Activity-based protein profiling of diacylglycerol lipases

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CHAPTER 5

Structure Activity Relationship of LEI105*

Introduction

Diacylglycerol lipase-α and -β (DAGL-α and DAGL-β) are the two main enzymes responsible for the production of the endocannabinoid 2-arachidonoylglycerol (2-AG). Both isoforms have a tissue specific contribution to 2-AG biosynthesis. DAGL-α is the main enzyme responsible for the production of 2-AG in the CNS, while DAGL-β has a more pronounced role in the periphery.[1,2] In addition to congenital knockout of DAGL-α, small molecule inhibitors that acutely inhibit DAGL-α activity are required to study the physiological role of DAGL-α. The DAGL inhibitors known to date can be divided in six different chemotypes (for review see Janssen *et al.*)[3]. Bis-oximino-carbamates,[4] β-lactones,[4,5] fluorophosphonates[6,7] and 1,2,3-triazole ureas[8,9] can be classified as irreversible inhibitors. Glycine sulfonamides[10,11] and α-ketoheterocycles[12-14] are reversible inhibitors. In chapters 3 and 4, LEI104 and LEI105 are described as α-ketoheterocycle-based DAGL inhibitors, which display high potency and selectivity over other serine hydrolases.[13,14] They have a reversible mode-of-action, which results in a lower probability of inducing idiosyncratic toxic effects. This makes the α-ketoheterocycles a promising chemotype for further development of DAGL inhibitors.

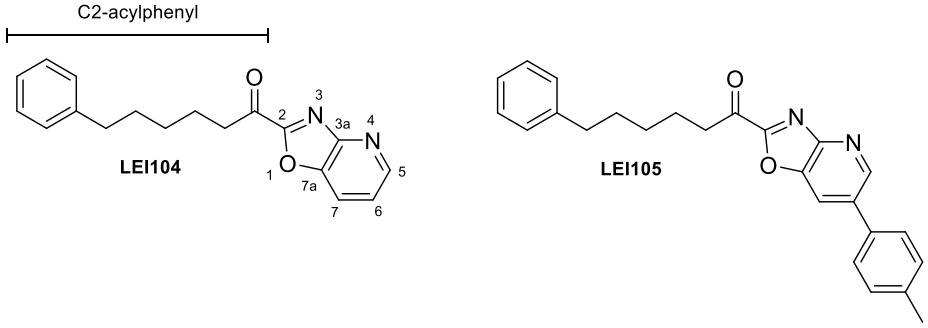

Figure 1. Structure of α-ketoheterocyclic DAGL inhibitors LEI104 and LEI105.

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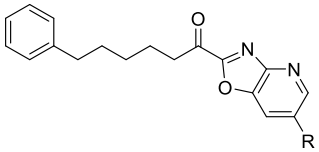
Janssen *et al.*[14] investigated the structural requirements for the interaction of α-ketoheterocycles with DAGL-α. A 1040-membered focused library of FAAH inhibitors, mainly consisting of previously published α-ketoheterocycles and their corresponding precursors, was screened for DAGL-α inhibition. In addition, 19 new α-ketoheterocylces were synthesized to complement the structure activity relationships. The focused library included benzoxazole, benzothiazole, benzimidazole and their 4-pyridine analogs. The screen revealed that the oxazolo-4N-pyridine was the most potent scaffold for DAGL-α inhibition. Investigation of the C2-acylphenyl spacer length revealed that the inhibitor with a 5-carbon spacer with a distal phenyl group showed the highest lipophilic efficiency (LipE: calculated as LipE = pIC_{50} – cLogP). In summary, this study revealed that LEI104 was the most optimal DAGL-α inhibitor from a 1059 member α-ketoheterocycle-based focused library (Figure 1). Introduction of a *para*-tolyl group at the 6-position of the oxazolopyridine of LEI104 produced the highly potent and selective dual DAGL-α/β inhibitor LEI105,[13] as described in the previous chapter. It was observed that a *p*-tolyl group completely removed cross-reactivity with fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of the other endocannabinoid anandamide. However, little is known about the effect of different substituents at the C6 position of LEI104. Therefore, the structure activity relationship of substituents at this position was investigated.

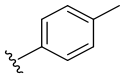
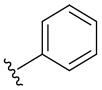
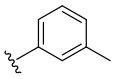
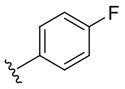
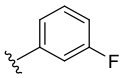
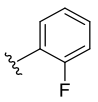
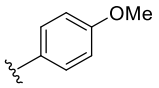
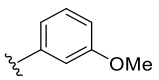
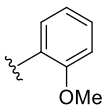
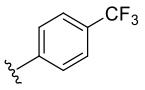
Results and Discussion

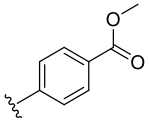
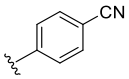
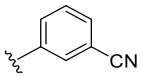
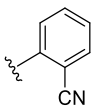
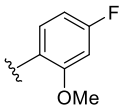
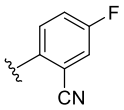
A similar strategy as the synthesis towards LEI105, as described in chapter 4, was used for the synthesis of α-ketoheterocycles **1** to **18.** The inhibitory potency of the the compounds against *h*DAGL-α was tested in a concentration-response experiment using a colorimetric DAGL-α activity assay based on the hydrolysis of para-nitrophenylbutyrate.[12] The activity of the inhibitors against native mouse DAGL-α was evaluated in an orthogonal assay using activity-based protein profiling with MB064 in mouse brain membrane proteome.[12] Inhibitor selectivity was investigated by pre-incubation of mouse brain membrane proteome with inhibitor (10 µM) and subsequent labeling with MB064 (250 nM) or TAMRA-FP (500 nM) as described in chapter 4. A summary of the results of these assays is given in Table 1.

The biological characterization revealed that replacing the *p*-tolyl group of LEI105 with a phenyl **1** had no effect on the inhibitory potency on DAGL-α in the ABPP assay. However, this inhibitor (10 µM) showed slightly reduced labeling of FAAH as measured with TAMRA-FP in mouse brain proteome. This is in line with the hypothesis in the previous chapter that the methyl group of LEI105 has a steric clash with FAAH. Changing the position of the methyl group to the *ortho*- **3** or *meta*- **2** position, or replacement of the methyl groups by fluorides on the *para*- **4**, *meta*- **5** or *ortho*-position **6** had little effect on the inhibitory potency against DAGL-α compared to LEI105 in the ABPP and PNP-assay. A methoxy group at the *meta*- or *para*-position reduced the potency of the inhibitors in the

Table 1. Summary of the biological characterization of the inhibitors described in this chapter.



Compound	R	PNP assay pIC_{50} ± SEM (N = 2, n = 2)	ABPP pIC_{50} ± SEM (n = 3)	ABPP FAAH % inhibition ± SD [10 µM] (n = 3)	cLogP*	LipE (PNP-assay)
LEI105		8.5 ± 0.06	7.5 ± 0.1	2 ± 5	6.5	2.1
1		9.2 ± 0.05	7.5 ± 0.1	20 ± 2	6.0	3.2
2		8.8 ± 0.03	7.2 ± 0.1	15 ± 6	6.5	2.3
3		8.5 ± 0.06	7.5 ± 0.1	41 ± 1	6.2	2.3
4		8.4 ± 0.08	7.5 ± 0.1	3 ± 4	6.1	2.3
5		8.7 ± 0.10	7.4 ± 0.1	5 ± 8	6.1	2.6
6		8.7 ± 0.08	7.4 ± 0.1	23 ± 2.8	6.1	2.6
7		8.6 ± 0.04	7.1 ± 0.1	11 ± 4	6.0	2.6
8		8.7 ± 0.05	7.1 ± 0.1	29 ± 35	6.0	2.7
9		8.5 ± 0.06	7.6 ± 0.1	50 ± 4	5.4	3.1
10		9.0 ± 0.03	7.5 ± 0.1	12 ± 13	6.8	2.2

Compound	R	PNP assay pIC_{50} ± SEM (N = 2, n = 2)	ABPP pIC_{50} ± SEM (n = 3)	ABPP FAAH % inhibition ± SD [10 µM] (n = 3)	cLogP*	LipE (PNP-assay)
11		8.5 ± 0.10	6.5 ± 0.2	3 ± 5	5.9	2.6
12		8.7 ± 0.04	7.3 ± 0.1	67 ± 9	5.4	3.3
13		8.5 ± 0.03	7.1 ± 0.1	31 ± 12	5.4	3.1
14		8.8 ±0.03	7.6 ± 0.1	24 ± 11	5.4	3.4
15		9.0 ± 0.05	7.5 ± 0.08	40 ± 4	5.6	3.4
16		9.0 ± 0.04	7.7 ± 0.05	17 ± 4	5.5	3.5
17		6.5 ± 0.26	6.9 ± 0.1	66 ± 3	4.5	2.0
18		8.7 ± 0.04	6.9 ± 0.03	84 ± 2	5.3	3.4

*cLogP was estimated with ChemBioDraw Ultra, version 14.0.0.117.

