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Lead Optimization of Benzoxazolone Carboxamides as Orally Bioavailable and CNS Penetrant Acid Ceramidase Inhibitors.

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ABSTRACT

Sphingolipids (SphLs) are a diverse class of molecules that are regulated by a complex network of enzymatic pathways. A disturbance in these pathways leads to lipid accumulation and initiation of several SphL- related disorders. Acid ceramidase is one of the key enzymes that regulate the metabolism of ceramides and glycosphingolipids, which are important members of the SphL family. Herein, we describe the lead optimization studies of benzoxazolone carboxamides resulting in piperidine **22m**, where we demonstrated target engagement in two animal models of neuropathic lysosomal storage diseases (LSDs), Gaucher's and Krabbe's diseases. After daily intraperitoneal administration at 90 mg Kg^{-1}, **22m** significantly reduced the brain levels of the toxic lipids glucosylsphingosine (GluSph) in 4L;C* mice and galactosylsphingosine (GalSph) in Twitcher mice. We believe that **22m** is a lead molecule that can be further developed for the correction of severe neurological LSDs, where GluSph or GalSph play a significant role in disease pathogenesis.

INTRODUCTION

Sphingolipids (SphLs) are a large class of diverse amphipathic molecules found in abundance in plasma membranes.[1-2] Besides being important as structural cellular components, SphLs play a central role in different biological processes, which are essential to maintain the homeostasis and the development of eukaryotic cells. These processes include signaling, angiogenesis, cell growth, proliferation and death, senescence, inflammation, immune responses, metabolism, autophagy, brain development and function.[2] Aided by recent technological advances, much has been accomplished in terms of the identification of the basic biological components of the complex network in dynamic and interconnected enzymatic pathways that regulate the biosynthesis of SphLs and the formation of a variety of bioactive metabolites in distinct cellular compartments.[1]

In recent years, both academia and industry have shown a growing interest in advancing our understanding of the multifaceted roles of SphL species under physiological and pathological conditions.[2] Collected evidence suggests that a disturbance between the synthesis and catabolism of SphLs leads to their accumulation in specific cellular compartments, such as the lysosomes, and the initiation of several SphL- related disorders. Lysosomes are critical organelles responsible for cellular homeostasis.[3] They contain different degradative enzymes that can hydrolyze proteins, DNA, RNA, polysaccharides, and lipids.[4]

Acid ceramidase (AC, also known as *N*-acylsphingosine amidohydrolase-1, ASAH-1) is a lysosomal cysteine amidase that catalyzes the hydrolysis of ceramides (Cer) into fatty acids and sphingosine, which is then converted into sphingosine 1-phosphate (Sph1P) by sphingosine kinase.[5-7] Cer and Sph1P are important members of the SphLs class and have opposing actions in the control of the cellular fate:[8-10] while Cer mediate cellular senescence[11] and apoptosis,[12-13] Sph1P promotes cell survival and proliferation.[14-17] Recent studies have shown that AC is abnormally expressed in various types of human cancer (for example, prostate, head and neck, colon and glioblastoma) and serum AC levels are elevated in patients with melanoma relative to control subjects.[18] Therefore, inhibition of AC has been envisaged as a potential cancer drug target (**Figure 1**). Aberrant AC activity has also been described in several other common diseases, including, inflammation, pain and various pulmonary disorders.[19-20]

Over the recent years, the multifaceted catabolic role of AC has attracted much attention for its potential therapeutic applications in many other altered conditions. Important genetic studies have identified specific mutations in several genes that encode defective expressions of some lysosomal enzymes as the causes of the onset and progression of severe pathological conditions, called lysosomal storage diseases (LSDs).[21-24] For example, Gaucher's disease (GD) is caused by a defective function of acid β-glucocerebrosidase (GCase), a lysosomal membrane-associated protein responsible for the hydrolysis of glucosylceramide (GluCer) to glucose and ceramides.[25-27] Krabbe's disease (KD) is associated with defective β-galactosyl-ceramidase (GALC) activity, a lysosomal enzyme responsible for the hydrolysis of galactosylceramide (GalCer).[28]

As a result of either enzyme absences or deficiencies, these metabolic lysosomal disorders are characterized by an abnormal storage of substrates or metabolites to concentration levels that are toxic or otherwise detrimental to the cells in various compartments, including the skeleton, skin, liver, spleen, lung, heart, and central nervous system (CNS). The substrate or metabolite accumulations are believed to be responsible for the disease progression.[24] In GD patients, for example, the accumulation of GluCer (x 3 fold) and/or glucosylsphingosine (GluSph) (x 200 fold) has been related to the brain pathogenesis of neuronopathic GD patients, due to neuronal death, which is propagated by the toxic effects of GluCer and /or GluSph.[29-30] Recent evidence demonstrates an active role of AC in an alternative catabolic pathway, which causes GluSph accumulation through the deacylation of the lysosomal GluCer.[31-32] In KD patients, deficiency of GALC activity leads to accumulation of neurotoxic galactosylsphingosine (GalSph or psychosine) in tissues, especially in the brain. It is possible that accumulation of GalSph mediates pathology of KD. A very recent report suggests that genetic ablation of AC or pharmacological inhibition of AC could eliminate psychosine accumulation and prolong the life span of Twitcher mice, a model of KD.[33-34] No approved therapeutic approaches are available to treat neuropathic GD and KD; inhibiting AC may provide an efficacious strategy for treating these two devastating diseases.

Efforts over the last decade to develop potent AC inhibitors, has resulted in limited success. The first structural analysis of mammalian AC was recently solved by Gebai and co-workers,[35] which may aid future medicinal chemistry programs. In 2013, Realini et al. reported the discovery of carmofur **1** and some close uracil analogs, as the first class of single-digit nanomolar inhibitors of intracellular AC activity and studied their potential use as chemosensitizing agents (**Figure 2**).[36-37] Despite being potent AC inhibitors, the uracil derivatives suffered from low chemical and metabolic stability. Subsequently, Diamanti et al. selected compound **1** as a ligand template for a computational-assisted virtual screening approach, leading to the identification of a new class of potent AC inhibitors, the pyrazole carboxamides.[38] However, although very potent against AC activity, as exemplified by pyrazole **3**, this class of molecules suffered from low metabolic stability, limiting their therapeutic potential (**Figure 2**).

