Discovery of novel inhibitors to investigate diacylglycerol lipases and α/β hydrolase domain 16A

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CHAPTER 3

Comprehensive analysis of structure-activity relationships of α-ketoheterocycles as *sn*-1-diacylglycerol lipase α inhibitors*

Introduction

Sn-1-specific diacylglycerol lipases (DAGLs), of which two isoforms exist (DAGLα and β), catalyse the formation of the signaling lipid 2-arachidonoylglycerol (2-AG) from diacylglycerols.[1] 2-AG is a full cannabinoid CB1 receptor agonist and modulates synaptic plasticity at GABAergic and glutamatergic synapses by regulating neurotransmitter release.[2, 3] In the periphery, 2-AG is mainly produced by DAGLβ, a key enzyme involved in the regulation of macrophage pro-inflammatory responses.[4] Disruption of 2-AG signaling is linked to diet-induced obesity and related metabolic disorders, as well as to addiction and (neuro)inflammation.[2, 3, 5]

2-AG is hydrolysed by monoacylglycerol lipase (MAGL), α/β hydrolase domain 6 and 12 (ABHD6, ABHD12) to give arachidonic acid. Both 2-AG and arachidonic acid can be converted by cyclooxygenase-2 into eicosanoids, including pro-inflammatory prostaglandins (and their ester derivatives) that contribute to neuroinflammation.[6-9] The many players involved in 2-AG metabolism signify that 2-AG and its metabolic products have a wide array of physiological functions, of which many are still poorly understood.[10] For a better understanding of 2-AG-mediated physiological processes, the development of inhibitors that selectively perturb DAGL activity, and hence 2-AG biosynthesis, are of great importance.[11] In addition, these inhibitors may serve as valuable probes to evaluate DAGLα as a novel target to treat human conditions like obesity, diabetes, cardiovascular and neurodegenerative diseases.[7] Recently, α-ketoheterocycles were discovered as a novel and highly potent class of DAGLα inhibitors.[12, 13] α-Ketoheterocycles have previously been applied to the discovery of potent inhibitors of diverse serine hydrolases and cysteine proteases such as fatty acid amide (FAAH),[14] elastase,[15-17] thrombin,[18, 19] factor Xa,[20]

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chymase,[21] tryptase,[22] cathepsin K and cathepsin S.[23, 24] The α-ketoheterocycle scaffold provides an electrophilic ketone group with tunable reactivity, as well as a structural template to introduce important interactions with key amino acids in the binding site to obtain potency and selectivity.[24] α-Ketoheterocycles have been shown to be orally bioavailable and have entered human clinical trials, and thus provide an interesting scaffold for probe and drug discovery purposes. 1-(Oxazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-one (LEI104, OL-100)[25] was identified via pharmocophore-based screening approach, as the first covalent and reversible inhibitor for DAGLα (Chapter 2). Compound **1** (LEI104) had an IC_{50} of 37 ± 5 nM in a colorimetric surrogate DAGLα substrate assay and was highly selective over a panel of serine hydrolases as assessed by gel-based competitive activity-based protein profiling (ABPP). FAAH was identified as its only detected off-target, which was not surprising, because **1** was originally designed as a potent inhibitor of FAAH,[14] an enzyme that inactivates the other endocannabinoid anandamide.

Here, the first extensive SAR study is reported of α-ketoheterocycles as DAGLα inhibitors, by screening of a 1040-member, focused library of FAAH inhibitors, which is mainly based on the α-ketoheterocycle scaffold. Newly synthesized analogues are included in the screens, and thereby the structural requirements for interaction of α-ketoheterocycles with DAGLα were systematically investigated.

Results and Discussion

To investigate the structure-activity relationship (SAR) of **1** a focused library consisting of 1040 previously published α-ketoheterocycles and their corresponding precursors[14, 26-33] was screened using a colorimetric DAGLα activity assay (Figure 1).[12] In total, 64 active compounds were identified with more than 50% inhibitory activity at 10 μM final inhibitor concentration. These active compounds were further analysed in concentration response experiments. To complement the SAR analysis of the focused library, 19 additional α-ketoheterocycles (**3**-**16**, **106-110**) were synthesized and tested.[12] The combined structure-activity-relationships are described in a topological fashion below.

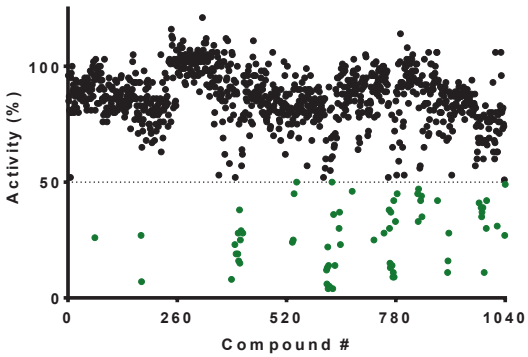


Figure 1. Screening results of the focused library of serine hydrolase inhibitors. 1040 Compounds were screened in duplicate (N = 2) at 10 μM using a colorimetric 96-well assay in which *para*-nitrophenylbutyrate

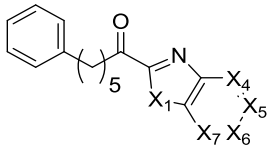
was used as a surrogate substrate on membranes of HEK293T cells overexpressing recombinant human DAGLα.[12] A total of 64 actives (indicated in green) showed >50% inhibitory activity and were selected for further concentration response analysis.

Central heterocyclic scaffold modifications

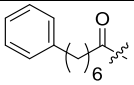
First, the influence of the central heterocyclic scaffold on DAGLα activity was explored. A diverse set of α-ketoheterocycles, including benzoxazole (**2**), benzimidazole (**3**), benzothioazole (**4**) and their 4-pyridine analogues (**1**, **5** and **6**, respectively) were analysed. *N4*-oxazolopyridine (**1**) was identified as the most potent scaffold with a pIC_{50} of 7.4 ± 0.05, whereas the other scaffolds have a pIC_{50} < 6 (Table 1) or are completely inactive (**17**-**42**). The imidazolopyridine (**5**) and thiazolopyridine (**6**) are predicted to reduce electrophilicity of the C-2 carbonyl (lower σ_i),[12, 13] thereby possibly reducing inhibitor activity.[15, 34] The basic nitrogen of the oxazolopyridine is another important feature of the scaffold, because its removal resulted in a 100-fold drop in potency (compare **1** and **2**). This observation is corroborated by the fact that other regioisomers of the oxazolopyridine scaffold (**7** and **8**) are also less potent (Table 2). Introduction of other electron withdrawing groups such as fluorine (**9**-**12**) and nitro-substituents (**13**, **14**) resulted in a 5-50 fold increased potency compared to their benzoxazole analogue (**2**), but they did not reach the same extent of inhibition as observed with the oxazolopyridine (**1**). This indicates that the nitrogen at the 4-position is not only required for its electron withdrawing properties, but may also have a specific interaction, likely an H-bond acceptor, with amino acid residues in the binding site. Interestingly, halogens at the 7-position in the heterocyclic scaffold (**12**, **16**) are not allowed.

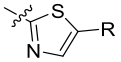
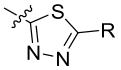
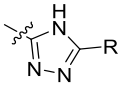
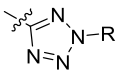
Deconstruction of the oxazolopyridine scaffold by removal of the pyridyl moiety is not allowed, because using a simple oxazole (**43**) or oxadiazole (**44**,**61**) as scaffolds results in inactive compounds. These results align with the observation, noted above, that the pyridyl provides important interactions with amino acids in the binding pocket. Activity of the oxazole scaffold can (partly) be rescued, however, by introducing small electron withdrawing groups at the *meta*-position (X_4: **45**-**55**), but not at the *para*-position (X_5: **62**-**75**, Table 2). A clear correlation between the electron withdrawing effect of the substituents and pIC_{50} was observed. A plot of the inhibition (pIC_{50}-values) versus the Hammet σ_m constants for the substituents (Figure 2) was found to follow a well-defined correlation (r^2 = 0.78) and the substituent effect was large (ρ = 2.95). This resulted in an almost 1000-fold increase in potency per unit change in σ_m, which indicates that the electron withdrawing effect of the substituent is the dominant factor contributing to the rescue of inhibitory activity. This may be explained by the increased electrophilic character of the reacting C-2 ketone imparted by the electron withdrawing C-4 substituent that leads to an increased strength of the covalent transient state in which Ser472 of the enzyme forms a hemiketal with the inhibitor, thereby increasing its affinity. The magnitude of the effect is similar as that reported previously for the activity of the α-ketoheterocycle inhibitors on FAAH, which indicates that this is a fundamental relationship for α-ketoheterocycles as serine hydrolase inhibitors.[32]

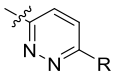
Table 1. Structure-activity relation of compounds with a varying central heterocyclic scaffold (**1-42**)



Entry	X_1	X_4	X_5	X_6	X_7	pIC_{50} ± SEM
1[12]	O	N	CH	CH	CH	7.43 ± 0.05
2[12]	O	CH	CH	CH	CH	5.44 ± 0.05
3	NH	CH	CH	CH	CH	4.92 ± 0.21
4	S	CH	CH	CH	CH	< 5
5	NH	N	CH	CH	CH	5.91 ± 0.10
6	S	N	CH	CH	CH	< 5
7	O	CH	N	CH	CH	6.69 ± 0.08
-	O	CH	CH	N	CH	Unstable
8	O	CH	CH	CH	N	5.77 ± 0.12
9	O	CF	CH	CH	CH	6.93 ± 0.17
10	O	CH	CF	CH	CH	5.85 ± 0.12
11	O	CH	CH	CF	CH	6.15 ± 0.11
12	O	CH	CH	CH	CF	< 5
13	O	CH	CNO_2	CH	CH	6.05 ± 0.08
14	O	CH	CH	CNO_2	CH	7.06 ± 0.08
15	O	CH	CH	CBr	CH	5.23 ± 0.12
16	O	CH	CH	CH	CBr	< 5

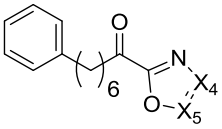


17-19		R = pyridin-2-yl, furan-2 yl, thiophen-2-yl	< 5
20-25		R = H, C(O)OMe, pyridine 2-yl, furan-2-yl, thiophen-2-yl, Ph	< 5
26-31		*R = H, C(O)OMe, pyridine 2-yl, furan-2-yl, thiophen-2-yl, Ph*	< 5
32-33		R = H, Me	< 5
34-38		R = H, Me, pyridin-2-yl, furan-2-yl, thiophen-2-yl	< 5