ABPP assay. Remarkably, the activity of the inhibitor with a methoxy group on the *ortho*-position **9** remained similar to LEI105 with a pIC_{50} of 7.6 ± 0.02 in the ABPP assay. However, **9** also showed the highest activity towards FAAH. A trend emerges that compounds featuring substituents at the *ortho*-position show the highest, and substituents at the *para*-position the lowest activity against FAAH (Figure 2). Introduction of an electron withdrawing CF_3 group at the *para*-position did not influence inhibitor activity, while introduction of an electron donating ester group at the same position decreased the inhibitory activity as measured with the ABPP assay. Introduction of a cyano substituent at the *ortho*- or *para*-position had little effect on the potency against DAGL-α, while at the

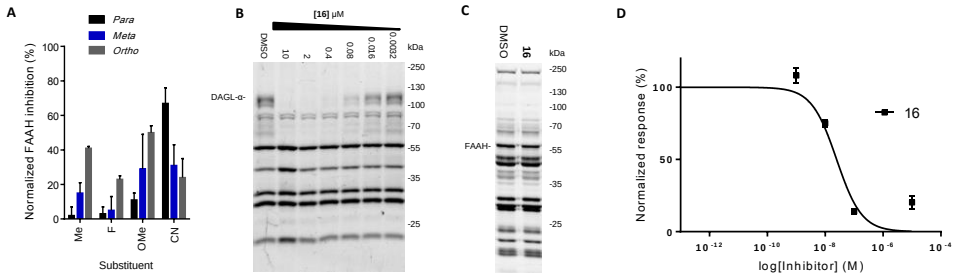


Figure 2. *A*) Effect of substituents at the ortho, meta or para position on the labeling of FAAH by the flurophosphonate-based ABP (TAMRA-FP). Substituents: Me = methyl, F = fluoro, OMe = methoxy, CN = Cyano. *B*) Example of a dose response gel using ABP MB064 (250 nM) and inhibitor **16** in the mouse brain membrane proteome. *C*) Example gel of the selectivity assay, 30 min. preincubation with **16** (10 µm) in the mouse brain membrane proteome, followed by labeling with TAMRA-FP (500 nM) for 15 minutes. *D*) Dose response curve of **16** in in a real-time, fluorescent natural substrate-based assay.

meta-position it reduced the pIC_{50} to 7.1 ± 0.03 in the ABPP assay. Interestingly, the cyano group at the *para*-position showed the highest activity against FAAH, compared to the same substituent at the *ortho*- or *meta*-position (Figure 2). Double substituted inhibitor **15** showed good potency in both the ABPP assay and the colorimetric assay, but reduced FAAH labeling by 40%. Compound **16** showed an improved potency in both the colorimetric assay and the ABPP assay, compared to LEI105, while reducing FAAH activity with only 17% at 10 µM. Introduction of a pyridine **17** or a N-methyl pyrrole **18**, decreased activity against DAGL-α and increased activity against FAAH compared to LEI105. Compound **16** displayed the highest lipophilic efficiency (LipE) and reduced labeling of FAAH with less than 25% at 10 µM. Therefore, this compound was analyzed further in a real-time, fluorescent natural substrate-based assay as described previously.[15] The inhibitor showed high activity in the natural substrate assay with a pIC_{50} of 7.6 ± 0.1 (Figure 2).

Conclusions

This chapter described the synthesis and the structure activity relationship of a series of LEI105 analogues. Variations have been made on the *p*-toluyl group of LEI105. None of the substituents tested in this chapter completely abolished activity against DAGL-α, this might indicate that the *p*-toluyl group of LEI105 and the R-groups tested in this chapter reside in a large binding pocket of DAGL-α. A general trend for FAAH off-target activity (*ortho* > *meta* > *para*) was observed, except for cyano substituted phenyls.

Compound **16** showed the highest activity in the ABPP activity assay and had the highest LiPE in the colorimetric assay and reduced labeling of FAAH with less than 25% at 10 µM. The newly identified inhibitor has a LiPE of 3.5, which is a strong improvement compared to LEI105 with a LiPE of 2.1. The activity of **16** against *h*DAGL-α was tested in

a natural substrate assay and proved to be potent in this assay (pIC_{50} **16**: 7.6 ± 0.1). In view of this attractive pharmacological profile, further *in situ* and *in vivo* studies are warranted.

Experimental Methods

Synthetic procedures

General remarks

All reactions were performed using oven or flame-dried glassware and dry solvents. Reagents were purchased from Sigma Aldrich, Acros and Merck and used without further purification unless noted otherwise. All moisture sensitive reactions were performed under an argon atmosphere. Traces of water were removed from starting compounds by co-evaporation with toluene.

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 (^{1}H) and 100.6 (^{13}C) MHz or a Bruker DMX-600 spectrometer 600 (^{1}H) and 150 (^{13}C) MHz using $CDCl_3$ or CD_3OD as solvent, unless stated otherwise. Chemical shift values are reported in ppm with tetramethylsilane or solvent resonance as the internal standard ($CDCl_3$ δ 7.26 for ^{1}H, δ 77.0 for ^{13}C, CD_3OD: δ 3.31 for ^{1}H). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, td = triple doublet, t = triplet, q = quartet, quinted = quint, br = broad, m = multiplet), coupling constants *J* (Hz), and integration. HPLC purification was performed on a preparative LC-MS system (Agilent 1200 serie) with an Agilent 6130 Quadruple MS detector. High-resolution mass spectra (HRMS) were recorded on a Thermo Scientific LTQ Orbitrap XL. Flash chromatography was performed using SiliCycle silica gel type SiliaFlash P60 (230 – 400 mesh). TLC analysis was performed on Merck silica gel 60/Kieselguhr F254, 0.25 mm. Compounds were visualized using either Seebach's reagent (a mixture of phosphomolybdic acid (25 g), cerium (IV) sulfate (7.5 g), H_2O (500 mL) and H_2SO_4 (25 mL)) or a $KMnO_4$ stain (K_2CO_3 (40 g), $KMnO_4$ (6 g), H_2O (600 mL) and 10% NaOH (5 mL)).

2-amino-5-(o-tolyl)pyridin-3-ol (19): 2-methylphenylboronic acid (400 mg, 2.96 mmol), 2-amino-5-bromopyridin-3-ol (558 mg, 2 mmol) and Cs_2CO_3 (845 mg, 2.6 mmol) were dissolved in 10 mL DME/H_2O (10:1). The mixture was degassed under a flow of argon. $Pd(Ph_3)_4$ (116 mg, 0.1 mmol) was added and the mixture was stirred at 85 °C for 12 h. The reaction mixture was allowed to cool to rt and H_2O (10 mL) was added. The reaction mixture was extracted with EtOAc. The organic layer was dried $NaSO_4$ and concentrated under reduced pressure. The residue was taken up in pentane/ EtOAc (1:1) and filtered over a pad of silica. The filtrate was concentrated and the crude 3-(benzyloxy)-5-(o-tolyl)pyridin-2-amine was used for the next reaction.

The crude 3-(benzyloxy)-5-(o-tolyl)pyridin-2-amine was dissolved in MeOH (20 mL) and 0.1 equivanlent 10% Pd/C was added. The mixture was stirred under H_2 atmosphere for 12 h at rt. The reaction mixture was filtered over celite and concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel using CH_2Cl_2/MeOH (9:1). This yielded 2-amino-5-(o-tolyl)pyridin-3-ol (165 mg, 0.82 mmol,

41% over two steps). ^{1}H NMR (400 MHz, DMSO) δ 7.42 (d, *J* = 1.9 Hz, 1H), 7.32 – 7.20 (m, 3H), 7.19 – 7.13 (m, 1H), 7.06 (d, *J* = 1.9 Hz, 1H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 148.18, 140.20, 137.37, 135.13, 130.73, 130.47, 129.52, 127.50, 126.11, 125.65, 120.88, 20.15.

6-phenyl-1-(6-(o-tolyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-ol) (20): Compound **19** (70 mg, 0.35 mmol) was dissolved in EtOH (4 mL), Pyridine (28 µL,0.35 mmol) was added and the mixture was heated for 15 min to 85 ^{o}C using. Imidate **7** (174 mg, 0.70 mmol) dissolved in EtOH (1.0 mL) was added and the mixture was heated to 85 ^{o}C for 12 h. The reaction mixture was concentrated under reduced pressure and purified by flash chromatography over silica gel using
Pentane/EtOAC (3:1) → (2:1). This yielded **27** (16 mg, 0.04 mmol; 6.0 %) as a colorless oil.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 1.6 Hz, 1H), 7.78 (d, *J* = 1.8 Hz, 1H), 7.33 (dt, *J* = 7. , 3.8 Hz, 2H), 7.29 – 7.22 (m, 3H), 7.19 – 7.12 (m, 4H), 5.14 – 4.92 (m, 1H), 2.60 (t, *J* = 17.2, 9.4 Hz, 2H), 2.28 (s, 3H), 2.19 – 1.93 (m, 2H), 1.73 – 1.49 (m, 4H), 1.49 – 1.30 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.24, 153.65, 147.16, 142.84, 142.51, 137.42, 135.75, 134.96, 130.71, 130.21, 128.42 (2C), 128.36 (2C), 128.23, 126.20, 125.63, 119.10, 68.20, 35.78, 35.41, 31.21, 28.88, 24.76, 20.42.

6-phenyl-1-(6-(o-tolyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-one (3): To a solution of **20** (15 mg, 0.04 mmol) in CH_2Cl_2 (2 mL) was added Dess-Martin periodinane (25 mg, 0.06 mmol) and the reaction mixture was stirred under argon atmosphere overnight. The reaction mixture was quenched with saturated NaHCO3 (aq). The layers were separated and the organic layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried on $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel using toluene/ethyl acetate (90:10) with 1% Et3N. This yielded **3** (12 mg, 0.031 mmol, 78%) as acolorless oil. (^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.93 (d, *J* = 1.8 Hz, 1H), 7.41 – 7.26 (m, 6H), 7.19 (d, *J* = 7.3 Hz, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.71 – 2.59 (m, 2H), 2.30 (s, 3H), 1.95 – 1.83 (m, 2H), 1.70 (dd, *J* = 15.4, 7.7 Hz, 2H), 1.50 (dd, *J* = 12.5, 5.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.41, 158.86, 153.04, 149.86, 143.60, 142.54, 138.10, 137.11, 135.84, 131.03, 130.30, 128.96, 128.55 (2C), 128.44 (2C), 126.51, 125.85, 120.45, 39.89, 35.85, 31.29, 28.84, 23.89, 20.55. HRMS (ESI+) m/z: calculated for $C_{25}H_{24}N_2O_2$ (M + H) 385.1911; found 385.1914. Purity ≥ 95% as determined by LC/MS.