An alternative approach, consisting of a screening campaign of a small compound library, led to the identification of a novel and very promising class of covalent AC inhibitors, the benzoxazolone carboxamides (BOAs), exemplified by the hit **2a** (**Figure 2**).[39] Preliminary chemical exploration of this series led to the identification of **2b**[39] and **2c**[40] as more advanced and systematically active analogs. More recently, Ortega et al. reported a systematic computational investigation of the general pharmacophore model for AC inhibition, comprising a 6+5 fused ring heterocycle linked to an aliphatic substituent via a urea moiety. These studies resulted in the identification of the novel class of benzimidazole derivatives **4a-d** with promising activity in different melanoma cell lines (**Figure 2**).[41]

Although some of the molecules discussed above exhibited potent inhibitory effect toward AC, they generally suffer from low aqueous solubility and moderate chemical or metabolic stability, which hamper their further development. As part of our continued efforts to optimize the class of BOAs, we further extended the preliminary studies around **2b**[39] (and **2c**)[40] and performed a focused structure-activity relationship (SAR) study around this scaffold (compound **5**, **Figure 2**), with the aim of identifying an optimal compound with improved physicochemical and pharmacokinetic profiles favoring oral administration. The subject of this manuscript describes the lead optimization and medicinal chemistry strategies that led to the discovery of **22m** as a lead candidate with improved oral bioavailability and excellent distribution to the CNS.

CHEMISTRY

All target compounds were prepared by synthetic routes outlined in **Schemes 1-11**. Compounds **8a-d** were synthetized under standard conditions by reacting **7a-d** with 4-phenylbutyl isocyanate (**Scheme 1**). The novel core scaffold of **12a** was prepared in three steps from the α-bromo ketone **9** (**Scheme 2A**). Reaction with TZD gave compound **10**, which was then converted in moderate yield to the fused bicyclic derivative **11** via an intramolecular cyclization under basic condition in anhydrous THF. Subsequent coupling of **11** to 4-phenylbutyl isocyanate gave **12a** which upon removal of the *N*-Boc protecting group, gave the key intermediate **12b** which was subsequently transformed to **12c-e**, via standard reductive amination and acetylation reactions. Alternatively, the isomeric key intermediate **17b** was prepared in four steps from the commercially available epoxide **13** (**Scheme 2B**). Ring opening[42] and subsequent oxidation of the corresponding alcohol **14a**, followed by intramolecular cyclization of **15** afforded compound **16.** Finally, as discussed for the synthesis of **12b**, standard reactions transformed **16** to **17b.**

We introduced cyclic and heterocyclic groups at C(5)-, C(6)- and C(7)- positions of the benzoxazolone cores by exploring different synthetic pathways (**Schemes 3-11**). The exploration at the C(4)- position of the benzoxazolone scaffold was abandoned, because, in accordance with previously reported results on the 4-Me and 4-Ph derivatives of **2a** (**Figure 2**),[40] we experienced a pronounced chemical instability of our targeted C(4)-derivatives.

The C(6)- substituted benzoxazolones **21a-d** were prepared in three steps starting from boronic esters **18a-c**, using Pd-catalyzed cross coupling reactions with the corresponding bromo-nitrophenols, followed by hydrogenation and intramolecular cyclization in the presence of CDI (**Scheme 3**). An additional step consisting of the *in situ* formation of boronic ester **29b**, from ketone **28** via enol triflate **29a**, was necessary for the preparation of the benzoxazolone **21l** (**Scheme 5**). A Pd-catalyzed cross coupling procedure was also used for the synthesis of the C(5)- substituted benzoxazolone **33** (**Scheme 6**) and other C(6)- substituted benzoxazolones, such as **37a** and **41a** (**Schemes 7-8**). In contrast, to overcome some synthetic problems in the Pd-catalyzed cross coupling reaction, we performed an alternative synthetic approach for the preparation of the benzoxazolone **21m** (**Scheme 9A**). Lithium-halogen exchange of 6-bromo-3*H*- 1,3-benzoxazol-2-one,[43] followed by the addition of the ketone **42a** afforded the alcohol **43**, which upon dehydration and hydrogenation, led to the key intermediate **21m**. A similar synthetic procedure was applied to insert the functionalization at the C(7)- position of the benzoxazolone, as in **47** (**Scheme 9B**).

Other C(6)-substituted benzoxazolones, as examples **23a-d** and **50j** (**Schemes 10** and **11**), were prepared in three steps and in satisfactory yield using a nucleophilic aromatic substitution (SNAr)[44] reaction of activated fluoro-phenyls with a set of heterocyclic amines, followed by hydrogenation and intramolecular cyclization reaction with CDI. An alternative approach was used for the synthesis of **50k** (**Scheme 11**). In this case, a Cu-catalyzed cross coupling *N*-arylation of *O*-Bn protected bromo-nitrophenol **51b** with 4-methylpiperazin-2-one afforded **52b** in acceptable yield,[45] which upon standard reactions, led to the benzoxazolone **50k**.

Finally, the carboxamide functionalities were introduced by standard conditions, which involved the reaction of the benzoxazolone intermediates with the corresponding commercially available isocyanates, as, for example, in the preparation of **22a-c** (**Schemes 3**) and **23a-d** (**Scheme 10**). Alternatively, the isocyanates were prepared *in situ*, upon activation of the corresponding amines by reaction with Boc_2O in the presence of DMAP in

MeCN,[46] as in the synthesis of **22j**, **l** and **22n-o** (**Scheme 4**) or by reaction with triphosgene in the presence of Et_3N in DCM,[47] as in the synthesis of **22m** (**Scheme 4**).

RESULTS AND DISCUSSION

The four most potent classes of AC inhibitors described to date are illustrated in **Figure 2**. Each class is defined by the presence of a common chemical warhead – the urea-like functionality – that can covalently react with the catalytic cysteine (Cys-143) of AC to form a thioester bond.[35] It has been reported that carboxamides **2a**[39] and **4a-b**[41] form, upon incubation experiments with the protein, the corresponding cysteine adducts. This was recently confirmed by Dementiev and coworkers who described the crystal structural analysis of the uracil **1** covalently bound to Cys-143 at 2.7 Å resolution.[48]

While potent and, in some cases, systemically active,[39-40] these molecules share two features that limit their use as oral drugs. First, the presence of a reactive warhead on the molecular scaffolds described to date contributes to their chemical and metabolic instability (*e.g.* uracil **1**)[37] and, secondly, the hydrophobic linear side chain, that ensures target recognition and some degree of specificity, negatively affects their drug-likeness (*e.g.* benzoxazolone **2a**).[39] Thus, the need for optimised AC inhibitors remains an important issue to be addressed.[49]

As previously reported, preliminary structural modifications of **2a** by variation of the lateral-side chain of the urea functionality (*Region A*) and substitution of the benzoxazolone moiety (*Region B*) led to the identification of **2b**[39] and **2c**[40] (**Figure 3**). Despite good potency and enhanced drug-likeness compared to the previous uracil[37] series, compounds **2b-c** suffer from low solubility in aqueous media, and moderate chemical and metabolic stability that limit their utility as oral drugs.[39-40] To address these issues, our lead optimization strategy focused on designing additional structural modifications on *Region A* and *B* (compound **5**, **Figure 2**) with the aim of improving the physicochemical and metabolic properties, while maintaining inhibitory potency.