39-42		R = H, pyridin-2-yl furan-2-yl, thiophen-2-yl	< 5

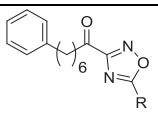
The aforementioned hypothesis establishes that both the aldehyde (**52**) and trifluoromethylketone (**45**) inhibit DAGLα as carbonyl active species and not as gem-diols, as previously observed for the FAAH inhibitors.[32] This is because the σ_m values for $CH(OH)_2$ and $C(OH)_2CF_3$, (0.02 and 0.33, respectively), do not explain the observed inhibition, while the σ_m values for C(O) and $C(O)CF_3$ (0.35 and 0.63, respectively) do correlate with DAGLα perturbation. The variation in assay buffer pH (7.4 vs 9.0 for the DAGLα assay and FAAH assay, respectively) may explain the observed differences in hydration state of the activated ketones. Of note, compounds featuring a methyl ketone (**46**) or a *t*-butyl ketone (**49**) display higher than expected activity-based on their σ_m values. This would indicate that these inhibitors exhibit additional H-bond or van der Waals interactions with the enzyme. Remarkably, all *para*-substituted compounds (**62**-**75**) that are able to directly conjugate with the electrophilic carbonyl, did not show any substantial inhibitor activity, whereas the *meta*-substituted derivatives (**45**-**60**), which exert their effects only through inductive electron-withdrawing properties, do inhibit the enzyme. This might be explained by steric hindrance of the *para*-substituents, thereby generating a steric clash that precludes their interaction with DAGLα (Figure 3B). The oxazoles could however, at least theoretically, simply flip their orientation in the active site reversing the location of the nitrogen and oxygen atom of the heterocycle in a manner that places the substituent in a comparable location as the *meta*-substituents. Apparently, this does not happen, which indicates that the electron-withdrawing effect is required, but not sufficient to inhibit DAGLα with *meta*- or *para*-substituted compounds. It also suggests that an additional interaction is required with the *meta*-substituted compounds to perturb DAGLα activity (Figure 3A), which cannot be formed by their *para*-substituted analogues (Figure 3C). Indeed, the nitrogen of the oxazole-scaffold has previously been implicated in H-bonding with the histidine residue of the catalytic triad of elastase.[17] It is, therefore, reasonable to suggest that the α-ketoheterocycle oxazole nitrogen is also required for its interaction with the catalytic His650 from DAGLα. Finally, substituted oxazoles with more sterically demanding side-groups (*e.g.* phenyl/ pyridine, entries **77**-**87**, such as potent FAAH inhibitor OL-135)[26] are not active on DAGLα, which suggest that the *meta*-substituents are located in a pocket that is restricted in size.

Table 2. Structure-activity relation of isoxazoles and oxadiazoles **43-105.**[26, 27, 31-33]



Entry	Substituent X	pIC_{50} ± SEM X_4 (X_5 = CH)	Entry	pIC_{50} ± SEM X_5 (X_4 = CH)
43	CH	< 5	-	-
44	N	5.57 ± 0.03	-	-
-	N	-	**61**	< 5
45	-CC(O)CF_3	6.91 ± 0.02	**62**	5.74 ± 0.03
46	-CC(O)CH_3	6.91 ± 0.03	**63**	< 5
47	-CCF_3	6.67 ± 0.03	**64**	< 5
48	-CSO_2Me	6.41 ± 0.03	**65**	< 5
49	-CC(O)^{t}Bu	6.32 ± 0.04	**-**	-
50	-CCN	6.24 ± 0.05	**66**	< 5
51	-CC(O)OMe	6.20 ± 0.04	**67**	< 5
52	-CCHO	5.82 ± 0.02	**68**	< 5
53	-Cl	5.63 ± 0.07	**-**	-
54	-CCl	5.62 ± 0.05	**69**	< 5
55	-$CCONMe_2$	5.47 ± 0.03	**70**	< 5
56	-COMe	< 5	**71**	< 5
57	-CBr	< 5	**72**	< 5
58	-CMe	< 5	**73**	< 5
59	-CSMe	< 5	**74**	< 5
60	-CC(O)NHMe	< 5	**75**	< 5

Entry	Substituent X	X_4	X_5	pIC_{50} ± SEM
76	-phenyl	CX	CH	5.06 ± 0.08
77	pyridin-2-yl	CX	CH	< 5
78	pyridin-4-yl	CX	CH	< 5
79	-phenyl	CH	CX	< 5
80-84	Mono substituted phenyl (*e.g.* 2, 3 or 4-F, -$COCF_3$, -OMe)	CH	CX	< 5
85-87	Mono substituted pyridine (*e.g.* 4-Me, -OMe, -CF_3)	CH	CX	< 5
88	furan-2-yl	CX	N	6.03 ± 0.03
89	6-cyanopyridin-2-yl	CX	N	5.98 ± 0.05
90	thiophen-2-yl	CX	N	5.71 ± 0.04
91	6-bromopyridin-2-yl	CX	N	5.69 ± 0.06
92	pyridin-2-yl	CX	N	5.56 ± 0.04

93-96	methyl-6-picolinate, 6-picolinic acid, 6-chloropyridin-2-yl, 6-iodopyridin-2-yl	CX	N	< 5
97-101	furan-2-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, Ph	N	CX	< 5
102-105	R = C(O)OMe, thiophen-2-yl, furan-2-yl, pyridin-2-yl			< 5

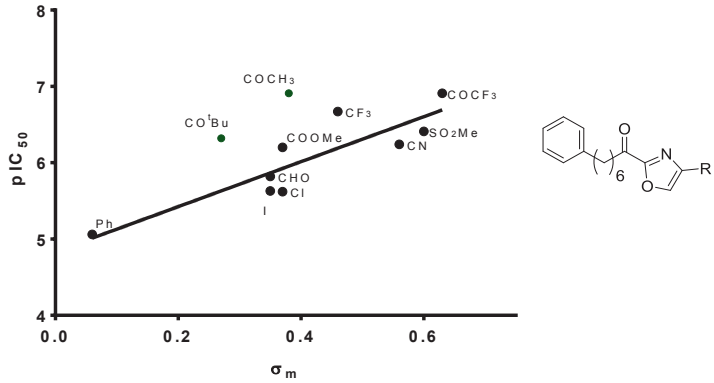


Figure 2. Effect of *meta* electron withdrawing substituent on potency of oxazoles. Electron withdrawing effect of *meta* (X_5) subsituted oxazoles, Hammet constant σ_m versus pIC_{50}. A linear correlation (R^2 = 0.78) with a slope ρ = 2.95 was found. Potency of inhibitors **46** and **49** (-CC(O)CH_3 and -CC(O)^{t}Bu) is higher than predicted solely by the electron withdrawing effect, indicating a potential additional interaction with the enzyme.

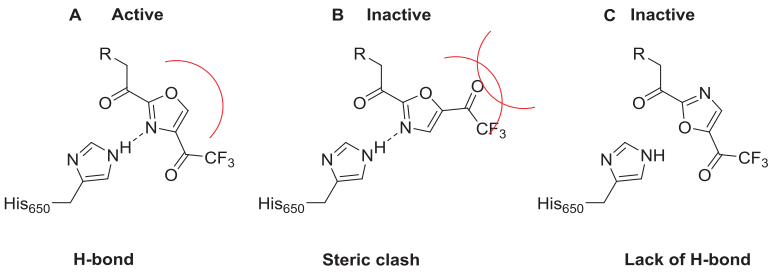
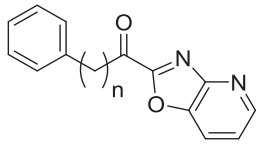


Figure 3. Proposed binding mode of substituted α-keto oxazoles. **A**. *Meta* (X_4) substituted oxazole (exemplified by **45**) forms a hydrogen bond with His650 and positions the substituent away from the highly sterically restricted pocket at X_5. **B**. *Para* (X_5) substituted oxazoles (exemplified by **62**) have a potential steric clash with the enzyme, possibly explaining their low potency on DAGLα. **C.** If the oxazole flips to avoid steric clash (*para* substituted), the oxazole nitrogen is excluded from hydrogen bond formation with His650.

Length of C2-acyl substituents

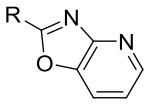
To investigate the influence of the C-2 acylphenyl spacer length the activity of analogues (**1**, **106-111**) was measured, in which the number of methylene groups was increased from 2 to 8 (Table 3). The inhibitory activity was higher with increasing number of methylene groups, and was highest at n = 8 (compound **111**), thereby making it the most potent inhibitor identified in this study with a pIC_{50} of 8.44 ± 0.04. Compound **109** (n = 6) exhibits a >10-fold drop in activity compared to compound **1** (n = 5). The reason for this reduced activity is not easily explained. When taking lipophilicity into account (i.e. lipophilic efficiency: LipE = pIC_{50} - cLogP), the most efficient linker length was n = 5 (compound **1**). A similar trend was also observed for α-ketoheterocycles in which the C-2 acyl chain consisted of either saturated or mono-unsaturated fatty acids (**112**-**123**, Table 4) Compounds bearing C_2-C_6 chains were inactive, but DAGLα inhibition increased upon further elongation of the acyl chain and was found to be optimal with an oleoyl chain ($C_{18:1}$) in compound **121**. Interestingly, compound **122** with an arachidonoyl substituent ($C_{20:4}$) displayed almost 100-fold less activity compared to its oleoyl analogue. This might indicate that the C-2 acyl chain is located in the hydrophobic channel that harbours the *sn*-1 acyl chain of the natural substrate of DAGLα.

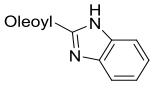
Table 3. Structure-activity relation of C2-acyl derivatives **106-111**.[12, 14]



Entry	n	pIC_{50} ± SEM	cLogP	LipE
106	2	4.74 ± 0.15	2.63	2.11
107	3	5.65 ± 0.10	3.01	2.64
108	4	6.69 ± 0.07	3.54	3.15
1	5	7.43 ± 0.05	4.07	3.35
109	6	6.28 ± 0.10	4.60	1.68
110	7	7.33 ± 0.07	5.13	2.20
111	8	8.44 ± 0.04	5.66	2.78

Table 4. Structure-activity relation of of C2-acyl derivatives **112-123**.[14]



Entry	R	pIC50 ± SEM
112	$(O)C_2H_3$	< 5
113	$(O)C_5H_9$	< 5
114	$(O)C_6H_{11}$	< 5
115	$(O)C_8H_{15}$	6.20 ± 0.03
116	$(O)C_{10}H_{19}$	7.13 ± 0.02
117	$(O)C_{10:1\ \Delta 9}$	6.26 ± 0.04
118	$(O)C_{12}H_{23}$	7.51 ± 0.02
119	$(O)C_{14}H_{27}$	7.13 ± 0.06
120	$(O)C_{16}H_{31}$	7.19 ± 0.04
121	Oleoyl ($C_{18:1}$)	7.58 ± 0.03
122	Arachidonoyl ($C_{20:4}$)	5.62 ± 0.08
123		6.71 ± 0.12

FAAH off-target activity

The compounds of the focused library were originally developed as FAAH inhibitors and therefore, the DAGLα pIC_{50} data of the hits was plotted against previously reported FAAH pK_i values (Figure 4). Most of the hits are dual FAAH/DAGLα inhibitors and display high FAAH activity ($pK_i > 8$). Oleoyl-based benzimidazole (**123**, Table 4) and compound **45** were the only two inhibitors selective over FAAH, but they were only moderately potent in the DAGLα assay ($pIC_{50} < 7$).