2-amino-5-phenylpyridin-3-ol (21): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (500 mg, 1.8 mmol) and phenylboronic acid (262 mg, 2.2 mmol) according to the procedures described for compound **19** This yielded 2-amino-5-phenylpyridin-3-ol (200 mg, 1.1 mmol, 61%). ^{1}H NMR (400 MHz, DMSO) δ 9.70 (s, 1H), 7.78 (d, *J* = 2.0 Hz, 1H), 7.51 (d, *J* = 7.3 Hz, 2H), 7.41 (t, *J* = 7.7 Hz, 2H), 7.34 – 7.19 (m,

2H), 7.13 (d, *J* = 2.0 Hz, 1H), 5.66 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 150.07, 139.32, 138.33, 135.28, 128.91 (2C), 126.31, 125.53 (2C), 124.97, 116.79.

6-phenyl-1-(6-phenyloxazolo[4,5-b]pyridin-2-yl)hexan-1-ol (22): The title compound was synthesized from **21** (67 mg, 0.36 mmol) according to the procedures described for **20**. This yielded 6-phenyl-1-(6-phenyloxazolo[4,5-b]pyridin-2-yl)hexan-1-ol (38 mg, 0.10 mmol, 28%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* = 1.9 Hz, 1H), 7.97 (d, *J* = 1.9 Hz, 1H), 7.59 – 7.37 (m, 3H), 7.28 – 7.02 (m, 6H), 5.09 – 4.93 (m, 1H), 2.68 – 2.50 (m, 2H), 2.13 – 1.92 (m, 2H), 1.70 – 1.30 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.04, 153.97, 145.48, 143.57, 142.63, 137.31, 134.94, 129.40 (2C), 128.48 (2C), 128.35 (2C), 127.66 (2C), 125.75, 125.73, 117.41, 68.23, 35.92, 35.46, 34.15, 31.38, 29.02.

6-phenyl-1-(6-phenyloxazolo[4,5-b]pyridin-2-yl)hexan-1-one (1): The title compound was synthesized from **22** (35 mg, 0.094 mmol) according to the procedure described for **3**. This yielded 6-phenyl-1-(6-phenyloxazolo[4,5-b]pyridin-2-yl)hexan-1-one (27 mg, 0.072 mmol, 78%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (d, *J* = 2.0 Hz, 1H), 8.12 (d, *J* = 2.0 Hz, 1H), 7.65 (dd, *J* = 5.3, 3.4 Hz, 2H), 7.58 – 7.51 (m, 2H), 7.48 (ddd, *J* = 7.3, 3.6, 1.3 Hz, 1H), 7.33 – 7.22 (m, 2H), 7.22 – 7.12 (m, 3H), 3.29 (t, *J* = 7.4 Hz, 2H), 2.71 – 2.60 (m, 2H), 1.88 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.71 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.57 – 1.41 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.35, 158.88, 153.41, 148.40, 144.16, 142.53, 137.65, 137.03, 129.54 (2C), 129.00, 128.54 (2C), 128.43 (2C), 127.84 (2C), 125.84, 118.18, 39.87, 35.84, 31.29, 28.84, 23.86. HRMS (ESI+) m/z: calculated for $C_{24}H_{22}N_2O_2$ (M + H) 371.1754; found 371.1754. Purity ≥ 95% as determined by LC/MS.

2-amino-5-(m-tolyl)pyridin-3-ol (23): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (418 mg, 1.5 mmol) and *m*-tolylboronic acid (300 mg, 2.2 mmol) according to the procedures described for compound **19** This yielded 2-amino-5-(m-tolyl)pyridin-3-ol (220 mg, 1.1 mmol, 73%). ^{1}H NMR (400 MHz, DMSO) δ 9.65 (s, 1H), 7.77 (d, *J* = 1.8 Hz, 1H), 7.47 – 7.21 (m, 3H), 7.21 – 6.97 (m, 2H), 5.63 (s, 2H), 2.35 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 149.95, 139.25, 138.22, 137.91, 135.20, 128.75, 126.94, 126.16, 125.05, 122.63, 116.82, 21.12.

6-phenyl-1-(6-(m-tolyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-ol (24): The title compound was synthesized from **23** (107 mg, 0.53 mmol) according to the procedures described for **20**. This yielded 6-phenyl-1-(6-(m-tolyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-ol (76 mg, 0.20 mmol, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 1.7 Hz, 1H), 7.91 (d, *J* = 1.7 Hz, 1H), 7.43 – 7.28 (m, 3H), 7.28 – 7.18 (m, 3H), 7.14 (t, *J* = 6.9 Hz, 3H), 5.13 – 4.98 (m, 1H), 2.65 – 2.52 (m, 2H), 2.42 (s, 3H), 2.11 – 1.91 (m, 2H), 1.73 – 1.46 (m, 4H), 1.46 – 1.30 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.65, 154.05, 145.56, 143.41, 142.63,

139.02, 137.40, 134.76, 129.20, 129.10, 128.43 (2C), 128.32, 128.30 (2C), 125.68, 124.71, 117.02, 68.14, 35.88, 35.42, 31.33, 29.03, 24.95, 21.59.

6-phenyl-1-(6-(m-tolyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-one (2): The title compound was synthesized from **24** (60 mg, 0.16 mmol) according to the procedure described for **3**. This yielded 6-phenyl-1-(6-(m-tolyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-one (45 mg, 0.12 mmol, 76%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (d, *J* = 1.9 Hz, 1H), 8.09 (d, *J* = 2.0 Hz, 1H), 7.47 – 7.39 (m, 3H), 7.32 – 7.23 (m, 3H), 7.17 (dd, *J* = 10.9, 4.4 Hz, 3H), 3.28 (dd, *J* = 9.4, 5.4 Hz, 2H), 2.69 – 2.57 (m, 2H), 2.46 (s, 3H), 1.87 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.70 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.55 – 1.43 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.30, 158.80, 153.29, 148.38, 144.13, 142.50, 139.29, 137.76, 136.97, 129.71, 129.40, 128.51 (2C), 128.40 (2C), 125.81, 124.93, 118.09, 39.83, 35.81, 31.26, 28.81, 23.83, 21.64. HRMS (ESI+) m/z: calculated for $C_{25}H_{24}N_2O_2$ (M + H) 385.1911; found 385.1914. Purity ≥ 95% as determined by LC/MS.

2-amino-5-(4-fluorophenyl)pyridin-3-ol (35): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (300 mg, 1.08 mmol) and 4-fluorophenylboronic acid (180 mg, 1.3 mmol) according to the procedures described for compound **19** This yielded 2-amino-5-(4-fluorophenyl)pyridin-3-ol (100 mg, 0.5 mmol, 50%)**.** ^{1}H NMR (400 MHz, DMSO) δ 9.67 (s, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.62 – 7.44 (m, 2H), 7.37 – 7.13 (m, 2H), 7.07 (d, *J* = 2.1 Hz, 1H), 5.64 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 161.16 (d, *J* = 242.9 Hz), 150.05, 139.28, 135.24, 134.87, 127.40 (d, *J* = 8.0 Hz, 2C), 124.02, 116.75, 115.65 (d, *J* = 21.2 Hz, 2C).

1-(6-(4-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (36): The title compound was synthesized from **25** (70 mg, 0.35 mmol) according to the procedures described for **20**. This yielded 1-(6-(4-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (40 mg, 0.10 mmol, 29%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 1.8 Hz, 1H), 7.91 (d, *J* = 1.8 Hz, 1H), 7.60 – 7.46 (m, 2H), 7.31 – 7.07 (m, 7H), 5.11 – 4.96 (m, 1H), 2.70 – 2.50 (m, 2H), 2.04 (pd, *J* = 13.8, 6.9 Hz, 2H), 1.73 – 1.47 (m, 4H), 1.47 – 1.32 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.71, 163.14 (d, *J* = 248.6 Hz), 154.24, 145.58, 143.49, 142.65, 133.81, 133.64 (d, *J* = 3.4 Hz), 129.38 (d, *J* = 8.2 Hz, 2C), 128.49 (2C), 128.37 (2C), 125.77, 117.02, 116.52, 116.30, 68.29, 35.92, 35.49, 31.35, 29.02, 24.91.

1-(6-(4-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (4): The title compound was synthesized from **36** (39 mg, 0.1 mmol) according to the procedures described for **3**. This yielded 1-(6-(4-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (31 mg, 0.08 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (d, *J* = 2.0 Hz, 1H), 8.07 (d, *J* = 2.0 Hz, 1H), 7.70 – 7.53 (m, 2H), 7.35 – 7.10 (m, 7H), 3.29 (dd, *J* = 9.4, 5.4 Hz, 2H), 2.72 – 2.54 (m, 2H), 1.97 – 1.79 (m, 2H), 1.71 (dt, *J* = 15.5, 7.6 Hz, 2H), 1.56 – 1.41 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.29, 163.46 (d, *J* = 249.5 Hz),

158.96, 153.45, 148.18, 144.09, 142.52, 136.63, 133.17 (d, *J* = 3.3 Hz), 129.61 (d, *J* = 8.4 Hz, 2C), 128.53 (2C), 128.43 (2C), 125.84, 118.05, 116.63 (d, *J* = 21.8 Hz, 2C), 39.88, 35.84, 31.28, 28.83, 23.84. HRMS (ESI+) m/z: calculated for $C_{24}H_{21}FN_2O_2$ (M + H) 389.1660; found 389.1662. Purity ≥ 95% as determined by LC/MS.