We initially investigated modifications of the lateral side chain (*Region A*) of **2c** confirming that, as previously reported with **2a** analogs,[40] this region is involved in lipophilic interactions important for target recognition (**Figure 3**). In fact, different attempts to improve solubility and metabolic stability by reducing lipophilicity of the side chain was detrimental regarding potency (**Figure 3**). Although, the removal of the phenyl ring was tolerated, as for the *n*-pentyl analog **2e** (*h*AC IC_{50} = 60 nM), no enhancement of solubility was observed (< 1 μM, PBS, pH 7.4). Replacement of one methylene unit with an oxygen (*e.g.* ethers **2d** and **2f-g**) to increase the hydrophilicity significantly reduced the inhibitory potency to the μM range. A similar trend was observed for the corresponding analogs in the **2b** series (data not shown), indicating that the lipophilic side chain of the urea was very likely occupying a hydrophobic pocket.

We then shifted our attention to the left-hand side (*Region B*) of the scaffold by evaluating the replacement of the benzoxazolone moiety with some bioisosteric 6+5 fused ring heterocyclic systems (**Figure 3**), alternative to those already reported by Ortega et al.[41]

However, both the isatin analog **8c** and the oxindole analog **8d** were inactive at concentrations up to 10 μM. We then investigated the bioisosteric insertion of an aza-group in the phenyl ring of the benzoxazolone moiety and this change resulted in very potent compounds. For example, compounds **8a** and **8b** gave *h*AC IC_{50}'s of 6 and 3 nM, respectively, compared to the earlier compound **2a**,[39] which has an *h*AC IC_{50} of 64 nM. We envisaged that the insertion of a polar group on the left-hand side of the scaffold (*Region B*) could have an impact on the solubility of this series in aqueous buffer (PBS, pH 7.4), but, unfortunately, both **8a** and **8b** had very poor chemical stability in these conditions ($t_{1/2}$ <15 min).

These findings prompted us to evaluate the inhibitory potency of the fused bicyclic derivatives **12b** and **17b** (**Figure 3**). We speculated that changing the left hand side leaving group at the urea functionality could have an effect on the chemical stability of the scaffold. Although, we generally observed a loss in potency to the sub-μM range, regardless of the substituent (**12b-e**) or the position of the nitrogen atom (**17b**), we were pleased to notice that, as for **12b** and **17b**, this novel class of *h*AC inhibitors showed improved chemical stability in PBS at pH 7.4 ($t_{1/2}$ > 8 h) and improved aqueous solubility (82 μM and 230 μM, respectively). Despite the novel chemotype of these AC inhibitors with promising physicochemical properties, our attempts to improve the potency of this series were unsuccessful (data not shown). In addition, although these compounds exhibited high mouse plasma stability (e.g. $t_{1/2}$ > 2 h, for **12b** and **17b**), this class of molecules also suffered from poor mouse liver microsomal stability ($t_{1/2}$ < 15 min).

Overall, these results confirmed the benzoxazolone moiety as a 'privileged scaffold' thus focusing our SAR strategy on *region B* with the intention of reducing the lipophilicity by replacing the phenyl ring of **2c** at the C(6)-position with aliphatic heterocyclic rings (**5**, **Figure 2**). We envisaged that the reduction of the number of sp^2-hybridized carbon atoms and the insertion of heteroatoms in this region could improve the overall physicochemical and metabolic stability of this class of inhibitors.[50-51]

We were pleased to observe that both the cyclohexyl analog **22a** and the tetrahydropyrane analog **22b** resulted in equipotent inhibition (*h*AC IC_{50} = 0.089 and 0.068 μM, respectively) compared to the corresponding phenyl derivative **2c**[39] (**Table 1** and **Figure 2**). With these results in hand, we were then interested in evaluating the effect of increasing the hydrophilicity by the addition of a polar basic amine, as in the piperidine analogs **22d** and **22e.** With this modification, we observed that both compounds showed only a slight loss of potency compared to the aliphatic analog **22a** (*h*AC IC_{50} = 0.134 and 0.129 μM, respectively). Encouraged by these results, we explored the effect of other *N*-containing heterocyclic systems, such as the piperidine **23a**, the morpholine **23b**, the 1,1-dioxothiomorpholine **23c** and the piperazines **23e-f.** Overall, this set of compounds showed similar potency compared to the initial cyclohexyl analog **22a.** Notably, the piperidine **23a** and the piperazine **23f** were the most potent compounds, showing IC_{50} values of 0.080 μM and 0.116 μM, respectively (**Table 1**). Based on these promising results, we selected the piperidine and piperazine series, exemplified by **22d** and **23e**, respectively, as novel scaffolds for further studies, exploiting the presence of a distal nitrogen atom as an anchor point for additional structural modifications (**Table 2**). First, we evaluated the SAR exploration around the piperidine series, by introducing both linear and branched alkyl chains on the nitrogen atom, such as the ethyl **22f**, isopropyl **22g** and isobutyl **22h** analogs (**Table 2**). In general, no significant differences were observed on the inhibitory potency of these derivatives, **22f-h** almost being equipotent to the unsubstituted **22d.** Moreover, the removal of the basic center by the introduction of either an exocyclic (**22i**) or endocyclic *N*-acyl group (**22q**) was tolerated, showing IC_{50} values of 0.064 μM and 0.105 μM, respectively. These results further confirmed that different polar groups were tolerated in this region of the scaffold.

The same strategy was applied to the piperazine series (**Table 2**). Specifically, both the *N*–alkyl derivatives **23g-i** and the piperazinones **23j-k** resulted in more potent AC inhibition compared to the parent **23e**. For examples, the *N*-ethyl piperazine **23g** and the piperazinone **23k** were almost 7-fold more potent than **23e** (*h*AC IC_{50} = 0.363 μM), showing IC_{50} values of 0.056 and 0.052 μM, respectively.