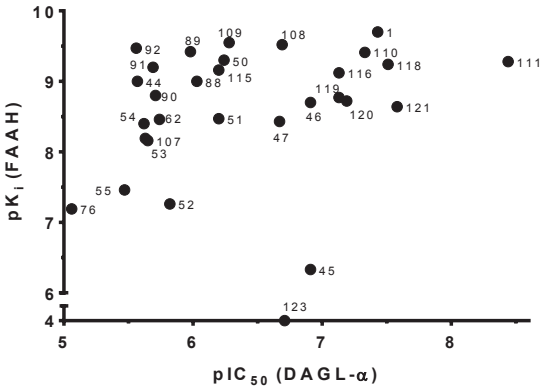


Figure 4. Graphical representation of DAGLα inhibition (pIC_{50}) versus FAAH activity (pK_i). FAAH activity is depicted as reported in literature.[14, 26-33]

Binding mode

Previously, the development of a homology model for DAGLα was reported together with a molecular dynamics simulation with **1** to understand its interaction with hDAGLα at a molecular level (Chapter 2).[12] The model represented the typical α,β-hydrolase fold and the catalytic triad, represented by Ser472, His650, and Asp524, was appropriately aligned in the binding cavity. The tetrahedral transition state of **1**, which is formed through the nucleophilic attack of Ser472 on the α-carbonyl, was minimized and subjected to a short molecular dynamics refinement. The oxyanion intermediate was stabilized by the backbone N–H of Leu473, as well as by the backbone N–H and side chain O–H of Thr400. The oxazole nitrogen of **1** formed hydrogen-bond interactions with His650 and the pyridine nitrogen showed hydrogen-bond interactions with His471 and His650, both of which could further stabilize the tetrahedral intermediate. These proposed hydrogen bonds are in line with the *4N*-oxazolopyridine being the optimal heterocycle. In addition, aliphatic amino acids like Leu427 and Val419 line the hydrophobic pocket accommodating the flexible acyl chain of **1**. This study indicates that this large pocket might normally accommodate *sn*-1 acyl chain of the natural substrate. The proposed binding mode is thus consistent with the observed structure–activity relationships reported in this study.

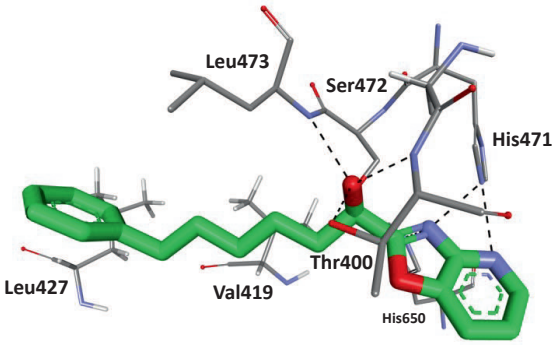


Figure 5. Proposed binding mode of **1** in DAGLα

Conclusions

Screening of an extensive focused library of α-ketoheterocyclic FAAH inhibitors, combined with the synthesis and analysis of novel α-ketoheterocycles, resulted in the rapid generation of a comprehensive and detailed set of structure-activity relationships for DAGLα inhibitors. It was shown that the binding site of DAGLα is remarkably sensitive to the type of α-ketoheterocycle with oxazolo-*4N*-pyridine as the optimal scaffold. The potency of the α-ketoheterocycle is also strongly influenced by a fundamental substituent effect. The electron-withdrawing character of the functional group on the *meta*-position of substituted oxazoles, but not on the *para*-position, increased to large extent inhibitor potency. As previously observed, the C-2 carbonyl (*i.e.* site of reversible covalent attachment) is key to inhibitor activity and its reduction to an hydroxyl group abolished DAGLα inhibition.[12,13] Increasing C-2 acyl chain length enhanced inhibitor activity and was optimal for an oleoyl ($C_{18:1}$) group, while an arachidonoyl ($C_{20:4}$) chain was less preferred. C_6-C_9 acyl chains with a distal phenyl group yielded the most potent inhibitors. These detailed SAR results provided valuable insight in the structural requirements for DAGLα inhibition by α-ketoheterocycles and was fully consistent with the proposed binding pose of **1** in the homology model. The homology mode was successfully applied to guide the design of new DAGLα inhibitors, which led to in the identification of LEI105 as a highly selective, reversible and dual DAGLα/DAGL-β inhibitor that was active in cells and reduced cannabinoid CB_1-receptor dependent synaptic plasticity.[13] Current efforts are directed towards optimizing the physicochemical properties of the α-ketoheterocycles to improve their pharmacokinetic properties. Of note, the reversible character of the α-ketoheterocycle inhibitors may have less probability to induce idiosyncratic toxic side effects, which may be associated with covalent irreversible inhibitors. Consequently, α-ketoheterocycles may provide potential leads for small molecule therapies to treat human conditions like obesity, diabetes, cardiovascular and neurodegenerative diseases where high 2-AG signaling and/or 2-AG metabolite levels play a crucial role.

Experimental

Experimental Procedures Computational Chemistry

The homology model was constructed as previously reported, based on the S146A Mutant Of Thermomyces (Humicola) Lanuginosa Lipase in Complex With Oleic Acid (PDB code 1GT6) as template (Chapter 2).[12]

Experimental Procedures Biochemistry

Cloning Procedures

Cloning procedures were performed as previously reported.[12] In brief, full-length human hDAGL-α cDNA was purchased from Biosource and cloned into mammalian expression vector pcDNA3.1, containing genes for ampicillin and neomycin resistance. The empty vector was used as a negative control (mock). All plasmids were grown in XL-10 Z-competent cells and prepped (Maxi Prep, Qiagen). The sequences were confirmed by sequence analysis at the Leiden Genome Technology Centre.

Cell Culture and Membrane Preparation

Cell culture and membrane preperations were performed as previously reported.[12] In brief, HEK293T cells were grown in DMEM with stable glutamine and phenol red (PAA) with 10% new born calf serum, penicillin, and streptomycin. Cells were passaged every 2−3 days by resuspension in medium and seeding to the appropriate confluence. Membranes were prepared from transiently transfected HEK293T cells. 24 Hours prior to transfection, 10^7 cells were seeded in a 15 cm Petri dish. Cells were transfected by the addition of a 3:1 mixture of polyethyleneimine (60 μg) and plasmid DNA (20 μg) in 2 mL of serum free medium. The medium was refreshed after 24 h, and after 72 h the cells were harvested by suspending them in 20 mL of medium. The supernatant was removed by centrifuge for 10 min at 1000 rpm. The cell pellet was flash frozen in liquid nitrogen and stored at −80 °C until use. Cell pellets were thawed on ice and suspended in lysis buffer A (20 mM HEPES, pH 7.2, 2 mM DTT, 0.25 M sucrose, 1 mM $MgCl_2$, 1× cocktail (Roche cOmplete EDTA free), 25 U/mL Benzonase). The suspension was homogenized by polytrone (3 × 7 s) and incubated for 30 min on ice. The membrane fraction was separated by ultracentrifuge (100.000g, 30 min, 4 °C, Beckman Coulter, type Ti70 rotor) and the pellet was resuspended in lysis buffer B (20 mM HEPES, pH 7.2, 2 mM DTT, 1× cocktail (Roche cOmplete EDTA free)). The protein concentration was determined with Qubit protein assay (Invitrogen). The total protein membrane was diluted to 1.0 mg/mL and the samples were flash frozen in liquid nitrogen and stored in small aliquots at −80 °C until use.

Biochemical hDAGL-α Activity Assay

The biochemical hDAGL-α activity assay was performed as previously reported.[12] In brief, the biochemical hDAGL-α activity assay is based on the hydrolysis of *para*-nitrophenylbutyrate (PNP-butyrate) by membrane preparations from HEK293T cells transiently transfected with hDAGL-α. Reactions (200 μL) were performed in a clear flat bottom Greiner 96-well plates, 50 mM HEPES pH 7.2 buffer with 0.05 μg/μL (final protein concentration) hDAGL-α transfected membrane fractions.

The focused library hit identification was performed using the 96-well plate protocol. Compound plates (13 plates, N = 2) were screened over a total of 4 days. A total of 68 actives were identified (< 50% activity at 10 μM inhibitor concentration, 6.54%).

Focused Library Dose Response Analysis

Dose response analysis was performed on the 64 hits of the hit identification screen. The hits were analysed (ten-fold serial dilution) using the above protocol[12] with minor adjustments for high throughput; 384-well plate, 50 µL total volume, OD_{405} was measured after 60 minutes incubation with PNP-butyrate (final concentration 0.3 mM) on an Envision plate reader.

Experimental Procedures Chemistry

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 using the reported deuterated solvent. 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 77.16 for ^{13}C; CD_3OD: δ 3.31 for 1H, δ 49.00 for ^{13}C). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, td = triple doublet, t = triplet, q = quartet, quintet = quint, b = broad, m = multiplet), coupling constants J (*Hz*), and integration. High resolution mass spectra were recorded on a Thermo Scientific LTQ Orbitrap XL. Compound purity (>95% unless stated otherwise) was measured by liquid chromatography on a Finnigan Surveyor LC-MS system, equipped with a C18 column. 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), and H_2O (600 mL)).