2-amino-5-(3-fluorophenyl)pyridin-3-ol (27): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (300 mg, 1.08 mmol) and 3-fluorophenylboronic acid (179 mg, 1.3 mmol) according to the procedures described for compound **19** This yielded 2-amino-5-(3-fluorophenyl)pyridin-3-ol (120 mg, 0.59 mmol, 54%)**.** ^{1}H NMR (400 MHz, DMSO) δ 9.70 (s, 1H), 7.81 (d, *J* = 2.1 Hz, 1H), 7.49 – 7.38 (m, 1H), 7.38 – 7.26 (m, 2H), 7.19 – 6.93 (m, 2H), 5.73 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 162.73 (d, *J* = 242.9 Hz), 150.58, 140.89 (d, *J* = 8.0 Hz), 139.21, 135.70, 130.75 (d, *J* = 8.8 Hz), 123.45, 121.43 (d, *J* = 2.4 Hz), 116.58, 112.82 (d, *J* = 21.1 Hz), 111.95 (d, *J* = 21.9 Hz).

1-(6-(3-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (28): The title compound was synthesized from **27** (70 mg, 0.35 mmol) according to the procedures described for **20**. This yielded 1-(6-(3-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (34 mg, 0.08 mmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, *J* = 1.9 Hz, 1H), 7.95 (d, *J* = 1.9 Hz, 1H), 7.56 – 7.41 (m, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.32 – 7.21 (m, 3H), 7.21 – 7.03 (m, 4H), 5.04 (dd, *J* = 13.7, 6.5 Hz, 1H), 2.70 – 2.44 (m, 2H), 2.20 – 1.91 (m, 2H), 1.74 – 1.48 (m, 4H), 1.47 – 1.31 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.04, 163.39 (d, *J* = 247.2 Hz), 154.64, 145.64, 143.50, 142.65, 139.67 (d, *J* = 7.7 Hz), 133.54, 131.01 (d, *J* = 8.5 Hz), 128.50 (2C), 128.38 (2C), 125.77, 123.37 (d, *J* = 2.9 Hz), 117.22, 115.39 (d, *J* = 21.1 Hz), 114.66 (d, *J* = 22.4 Hz), 68.31, 35.92, 35.50, 31.36, 29.02, 24.9.

1-(6-(3-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (5): The title compound was synthesized from **28** (33 mg, 0.085 mmol) according to the procedure described for **3**. This yielded 1-(6-(3-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (28 mg, 0.07 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (d, *J* = 1.8 Hz, 1H), 8.11 (d, *J* = 2.0 Hz, 1H), 7.51 (td, *J* = 8.0, 5.8 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.37 – 7.31 (m, 1H), 7.28 (dt, *J* = 6.7, 2.5 Hz, 2H), 7.22 – 7.11 (m, 4H), 3.29 (dd, *J* = 9.4, 5.4 Hz, 2H), 2.70 – 2.58 (m, 2H), 1.88 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.71 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.56 – 1.42 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.25, 163.42 (d, *J* = 247.7 Hz), 159.12, 153.88, 148.19, 144.05, 142.51, 139.18 (d, *J* = 7.8 Hz), 136.29, 131.19 (d, *J* = 8.4 Hz), 128.53 (2C), 128.43 (2C), 125.84, 123.55 (d, *J* = 3.0 Hz), 118.30, 115.93 (d, *J* = 21.1 Hz), 114.85 (d, *J* = 22.6 Hz), 39.90, 35.83, 31.27, 28.82, 23.83. HRMS (ESI+) m/z: calculated for $C_{24}H_{21}FN_2O_2$ (M + H) 389.1660; found 389.1664. Purity ≥95% as determined by LC/MS.

2-amino-5-(2-fluorophenyl)pyridin-3-ol (29): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (509 mg, 1.9 mmol) and 2-flourophenylboronic acid (400 mg, 2.8 mmol) according to the procedures described for compound **19** This yielded 2-amino-5-(2-fluorophenyl)pyridin-3-ol (70 mg, 0.34 mmol, 18%). ^{1}H NMR (400 MHz, DMSO) δ 9.70 (s, 1H), 7.64 (s, 1H), 7.44 (td, *J* = 7.8, 1.5 Hz, 1H), 7.37 – 7.14 (m, 3H), 7.06 (s, 1H), 5.70 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 159.07 (d, *J* = 244.6 Hz), 150.19, 138.74, 137.12 (d, *J* = 3.4 Hz), 129.81 (d, *J* = 3.8 Hz), 128.41 (d, *J* = 8.3 Hz), 126.14 (d, *J* = 13.2 Hz), 124.88 (d, *J* = 3.4 Hz), 119.58, 118.61 (d, *J* = 3.7 Hz), 116.02 (d, *J* = 22.7 Hz).

1-(6-(2-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (30): The title compound was synthesized from **29** (66 mg, 0.32 mmol) according to the procedures described for **20**. This yielded 1-(6-(2-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (20 mg, 0.05 mmol, 16%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.04 (d, *J* = 18.2 Hz, 1H), 7.56 – 7.36 (m, 2H), 7.34 – 6.99 (m, 7H), 5.10 – 4.96 (m, 1H), 2.65 – 2.57 (m, 2H), 2.18 – 1.91 (m, 2H), 1.63 (tt, *J* = 12.6, 6.2 Hz, 2H), 1.58 – 1.47 (m, 2H), 1.48 – 1.31 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.88, 159.92 (d, *J* = 248.7 Hz), 154.33, 146.95, 143.15, 142.67, 130.93 (d, *J* = 2.8 Hz), 130.44 (d, *J* = 8.2 Hz), 129.15, 128.52 (2C), 128.39 (2C), 125.78, 125.02 (d, *J* = 3.7 Hz), 119.26, 116.58 (d, *J* = 22.3 Hz), 68.34, 35.93, 35.55, 31.36, 29.03, 24.90.

1-(6-(2-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (6): The title compound was synthesized from **30** (19 mg, 0.049 mmol) according to the procedure described for **3**. This yielded 1-(6-(2-fluorophenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (17 mg, 0.04 mmol, 89%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.16 (t, *J* = 1.7 Hz, 1H), 7.48 (dddd, *J* = 9.9, 7.1, 6.4, 1.7 Hz, 2H), 7.35 – 7.24 (m, 5H), 7.20 – 7.13 (m, 3H), 3.29 (t, *J* = 7.4 Hz, 2H), 2.70 – 2.60 (m, 2H), 1.88 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.71 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.49 (ddd, *J* = 18.4, 8.9, 6.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.34, 159.94 (d, *J* = 249.3 Hz), 153.55, 149.44 (d, *J* = 2.9 Hz), 143.73, 143.60, 142.54, 132.02, 131.01, 130.92, 128.55 (2C), 128.44 (2C), 125.85, 125.16 (d, *J* = 3.7 Hz), 124.83 (d, *J* = 13.4 Hz), 120.43 (d, *J* = 4.1 Hz), 116.74 (d, *J* = 22.3 Hz), 39.92, 35.85, 31.29, 28.84, 23.86. HRMS (ESI+) m/z: calculated for $C_{24}H_{21}FN_2O_2$ (M + H) 389.1660; found 389.1662. Purity ≥ 95% as determined by LC/MS.

2-amino-5-(4-methoxyphenyl)pyridin-3-ol (31): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (300 mg, 1.08 mmol) and 4-methoxyphenylboronic acid (198 mg, 1.3 mmol) according to the procedures described for compound **19**. This yielded 2-amino-5-(4-methoxyphenyl)pyridin-3-ol (148 mg, 0.69 mmol, 63%). ^{1}H NMR (400 MHz, DMSO) δ 9.62 (s, 1H), 7.71 (d, *J* = 2.1 Hz, 1H), 7.53 – 7.28 (m, 2H), 7.07 (d, *J* = 2.1 Hz, 1H), 7.02 – 6.89 (m, 2H), 5.54 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 158.07, 149.49, 139.27, 134.73, 130.79, 126.63 (2C), 124.89, 116.68, 114.34 (2C), 55.09.

1-(6-(4-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (32): The title compound was synthesized from **31** (52 mg, 0.24 mmol) according to the procedures described for **20**. This yielded 1-(6-(4-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (28 mg, 0.07 mmol, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 1.9 Hz, 1H), 7.90 (d, *J* = 1.9 Hz, 1H), 7.49 (t, *J* = 5.8 Hz, 2H), 7.25 (dd, *J* = 8.7, 6.5 Hz, 2H), 7.15 (t, *J* = 6.8 Hz, 3H), 7.06 – 6.92 (m, 2H), 5.09 – 4.92 (m, 1H), 3.86 (s, 3H), 2.66 – 2.53 (m, 2H), 2.14 – 1.89 (m, 2H), 1.72 – 1.58 (m, 2H), 1.58 – 1.45 (m, 2H), 1.46 – 1.33 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.27, 160.02, 153.70, 145.42, 143.57, 142.67, 134.46, 129.90, 128.76 (2C), 128.49 (2C), 128.36 (2C), 125.75, 116.56, 114.83 (2C), 68.26, 55.53, 35.92, 35.48, 31.36, 29.03, 24.92.

1-(6-(4-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (7): The title compound was synthesized from **32** (25 mg, 0.062 mmol) according to the procedure described for **3**. This yielded 1-(6-(4-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (18 mg, 0.045 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (d, *J* = 1.3 Hz, 1H), 8.05 (d, *J* = 2.0 Hz, 1H), 7.63 – 7.53 (m, 2H), 7.28 (dt, *J* = 6.7, 2.9 Hz, 2H), 7.17 (dd, *J* = 10.7, 4.3 Hz, 3H), 7.09 – 7.02 (m, 2H), 3.88 (s, 3H), 3.28 (dd, *J* = 9.4, 5.4 Hz, 2H), 2.70 – 2.58 (m, 2H), 1.87 (dt, *J* = 15.2, 7.5 Hz, 2H), 1.70 (dt, *J* = 15.5, 7.6 Hz, 2H), 1.55 – 1.43 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.33, 160.48, 158.66, 152.87, 148.10, 144.26, 142.53, 137.35, 129.34, 128.97 (2C), 128.53 (2C), 128.41 (2C), 125.82, 117.41, 115.01 (2C), 55.59, 39.82, 35.83, 31.28, 28.83, 23.86. HRMS (ESI+) m/z: calculated for $C_{25}H_{24}N_2O_3$ (M + H) 401.1860; found 401.1852. Purity ≥95% as determined by LC/MS.