However, a comparison of the piperazine and piperidine series in terms of aqueous kinetic solubility (PBS, pH 7.4), *in vitro* metabolism highlighted some significant differences (**Table 3**). Interestingly, the piperidine analogs, bearing small linear alkyl groups (**22d-f**), were highly soluble (kinetic solubility > 100 μM), and, in some cases (**22d**), had acceptable stability profiles both in mouse plasma and in liver microsomes. On the other hand, the piperidine derivatives, bearing more sterically hindered lipophilic alkyl groups, such as the isopropyl **22g** and isobutyl **22h,** or the acyls **22i** and **22q** suffered from low solubility and, with the exception of **22h**, poor stability in mouse plasma (**Table 3**). Conversely, all the piperazine derivatives generally suffered from poor aqueous solubility, poor microsomal and plasma stability (**Table 3**). As illustrative examples, the piperazines **23f-g** showed poor solubility in water (< 1 μM), rapid metabolism in liver microsomes and poor plasma stability (*m*-plasma and *m*-liver microsomes, $t_{1/2}$ = < 5 min). Some improvement in microsomal stability was observed with the *des*-methylated **23e** and with **23k**, which bears a heterocyclic ring at a higher oxidative state compared to **23f.**

With these results in hand, we focused our efforts on exploring *regions A* and *B* of the *N*-methyl piperidine **22e**, as reported in **Table 4.** In order to reduce the lipophilicity and improve metabolic stability of this scaffold, we followed different strategies: a) insertion of a heteroatom, removal of the phenyl ring and reduction of the side chain length (*Region A*); and b) removal of potential metabolic soft spots (*Region A* and *B*).

An immediate loss in potency was observed with the removal of the lipophilic phenyl ring (**22k**), or the insertion of an oxygen on the lateral chain (**22j** and **22l**); whilst the bioisosteric replacement of a fluorine on the distal phenyl ring resulted in **22o**, being almost equipotent to **22e** (**Table 4**). Nonetheless, exploration of *region A* continued with the insertion of branched alkyl groups. We were pleased that the isobutyl analog **22m** (*h*AC IC_{50} = 0.166 μM) was equipotent to the corresponding butyl phenyl **22e**, demonstrating that it was possible to remove the phenyl group and reduce the overall lipophilicity without compromise potency. On the other hand, a methyl group adjacent to the urea functionality, such as the *sec*-butyl analog **22n**, was detrimental for potency, with an IC_{50} of 2.1 μM. Moving the SAR exploration back to *region B*, insertion of a fluorine on the benzoxazolone ring **22p** boosted the inhibitory potency (IC_{50} = 0.024 μM), while the difluoro ethyl analog **22r** showed similar potency (IC_{50} = 0.095 μM) compared to **22e**. The kinetic aqueous solubility and *in vitro* metabolic stability of a selection of compounds in the piperidine series are summarized in **Table 4**. Notably, while the insertion of an oxygen did not affect either the solubility or metabolic stability in microsomes of **22j** compared to **22e**, reducing lipophilicity with small aliphatic groups (**22k** and **22m**) was particularly beneficial. For example, **22k** and **22m**, showed high aqueous solubility (240 μM) and improved plasma and liver microsomal stabilities ($t_{1/2} > 60$ min). On the other hand, attempts to improve the liver microsomal stability of **22e** by inserting a fluorine atom at different potential metabolic soft spots of both *Region A* and *B* (compounds **22o-p**, **22r**) was not successful.

Not surprisingly, these bioisosteric replacements negatively affected the aqueous solubility of **22o-p**, **22r**, without a substantial improvement of the metabolic stability in microsomes.

With these results in hand, SAR studies continued on the scaffold of compound **22m** (**Table 5**). Specifically, we evaluated the effect of the location of both - the *N-methyl piperidine ring* -at C(5) and C(7) positions of the benzoxazolone moiety (compounds **24c** and **25**) and - the *N-methylated nitrogen atom*- within the piperidine nucleus (compounds **26** and **27**). Overall, we generally observed a loss in the inhibitory potency of these targeted analogs compared to **22m**, which was even more pronounced with compounds **26** and **27**, showing IC_{50} values in the μM range. Finally, the evaluation of the kinetic aqueous solubility and *in vitro* metabolism of **22m** and close analogs was completed (**Table 5**). In general, all the targeted compounds showed high solubility values in aqueous media, except for **24c**, which bears the piperidine ring at the C(5) position of the benzoxazolone system. On the other hand, major differences were observed comparing their metabolic stability properties. In particular, we observed that substitution at the C(6) position was critical to maintaining acceptable mouse plasma and liver microsomal stabilities (compound **22m**, *m*-plasma $t_{1/2}$ = 80 min and *m*-liver microsomes $t_{1/2} > 60$ min (76% remaining at 1 h)). On the other hand, both derivatives with the piperidine ring at the C(5) and C(7) positions, **24c** and **25** showed reduced mouse plasma and liver microsomal stability. A similar trend was observed by moving the nitrogen atom to a different position on the piperidine ring, except for **27**, which showed a similar liver microsomal stability compared to **22m.** Due to its inhibitory potency and improved overall drug-likeness profile, the piperidine **22m** was selected for further biological and pharmacological investigations.

We firstly envisaged that the inhibition of **22m**, belonging to the same class of the benzoxazolone carboxamide **2c**,[39] should occur through the same covalent AC modification. According to our hypothesis, the corresponding benzoxazolone **21g** (**Scheme 4**), tested at 1 and 10 μM, was not able to inhibit *h*AC, due to the lack of the reactive urea -like functionality. Moreover, kinetic studies on *h*AC –enriched lysates showed that **22m** causes a concentration-dependent reduction in the maximal catalytic velocity of AC (V_{max}) without influencing the Michaelis-Menten constant (K_M) (**Figure 4B** and **Table S1**) and time dependent inhibition at different **22m** concentrations with a $k_i/K_I = 0.02$ μM^{-1} min^{-1} and $k_i = 0.15$ min^{-1} (**Figure 4C** and **Figure 4D**), suggesting a very fast covalent bond formation to the enzyme.[52-53]

The selectivity of **22m** was evaluated against a set of related lysosomal enzymes. The compound showed only a weak inhibitory effect (IC_{50} = 8.0 μM) on human *N*-acylethanolamine acid amidase (*h*NAAA), a lysosomal cysteine amidase that shares 33–34% sequence identity and a very similar reactive site with AC.[54] **22m** had no effect at the concentrations tested (1 and 10 μM) on the activity of either acid sphingomyelinase (ASM) and GCase. We next assessed the selectivity of **22m** against two of the most representative members of serine hydrolases, human fatty acid amide hydrolyase (FAAH)[55] and monoacylglycerol lipase (MAGL)[56]: **22m** showed inhibitory activity on FAAH with an IC_{50} of 0.070 μM and no effect on monoacylglycerol lipase (MAGL) at the concentrations tested (1 and 10 μM). Although off-target activity of **22m** against FAAH is observed, to our knowledge, no evidence for biological cross-talk between the sphingolipid-signaling pathways[2] and the FAAH-signaling pathway[55, 57] has been reported that could preclude further development of **22m**.