2-Hydroxy-7-phenylheptanenitrile (124)

The title compound was synthesized from commercially available 6-phenylhexan-1-ol (1.70 g, 9.51 mmol) to yield 2-hydroxy-7-phenylheptanenitrile (1.67 g, 8.22 mmol, 86% over 2 steps) using previously reported procedures. Spectroscopic data are in agreement with those previously reported.[12]

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (125)

To a dry round bottom flask containing a solution of dry EtOH (1.4 mL, 17 mmol) and dry $CHCl_3$ (1.4 mL) was added dropwise AcCl (1.4 mL, 19 mmol) at 0°C under argon. The reaction mixture was stirred for 30 minutes after which a solution of 2-hydroxy-7-phenylheptanenitrile (**124**, 117 mg, 0.58 mmol) in dried $CHCl_3$ (1.0 mL) was added dropwise at 0°C under argon. The reaction mixture was stirred for 2 h, slowly warmed up to rt and concentrated at 25°C *in vacuo*. The crude mixture was coevaporated with toluene (3x5 mL) until the white solid imidate was obtained. The solid was dissolved in dry EtOH (1.0 mL) and was added under argon to a sealed and dried microwave tube containing a prestirred solution (80˚C for 30 minutes, then to rt) of commercially available 2-amino-3-hydroxypyridine (68.8 mg, 0.63 mmol) with pyridine (50 µL, 0.63 mmol) in dry EtOH (4.0 mL). The reaction mixture was heated to reflux (80˚C) for 8 h. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography to yield 1-(oxazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (34 mg, 0.58 mmol, 20%). Spectroscopic data are in agreement with those previously reported.[12]

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-one (1)

The title compound was synthesized from 1-(oxazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (**125**) as previously reported.[12]

1-(Benzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (2)

The title compound was synthesized from 1-(benzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol as previously reported.[12]

1-(1*H*-Benzo[*d*]imidazol-2-yl)-6-phenylhexan-1-ol (126)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 100 mg, 0.49 mmol) and commercially available benzene-1,2-diamine (55 mg, 0.51 mmol) according to the procedure described for compound **125**. This yielded 1-(1*H*-benzo[d]imidazol-2-yl)-6-phenylhexan-1-ol (136 mg, 0.46 mmol, 94%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.47 (dd, *J* = 7.0, 3.4 Hz, 2H), 7.25 – 7.11 (m, 3H), 7.06 (d, *J* = 7.4 Hz, 2H), 6.77 – 6.65 (m, 2H), 5.29 (bs, 2H), 4.92 (d, *J* = 6.7 Hz, 2H), 2.43 (t, *J* = 7.7 Hz, 2H), 1.94 – 1.56 (m, 4H), 1.55 – 1.04 (m, 4H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 157.55, 142.67, 137.57, 134.67, 128.44(2C), 128.31(2C), 125.69, 122.81, 120.49, 116.92, 115.00, 68.43, 36.90, 35.86, 31.31, 29.04, 25.23.

1-(1*H*-Benzo[*d*]imidazol-2-yl)-6-phenylhexan-1-one (3)

The title compound was synthesized from 1-(1*H*-benzo[*d*]imidazol-2-yl)-6-phenylhexan-1-ol (**126**, 65 mg, 0.22 mmol) according to the procedure described for compound **1**. This yielded 1-(1*H*-benzo[*d*]imidazol-2-yl)-6-phenylhexan-1-one (22 mg, 0.075 mmol, 34%). HRMS (ESI+) m/z: calculated for $C_{19}H_{21}N_2O$ ([M + H]), 293.1648; found, 293.1648. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.75 (bs, 1H), 7.91 (bs, 1H), 7.55 (bs, 1H), 7.40 (bs, 2H), 7.29 – 7.23 (m, 2H), 7.21 – 7.13 (m, 3H), 3.31 (t, *J* = 7.5 Hz, 2H), 2.62 (t, *J* = 7.7 Hz, 2H), 1.85 (p, *J* = 7.5 Hz, 2H), 1.70 (p, *J* = 7.7 Hz, 2H), 1.55 – 1.41 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 194.82, 147.60, 142.86 (bs), 142.52, 134.18 (bs), 128.40(2C), 128.27(2C), 125.66, 124.83 (bs, 2C), 121.42 (bs), 112.62 (bs), 38.36, 35.77, 31.30, 28.84, 23.82. Purity of >95% as determined by LC-MS.

1-(Benzo[*d*]thiazol-2-yl)-6-phenylhexan-1-ol (127)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 50 mg, 0.25 mmol) and commercially available 2-aminobenzenethiol (0.03 mL, 0.28 mmol) according to the procedure described for compound **125**. This yielded 1-(benzo[*d*]thiazol-2-yl)-6-phenylhexan-1-ol (63 mg, 0.20 mmol, 83%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97 (d, *J* = 8.2 Hz, 1H), 7.87 (d, *J* = 8.2, 1H), 7.51 – 7.41 (m, 1H), 7.41 – 7.32 (m, 1H), 7.31 – 7.21 (m, 2H), 7.21 – 7.10 (m, 4H), 5.08 (t, *J* = 7.9, 1H), 2.59 (t, *J* = 7.8, 2H), 2.11 – 1.26 (m, 8H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 152.76, 142.61, 134.81, 130.91, 128.41(2C), 128.26(2C), 126.11, 125.64, 125.04, 122.86, 121.86, 72.29, 38.05, 35.83, 31.30, 29.01, 24.98.

1-(Benzo[*d*]thiazol-2-yl)-6-phenylhexan-1-one (4)

The title compound was synthesized from 1-(benzo[*d*]thiazol-2-yl)-6-phenylhexan-1-ol (**127**, 50 mg, 0.16 mmol) according to the procedure described for compound **1**. This yielded 1-(1*H*-imidazo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (16 mg, 0.05 mmol, 32%). HRMS (ESI+) m/z: calculated for $C_{19}H_{20}NOS$ ([M + H]), 310.1260; found, 310.1260. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 – 8.13 (m, 1H), 8.01 – 7.93 (m, 1H), 7.61 – 7.48 (m, 2H), 7.31 – 7.23 (m, 2H), 7.21 – 7.13 (m, 3H), 3.27 (t, *J* = 7.4 Hz, 2H), 2.63 (t, *J* = 7.8 Hz, 2H), 1.84 (p, *J* = 7.8 Hz, 2H), 1.70 (p, *J* = 7.8 Hz, 2H), 1.54 – 1.42 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 195.65, 166.67, 153.69, 142.65, 137.38, 128.53(2C), 128.39(2C), 127.73, 127.07, 125.78, 125.51, 122.57, 38.65, 35.88, 31.37, 28.96, 23.94. Purity of >95% as determined by LC-MS.

1-(1*H*-Imidazo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (128)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 254 mg, 1.25 mmol) and commercially available pyridine-2,3-diamine (54 mg, 0.40 mmol) according to the procedure described for compound **125**. This yielded 1-(1*H*-imidazo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (24 mg, 0.08 mmol, 16%). ^{1}H NMR (MeOD, 400 MHz): δ 8.33 (d, *J* = 4.7 Hz, 1H), 7.95 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.28 (dd, J = 8.0, 4.9 Hz, 1H), 7.23 – 7.17 (m, 2H), 7.16 – 7.05 (m, 3H), 4.98 – 4.91 (m, 1H), 2.57 (t, *J* = 8.0 Hz, 2H), 2.06 – 1.82 (m, 2H), 1.61

(p, *J* = 7.4 Hz, 2H), 1.51 – 1.42 (m, 2H), 1.41 – 1.31 (m, 2H). ^{13}C APT NMR (MeOD, 101 MHz): δ 162.22, 153.23 (bs), 144.53, 143.79, 131.27 (bs), 129.36(2C), 129.22(2C), 126.60, 124.00 (bs), 119.27, 69.40, 37.70, 36.76, 32.58, 29.99, 25.97.

1-(1*H*-Imidazo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-one (5)

The title compound was synthesized from 1-(1*H*-imidazo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (**128**, 23.7 mg, 0.08 mmol) according to the procedure described for compound **1**. This yielded 1-(1*H*-imidazo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (10 mg, 0.034 mmol, 43%). HRMS (ESI+) m/z: calculated for $C_{18}H_{20}N_3O$ ([M + H]), 294.1601; found, 294.1600. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 14.92 (bs, 1H), 8.91 (d, *J* = 4.3 Hz, 1H), 8.30 (d, *J* = 8.1 Hz, 1H), 7.43 (dd, *J* = 8.2, 4.7 Hz, 1H), 7.29 – 7.24 (m, 2H), 7.21 – 7.14 (m, 3H), 3.32 (t, *J* = 8.0, 7.0 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 1.89 (p, *J* = 7.5 Hz, 2H), 1.78 – 1.65 (m, 2H), 1.57 – 1.45 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 194.41, 149.12, 147.18, 142.52, 136.08, 136.10, 130.78, 128.42(2C), 128.27(2C), 125.67, 119.55, 38.17, 35.78, 31.28, 28.87, 23.75. Purity of >95% as determined by LC-MS.

2-Aminopyridine-3-thiol (129)

Commercially available 3-(*tert*-butylthio)pyridin-2-amine (235.4 mg, 1.291 mmol) was refluxed in 37% aq. HCl (5 mL, 60.0 mmol) for 14 h until completion. The mixture was concentrated *in vacuo* and coevaporated with toluene (3 x 20 mL). The resulting solid was taken up in sat. $NaHCO_3$ (40 mL), extracted with EtOAc (3 x 20 mL), washed with brine, dried and concentrated *in vacuo* to obtain 2-aminopyridine-3-thiol (155 mg, 1.228 mmol, 95 % yield) without further purification. ^{1}H NMR (MeOD, 400 MHz): δ 7.95 (dd, *J* = 5.0, 1.8 Hz, 1H), 7.32 (dd, *J* = 7.4, 1.8 Hz, 1H), 6.51 (dd, *J* = 7.5, 5.0 Hz, 1H). ^{13}C BBDEC NMR (MeOD, 101 MHz): 161.06, 150.74, 146.34, 114.42, 114.36.

1-(Thiazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (130)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 52.3 mg 0.257 mmol) and 2-aminopyridine-3-thiol (**129**, 41.9 mg, 0.332 mmol) according to the procedure described for compound **125**. This yielded 1-(thiazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (15.4 mg, 0.049 mmol, 19%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.68 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.22 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.30 (dd, *J* = 8.0, 4.7 Hz, 1H), 7.28 – 7.22 (m, 2H), 7.19 – 7.13 (m, 3H), 5.19 (dd, *J* = 8.0, 4.4 Hz, 1H), 3.97 (bs, 1H), 2.58 (t, *J* = 7.6 Hz, 2H), 2.12 – 1.86 (m, 2H), 1.69 – 1.48 (m, 4H), 1.46 – 1.35 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 181.08, 163.86, 148.00, 142.72, 131.12, 128.65, 128.51(2C), 128.36(2C), 125.74, 119.85, 72.49, 37.88, 35.95, 31.40, 29.12, 24.99.

1-(Thiazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-one (6)

The title compound was synthesized from 1-(thiazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (**130**, 15.4 mg, 0.049 mmol) according to the procedure described for compound **1**. This yielded 1-(thiazolo[4,5-*b*]pyridin-2-yl)-6-phenylhexan-1-one (10.9 mg, 0.035 mmol, 71%). HRMS (ESI+) m/z: calculated for $C_{18}H_{19}N_2OS$ ([M + H]), 311.1213; found, 311.1213. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.89 (dd, *J* = 4.5, 1.7 Hz, 1H), 8.38 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.47 (dd, *J* = 8.2, 4.6 Hz, 1H), 7.31 – 7.23 (m, 2H), 7.18 (d, *J* = 7.3 Hz, 3H), 3.35 (t, *J* = 7.4 Hz, 2H), 2.63 (t, *J* = 7.8 Hz, 2H), 1.86 (p, *J* = 7.5 Hz, 2H), 1.75 – 1.64 (m, 2H), 1.54 – 1.43 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 195.55, 169.14, 163.78, 149.94, 142.60, 131.94, 131.48, 128.53(2C), 128.40(2C), 125.79, 122.10, 38.89, 35.87, 31.32, 28.96, 23.96. Purity of >95% as determined by LC-MS.