2-amino-5-(3-methoxyphenyl)pyridin-3-ol (33): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (367 mg, 1.3 mmol) and 3-methoxyphenylboronic acid (237 mg, 1.6 mmol) according to the procedures described for compound **19**. This yielded 2-amino-5-(3-methoxyphenyl)pyridin-3-ol (80 mg, 0.37 mmol, 28%). ^{1}H NMR (400 MHz, MeOD) δ 7.63 (d, *J* = 2.0 Hz, 1H), 7.26 (t, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 2.0 Hz, 1H), 7.06 – 6.90 (m, 2H), 6.81 (dd, *J* = 8.2, 2.4 Hz, 1H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 152.09, 141.65, 132.98, 131.49, 124.65, 121.42, 119.11, 110.05, 109.59, 103.66, 103.36, 46.17.

1-(6-(3-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (34): The title compound was synthesized from **33** (96 mg, 0.45 mmol) according to the procedures described for **20**. This yielded 1-(6-(3-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (35 mg, 0.09 mmol, 19%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, *J* = 1.9 Hz, 1H), 7.95 (d, *J* = 2.0 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.31 – 7.21 (m, 2H), 7.20 – 7.04 (m, 5H), 7.03 – 6.92 (m, 1H), 5.10 – 4.93 (m, 1H), 3.87 (s, 3H), 2.69 – 2.57 (m, 2H), 2.13 –

1.93 (m, 2H), 1.71 – 1.46 (m, 4H), 1.45 – 1.32 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.73, 160.30, 154.22, 145.69, 143.48, 142.65, 138.88, 134.65, 130.44, 128.48 (2C), 128.35 (2C), 125.74, 125.71, 120.07, 117.24, 113.63, 113.55, 68.25, 35.92, 35.48, 31.37, 29.03, 24.93.

1-(6-(3-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (8): The title compound was synthesized from **34** (35 mg, 0.087 mmol) according to the procedure described for **3**. This yielded 6-phenyl-1-(6-phenyloxazolo[4,5-b]pyridin-2-yl)hexan-1-one (20 mg, 0.05 mmol, 57%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 8.11 (d, *J* = 2.0 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.28 (dt, *J* = 6.6, 1.6 Hz, 2H), 7.24 – 7.12 (m, 5H), 7.01 (ddd, *J* = 8.3, 2.5, 0.7 Hz, 1H), 3.90 (s, 3H), 3.29 (t, *J* = 7.4 Hz, 2H), 2.71 – 2.55 (m, 2H), 1.87 (dt, *J* = 15.2, 7.5 Hz, 2H), 1.70 (dq, *J* = 15.7, 7.8 Hz, 2H), 1.55 – 1.43 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.33, 160.43, 158.91, 153.51, 148.40, 144.12, 142.53, 138.45, 137.53, 130.63, 128.54 (2C), 128.43 (2C), 125.84, 120.23, 118.24, 114.14, 113.75, 55.59, 39.87, 35.84, 31.29, 28.83, 23.85. Purity ≥95% as determined by LC/MS, m/z: calculated for $C_{25}H_{24}N_2O_3$ (M + H) 401.19; found 401.00. HRMS (ESI+) m/z: calculated for $C_{25}H_{24}N_2O_3$ (M + H) 401.1860; found 401.1852.

2-amino-5-(2-methoxyphenyl)pyridin-3-ol (35): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (300 mg, 1.08 mmol) and 2-methoxyphenylboronic acid (198 mg, 1.3 mmol) according to the procedures described for compound **20**. This yielded 2-amino-5-(2-methoxyphenyl)pyridin-3-ol (135 mg, 0.63 mmol, 58%). ^{1}H NMR (400 MHz, DMSO) δ 9.52 (s, 1H), 7.54 (d, *J* = 1.7 Hz, 1H), 7.25 (ddd, *J* = 13.0, 8.1, 3.8 Hz, 2H), 7.01 (dt, *J* = 14.6, 5.7 Hz, 3H), 5.51 (s, 2H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 156.18, 149.35, 138.28, 137.20, 129.64, 128.03, 127.55, 122.87, 120.77, 119.85, 111.66, 55.43.

1-(6-(2-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (36): The title compound was synthesized from **35** (77 mg, 0.36 mmol) according to the procedures described for **20**. This yielded 1-(6-(2-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (25 mg, 0.06 mmol, 17%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 1.8 Hz, 1H), 8.02 (d, *J* = 1.8 Hz, 1H), 7.48 – 6.95 (m, 9H), 5.01 (dt, *J* = 20.7, 10.3 Hz, 1H), 3.81 (d, *J* = 9.2 Hz, 3H), 2.70 – 2.43 (m, 2H), 2.17 – 1.90 (m, 2H), 1.76 – 1.33 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.39, 156.61, 153.55, 147.45, 142.99, 131.83, 131.07, 130.00, 128.50 (2C), 128.36 (2C), 126.43, 125.74, 125.71, 121.30, 119.84, 111.47, 68.28, 55.66, 35.93, 35.53, 31.37, 29.04, 24.91.

1-(6-(2-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (9): The title compound was synthesized from **36** (23 mg, 0.057 mmol) according to the procedure described for **3**. This yielded 1-(6-(2-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (15 mg, 0.037 mmol, 66%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, *J* =

1.9 Hz, 1H), 8.17 (d, *J* = 1.9 Hz, 1H), 7.48 – 7.36 (m, 2H), 7.33 – 7.23 (m, 2H), 7.17 (dd, *J* = 10.8, 4.4 Hz, 3H), 7.11 (td, *J* = 7.5, 1.0 Hz, 1H), 7.05 (d, *J* = 8.3 Hz, 1H), 3.85 (s, 3H), 3.29 (t, *J* = 7.4 Hz, 2H), 2.69 – 2.58 (m, 2H), 1.87 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.70 (dq, *J* = 14.8, 7.3 Hz, 2H), 1.55 – 1.42 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.47, 158.68, 156.66, 152.76, 150.19, 143.66, 142.54, 134.82, 131.06, 130.49, 128.53 (2C), 128.41 (2C), 125.98, 125.82, 121.40, 120.87, 111.56, 55.70, 39.84, 35.85, 31.30, 28.84, 23.90. HRMS (ESI+) m/z: calculated for $C_{25}H_{24}N_2O_3$ (M + H) 401.1860; found 401.1858. Purity ≥ 95% as determined by LC/MS.

2-amino-5-(4-(trifluoromethyl)phenyl)pyridin-3-ol (37): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (300 mg, 1.08 mmol) and 4-(trifluoromethyl)phenylboronic acid (244 mg, 1.3 mmol) according to the procedures described for compound **19.** This yielded 2-amino-5-(4-(trifluoromethyl)phenyl)pyridin-3-ol (140 mg, 0.55 mmol, 51%)**.** ^{1}H NMR (400 MHz, DMSO) δ 9.80 (s, 1H), 7.95 – 7.82 (m, 1H), 7.74 (s, 4H), 7.18 (d, *J* = 1.8 Hz, 1H), 5.85 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 150.95, 142.41, 139.34, 136.06, 125.90, 125.74, 125.71, 123.07, 116.45. ^{13}C NMR (101 MHz, DMSO) δ 150.95, 142.41, 139.34, 136.06, 126.63, 126.32, 125.90 (2C), 125.72 (q, *J* = 3.6 Hz, 2C), 123.07, 116.45.

6-phenyl-1-(6-(4-(trifluoromethyl)phenyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-ol (38): The title compound was synthesized from **37** (96 mg, 0.38 mmol) according to the procedures described for **20**. This yielded 6-phenyl-1-(6-(4-(trifluoromethyl)phenyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-ol (35 mg, 0.08 mmol, 21%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, *J* = 1.9 Hz, 1H), 8.00 (d, *J* = 1.9 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.73 – 7.65 (m, 2H), 7.33 – 7.02 (m, 5H), 5.12 – 4.98 (m, 1H), 2.68 – 2.50 (m, 2H), 2.15 – 1.93 (m, 2H), 1.71 – 1.32 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.42, 154.81, 145.70, 143.50, 142.64 (d, *J* = 5.4 Hz), 140.95, 133.35, 130.75, 130.48, 128.49 (2C), 128.39 (2C), 128.03 (2C), 126.37 (q, *J* = 3.7 Hz, 2C), 125.78 (d, *J* = 2.4 Hz), 117.46, 68.32, 35.92, 35.50, 31.35, 29.00, 24.92.

6-phenyl-1-(6-(4-(trifluoromethyl)phenyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-one (10): The title compound was synthesized from **38** (33 mg, 0.075 mmol) according to the procedure described for **3**. This yielded 6-phenyl-1-(6-(4-(trifluoromethyl)phenyl)oxazolo[4,5-b]pyridin-2-yl)hexan-1-one (28 mg, 0.064 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (d, *J* = 2.0 Hz, 1H), 8.15 (d, *J* = 2.0 Hz, 1H), 7.78 (q, *J* = 8.4 Hz, 4H), 7.33 – 7.23 (m, 2H), 7.17 (dd, *J* = 10.4, 4.3 Hz, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.69 – 2.60 (m, 2H), 1.88 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.71 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.55 – 1.43 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.22, 159.26, 154.16, 148.21, 144.02, 142.50, 140.56, 136.05, 131.10 (q, *J* = 32.9 Hz), 128.54 (2C), 128.44 (2C), 128.24 (2C), 126.51 (q, *J* = 3.6 Hz, 2C), 125.86, 125.40, 118.54, 39.93, 35.84, 31.28, 28.82, 23.81.