The favorable overall profile of **22m** prompted us to test its ability to inhibit AC in intact cells. Human neuroblastoma SH-SY5Y cells were incubated in the presence of **22m** at different doses (1, 2.5, 5, 10 μM). AC activity was measured with a liquid chromatography/mass spectrometry (LCMS)-based activity assay after different incubation times (30 min, 1, 3, 6 h) and SphL levels were identified and quantified by LC/MS, showing that **22m** effectively engages AC in these cells leading to the expected variations in the SphL levels, as reported in **Figure 5** and **6**. Treatment of cultures of human neuroblastoma SH-SY5Y cells with **22m** caused a concentration (**Figure 5A**) and time dependent reduction of AC activity (**Figure 6A**). After 3 h incubation, this effect resulted in an intracellular accumulation of various ceramide species, including Cer (d18:0/16:0) and Cer (d18:1/16:0) (**Figure 5B-C**) and a corresponding decrease in the levels of sphingosine (**Figure 5D**) in a concentration dependent manner. The effect of **22m** (10 μM) on AC activity inhibition and SphL persisted for up to 6 h under our experimental conditions (**Figure 6B-D**). The results indicated that **22m** inhibits AC in the complex cellular environment leading to an increased Cer (d18:0/16:0) and Cer (d18:1/16:0) (**Figure 6B-C**) and decreased sphingosine levels with a partial recovery of sphingosine levels after 3-6 h (**Figure 6D**). Conversely, as expected, no major variations were observed in the levels of sphingomyelin (SM) (d18:1/16:0) (**Figure 5E** and **6E**) and hexosylceramide (HexCer) (d18:1/16:0) (**Figure 5F** and **Figure 6F**).

Pharmacokinetic studies of **22m** were determined in CD1 mice and relevant pharmacokinetic parameters are reported in **Table 6.** Values of plasma clearance (Cl_p), volume of distribution (Vd_{ss}) and plasma half-life ($t_{1/2}$) were calculated after intravenous administration of **22m** at 3 mg Kg^{-1}. Clearance was moderately high (14.1 L/h/Kg), with a relative short plasma half-life (1 h) and high Vd_{ss} (12.5 L/Kg) indicating **22m** well distributed out of the circulating plasma compartment. Good oral bioavailability was observed dosing **22m** at 10 mg Kg^{-1} (F = 58%), with significant exposures in plasma, brain and cerebrospinal fluid (CSF) (AUC values = 412, 14648 and 119 (h x ng/mL), respectively). A maximum tolerated dose (MTD) study in mice was also conducted in the same background as the pharmacodynamic model using C57BL/6 mice at intraperitoneal dose escalation of 20, 40, 80, and 120 mg Kg^{-1} during a time range of 4 days and no clinical abnormalities were observed in any animals within the doses and time range used.

Based on these results, we decided to study the effect of dosing **22m** in 4L;C* mice, a validated genetic mutated animal model for neuropathic GD.[58] 4L;C* mice have marked increase (20- to 30-fold) of GluSph and moderate elevation (1.5- to 3-fold) of GluCer in the brain, therefore they are a unique model suitable for testing GluSph reduction therapy. **22m** was administered at the selected doses of 30 and 90 mg Kg^{-1} by intraperitoneal injection (i.p.), once a day for 14 days starting at postnatal day 5. Preliminary results showed that compound **22m** significantly reduces GluSph (d18:1) in the brain of 4L;C* mice in a dose dependent manner (**Figure 7**). Target engagement was demonstrated at the high dose of 90 mg Kg^{-1} with 54% reduction of the GluSph levels relative to control.

Next, we evaluated **22m** in Twitcher mouse, an animal model of Krabbe's disease. The Twitcher mice naturally carry a GALC mutation that contains a premature stop codon in GALC and leads to a complete loss of GALC activity. As a result, dramatic increase of the extremely toxic lipid GalSph is observed in Twitcher mouse brains. After i.p. administration at 30 and 90 mg Kg^{-1} once daily for a treatment period of 20 days starting at postnatal day

10, **22m** showed dose dependent reduction of the toxic lipid GalSph (d18:1) levels in the brain of Twitcher mice by 72% and 41% at the high and low doses, respectively (**Figure 8**).

In the group of 4L;C* mice at 90 mg Kg^{-1} doses, the unbounded drug levels in the brain 1 h post last dose (day 14) are 2.6 μM (6.4- fold higher than the EC_{50} value) (**Table 7**); whilst at the lower dose of 30 mg Kg^{-1} the unbounded drug levels are 0.77 μM (1.9- fold higher than the EC_{50} value). In the group of Twitcher mice at 90 mg Kg^{-1} doses, the unbounded drug levels in the brain 1 h post last dose (day 20) are 2.15 μM (5.2- fold higher than the EC_{50} value); whilst at the lower dose of 30 mg Kg^{-1} the unbounded drug levels are 0.80 μM (2.0- fold higher than the EC_{50} value). Overall, these data support the observed dose responses in the two animal models.

To our knowledge, this is the first report showing the efficacy of inhibiting AC on reducing the neurotoxic lipids GluSph in the brains of 4L;C* mice. Our result that inhibiting AC reduces neurotoxic lipid GalSph level in the brains of Twitcher mice is consistent with the recent report.[34]

Further pharmacological studies of **22m** will be reported in due course.

CONCLUSIONS

The present work outlines the lead optimization studies of a class of benzoxazolone carboxamides as AC inhibitors. We further extended the preliminary studies around **2b** (and **2c**)[39-40] and performed a focused structure-activity relationship (SAR) study on *Region A* and *B* of this scaffold with the aim of improving the physicochemical and metabolic properties of the series, while maintaining the inhibitory potency. Introduction of different heterocyclic groups on the benzoxazolone moiety were tolerated regarding inhibitory potency, as for the tetrahydropyrane **22b**, the piperidines **22d**, **23a** and the piperazines **23e**, **23f**. A more focused exploration around **22d** and **23e** by changing the nature of substitution on the distal nitrogen atom led to the identification of novel potent analogs with improved solubility, as, for example, the piperidines **22e** and **22f**. Targeted modifications on different positions of *Region A* and *B* of the *N*-methylated piperazine series led to compound **22m** as a potent and oral bioavailable AC inhibitor with excellent brain penetration in mice. Preliminary results demonstrated target engagement of **22m** both in the 4L;C* and Twitcher mouse models, where dose dependent reductions in GluSph and GalSph were observed, supporting further optimized AC inhibitors may be used in the correction of severe pathological neurological states of LSD, where these toxic lipids may play a significant role in the pathology, such as GD and KD.