3-Amino-4-hydroxypyridine (131)

To a solution of commercially available 4-hydroxy-3-nitropyridine (500 mg, 3.58 mmol) in methanol (25 mL) was added 100 mg of 10% Pd/C. The reaction mixture was stirred under hydrogen atmosphere for 10 h. Upon completion the solution was filtered and concentrated *in vacuo* to obtain 3-amino-4-hydroxypyridine (350 mg,

3.18 mmol, 89%). ^{1}H NMR (DMSO-d6, 400 MHz): δ 7.34 (dd, *J* = 6.7, 1.6 Hz, 1H), 7.12 (s, 1H), 5.99 (d, *J* = 6.6 Hz, 1H), 4.52 (s, 2H).

1-(Oxazolo[4,5-*c*]pyridin-2-yl)-6-phenylhexan-1-ol (132)

To a dry round bottom flask containing a solution of dry EtOH (1.0 mL, 17 mmol) and dry $CHCl_3$ (1.0 mL) was added dropwise AcCl (1.0 mL, 14 mmol) at 0°C under argon. The reaction mixture was stirred for 30 minutes after which a solution of 2-hydroxy-7-phenylheptanenitrile (**124**, 213 mg, 1.05 mmol) in dry $CHCl_3$ (1.0 mL) was added dropwise at 0°C under argon. The reaction mixture was stirred for 2 h, slowly warmed up to rt and concentrated at 25°C *in vacuo*. The imidate was dissolved in dry EtOH (1.0 mL) and was added under argon to a sealed microwave tube containing 3-amino-4-hydroxypyridine (**131**, 121 mg, 1.1 mmol) in dry EtOH (4.0 mL). The reaction mixture was heated to reflux (80˚C) for 8 h using microwave irradiation. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography to yield 1-(oxazolo[4,5-*c*]pyridin-2-yl)-6-phenylhexan-1-ol (16 mg, 0.054 mmol, 5%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.03 (s, 1H), 8.56 (dd, *J* = 5.6, 1.4 Hz, 1H), 7.50 (dt, *J* = 5.6, 1.0 Hz, 1H), 7.28-7.24 (m, 2H), 7.21 – 7.10 (m, 3H), 5.00 (dd, *J* = 7.6, 5.3 Hz, 1H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.13 – 1.93 (m, 2H), 1.64 (p, 2H), 1.58 – 1.47 (m, 2H), 1.46 – 1.39 (m, 2H).^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 168.81, 156.07, 145.56, 142.85, 142.59, 138.46, 128.49(2C), 128.38(2C), 125.80, 106.91, 68.04, 35.91, 35.52, 31.33, 28.97, 24.92.

1-(Oxazolo[4,5-*c*]pyridin-2-yl)-6-phenylhexan-1-one (7)

To a solution of 1-(oxazolo[4,5-*c*]pyridin-2-yl)-6-phenylhexan-1-ol (**132**, 15 mg, 0.050 mmol) in dry CH_2Cl_2 (3 mL) was added Dess-Martin periodinane (43 mg, 0.1 mmol). The reaction mixture was stirred for 10 h and quenched with 5 mL sat. $NaHCO_3$ (aq.) upon completion. The organic layer was washed with sat. $NaHCO_3$ (aq.), brine, dried on $MgSO_4$, filtered, concentrated *in vacuo* and purified by flash chromatography to obtain 1-(oxazolo[4,5-*c*]pyridin-2-yl)-6-phenylhexan-1-one (9.7 mg, 0.033 mmol, 66%). HRMS (ESI+) m/z: calculated for $C_{18}H_{19}N_2O_2$ (M + H^+) 295.1441; found 295.1440. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.26 (s, 1H), 8.77 – 8.70 (d, *J* = 3.6 Hz, 1H), 7.64 (d, *J* = 5.6 Hz, 1H), 7.29-7.25 (m, 2H), 7.19 – 7.15 (m, 3H), 3.23 (t, *J* = 7.4 Hz, 2H), 2.64 (t, *J* = 7.7 Hz, 2H), 1.87 (p, *J* = 7.5 Hz, 2H), 1.69 (p, *J* = 7.7 Hz, 2H), 1.53 – 1.42 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 189.78, 157.42, 155.66, 148.03, 145.64, 142.46, 128.51(2C), 128.42(2C), 125.85, 107.73, 39.77, 35.82, 31.26, 28.78, 23.71. Purity of 90% as determined by LC-MS.

1-(Oxazolo[5,4-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (133)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 59 mg, 0.29 mmol) and commercially available 3-amino-2-hydroxypyridine (35 mg, 0.31 mmol) according to the procedure described for compound **132**. This yielded 1-(oxazolo[5,4-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (5 mg, 0.018 mmol, 6%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.36 (dd, *J* = 5.0, 1.6 Hz, 1H), 8.03 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.35 (dd, *J* = 7.8, 5.0 Hz, 1H), 7.30 – 7.27 (m, 2H), 7.20 – 7.14 (m, 3H), 5.01 – 4.95 (m, 1H), 2.60 (t, *J* = 7.6 Hz, 2H), 2.14 – 1.90 (m, 2H), 1.82 – 1.73 (m, 2H), 1.60 – 1.50 (m, 4H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 168.19, 159.96, 144.98, 142.65, 132.46, 128.82(2C), 128.51(2C), 128.39, 125.78, 121.16, 68.30, 35.93, 35.45, 31.37, 29.02, 24.85.

1-(Oxazolo[5,4-*b*]pyridin-2-yl)-6-phenylhexan-1-one (8)

The title compound was synthesized from 1-(oxazolo[5,4-*b*]pyridin-2-yl)-6-phenylhexan-1-ol (**133**, 5 mg, 0.018 mmol) according to the procedure described for compound **7**. This yielded 1-(oxazolo[5,4-*b*]pyridin-2-yl)-6-phenylhexan-1-one (3 mg, 0.010 mmol, 57%). HRMS (ESI+) m/z: calculated for $C_{18}H_{19}N_2O_2$ (M + H^+) 295.1441; found 295.1441. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.24 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.48 (dd, *J* = 8.0, 4.9 Hz, 1H), 7.30 – 7.24 (m, 2H), 7.19 – 7.16 (m, 3H), 3.21 (t, *J* = 7.4 Hz, 2H), 2.64 (t, *J* = 7.6 Hz, 2H), 1.85 (p, *J* = 7.5 Hz, 2H), 1.75 – 1.66 (m, 2H), 1.50 – 1.44 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 189.73, 156.64, 148.66, 142.39, 132.41, 131.24, 128.40(2C), 128.30(2C), 125.72, 122.15, 39.40, 35.71, 31.15, 28.68, 23.56. Purity of >95% as determined by LC-MS.

1-(4-Fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (134)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 100 mg, 0.49 mmol) and commercially available 2-amino-3-fluorophenol (62 mg, 0.49 mmol) according to the procedure described for compound **125**. This yielded 1-(4-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (99 mg, 0.32 mmol, 65%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34 – 7.29 (m, 1H), 7.29 – 7.23 (m, 3H), 7.19 – 7.13 (m, 3H), 7.05 (ddd, *J* = 9.3, 7.8, 1.3 Hz, 1H), 4.97 (dd, *J* = 7.7, 5.2 Hz, 1H), 2.60 (t, *J* = 7.6 Hz, 2H), 2.09 – 1.91 (m, 2H), 1.68 – 1.60 (m, 2H), 1.56 – 1.46 (m, 2H), 1.44 – 1.37 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 168.28, 153.47 (d, *J* = 256.54 Hz), 153.03 (d, *J* = 7.1 Hz), 142.67, 129.23 (d, *J* = 16.2 Hz), 128.49(2C), 128.36(2C), 125.76 (d, *J* = 7.1 Hz), 125.74, 110.96 (d, *J* = 17.6 Hz), 107.07 (d, *J* = 4.5 Hz), 68.09, 35.91, 35.55, 31.36, 28.99, 24.90.

1-(4-Fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (9)

The title compound was synthesized from 1-(4-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (**134**, 63 mg, 0.20 mmol) according to the procedure described for compound **7**. This yielded 1-(4-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (41 mg, 0.13 mmol, 66%). HRMS (ESI+) m/z: calculated for $C_{19}H_{19}FNO_2$ ([M + H]), 312.1394; found, 312.1393. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 – 8.13 (m, 1H), 8.01 – 7.93 (m, 1H), 7.61 – 7.48 (m, 2H), 7.31 – 7.23 (m, 2H), 7.21 – 7.13 (m, 3H), 3.27 (t, *J* = 7.4 Hz, 2H), 2.63 (t, *J* = 7.8 Hz, 2H), 1.84 (p, *J* = 7.8 Hz, 2H), 1.70 (p, *J* = 7.8 Hz, 2H), 1.54 – 1.42 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 190.01, 157.23, 154.85 (d, *J* = 260.6 Hz), 152.67 (d, *J* = 6.1 Hz), 142.52, 129.79 (d, *J* = 20.2 Hz), 129.16 (d, *J* = 7.1 Hz), 128.51(2C), 128.40(2C), 125.81, 111.73 (d, *J* = 17.2 Hz), 108.16 (d, J = 5.1 Hz), 39.56, 35.83, 31.29, 28.78, 23.72. Purity of >95% as determined by LC-MS.

1-(5-Fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (135)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 124 mg, 0.61 mmol) and commercially available 2-amino-4-fluorophenol (72 mg, 0.57 mmol) according to the procedure described for compound **132**. This yielded 1-(5-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (133 mg, 0.42 mmol, 75%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45 (dd, *J* = 8.9, 4.2 Hz, 1H), 7.39 (dd, *J* = 8.3, 2.6 Hz, 1H), 7.30 – 7.22 (m, 2H), 7.20 – 7.12 (m, 3H), 7.08 (td, *J* = 9.1, 2.6 Hz, 1H), 4.94 (dd, *J* = 7.6, 5.1 Hz, 1H), 2.70 (bs, 1H), 2.60 (t, *J* = 7.8 Hz, 2H), 2.12 – 1.87 (m, 2H), 1.70 – 1.57 (m, 2H), 1.56 – 1.45 (m, 2H), 1.45 – 1.33 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 169.65, 160.15 (d, *J* = 241.4 Hz), 147.24 (d, *J* = 1.0 Hz), 142.65, 141.35 (d, *J* = 13.1 Hz), 128.51(2C), 128.39(2C), 125.78, 113.05 (d, *J* = 26.3 Hz), 111.26 (d, *J* = 10.1 Hz), 106.66 (d, *J* = 25.3 Hz), 68.21, 35.93, 35.58, 31.39, 29.01, 24.89.