HRMS (ESI+) m/z: calculated for $C_{25}H_{21}F_3N_2O_2$ (M + H) 439.1628; found 439.1626. Purity ≥ 95% as determined by LC/MS.

methyl 4-(6-amino-5-hydroxypyridin-3-yl)benzoate (39): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (300 mg, 1.08 mmol) and 4-methoxycarbonylphenylboronic acid (234 mg, 1.3 mmol) according to the procedures described for compound **19** This yielded methyl 4-(6-amino-5-hydroxypyridin-3-yl)benzoate (150 mg, 0.61 mmol, 57%). ^{1}H NMR (400 MHz, DMSO) δ 9.85 (s, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.88 (d, *J* = 2.1 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.19 (d, *J* = 2.1 Hz, 1H), 5.90 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 166.08, 150.80, 142.87, 139.41, 135.70, 129.85, 127.09, 125.33, 123.24, 116.43, 52.02.

methyl 4-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzoate (40): The title compound was synthesized from **39** (34 mg, 0.14 mmol) according to the procedures described for **20**. This yielded methyl 4-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzoate (14 mg, 0.032 mmol, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.16 (d, *J* = 8.3 Hz, 2H), 8.01 (d, *J* = 1.3 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.26 (t, *J* = 7.5 Hz, 2H), 7.16 (t, *J* = 6.9 Hz, 3H), 5.10 – 4.95 (m, 1H), 4.00 – 3.88 (m, 3H), 2.67 – 2.55 (m, 2H), 2.18 – 1.95 (m, 2H), 1.74 – 1.48 (m, 4H), 1.48 – 1.34 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.98, 166.76, 154.88, 145.88, 143.51, 142.64, 141.89, 133.57, 130.64 (2C), 130.06, 128.50 (2C), 128.39 (2C), 127.62 (2C), 125.79, 117.24, 68.35, 52.46, 35.92, 35.52, 31.36, 29.01, 24.89.

Methyl 4-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzoate (11): The title compound was synthesized from **40** (13 mg, 0.03 mmol) according to the procedure described for **3**. This yielded methyl 4-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzoate (7.2 mg, 0.017 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.22 – 8.18 (m, 2H), 8.16 (d, *J* = 2.0 Hz, 1H), 7.77 – 7.66 (m, 2H), 7.28 (dt, *J* = 6.5, 1.5 Hz, 2H), 7.17 (dd, *J* = 10.4, 4.3 Hz, 3H), 3.97 (s, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.70 – 2.56 (m, 2H), 1.88 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.71 (dt, *J* = 15.5, 7.6 Hz, 2H), 1.49 (ddd, *J* = 18.2, 8.8, 6.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.24, 166.63, 159.20, 154.05, 148.28, 144.06, 142.51, 141.31, 136.39, 130.75 (2C), 130.57, 128.53 (2C), 128.43 (2C), 127.83 (2C), 125.85, 118.44, 52.53, 39.93, 35.85, 31.29, 28.84, 23.84.HRMS (ESI+) m/z: calculated for $C_{26}H_{24}N_2O_4$ (M + H) 429.1809; found 429.1809. Purity ≥ 95% as determined by LC/MS.

4-(6-amino-5-hydroxypyridin-3-yl)benzonitrile (41): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (350 mg, 1.25 mmol) and 4-cyanophenylboronic acid (276 mg, 1.9 mmol) according to the procedures described for compound **19** This yielded 4-(6-amino-5-hydroxypyridin-3-yl)benzonitrile (87 mg, 0.41 mmol, 33%). ^{1}H NMR (400 MHz, DMSO) δ 9.81 (s, 1H), 7.89 (d, *J* = 2.0 Hz, 1H), 7.82 (d,

J = 8.3 Hz, 2H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 2.0 Hz, 1H), 5.90 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 151.22, 142.96, 139.29, 136.44, 132.79, 125.88 (2C), 122.61, 119.13, 116.18, 108.33.

4-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (42): The title compound was synthesized from **41** (87 mg, 0.41 mmol) according to the procedures described for **20**. This yielded 4-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (33 mg, 0.08 mmol, 20%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, *J* = 2.0 Hz, 1H), 8.00 (d, *J* = 2.0 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.34 – 7.20 (m, 2H), 7.20 – 7.08 (m, 3H), 5.03 (ddd, *J* = 19.1, 12.3, 3.6 Hz, 1H), 2.65 – 2.53 (m, 2H), 2.15 – 1.95 (m, 2H), 1.72 – 1.58 (m, 2H), 1.58 – 1.48 (m, 2H), 1.48 – 1.34 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.47, 155.25, 145.65, 143.41, 142.57, 141.94, 133.12 (2C), 132.61, 128.45 (2C), 128.35 (2C), 128.27 (2C), 125.76, 118.51, 117.26, 112.21, 68.25, 35.88, 35.46, 31.34, 28.98, 24.91.

4-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (12): The title compound was synthesized from **42** (33 mg, 0.83 mmol) according to the procedure described for **3**. This yielded (3-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (26 mg, 0.066 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (t, *J* = 7.4 Hz, 1H), 8.15 (d, *J* = 2.0 Hz, 1H), 7.91 – 7.81 (m, 2H), 7.80 – 7.73 (m, 2H), 7.33 – 7.22 (m, 2H), 7.17 (dd, *J* = 10.2, 4.2 Hz, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.73 – 2.58 (m, 2H), 1.88 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.71 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.56 – 1.43 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.15, 159.41, 155.96, 154.46, 148.05, 143.96, 142.47, 141.44, 135.42, 133.27 (2C), 128.51 (2C), 128.42 (2C), 125.85 (2C), 118.53, 118.38, 112.85, 39.94, 35.81, 31.26, 28.79, 23.77. Purity ≥95% as determined by LC/MS, m/z: calculated for $C_{25}H_{21}N_3O_2$ (M + H) 396.17; found 396.00. HRMS (ESI+) m/z: calculated for $C_{25}H_{21}N_3O_2$ (M + H) 396.1707; found 396.1703.

3-(6-amino-5-hydroxypyridin-3-yl)benzonitrile (43): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (347 mg, 1.24 mmol) and 3-cyanophenylboronic acid (181 mg, 1.24 mmol) according to the procedures described for compound **19** This yielded 3-(6-amino-5-hydroxypyridin-3-yl)benzonitrile (82 mg, 0.39 mmol, 31%). ^{1}H NMR (400 MHz, DMSO) δ 9.75 (s, 1H), 7.98 (s, 1H), 7.85 (t, *J* = 4.9 Hz, 2H), 7.70 (d, *J* = 7.7 Hz, 1H), 7.58 (dd, *J* = 17.6, 9.8 Hz, 1H), 7.13 (d, *J* = 2.0 Hz, 1H), 5.80 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 150.88, 139.59, 139.26, 136.02, 130.15, 130.09, 129.72, 128.80, 122.62, 118.94, 116.46, 112.00.

3-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (44): The title compound was synthesized from **43** (82 mg, 0.39 mmol) according to the procedures described for **20**. This yielded 3-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (10 mg, 0.025 mmol, 6.4%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (t, *J* = 6.1

Hz, 1H), 7.99 (dd, *J* = 9.4, 1.9 Hz, 1H), 7.93 – 7.79 (m, 2H), 7.78 – 7.69 (m, 1H), 7.31 – 7.21 (m, 2H), 7.17 (dd, *J* = 10.4, 4.6 Hz, 4H), 5.11 – 4.95 (m, 1H), 2.67 – 2.53 (m, 2H), 2.14 – 1.93 (m, 2H), 1.74 – 1.60 (m, 2H), 1.60 – 1.48 (m, 2H), 1.47 – 1.35 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.27, 172.22, 155.19, 145.69, 143.47, 138.94, 132.00, 131.86, 131.16, 130.31, 128.51 (2C), 128.40 (2C), 125.81, 118.44, 117.19, 115.64, 113.74, 68.35, 35.92, 35.53, 31.36, 29.00, 24.89.

3-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (13): The title compound was synthesized from **44** (10 mg, 0.025 mmol) according to the procedure described for **3**. This yielded (3-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (5 mg, 0.013 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (d, *J* = 1.6 Hz, 1H), 8.12 (d, *J* = 2.0 Hz, 1H), 7.92 (d, *J* = 1.3 Hz, 1H), 7.90 – 7.85 (m, 1H), 7.78 (dd, *J* = 6.5, 1.2 Hz, 1H), 7.72 – 7.63 (m, 1H), 7.31 – 7.22 (m, 2H), 7.17 (dd, *J* = 10.1, 4.3 Hz, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.70 – 2.59 (m, 2H), 1.88 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.71 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.49 (ddd, *J* = 18.4, 8.9, 6.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.19, 159.39, 154.35, 147.99, 143.98, 142.50, 138.43, 135.17, 132.36, 132.13, 131.31, 130.48, 128.54 (2C), 128.45 (2C), 125.87, 118.48, 118.27, 113.98, 39.96, 35.84, 31.28, 28.82, 23.80. Purity ≥95% as determined by LC/MS, m/z: calculated for $C_{25}H_{21}N_3O_2$ (M + H) 396.17; found 396.00. HRMS (ESI+) m/z: calculated for $C_{25}H_{21}N_3O_2$ (M + H) 396.1707; found 396.1704.