EXPERIMENTAL SECTION

Chemicals, Materials, and Methods. Solvents and reagents were obtained from commercial suppliers and were used without further purification. Automated column chromatography purifications were done using a Teledyne ISCO apparatus (CombiFlash® Rf) with pre-packed SiO_2 columns of different sizes (from 4 g until 40 g). Mixtures of increasing polarity of Cy and EtOAc or DCM and MeOH were used as eluents. TLC analyses were performed using Supelco on TLC Al foils 0.2 mm with fluorescence indicator 254 nm. Purifications of basic compounds were done using IST ISOLUTE® SCX packed into SPE cartridges (SCX). Hydrogenation reaction were performed using H-Cube continuous hydrogenation equipment (SS-reaction line version), employing disposable catalyst cartridges (CatCart) preloaded with the required heterogeneous catalyst. Microwave heating was performed using Explorer®-48 positions instrument (CEM). NMR experiments of all the intermediates and final compounds were run on a Bruker Avance III 400 system (400.13 MHz for ^{1}H, and 100.62 MHz for ^{13}C), equipped with a BBI probe and Z-gradients. Spectra were acquired at 300 K, using deuterated dimethylsulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as solvents. Chemical shifts for ^{1}H and ^{13}C spectra were recorded in parts per million using the residual non-deuterated solvent as the internal standard (for DMSO-d_6: 2.50 ppm, ^{1}H; 39.52 ppm, ^{13}C; for $CDCl_3$: 7.26 ppm, ^{1}H and 77.16 ppm, ^{13}C). Data are reported as follows: chemical shift (ppm), multiplicity (indicated as: bs, broad singlet; s, singlet; d, doublet; t, triplet; q, quartet; p, quintet, sx, sextet; m, multiplet and combinations thereof), coupling constants (*J*) in Hertz (Hz) and integrated intensity. Quantitative ^{1}H-NMR analyses of the freshly prepared 10 mM DMSO-d_6 stock solutions (used for biological screenings) of the final compounds were performed using PULCON method (PUlse Length based CONcentration determination, Bruker software, topspin 3.0. References: a) Wider G., Reires L. *J. Am. Chem. Soc*. **2006**, *128* (8), 2571-2576; b) Burton I. W., Quilliam M. A., Valter J. A., *Anal. Chem*. **2005**, *77*, 3123-3131). UPLC/MS analyses of all the intermediates and final compounds were performed on a Waters ACQUITY UPLC/MS system consisting of a Single Quadrupole Detector (SQD) Mass Spectrometer (MS) equipped with an Electrospray Ionization (ESI) interface and a Photodiode Array Detector (PDA). PDA range was 210-400 nm. Analyses were performed on an ACQUITY UPLC BEH C18 column (50 x 2.1 mm ID, particle size 1.7 µm) with a VanGuard BEH C18 pre-column (5x 2.1 mmID, particle size 1.7 µm). Mobile phase was 10 mM NH_4OAc in H_2O at pH 5 adjusted with AcOH (A) and 10 mM NH_4OAc in MeCN-H_2O (95: 5) at pH 5 (B). ESI in both positive and negative modes was used in the mass scan range 100-650Da. Analyses were performed with *method A*, *B*, *C* or

D. *Method A*: Gradient 5 to 95% B over 2.5 min. Flow rate 0.5 mL/min. Temperature 40 °C. *Method B*: Gradient 50 to 100% B over 2.5 min. Flow rate 0.5 mL/min. Temperature 40 °C. *Method C*: Gradient 0 to 100% B over 2.5 min. Flow rate 0.5 mL/min. Temperature 40 °C. *Method D*: Isocratic 55% B over 5 min. Flow rate 0.5 mL/min. Temperature 40 °C. UPLC/MS analyses of the final compounds were performed with *method E* or *F* using freshly prepared 10 mM DMSO-d_6 stock solutions (used for biological screenings), diluted 20- fold or 100 fold in MeCN/H_2O (1:1) and directly analyzed. An ACQUITY UPLC BEH C18 (100x 2.1 mmID, particle size 1.7μm) with a VanGuard BEH C18 pre-column (5x 2.1 mm ID, particle size 1.7 μm). Mobile phase was 10 mM NH_4OAc in H_2O at pH 5 adjusted with AcOH (A) and 10 mM NH_4OAc in MeCN-H_2O (95: 5) at pH 5 (B). ESI in both positive and negative modes was used in the mass scan range 100-650Da. *Method E*: Gradient: 10 to 90% B over 6 min. Flow rate 0.5 mL/min. Temperature 40 °C. *Method F*: Gradient: 50 to 100% B over 6 min. Flow rate 0.5 mL/min. Temperature 40 °C. The detection wavelength (λ) was set at 215 nm for relative purity determination. Rt of the final compounds are reported in **Table S2**. Accurate mass measurements were performed on a Synapt G2 Quadrupole-ToF Instrument (Waters, USA), equipped with an ESI ion source; compounds were diluted to 50 μM in H_2O/ MeCN and analyzed. Leucine Enkephalin (2 ng/mL) was used as lock mass reference compound for spectra recalibration. All final compounds displayed ≥ 95% purity as determined by NMR and UPLC/MS analysis.

General procedure for palladium-catalyzed cross coupling reaction (Procedure A).

To a solution of the appropriate phenyl bromide (1.0 eq.) in dry 1,4-dioxane (0.5 M, previously degassed under nitrogen atmosphere) was added the appropriate boronic acid or its corresponding boronic ester (1.1 eq.), followed by the addition of $Pd(PPh_3)_4$ or

$Pd(dppf)Cl_2$ (0.05- 0.2 eq.) and 2M Na_2CO_3 (2.5 eq.). The dark reaction mixture was stirred at reflux for 15 h, then diluted with EtOAc and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure and the crude was purified by column chromatography, eluting with Cy/ EtOAc as indicated in each case.

General procedure for catalytic hydrogenation reaction (Procedure B).

Method A: To a suspension of the appropriate 2-nitrophenol (1.0 eq.) in MeOH, or EtOH, or EtOAc (0.4 M) were added 10% Pd/C (0.25 eq.) and cyclohexene (30 eq.) and the reaction mixture was stirred at reflux until disappearance of the starting material, as indicated by UPLC/MS analysis. The suspension was filtered through a pad of Celite and the filtrate was quickly evaporated under reduced pressure. The crude was used in the next step without further purification.

Method B: A suspension of the appropriate 2-nitrophenol (1.0 eq.) in MeOH (0.4 M) was hydrogenated with the H-Cube apparatus using 10% Pd/C at 60 °C and full H_2 mode. After complete conversion (UPLC/MS analysis monitoring), the solvent was evaporated under reduced pressure. The crude was used in the next step without further purification.

General procedure for intramolecular cyclization using CDI (Procedure C).