1-(5-Fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (10)

The title compound was synthesized from 1-(5-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (**135**, 44 mg, 0.14 mmol) according to the procedure described for compound **7**. This yielded 1-(5-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (33 mg, 0.10 mmol, 74%). HRMS (ESI+) m/z: calculated for $C_{19}H_{19}FNO_2$ ([M + H]), 312.1394; found, 312.1395. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.61 (dd, *J* = 9.0, 4.2 Hz, 1H), 7.56 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.32 – 7.24 (m, 2H), 7.21 – 7.13 (m, 3H), 3.20 (t, *J* = 7.4 Hz, 2H), 2.64 (t, *J* = 7.5 Hz, 2H), 1.84 (p, *J* = 7.5 Hz, 2H), 1.75 – 1.63 (m, 2H), 1.52 – 1.41 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 190.10, 160.66 (d, *J* = 244.4 Hz), 158.69, 147.20 (d, *J* = 1.0 Hz), 142.53, 141.35 (d, *J* = 14.1 Hz), 128.53(2C), 128.42(2C), 125.84, 116.97 (d, *J* = 27.3 Hz), 112.71 (d, *J* = 10.1 Hz), 108.28 (d, *J* = 25.3 Hz), 39.63, 35.85, 31.31, 28.82, 23.75. Purity of >95% as determined by LC-MS.

2-Amino-5-fluorophenol (136)

The title compound was synthesized from commercially available 5-fluoro-2-nitrophenol (500 mg, 3.18 mmol) according to the procedure described for compound **131**. This yielded 2-amino-5-fluorophenol (388 mg, 3.05 mmol, 96%). ^{1}H NMR (DMSO-d6, 400 MHz): δ 6.53 (dd, *J* = 8.3, 6.3 Hz, 1H), 6.46 (dd, *J* = 10.3, 2.6 Hz, 1H), 6.35

(td, *J* = 8.7, 2.7 Hz, 1H). ^{13}C APT NMR (DMSO-d6, 101 MHz): δ 154.63 (d, *J* = 230.8 Hz), 144.99, 133.47, 114.32 (d, *J* = 9.3 Hz), 105.16 (d, *J* = 21.3 Hz), 102.28 (d, *J* = 24.9 Hz).

1-(6-Fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (137)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 110 mg, 0.54 mmol) and 2-amino-5-fluorophenol (**136**, 72 mg, 0.57 mmol) according to the procedure described for compound **132**. This yielded 1-(6-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (66 mg, 0.21 mmol, 39%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.62 (dd, *J* = 8.8, 4.8 Hz, 1H), 7.29 – 7.25 (m, 2H), 7.24 – 7.21 (m, 1H), 7.19 – 7.13 (m, 3H), 7.08 (ddd, *J* = 9.5, 8.7, 2.4 Hz, 1H), 4.93 (dd, *J* = 7.6, 5.2 Hz, 1H), 2.59 (t, *J* = 6.4 Hz, 2H), 2.08 – 1.89 (m, 2H), 1.63 (p, *J* = 7.8 Hz, 2H), 1.55 – 1.46 (m, 2H), 1.44 – 1.36 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 168.45 (d, *J* = 4.0 Hz), 160.72 (d, *J* = 244.4 Hz), 150.84 (d, J = 15.2 Hz), 142.65, 136.83 (d, *J* = 1.0 Hz), 128.50(2C), 128.38(2C), 125.78, 120.41 (d, *J* = 10.1 Hz), 112.69 (d, *J* = 24.2 Hz), 99.01 (d, *J* = 28.3 Hz), 68.11, 35.93, 35.52, 31.38, 29.01, 24.92.

1-(6-Fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (11)

The title compound was synthesized from 1-(6-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (**137**, 44 mg, 0.14 mmol) according to the procedure described for compound **7**. This yielded 1-(6-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (37 mg, 0.12 mmol, 85%). HRMS (ESI+) m/z: calculated for $C_{19}H_{19}FNO_2$ ([M + H]), 312.1394; found, 312.1393. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 – 8.13 (m, 1H), 8.01 – 7.93 (m, 1H), 7.61 – 7.48 (m, 2H), 7.31 – 7.23 (m, 2H), 7.21 – 7.13 (m, 3H), 3.27 (t, *J* = 7.4 Hz, 2H), 2.63 (t, *J* = 7.8 Hz, 2H), 1.84 (p, *J* = 7.8 Hz, 2H), 1.70 (p, *J* = 7.8 Hz, 2H), 1.54 – 1.42 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 189.79, 162.71 (d, *J* = 250.5 Hz), 158.04 (d, *J* = 4.0 Hz), 151.03 (d, *J* = 15.2 Hz), 142.50, 136.98 (d, *J* = 1.0 Hz), 128.49(2C), 128.38(2C), 125.80, 123.11 (d, *J* = 10.1 Hz), 114.66 (d, *J* = 25.3 Hz), 99.73 (d, *J* = 27.3 Hz), 39.48, 35.82, 31.25, 28.81, 23.79. Purity of >95% as determined by LC-MS.

1-(7-Fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (138)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 124 mg, 0.61 mmol) and commercially available 2-amino-6-fluorophenol (75 mg, 0.59 mmol) according to the procedure described for compound **125**. This yielded 1-(7-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (157 mg, 0.50 mmol, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.50 (dd, *J* = 8.0, 0.9 Hz, 1H), 7.33 – 7.22 (m, 3H), 7.20 – 7.13 (m, 3H), 7.13 – 7.06 (m, 1H), 4.97 (dd, *J* = 7.7, 5.1 Hz, 1H), 3.06 (bs, 1H), 2.60 (t, *J* = 7.9 Hz, 2H), 2.19 – 1.88 (m, 2H), 1.73 – 1.58 (m, 2H), 1.58 – 1.46 (m, 2H), 1.46 – 1.33 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 168.34, 147.16 (d, *J* = 253.5 Hz), 143.82 (d, *J* = 2.0 Hz), 142.66, 138.05 (d, *J* = 11.1 Hz), 128.51(2C), 128.38(2C), 125.78, 125.15 (d, *J* = 5.0 Hz), 115.93 (d, *J* = 4.0 Hz), 112.17 (d, *J* = 16.2 Hz), 68.13, 35.93, 35.60, 31.38, 29.00, 24.90.

1-(7-Fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (12)

The title compound was synthesized from 1-(7-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (**138**, 46 mg, 0.15 mmol) according to the procedure described for compound **1**. This yielded 1-(7-fluorobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (39 mg, 0.13 mmol, 86%). HRMS (ESI+) m/z: calculated for $C_{19}H_{19}FNO_2$ ([M + H]), 312.1394; found, 312.1395. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.68 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.41 (td, *J* = 8.2, 4.5 Hz, 1H), 7.31 – 7.24 (m, 3H), 7.21 – 7.14 (m, 3H), 3.22 (t, *J* = 7.4 Hz, 2H), 2.64 (t, *J* = 7.9 Hz, 2H), 1.85 (p, *J* = 7.5 Hz, 2H), 1.74 – 1.65 (m, 2H), 1.52 – 1.41 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 189.70, 157.49, 147.54 (d, *J* = 255.5 Hz), 143.65 (d, *J* = 1.0 Hz), 142.53, 138.34 (d, *J* = 11.1 Hz) 128.53(2C), 128.42(2C), 126.26 (d, *J* = 6.1 Hz), 125.84, 118.10 (d, *J* = 4.04 Hz), 114.89 (d, *J* = 16.2 Hz), 39.84, 35.85, 31.31, 28.81, 23.72. Purity of >95% as determined by LC-MS.

1-(5-Nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (139)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 131 mg, 0.64 mmol) and commercially available 2-amino-4-nitrophenol (99 mg, 0.64 mmol) according to the procedure described for compound **125**. This yielded 1-(5-nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (120 mg, 0.35 mmol, 55%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (d, *J* = 2.2 Hz, 1H), 8.30 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.62 (d, *J* = 8.9 Hz, 1H), 7.30 – 7.23 (m, 2H), 7.18 – 7.13 (m, 3H), 5.01 (dd, *J* = 7.7, 5.2 Hz, 1H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.11 – 1.93 (m, 2H), 1.64 (p, *J* = 7.8 Hz, 2H), 1.57 – 1.50 (m, 2H), 1.45 – 1.37 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 171.10, 154.28, 145.41, 142.49, 140.96, 128.42(2C), 128.33(2C), 125.75, 121.43, 116.49, 111.18, 68.06, 35.84, 35.41, 31.27, 28.90, 24.87.

1-(5-Nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (13)

The title compound was synthesized from 1-(5-nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (**139**, 74 mg, 0.22 mmol) according to the procedure described for compound **7**. This yielded 1-(5-nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (55 mg, 0.16 mmol, 74%). HRMS (ESI+) m/z: calculated for $C_{38}H_{37}N_4O_8$ ([2M + H]), 677.2606; found, 677.2605. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.79 (d, *J* = 2.2 Hz, 1H), 8.47 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.29 – 7.24 (m, 2H), 7.21 – 7.15 (m, 3H), 3.24 (t, *J* = 7.4 Hz, 2H), 2.64 (t, *J* = 7.2 Hz, 2H), 1.86 (p, *J* = 7.5 Hz, 2H), 1.76 – 1.66 (m, 2H), 1.53 – 1.43 (m, 2H) ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 189.60, 159.38, 153.98, 146.13, 142.38, 140.83, 128.45(2C), 128.36(2C), 125.80, 124.05, 118.84, 112.55, 39.74, 35.76, 31.19, 28.71, 23.61. Purity of >95% as determined by LC-MS.

1-(6-Nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (140)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 100 mg, 0.49 mmol) and commercially available 2-amino-5-nitrophenol (76 mg, 0.49 mmol) according to the procedure described for compound **125**. This yielded 1-(6-nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (59 mg, 0.17 mmol, 35%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.42 (d, *J* = 2.1 Hz, 1H), 8.30 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.28 – 7.22 (m, 2H), 7.18 – 7.12 (m, 3H), 5.01 (dd, *J* = 7.7, 5.1 Hz, 1H), 2.60 (t, *J* = 7.6 Hz, 2H), 2.12 – 1.93 (m, 2H), 1.64 (p, *J* = 7.8 Hz, 2H), 1.57 – 1.49 (m, 2H), 1.45 – 1.38 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 172.53, 150.10, 145.85, 145.50, 142.54, 128.48(2C), 128.40(2C), 125.82, 120.85, 120.21, 107.65, 68.25, 35.90, 35.55, 31.32, 28.94, 24.87.