2-(6-amino-5-hydroxypyridin-3-yl)benzonitrile (45): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (380 mg, 1.4 mmol) and 2-cyanophenylboronic acid (300 mg, 2.0 mmol) according to the procedures described for compound **19** This yielded 2-(6-amino-5-hydroxypyridin-3-yl)benzonitrile (90 mg, 0.42 mmol, 30%). ^{1}H NMR (400 MHz, DMSO) δ 9.90 (s, 1H), 8.02 – 7.82 (m, 1H), 7.74 (td, *J* = 7.7, 1.4 Hz, 1H), 7.66 (d, *J* = 2.1 Hz, 1H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.50 (td, *J* = 7.6, 1.1 Hz, 1H), 7.09 (t, *J* = 4.6 Hz, 1H), 5.90 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 151.38, 142.88, 139.22, 137.62, 134.38, 133.98, 129.97, 127.75, 122.78, 119.35, 118.53, 109.99.

2-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (46): The title compound was synthesized from **45** (45 mg, 0.21 mmol) according to the procedures described for **20**. This yielded 2-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (22 mg, 0.06 mmol, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 1.7 Hz, 1H), 8.08 (d, *J* = 1.8 Hz, 1H), 7.89 – 7.78 (m, 1H), 7.78 – 7.67 (m, 1H), 7.56 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.21 (m, 2H), 7.21 – 7.04 (m, 3H), 5.03 (dd, *J* = 23.5, 18.1 Hz, 1H), 2.67 – 2.59 (m, 2H), 2.12 – 1.95 (m, 2H), 1.73 – 1.51 (m, 4H), 1.50 – 1.36 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.59, 155.28, 146.72, 142.84, 142.66, 141.30, 134.15, 133.43, 131.31, 130.60, 128.86, 128.50 (2C), 128.36 (2C), 125.74, 119.06, 118.15, 111.93, 68.31,55.51, 35.93, 35.51, 31.37, 29.03, 24.92.

2-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (14): The title compound was synthesized from **46** (22 mg, 0.055 mmol) according to the procedure described for **3**. This yielded 2-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (15 mg, 0.038 mmol, 69%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.24 (t, *J* = 7.2 Hz, 1H), 7.93 – 7.84 (m, 1H), 7.77 (td, *J* = 7.8, 1.3 Hz, 1H), 7.60 (ddd, *J* = 7.6, 4.0, 1.2 Hz, 2H), 7.34 – 7.24 (m, 2H), 7.17 (dd, *J* = 11.1, 4.5 Hz, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.72 – 2.53 (m, 2H), 1.88 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.71 (dt, *J* = 15.5, 7.6 Hz, 2H), 1.50 (ddd, *J* = 18.5, 8.9, 6.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.19, 159.52, 154.52, 149.04, 142.52, 140.80, 134.28, 134.11, 133.51, 130.60, 129.31, 128.55 (2C), 128.44 (2C), 125.85, 120.45, 117.91, 112.16, 39.98, 35.84, 31.29, 28.81, 23.82. Purity ≥95% as determined by LC/MS, m/z: calculated for $C_{25}H_{21}N_3O_2$ (M + H) 396.17; found 396.13. HRMS (ESI+) m/z: calculated for $C_{25}H_{21}N_3O_2$ (M + H) 396.1707; found 396.1700.

2-Amino-5-(4-fluoro-2-methoxyphenyl)pyridin-3-ol (47) The title compound was synthesized from 3-(benzyloxy)-5-bromopyridin-2-amine (320 mg, 1.15 mmol according to the procedures described for compound **19.** This yielded 2-amino-5-(4-fluoro-2-methoxyphenyl)pyridin-3-ol (129 mg, 0.55 mmol, 48%, 2 steps). ^{1}H NMR (MeOD, 400 MHz): δ 7.47 (d, *J* = 1.9 Hz, 1H), 7.23 (dd, *J* = 8.4, 6.7 Hz, 1H), 7.12 (d, *J* = 1.9 Hz, 1H), 6.84 (dd, *J* = 11.1, 2.5 Hz, 1H), 6.76 – 6.68 (m, 1H), 3.80 (s, 3H). ^{13}C BBDEC NMR (MeOD, 101 MHz): δ 164.43 (d, *J* = 244.6 Hz), 159.20 (d, *J* = 9.9 Hz), 150.24, 141.75, 135.00, 133.04 (d, *J* = 10.1 Hz), 131.83 (d, *J* = 9.9 Hz), 125.31, 120.67, 114.60, 107.93 (d, *J* = 21.4 Hz), 100.51 (d, *J* = 26.2 Hz), 56.28.

1-(6-(4-fluoro-2-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (48): The title compound was synthesized from **47** (150 mg, 0.69 mmol) according to the procedures described for **20**. This yielded 1-(6-(4-fluoro-2-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (66 mg, 0.16 mmol, 25%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (d, *J* = 1.8 Hz, 1H), 7.99 (d, *J* = 1.8 Hz, 1H), 7.32 – 7.13 (m, 6H), 6.83 – 6.74 (m, 2H), 5.16 – 4.88 (m, 1H), 3.84 (d, *J* = 8.1 Hz, 3H), 2.64 (dd, *J* = 21.0, 13.8 Hz, 2H), 2.22 – 1.94 (m, 2H), 1.75 – 1.33 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.63, 163.88 (d, *J* = 248.0 Hz), 157.81 (d, *J* = 9.9 Hz), 153.56, 147.34, 142.81 (d, *J* = 35.4 Hz), 131.85 (d, *J* = 10.0 Hz), 131.03, 128.50, 128.37, 125.76, 122.41 (d, *J* = 3.4 Hz), 119.79, 111.07, 107.78 (d, *J* = 21.4 Hz), 99.82 (d, *J* = 25.9 Hz), 68.25, 55.93, 35.92, 35.50, 31.36, 29.03, 24.92.

1-(6-(4-fluoro-2-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (15): The title compound was synthesized from **48** (66 mg, 0.16 mmol) according to the procedure described for **3**. This yielded 1-(6-(4-fluoro-2-methoxyphenyl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (19 mg, 0.045 mmol, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (d, *J* = 18.6 Hz, 1H), 7.93 (t, *J* = 5.8 Hz, 1H), 7.34 – 7.23 (m, 2H), 7.21 – 7.09 (m, 3H), 6.88 – 6.81 (m, 1H), 6.41 (dt, *J* = 12.8, 6.4 Hz, 1H), 6.33 – 6.25 (m, 1H), 3.75

(s, 3H), 3.28 (t, *J* = 7.4 Hz, 2H), 2.63 (dd, *J* = 17.9, 10.2 Hz, 2H), 1.87 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.69 (dq, *J* = 15.8, 7.8 Hz, 2H), 1.55 – 1.45 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.41, 164.18 (d, *J* = 248.8 Hz), 158.77, 157.95 (d, *J* = 10.0 Hz), 152.82, 150.00, 143.69, 142.53, 133.99, 131.91 (d, *J* = 10.1 Hz), 128.53 (2C), 128.42 (2C), 125.83, 122.05 (d, *J* = 3.4 Hz), 120.80, 108.00 (d, *J* = 21.5 Hz), 99.99 (d, *J* = 25.9 Hz), 56.01, 39.86, 35.85, 31.29, 28.84, 23.90. Purity ≥95% as determined by LC/MS, m/z: calculated for $C_{25}H_{23}FN_2O_3$ (M + H) 419.18; found 419.13. HRMS (ESI+) m/z: calculated for $C_{25}H_{23}FN_2O_3$ (M + H) 419.1765; found 419.1762.

2-(6-amino-5-hydroxypyridin-3-yl)-5-fluorobenzonitrile (49): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (279 mg, 1.0 mmol) and 2-cyano-4-fluorobenzeneboronic acid pinacol ester (250 mg, 1.0 mmol) according to the procedures described for compound **19.** This yielded 2-(6-amino-5-hydroxypyridin-3-yl)-5-fluorobenzonitrile (80 mg, 0.35 mmol, 35%). ^{1}H NMR (400 MHz, MeOD) δ 7.66 – 7.52 (m, 3H), 7.48 (td, *J* = 8.5, 2.7 Hz, 1H), 7.11 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ 162.58 (d, *J* = 248.0 Hz), 152.21, 141.52, 140.47, 136.96, 133.10 (d, *J* = 8.4 Hz), 124.44, 121.87 (d, *J* = 21.6 Hz), 121.25 (d, *J* = 25.2 Hz), 119.97, 118.48, 113.83 – 112.52.

5-fluoro-2-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (50): The title compound was synthesized from **49** (78 mg, 0.34 mmol) according to the procedures described for **20**. This yielded 5-fluoro-2-(2-(1-hydroxy-6-phenylhexyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (48 mg, 0.12 mmol, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, *J* = 1.8 Hz, 1H), 8.04 (d, *J* = 1.9 Hz, 1H), 7.54 (dt, *J* = 7.4, 3.7 Hz, 2H), 7.44 (td, *J* = 8.2, 2.6 Hz, 1H), 7.31 – 7.20 (m, 2H), 7.20 – 7.08 (m, 3H), 5.15 – 4.96 (m, 1H), 2.59 (dd, *J* = 17.8, 10.1 Hz, 2H), 2.18 – 1.94 (m, 2H), 1.71 – 1.29 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.83, 161.98 (d, *J* = 252.6 Hz), 155.36, 142.78, 142.64, 137.70 (d, *J* = 3.8 Hz), 132.63 (d, *J* = 8.4 Hz), 130.31, 128.49 (2C), 128.35 (2C), 125.74, 121.18 (d, *J* = 21.3 Hz), 120.86 (d, *J* = 24.8 Hz), 119.06, 116.95 (d, *J* = 2.8 Hz), 113.36 (d, *J* = 9.4 Hz), 68.27, 35.90, 35.47, 31.36, 29.00, 24.89.