To a solution of the appropriate 2-amino phenol (1.0 eq.) in MeCN (0.1M) was added CDI (1.0 – 1.5 eq.). The reaction mixture was stirred at rt for 2 h. Then, the solvent was evaporated under reduced pressure and the crude was re-dissolved in EtOAc, washed with H_2O, brine and dried over Na_2SO_4. After evaporation of the solvent, the crude was purified by column chromatography, eluting with Cy/ EtOAc, or DCM/ MeOH, or used in the next step without further purification, as indicated in each case.

General Procedure for carboxamide synthesis (Procedure D).

Method A: To a stirred solution of the appropriate oxazolone (1.0 eq.) and DMAP (1.1 eq.) in dry MeCN was added the appropriate isocyanate (1.1- 3.0 eq.). The reaction mixture was stirred at rt for 30 min under nitrogen atmosphere. After evaporation of the solvent, the crude was purified by column chromatography, eluting with Cy/ EtOAc or DCM/ MeOH as indicated in each case.

Method B: To a stirred solution of triphosgene (0.33 eq.) in dry DCM (0.2 M) were added the appropriate amine (1.5- 3.0 eq.) and dry Et_3N (3.0 eq.) at -15 °C. The resulting mixture was stirred at rt for 30 min under nitrogen atmosphere and then added to a solution of the appropriate oxazolone (1.0 eq.) and Et_3N (1.0 eq.) in dry DCM. The reaction mixture was stirred at rt for 30 min under nitrogen and then diluted with DCM. The organic phase was washed with saturated aqueous NH_4Cl solution, brine and dried over Na_2SO_4. After evaporation of the solvent, the crude was purified by column chromatography, eluting with Cy/ EtOAc, or DCM/ MeOH, as indicated in each case.

Method C: To a stirred solution of Boc_2O (2.0 eq.) in MeCN (0.4 M) were added DMAP (2.0 eq.) and the appropriate amine (2.0 eq.). The resulting solution was stirred at rt for 10 min then the appropriate oxazolone derivative (1.0 eq.) was added and the mixture was stirred at rt for 1 h. After evaporation of the solvent, the crude was purified by flash column chromatography, eluting with Cy/ EtOAc, or DCM/ MeOH, as indicated in each case.

General procedure for *N*-Boc removal (Procedure E).

To a suspension of the appropriate *N*-Boc protected derivative (1.0 eq.) in 1,4-dioxane or DCM (0.1 M) HCl (30 eq., 4M in 1,4-dioxane) was added and the reaction mixture was stirred at rt for 2 h. After evaporation of the solvent, the crude was triturated with Et_2O or used in the next step without further purification, as indicated in each case.

General procedure for reductive amination reaction (Procedure F).

To a solution of the appropriate secondary amine (1.0 eq.) in MeCN, or THF (0.1 M) were added the appropriate aldehyde or ketone (1.6– 5.0 eq.), AcOH (1.6 -5.0 eq.) and $NaBH(OAc)_3$ (1.6- 3.0 eq.). The mixture was stirred at rt for 2-16 h under nitrogen atmosphere. Then, the reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the crude was purified by SCX.

General procedure for nucleophilic aromatic substitution reaction (SNAr) (Procedure G).

To a solution of the appropriate 4-fluoro nitrobenzene (1.0 eq.) in MeCN were added the appropriate amine (2.0 eq.) and DIPEA (2.0 eq.). The reaction mixture was refluxed (or stirred under MW irradiation, 90 °C, power 200W) until disappearance of the starting material, as indicated by UPLC/MS analysis. After evaporation of the solvent, the crude was purified by flash column chromatography, eluting with Cy/ EtOAc, or DCM/ MeOH, as indicated in each case.

General procedure for intramolecular cyclization under basic condition (Procedure H)

To a solution of the appropriate thiazolidinedione derivative (1.0 eq.) in dry THF (0.1 M) was added *t*BuOK (2.0- 4.0 eq.) at rt under nitrogen atmosphere. After 30 min, the reaction mixture was diluted with EtOAc, washed with saturated aqueous NH_4Cl solution, brine and dried over Na_2SO_4. After evaporation of the solvent, the crude was used in the next step without further purification.

General procedure for lithium/halogen exchange - addition reaction (Procedure I).

To a solution of the appropriate bromobenzoxazolone (1.0 eq.) in dry THF (0.1M) was added MeMgBr (1.5 eq., 3.0 M in Et_2O) at -78 °C under nitrogen atmosphere for 30 min, followed by the addition of *n*-BuLi (1.2 eq., 2.5 M in hexanes). After 30 min, a solution of the appropriate piperidone (1.7 eq.) in dry THF (0.7 M) was added dropwise at -78 °C under nitrogen atmosphere and, then, the reaction mixture was allowed to warm to rt. After 30 min, the reaction was quenched by addition of saturated aqueous NH_4Cl solution, diluted with EtOAc, washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the crude was purified by column chromatography, eluting with Cy/ EtOAc, or DCM/ MeOH, as indicated in each case.

General procedure for dehydration reaction of tertiary alcohols (Procedure L).

To a suspension of the appropriate tertiary alcohol (1.0 eq.) in dry toluene (0.1 M) was added *p*-TsOH (3.0 eq.) and the reaction mixture was stirred at reflux for 2 h. After evaporation of the solvent, the crude was purified by SCX, or used in the next step without further purification, as indicated in each case.

Synthesis of *N*-(2-benzyloxyethyl)-6-(4-fluorophenyl)-2-oxo-1,3-benzoxazole-3-carboxamide (2d). Compound **2d** was prepared according to the general procedure D (method B), using 6-(4-fluorophenyl)-3*H*-1,3-benzoxazol-2-one[39] (0.060 g, 0.26 mmol), 2-(benzyloxy)-1-ethanamine hydrochloride (0.073 g, 0.39 mmol) and Et_3N (0.11 mL, 0.079 g, 0.78 mmol), in dry DCM (3 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **2d** as white solid (0.06 g, 57%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.35 (bs, 1H), 8.08 (d, J = 8.3 Hz, 1H), 7.52 (dd, J = 8.8, 5.2 Hz, 2H), 7.43 (dd, J = 8.3, 1.7 Hz, 1H), 7.40 (d, J = 1.7 Hz, 2H), 7.39 – 7.33 (m, 4H), 7.28 (tt, J = 7.0, 1.6 Hz, 1H), 7.14 (t, J = 8.6 Hz, 2H), 4.59 (s, 2H), 3.74 - 3.65 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.82 (d, J_{C-F} = 247.5 Hz), 153.12, 149.94, 142.43, 137.91, 137.51, 136.18, 136.16, 128.86 (d, J_{C-F} = 8.2 Hz), 128.62, 127.96, 127.95, 127.28, 123.87, 116.03 (d, J_{C-F} = 21.4 Hz), 115.78, 108.57, 73.40, 68.26, 40.37. UPLC/MS (*method A*): Rt 2.78 min. MS (ES) $C_{23}H_{19}FN_2O_4$ requires 406, found 407 $[M+H]^+$. HRMS $C_{23}H_{20}FN_2O_4$ $[M+H]^+$: calculated 407.1407, measured: 407.1424, Δppm 4.2.