1-(6-Nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (14)

The title compound was synthesized from 1-(6-nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (**140**, 40 mg, 0.12 mmol) according to the procedure described for compound **7**. This yielded 1-(6-nitrobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (35 mg, 0.10 mmol, 86%). HRMS (ESI+) m/z: calculated for $C_{38}H_{37}N_4O_8$ ([2M + H]), 677.2606; found, 677.2605. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.55 (s, 1H), 8.39 (d, *J* = 8.9 Hz, 1H), 8.02 (d, *J* = 8.9 Hz, 1H), 7.30 – 7.25 (m, 2H), 7.21 – 7.15 (m, 3H), 3.23 (t, *J* = 7.3 Hz, 2H), 2.64 (t, *J* = 7.7 Hz, 2H), 1.86 (p, *J* = 7.5 Hz, 2H), 1.71 (p, *J* = 7.6 Hz, 2H), 1.53 – 1.43 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 189.50, 160.31, 149.92, 147.49, 145.34, 142.38, 128.45(2C), 128.37(2C), 125.82, 122.69, 121.43, 108.76, 39.83, 35.76, 31.17, 28.72, 23.59. Purity of >95% as determined by LC-MS.

1-(6-Bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (141)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 124 mg, 0.61 mmol) and commercially available 2-amino-5-bromophenol (93 mg, 0.50 mmol) according to the procedure described for compound **132**. This yielded 1-(6-bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (116 mg, 0.31 mmol, 62%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84 (d, *J* = 1.9 Hz, 1H), 7.46 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.40 (d, *J* = 8.6 Hz, 1H), 7.30 – 7.22 (m, 2H), 7.21 – 7.11 (m, 3H), 4.94 (dd, *J* = 7.6, 5.1 Hz, 1H), 2.60 (t, *J* = 7.8 Hz, 2H), 2.60 (bs, 1H), 2.16 – 1.85 (m, 2H), 1.63 (p, *J* = 7.6 Hz, 2H), 1.57 – 1.45 (m, 2H), 1.45 – 1.34 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ

169.03, 149.94, 142.64, 142.18, 128.51(2C), 128.41, 128.39(2C), 125.79, 123.21, 117.44, 112.18, 68.16, 35.93, 35.57, 31.38, 29.00, 24.88.

1-(6-Bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (15)

The title compound was synthesized from 1-(6-bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (**141**, 43 mg, 0.11 mmol) according to the procedure described for compound **7**. This yielded 1-(6-bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (38 mg, 0.10 mmol, 89%). HRMS (ESI+) m/z: calculated for $C_{19}H_{19}BrNO_2$ ([M + H]), 372.0594 and 374.0573; found, 372.0596 and 374.0575. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.03 (d, *J* = 1.9 Hz, 1H), 7.64 (dd, *J* = 8.8, 1.8 Hz, 1H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.31 – 7.23 (m, 2H), 7.21 – 7.14 (m, 3H), 3.20 (t, *J* = 7.4 Hz, 2H), 2.64 (t, *J* = 7.7 Hz, 2H), 1.84 (p, *J* = 7.5 Hz, 2H), 1.75 – 1.63 (m, 2H), 1.52 – 1.41 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 190.09, 158.00, 149.77, 142.52, 142.13, 131.77, 128.53(2C), 128.42(2C), 125.85, 125.23, 118.67, 113.38, 39.68, 35.85, 31.30, 28.81, 23.74. Purity of >95% as determined by LC-MS.

2-Amino-6-bromophenol (142)

To a solution of commercially available 2-bromo-6-nitrophenol (200 mg, 0.92 mmol) in EtOH (3 mL) under argon atmosphere was added $SnCl_2$ (870 mg, 4.59 mmol) and the reaction mixture was heated to 70°C. After full conversion (5 minutes) the reaction mixture was cooled to rt and poured into ice water (20 mL). The pH was set to 10 (3M NaOH) and the mixture was stirred for thirty minutes. The water layer was extracted with 3× 30 mL EtOAc. The organic layer was washed with 50 mL brine, treated with charcoal and filtered, dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield 2-amino-6-bromophenol (42 mg, 0.22 mmol, 25%). ^{1}H NMR (MeOD, 400 MHz): δ 7.07 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.93 (dd, *J* = 7.9, 1.4 Hz, 1H), 6.67 (t, *J* = 8.0 Hz, 1H). ^{13}C APT NMR (MeOD, 101 MHz): δ 145.56, 132.00, 127.50, 122.52, 119.45, 111.94.

1-(7-Bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (143)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**124**, 98 mg, 0.49 mmol) and 2-amino-6-bromophenol (**142**, 92 mg, 0.49 mmol) according to the procedure described for compound **125**. This yielded 1-(7-bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (132 mg, 0.36 mmol, 73%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.61 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.46 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.29 – 7.22 (m, 2H), 7.22 – 7.11 (m, 4H), 4.97 (dd, *J* = 7.8, 5.1 Hz, 1H), 2.58 (t, *J* = 7.6 Hz, 2H), 2.11 – 1.91 (m, 2H), 1.67 – 1.58 (m, 2H), 1.56 – 1.47 (m, 2H), 1.43 – 1.37 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 168.30, 148.97, 142.60, 141.12, 128.44(2C), 128.31(2C), 125.80, 119.09, 102.74, 67.99, 35.86, 35.49, 31.31, 28.93, 24.95.

1-(7-Bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (16)

The title compound was synthesized from 1-(7-bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-ol (**143**, 37 mg, 0.10 mmol) according to the procedure described for compound **1**. This yielded 1-(7-bromobenzo[*d*]oxazol-2-yl)-6-phenylhexan-1-one (41 mg, 0.13 mmol, 66%). HRMS (ESI+) m/z: calculated for $C_{19}H_{19}BrNO_2$ ([M + H]), 372.0594 and 374.0573; found, 372.0596 and 374.0574. ^{1}H NMR (CDCl3, 400 MHz): δ 7.83 (dd, J = 8.1, 1.0 Hz, 1H), 7.68 (dd, J = 8.0, 1.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.30 – 7.25 (m, 2H), 7.20 – 7.16 (m, 3H), 3.20 (t, J = 7.4 Hz, 2H), 2.63 (t, J = 7.6 Hz, 2H), 1.85 (p, *J* = 7.5 Hz, 2H), 1.74 – 1.65 (m, 2H), 1.52 – 1.43 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 189.67, 157.18, 149.16, 142.54, 141.24, 131.57, 128.53(2C), 128.42(2C), 126.96, 125.84, 121.37, 103.90, 39.77, 35.85, 31.30, 28.82, 23.80. Purity of 95% as determined by LC-MS.

2-Hydroxy-4-phenylbutanenitrile (144)

The title compound was synthesized from commercially available 3-phenylpropanal (1.00 g 7.45 mmol) according to the previously reported procedure.[12] This yielded 2-hydroxy-4-phenylbutanenitrile (810 mg, 5.02 mmol, 68%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38 – 7.33 (m, 2H), 7.30 – 7.23 (m, 3H), 4.40 (t, *J* = 6.8 Hz, 1H), 4.19

(bs, 1H), 2.83 (t, *J* = 8.0 Hz, 2H), 2.18 – 2.07 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 139.56, 128.54(2C), 128.40(2C), 126.35, 119.96, 60.02, 36.28, 30.46.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-3-phenylpropan-1-ol (145)

The title compound was synthesized from 2-hydroxy-4-phenylbutanenitrile (**144**, 190 mg, 1.18 mmol) and commercially available 2-amino-3-hydroxypyridine (142 mg, 1.29 mmol) according to the procedure described for compound **125**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-3-phenylpropan-1-ol (44 mg, 0.17 mmol, 15%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.52 (dd, *J* = 4.9, 1.4 Hz, 1H), 7.78 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.30 – 7.25 (m, 2H), 7.24 – 7.19 (m, 3H), 7.19 – 7.13 (m, 1H), 5.06 (dd, *J* = 7.8, 5.0 Hz, 1H), 4.31 (bs, 1H), 2.92 – 2.79 (m, 2H), 2.47 – 2.26 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 171.12, 154.90, 146.51, 143.15, 140.90, 128.68(2C), 128.53(2C), 126.16, 120.40, 118.83, 67.33, 36.91, 31.17.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-3-phenylpropan-1-one (106)

The title compound was synthesized from 1-(oxazolo[4,5-*b*]pyridin-2-yl)-3-phenylpropan-1-ol (**145**, 43.8 mg, 0.17 mmol) according to the procedure described for compound **1**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-3-phenylpropan-1-one (40 mg, 0.16 mmol, 93%). HRMS (ESI+) m/z: calculated for $C_{15}H_{13}N_2O_2$ ([M + H]), 253.0972; found, 253.0970. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.76 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.00 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.50 (dd, *J* = 8.3, 4.7 Hz, 1H), 7.34 – 7.27 (m, 4H), 7.25 – 7.16 (m, 1H), 3.63 (t, *J* = 7.7 Hz, 2H), 3.16 (t, *J* = 7.6 Hz, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 189.38, 158.53, 154.20, 148.90, 143.77, 140.10, 128.72(2C), 128.58(2C), 126.53, 123.36, 120.44, 41.43, 29.70. Purity of >95% as determined by LC-MS.

2-Hydroxy-5-phenylpentanenitrile (146)

The title compound was synthesized commercially available 4-phenylbutan-1-ol (500 mg, 3.33 mmol) according to the previously reported 2 step procedure.[12] This yielded 2-hydroxy-5-phenylpentanenitrile (439 mg, 2.51 mmol, 75% over 2 steps). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.32 – 7.25 (m, 2H), 7.22 – 7.14 (m, 3H), 4.46 – 4.38 (m, 1H), 3.10 (bs, 1H), 2.69 – 2.65 (m, 2H), 1.90 – 1.74 (m, 4H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 141.24, 128.59(2C), 128.49(2C), 126.23, 120.04, 61.16, 35.10, 34.61, 26.27.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-4-phenylbutan-1-ol (147)

The title compound was synthesized from 2-hydroxy-5-phenylpentanenitrile (**146**, 206 mg, 1.18 mmol) and commercially available 2-amino-3-hydroxypyridine (129 mg, 1.17 mmol) according to the procedure described for compound **125**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-4-phenylbutan-1-ol (56 mg, 0.21 mmol, 18%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.48 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.77 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.27 – 7.21 (m, 3H), 7.19 – 7.12 (m, 3H), 5.08 (dd, *J* = 7.5, 5.5 Hz, 1H), 4.62 (bs, 1H), 2.67 (t, *J* = 7.6 Hz, 2H), 2.12 – 1.98 (m, 2H), 1.94 – 1.78 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 171.31, 154.87, 146.35, 143.05, 141.86, 128.48(2C), 128.39(2C), 125.90, 120.31, 118.84, 67.91, 35.51, 34.96, 26.80.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-4-phenylbutan-1-one (107)