5-fluoro-2-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (16): The title compound was synthesized from **50** (48 mg, 0.12 mmol) according to the procedure described for **3**. This yielded 5-fluoro-2-(2-(6-phenylhexanoyl)oxazolo[4,5-b]pyridin-6-yl)benzonitrile (12 mg, 0.029 mmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (d, *J* = 14.0 Hz, 1H), 8.22 (dd, *J* = 15.0, 1.9 Hz, 1H), 7.67 – 7.53 (m, 2H), 7.49 (td, *J* = 8.2, 2.7 Hz, 1H), 7.38 – 7.23 (m, 2H), 7.22 – 7.08 (m, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.64 (dd, *J* = 16.5, 8.7 Hz, 2H), 1.96 – 1.82 (m, 2H), 1.70 (dq, *J* = 14.8, 7.3 Hz, 2H), 1.49 (dt, *J* = 15.3, 7.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.12, 162.24 (d, *J* = 253.3 Hz), 159.55, 154.59, 148.89, 143.29, 142.48, 137.16 (d, *J* = 3.8 Hz), 133.05, 132.64 (d, *J* = 8.5 Hz), 128.52 (2C), 128.41 (2C), 125.83, 121.18, 121.17 (d, *J* = 45.3 Hz), 120.45, 116.73 (d, *J* = 2.7 Hz),

113.62 (d, *J* = 9.3 Hz), 39.97, 35.82, 31.29, 28.79, 23.77. Purity ≥95% as determined by LC/MS, m/z: calculated for $C_{25}H_{20}FN_3O_2$ (M + H) 414.16; found 414.13. HRMS (ESI+) m/z: calculated for $C_{25}H_{20}FN_3O_2$ (M + H) 414.1612; found 414.608.

5-(benzyloxy)-[3,3'-bipyridin]-6-amine (51): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (305 mg, 1.1 mmol) and 3-pyridineboronic acid (1.2 eq., 161 mg, 1.3 mmol) according to the procedures described for compound **19.** This yielded 5-(benzyloxy)-[3,3'-bipyridin]-6-amine (200 mg, 0.73 mmol, 66%).^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.72 (s, 1H), 8.52 (d, *J* = 4.8, 1.5 Hz, 1H), 7.84 (s, 1H), 7.74 (d, *J* = 8.0, 1.9 Hz, 1H), 7.48 – 7.26 (m, 6H), 7.15 (s, 1H), 5.42 (br s, 2H), 5.12 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 150.43, 147.79, 147.27, 141.62, 136.21, 135.76, 134.07, 133.60, 128.73, 128.43, 127.63, 123.64, 123.30, 115.26, 70.38. HR-MS (ESI+): *m/z* = calc. for C17H15N3O [M + H+] 278.1288. Found 278.1287.

6-phenyl-1-(6-(pyridin-3-yl)oxazolo[4,5-b]pyridin- 2-yl)hexan-1-ol (52): The title compound was synthesized from **51** (96 mg, 0.5 mmol) according to the procedures described for **20**. This yielded 6-phenyl-1-(6-(pyridin-3-yl)oxazolo[4,5-b]pyridin- 2-yl)hexan-1-ol (72 mg, 0.19 mmol, 38%).^{1}H NMR (400 MHz, MeOD): δ = 8.91 (d, *J* = 2.3 Hz, 1H), 8.81 (d, *J* = 2.0 Hz, 1H), 8.61 (dd, *J* = 4.9, 1.5 Hz, 1H), 8.41 (d, *J* = 2.0 Hz, 1H), 8.21 (dt, *J* = 8.1, 1.9 Hz, 1H), 7.59 (dd, *J* = 8.0, 4.9 Hz, 1H), 7.29 – 7.05 (m, 5H), 4.95 (t, *J* = 7.7, 5.8 Hz, 1H), 2.59 (t, *J* = 7.7 Hz, 2H), 1.80 – 1.69 (m, 2H), 1.68 – 1.52 (m, 2H), 1.52 – 1.24 (m, 4H). ^{13}C NMR (101 MHz, MeOD): δ = 180.63, 149.76, 148.86, 146.15, 143.86, 137.20, 132.49, 129.38, 129.23, 126.63, 125.68, 119.20, 68.79,36.81, 35.57, 32.67, 30.06, 25.95.

6-phenyl-1-(6-(pyridin-3-yl)oxazolo[4,5-b]pyridin- 2-yl)hexan-1-one(17): The title compound was synthesized from 6-phenyl-1-(6-(pyridin-3-yl)oxazolo[4,5-b]pyridin- 2-yl)hexan-1-ol (49 mg, 0.13mmol) according to the procedure described for **3**. This yielded 6-phenyl-1-(6-(pyridin-3-yl)oxazolo[4,5-b]pyridin- 2-yl)hexan-1-one (20 mg, 0.05 mmol, 39%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.98 (d, *J* = 2.0 Hz, 1H), 8.93 (s, 1H), 8.74 (d, *J* = 4.8 Hz, 1H), 8.16 (d, *J* = 2.0 Hz, 1H), 7.98 (dt, *J* = 8.1, 1.8 Hz, 1H), 7.51 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.32 – 7.25 (m, 2H), 7.22 – 7.14 (m, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.65 (t, *J* = 7.7 Hz, 2H), 1.88 (p, *J* = 7.5 Hz, 2H), 1.71 (p, *J* = 7.5 Hz, 2H), 1.55 – 1.44 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 190.18, 159.18, 154.14, 149.92, 148.48, 148.02, 143.99, 142.47, 135.25, 134.08, 132.93, 128.51, 128.41, 25.83, 124.21, 118.41, 39.92, 35.82, 31.28, 28.80, 23.78. HR-MS (ESI+): *m/z* = calc. for $C_{23}H_{21}N_3O_2$ [M + H] 372.1707. Found 372.1711. Purity ≥95% as determined by LC/MS.

2-amino-5-(1-methyl-1H-pyrrol-2-yl)pyridin-3-ol (53): The title compound was synthesized from 2-amino-5-bromopyridin-3-ol (1.59 g, 5.7 mmol) and 1-methyl-2-

pyrroleboronic acid pinacol ester (895 mg, 7.1 mmol) according to the procedures described for compound **19** using DMF as the solvent for the suzuki coupling**.** This yielded 2-amino-5-(1-methyl-1H-pyrrol-2-yl)pyridin-3-ol (117 mg, 0.61 mmol, 11%).^{1}H NMR (500 MHz, MeOD) δ 7.33 (d, *J* = 1.8 Hz, 1H), 7.06 (d, *J* = 1.8 Hz, 1H), 6.69 (dd, *J* = 2.5, 2.0 Hz, 1H), 6.04 (ddd, *J* = 6.4, 3.6, 2.3 Hz, 2H), 3.56 (s, 3H). ^{13}C NMR (126 MHz, MeOD) δ 149.38, 143.05, 130.69, 129.12, 125.20, 121.92, 121.15, 109.83, 108.67, 35.03.

1-(6-(1-methyl-1H-pyrrol-2-yl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (54): The title compound was synthesized from **53** (128 mg, 0.67 mmol) according to the procedures described for **20**. This yielded 1-(6-(1-methyl-1H-pyrrol-2-yl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-ol (44 mg, 0.12 mmol, 18%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.96 – 7.66 (m, 1H), 7.27 – 7.23 (m, 3H), 7.16 – 7.11 (m, 2H), 6.80 (d, *J* = 1.7 Hz, 1H), 6.40 – 6.16 (m, 2H), 5.13 – 4.89 (m, 1H), 3.69 (s, 3H), 2.65 – 2.51 (m, *J* = 17.1, 9.2 Hz, 2H), 2.12 – 1.92 (m, 2H), 1.72 – 1.32 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.61, 153.37, 146.65, 143.15, 142.65, 130.21, 128.50 (2C), 128.37 (2C), 126.97, 125.77, 125.23, 118.24, 110.71, 108.60, 68.26, 35.93, 35.49, 35.25, 31.38, 29.02, 24.93.

1-(6-(1-methyl-1H-pyrrol-2-yl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (18): The title compound was synthesized from **54** (44 mg, 0.12 mmol) according to the procedure described for **3**. This yielded (1-(6-(1-methyl-1H-pyrrol-2-yl)oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (19 mg, 0.051 mmol, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.81 (d, *J* = 23.2 Hz, 1H), 7.92 (dd, *J* = 14.2, 1.9 Hz, 1H), 7.41 – 7.23 (m, 2H), 7.23 – 7.10 (m, 3H), 6.84 (dd, *J* = 2.4, 1.9 Hz, 1H), 6.48 – 6.35 (m, 1H), 6.35 – 6.15 (m, 1H), 3.73 (s, 3H), 3.28 (t, *J* = 7.4 Hz, 2H), 2.70 – 2.56 (m, 2H), 1.92 – 1.81 (m, 2H), 1.75 – 1.66 (m, 2H), 1.49 (ddd, *J* = 18.1, 8.8, 6.6 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.25, 158.68, 152.51, 149.18, 143.84, 142.53, 129.97,129.76, 128.54 (2C), 128.43 (2C), 126.16, 125.84, 118.48, 111.58, 109.00, 39.85, 35.85, 35.46, 31.29, 28.85, 23.90. Purity ≥95% as determined by LC/MS, m/z: calculated for (M + H) $C_{23}H_{24}N_3O_2$ 374.18 found 374.20. HRMS (ESI+) m/z: calculated for $C_{23}H_{23}N_3O_2$ (M + H) 374.1863; found 374.1863.

Biochemical methods

The colorimetric hDAGL-α assay based on the hydrolysis of PNP-butyrate was performed as described in Chapter 4.

Concentration response analysis of the inhibitors against native DAGL-α in the mouse brain membrane proteome using competitive ABPP with MB064 (250 nM) were performed as described in chapter 4 and 5.

Selectivity in the mouse brain membrane proteome using TAMRA-FP (500 nM) and MB064 (250 nM) were performed as describes in chapter 4.

The fluorescence based DAGL-α natural substrate assay was performed as reported previously.[15]

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