CODES

Authors will release the atomic coordinates and experimental data upon publication.

Compound **6**: PDB ID code 5MZC
Compound **7**: PDB ID code 5MZI
Compound **9**: PDB ID code 5MZK

ANCILLARY INFORMATION

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SUPPORTING INFORMATION AVAILABLE

LCMS methods. Chiral SFC methods. Supplementary Table 1. X-ray data collection and refinement statistics. Supplementary Figure 1. Electron density difference maps in the inhibitor binding sites.

ABBREVIATIONS USED

ALT, alanine aminotransferase; AP, acute pancreatitis; Atm., Atmosphere; BAL, bronchoalveolar lavage; CDI, 1,1'-carbonyldiimidazole; CNS, central nervous system; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DMF, dimethyl formamide; DMPK, drug metabolism and pharmacokinetics; DMSO, dimethyl sulfoxide; FAD, flavin adenine dinucleotide; H&E, hematoxylin and eosin stain; HD, Huntington's disease; HEK, human embryonic kidney; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase; HWB, human whole blood; HSA, human serum albumin; IDO, indoleamine 2,3-dioxygenase; i.v., intravenous; KAT I / II, kynurenine aminotransferase I / II; KMO, kynurenine 3-monooxygenase; LCMS, liquid chromatography-mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate; NMR; nuclear magnetic resonance; Pf KMO, *Pseudomonas fluorescens* kynurenine 3-monooxygenase; PPB, plasma protein binding; R-CBS, (R)-2-methyl-CBS-oxazaborolidine; TDO, tryptophan 2,3-dioxygenase; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Tris, Tris(hydroxymethyl)aminomethane; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

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16) Rat iv DMPK. The study was carried out using n=3 han wistar rat. The compound was administered intravenously to fed animals at a target dose of 1mg/kg. Blood samples were collected at various time points. The blood samples were then assayed using a method based upon protein precipitation with acetonitrile followed by LC/MS/MS analysis (MRM:363.1>107.1.LLQ=1ng/mL.) Non-compartmental methods were used for pharmacokinetic analysis of blood concentration versus time data.

17) Experimental AP in rats. All AP experiments were performed after institutional ethical review and approval and under licence (PPL 60/4433). AP was induced in rats under terminal anaesthesia at laparotomy by intraductal infusion of glycodeoxycholic acid followed by i.v caerulein infusion for 6 hours as described in detail elsewhere (ref 2). One hour after the induction of AP, **28** was administered as a 20 mg/kg iv bolus followed by a 2.2 mg/kg/hr infusion. The compound was formulated as an in-situ prepared ethanolamine salt in 10%w/v (2-hydroxypropyl)-ß-cyclodextrin in Saline. Steady state plasma total drug levels were ~600µM which provides free drug levels >100x the rat cellular IC_{50}. Plasma metabolite levels were measured at

indicated times. Animals were sacrificed 6 hours after the induction of AP. AP was confirmed by elevated serum amylase levels and histological evidence of acute inflammation, oedema and necrosis on histological examination, as previously described (ref 2). Lung injury was assessed on formalin-fixed paraffin-embedded histological tissue sections stained with haematoxylin and eosin and visualized by light microscopy. Lung protein leak was measured by protein levels in bronchoalveolar lavage fluid. Kidney injury was assessed by serum creatinine level and by enumeration of TUNEL positive apoptotic cells in the outer medullary stripe on kidney tissue sections, as previously described (ref 2).

18) Dog i.v. DMPK. The study was carried out using n=3 beagle dog. The compound was administered intravenously to fed animals at a target dose of 1mg/kg DMSO/hydroxyprooyl-beta-Cyclodextrin 20% in phosphate buffer 60m/M pH7 (5/95). Blood samples were collected at various time points from the jugular / cephalic veins. The blood samples were assayed using a method based upon protein precipitation with acetonitrile followed by LC/MS/MS analysis. Non-compartmental methods were used for pharmacokinetic analysis of blood concentration versus time data.

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