The title compound was synthesized from 1-(oxazolo[4,5-*b*]pyridin-2-yl)-4-phenylbutan-1-ol (**147**, 52.4 mg, 0.20 mmol) according to the procedure described for compound **1**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-4-phenylbutan-1-one (22 mg, 0.08 mmol, 42%). HRMS (ESI+) m/z: calculated for $C_{16}H_{15}N_2O_2$ ([M + H]), 267.1128; found, 267.1126. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.76 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.99 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.49 (dd, *J* = 8.3, 4.8 Hz, 1H), 7.31 – 7.25 (m, 2H), 7.24 – 7.14 (m, 3H), 3.31 (t, *J* = 7.3 Hz, 2H), 2.76 (t, *J* = 7.6 Hz, 2H), 2.22 – 2.11 (m, 2H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 190.12, 158.58, 154.22, 148.85, 143.72, 141.19, 128.64(2C), 128.58(2C), 123.29, 120.40, 39.25, 35.12, 25.41. Purity of 94% as determined by LC-MS. Spectroscopic data are in agreement with reported literature.[14]

2-Hydroxy-6-phenylhexanenitrile (148)

The title compound was synthesized from commercially available 5-phenylpentanol (513 mg 3.12 mmol) according to the previously reported 2 step procedure.[12] This yielded 2-hydroxy-6-phenylhexanenitrile (318 mg, 1.68 mmol, 54% over 2 steps). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.33-7.19 (m, 5H), 4.43 (bs, 1H), 4.18-4.12 (m, 1H), 2.66 (t, *J* = 7.6 Hz, 2H), 1.86 (q, *J* = 7.2 Hz, 2H), 1.70 (p, *J* = 7.6 Hz, 2H), 1.55 (p, *J* = 7.6 Hz, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 141.96, 128.81(2C), 128.32(2C), 125.79, 120.20, 60.92, 35.56, 34.90, 30.72, 24.21.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-5-phenylpentan-1-ol (149)

The title compound was synthesized from 2-hydroxy-6-phenylhexanenitrile (**148**, 174 mg, 0.92 mmol) and commercially available 2-amino-3-hydroxypyridine (102 mg, 0.93 mmol) according to the procedure described for compound **125**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-5-phenylpentan-1-ol (51 mg, 0.18 mmol, 20%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.49 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.79 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.29 – 7.20 (m, 4H), 7.18 – 7.10 (m, 3H), 5.05 (dd, *J* = 7.4, 5.6 Hz, 1H), 2.60 (t, *J* = 7.6 Hz, 2H), 2.12 – 1.98 (m, 2H), 1.72 – 1.62 (m, 2H), 1.60 – 1.49 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 170.88, 154.90, 146.59, 143.08, 142.26, 128.38(2C), 128.30(2C), 125.74, 120.31, 118.68, 68.14, 35.71, 35.34, 31.12, 24.57.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-5-phenylpentan-1-one (108)

The title compound was synthesized from 1-(oxazolo[4,5-*b*]pyridin-2-yl)-5-phenylpentan-1-ol (**149**, 40 mg, 0.14 mmol) according to the procedure described for compound **1**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-5-phenylpentan-1-one (35 mg, 0.10 mmol, 86%). HRMS (ESI+) m/z: calculated for $C_{17}H_{17}N_2O_2$ ([M + H]), 281.1285; found, 281.1282. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.75 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.00 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.49 (dd, *J* = 8.3, 4.8 Hz, 1H), 7.30 – 7.26 (m, 2H), 7.23 – 7.16 (m, 3H), 3.31 (t, *J* = 7.2 Hz, 2H), 2.69 (t, *J* = 7.5 Hz, 2H), 1.93 – 1.83 (m, 2H), 1.81 – 1.72 (m, 2H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 190.23, 158.61, 154.26, 148.85, 143.73, 142.01, 128.66(2C), 128.44(2C), 125.92, 123.26, 120.35, 39.72, 35.69, 30.87, 23.53. Purity of >95% as determined by LC-MS. Spectroscopic data are in agreement with reported literature.[14]

2-Hydroxy-8-phenyloctanenitrile (150)

The title compound was synthesized from commercially available 7-phenylheptan-1-ol (500 mg, 2.60 mmol) according to the previously reported 2 step procedure.[12] This yielded 2-hydroxy-8-phenyloctanenitrile (430 mg, 1.98 mmol, 76% over 2 steps). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.29 – 7.20 (m, 2H), 7.18 – 7.10 (m, 3H), 4.34 (q, *J* = 6.1 Hz, 1H), 3.86 – 3.78 (bs, 1H), 2.57 (t, *J* = 7.7 Hz, 2H), 1.75 (q, J = 7.2 Hz, 2H), 1.59 (t, J = 7.5 Hz, 2H), 1.49 – 1.38 (m, 2H), 1.37 – 1.27 (m, 4H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 142.51, 128.31(2C), 128.20(2C), 125.58, 120.14, 60.97, 35.76, 34.85, 31.20, 28.88, 28.68, 24.40.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-7-phenylheptan-1-ol (151)

The title compound was synthesized from 2-hydroxy-8-phenyloctanenitrile (**150**, 231 mg, 1.06 mmol) and commercially available 2-amino-3-hydroxypyridine (101 mg, 0.92 mmol) according to the procedure described for compound **125**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-7-phenylheptan-1-ol (38 mg, 0.12 mmol, 13%). ^{1}H NMR ($CDCl_3$, 400 MHz): 8.53 (dd, *J* = 4.9, 1.4 Hz, 1H), 7.81 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.30 – 7.23 (m, 3H), 7.19 – 7.13 (m, 3H), 5.03 (dd, *J* = 7.6, 5.3 Hz, 1H), 3.91 (bs, 1H), 2.58 (t, *J* = 7.8 Hz, 2H), 2.10 – 1.91 (m, 2H), 1.65 – 1.54 (m, 2H), 1.54 – 1.44 (m, 2H), 1.41 – 1.29 (m, 4H). δ ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 171.29, 154.97, 146.54, 143.15, 142.82, 128.48(2C), 128.34(2C), 125.70, 120.37, 118.82, 68.21, 36.01, 35.54, 31.48, 29.27, 29.20, 24.96. Spectroscopic data are in agreement with reported literature.[14]

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-7-phenylheptan-1-one (109)

The title compound was synthesized from 1-(oxazolo[4,5-*b*]pyridin-2-yl)-7-phenylheptan-1-ol (**151**, 38 mg, 0.12 mmol) according to the procedure described for compound **1**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-7-

phenylheptan-1-one (23 mg, 0.07 mmol, 61%). HRMS (ESI+) m/z: calculated for $C_{19}H_{21}N_2O_2$ ([M + H]), 309.1598; found, 309.1596. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.77 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.00 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.50 (dd, *J* = 8.3, 4.8 Hz, 1H), 7.30 – 7.23 (m, 2H), 7.20 – 7.13 (m, 3H), 3.27 (t, *J* = 7.4 Hz, 2H), 2.61 (t, *J* = 7.5 Hz, 2H), 1.83 (p, *J* = 7.4 Hz, 2H), 1.70 – 1.59 (m, 2H), 1.51 – 1.35 (m, 4H). ^{13}C BBDEC NMR ($CDCl_3$, 101 MHz): δ 190.48, 158.68, 154.25, 148.81, 143.77, 142.75, 128.51(2C), 128.37(2C), 125.74, 123.30, 120.47, 39.91, 35.98, 31.38, 29.08, 29.05, 23.86. Purity of 94% as determined by LC-MS. Spectroscopic data are in agreement with reported literature.[14]

2-Hydroxy-9-phenylnonanenitrile (152)

The title compound was synthesized from commercially available 8-phenyloctan-1-ol (1.00 g 4.85 mmol) according to the previously reported 2 step procedure. This yielded 2-hydroxy-9-phenylnonanenitrile (850 mg, 4.11 mmol, 85% over 2 steps). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.32 – 7.23 (m, 2H), 7.22 – 7.13 (m, 3H), 4.46 (t, *J* = 6.7 Hz, 1H), 2.60 (t, *J* = 7.9 Hz, 2H), 2.44 (bs, 1H), 1.90 – 1.77 (m, 2H), 1.67 – 1.55 (m, 2H), 1.54 – 1.43 (m, 2H), 1.39 – 1.28 (m, 6H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 142.86, 128.52(2C), 128.38(2C), 125.74, 120.03, 61.50, 36.03, 35.32, 31.53, 29.31, 29.19, 28.95, 24.60.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-8-phenyloctan-1-ol (153)

The title compound was synthesized from 2-hydroxy-7-phenylheptanenitrile (**152**, 337 mg, 1.46 mmol) and commercially available 2-amino-3-hydroxypyridine (159 mg, 1.44 mmol) according to the procedure described for compound **125**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-8-phenyloctan-1-ol (104 mg, 0.32 mmol, 22%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.49 (dd, *J* = 4.9, 1.4 Hz, 1H), 7.79 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.28 – 7.23 (m, 3H), 7.18 – 7.13 (m, 3H), 5.05 (dd, *J* = 7.4, 5.6 Hz, 1H), 4.55 (bs, 1H), 2.57 (t, *J* = 8.0 Hz, 2H), 2.12 – 1.90 (m, 2H), 1.63 – 1.53 (m, 2H), 1.53 – 1.40 (m, 2H), 1.39 – 1.20 (m, 6H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 171.46, 154.92, 146.34, 143.05, 142.87, 128.44(2C), 128.28(2C), 125.62, 120.26, 118.79, 68.06, 35.99, 35.47, 31.53, 29.37, 29.30, 29.26, 25.05. Spectroscopic data are in agreement with reported literature.[14]

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-8-phenyloctan-1-one (110)

The title compound was synthesized from 1-(oxazolo[4,5-*b*]pyridin-2-yl)-8-phenyloctan-1-ol (**153**, 50 mg, 0.15 mmol) according to the procedure described for compound **1**. This yielded 1-(oxazolo[4,5-*b*]pyridin-2-yl)-8-phenyloctan-1-one (42 mg, 0.13 mmol, 84%). HRMS (ESI+) m/z: calculated for $C_{20}H_{23}N_2O_2$ ([M + H]), 323.1754; found, 323.1755. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.76 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.00 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.50 (dd, *J* = 8.3, 4.8 Hz, 1H), 7.30 – 7.24 (m, 2H), 7.20 – 7.13 (m, 3H), 3.27 (t, *J* = 7.4 Hz, 2H), 2.60 (t, *J* = 7.8 Hz, 2H), 1.82 (p, *J* = 7.4 Hz, 2H), 1.62 (p, *J* = 7.6 Hz, 2H), 1.48 – 1.30 (m, 6H). ^{13}C APT NMR ($CDCl_3$, 101 MHz): δ 190.58, 158.65, 154.30, 148.89, 143.74, 142.91, 128.53(2C), 128.37(2C), 125.71, 123.30, 120.41, 39.95, 36.06, 31.56, 29.33, 29.22, 29.14, 23.95. Purity of >95% as determined by LC-MS. Spectroscopic data are in agreement with reported literature.[14]

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