Synthesis of 6-(4-fluorophenyl)-2-oxo-*N*-pentyl-1,3-benzoxazole-3-carboxamide (2e). Compound **2e** was prepared according to the general procedure D (method A), using 6-(4-fluorophenyl)-3*H*-1,3-benzoxazol-2-one (0.080 g, 0.35 mmol), 1-pentyl isocyanate (0.05 mL, 0.040 g, 0.39 mmol) in dry MeCN (3 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **2e** as white solid (0.100 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ. 8.10 (d, *J* = 8.3 Hz, 1H), 8.04 (bs, 1H), 7.52 (dd, *J* = 8.6, 5.3 Hz, 2H), 7.47 – 7.42 (m, 1H), 7.41-7.38 (m, 1H), 7.14 (t, *J* = 8.6 Hz, 2H), 3.44 (q, *J* = 6.9 Hz, 2H), 1.66 (p, *J* = 7.2 Hz, 2H), 1.45-1.32 (m, 4H), 0.93 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.70 (d, $J_{C\text{-}F}$ = 247.6 Hz), 153.23, 149.71, 142.28, 137.38, 136.06, 128.73 (d, $J_{C\text{-}F}$ = 8.8 Hz), 123.78, 116.00 (d, $J_{C\text{-}F}$ = 25.3 Hz), 115.75, 108.42, 99.96, 40.34, 29.12, 28.96, 22.30, 13.94. UPLC/MS (*method A*): Rt 2.43 min. MS (ES) $C_{19}H_{19}FN_2O_3$ requires 342, found 343 $[M+H]^+$. HRMS $C_{19}H_{20}FN_2O_3$ $[M+H]^+$: calculated 343.1458, measured: 343.1449, Δppm -2.6.

Synthesis of *N*-(2-ethoxyethyl)-6-(4-fluorophenyl)-2-oxo-1,3-benzoxazole-3-carboxamide (2f). Compound **2f** was prepared according to the general procedure D (method B), using 6-(4-fluorophenyl)-3*H*-1,3-benzoxazol-2-one (0.130 g, 0.57 mmol), 2-ethoxyethylamine (0.09 mL, 0.080 g, 0.85 mmol) and Et_3N (0.20 mL, 0.140 g, 1.42 mmol) in dry DCM (15 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **2f** as white solid (0.03 g, 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (bs, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 7.57-7.49 (m, 2H), 7.46 – 7.38 (m, 2H), 7.18 – 7.10 (m, 2H), 3.68 - 3.61 (m, 4H), 3.56

(q, *J* = 7.0 Hz, 2H), 1.24 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.69 (d, J_{C-F} = 247.7 Hz), 153.16, 149.98, 142.47, 137.54, 136.20, 128.88 (d, J_{C-F} = 8.2 Hz), 127.34, 123.89, 116.04 (d, J_{C-F} = 21.7 Hz), 115.81, 108.60, 68.63, 66.81, 40.43, 15.25. UPLC/MS (*method A*): Rt 2.56 min. MS (ES) $C_{18}H_{17}FN_2O_4$ requires 344, found 345 $[M+H]^+$. HRMS $C_{18}H_{18}FN_2O_4$ $[M+H]^+$: calculated 345.1251, measured: 345.1258, Δppm 2.

Synthesis of 6-(4-fluorophenyl)-*N*-(3-methoxypropyl)-2-oxo-1,3-benzoxazole-3-carboxamide (2g). Compound **2g** was prepared according to the general procedure D (method B), using 6-(4-fluorophenyl)-3*H*-1,3-benzoxazol-2-one (0.08 g, 0.35 mmol), 3-methoxypropylamine (0.06 mL, 0.050 g, 0.52 mmol) and Et_3N (0.12 mL, 0.09 g, 0.88 mmol) in dry DCM (15 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **2g** as white solid (0.020 g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (bs, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 7.52 (dd, *J* = 8.6, 5.3 Hz, 2H), 7.43 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.41-7.39 (m, 1H), 7.14 (t, *J* = 8.6 Hz, 2H), 3.55 (dt, *J* = 16.4, 6.0 Hz, 4H), 3.39 (s, 3H), 1.92 (p, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.67 (d, J_{C-F} = 247.4 Hz), 153.16, 142.84, 142.43, 137.44, 136.23, 128.70 (d, J_{C-F} = 8.1 Hz, 2C), 127.40, 123.84, 116.12, 115.88 (d, J_{C-F} = 11.3 Hz, 2C), 108.53, 70.95, 58.95, 38.60, 29.28. UPLC/MS (*method A*): Rt 2.49 min. MS (ES) $C_{18}H_{17}FN_2O_4$ requires 344, found 345 $[M+H]^+$. HRMS $C_{18}H_{18}FN_2O_4$ $[M+H]^+$: calculated 345.1251, measured: 345.1258, Δppm 2.

Synthesis of 3*H*-oxazolo[4,5-*c*]pyridin-2-one (7a). Compound **7a** was prepared according to the general procedure C using **6a** (0.10 g, 0.91 mmol) and CDI (0.290 g, 1.82 mmol, 2.0 eq.) in a mixture of MeCN/ DMF (9 mL, 2: 1). The crude was triturated with DCM to afford **7a** as a whitish solid (0.100 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.32 (d, *J* = 5.3

Hz, 1H), 7.38 (d, *J* = 5.3 Hz, 1H). UPLC/MS (*method A*): Rt 0.61 min. MS (ES) $C_6H_4N_2O_2$ requires 136, found 137 $[M+H]^+$, 135 $[M-H]^-$.

Synthesis of 1*H*-oxazolo[5,4-*c*]pyridin-2-one (7b). Compound **7b** was prepared according to the general procedure C using **6b** (0.100 g, 0.91 mmol) and CDI (0.441 g, 2.72 mmol) in a mixture of MeCN/ DMF (9 mL, 1: 4). The crude was triturated with Et_2O to afford **7b** as a brown solid (0.123 g, quant.). ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 12.51 (bs, 1H), 8.38 (s, 1H), 8.23 (d, *J* = 5.5 Hz, 1H), 7.16 (d, *J* = 5.5 Hz, 1H). UPLC/MS (*method C*): Rt 1.06 min. MS (ES) $C_6H_4N_2O_2$ requires 136, found 137 $[M+H]^+$, 135 $[M-H]^-$.

Synthesis of 2-oxo-N-(4-phenylbutyl)oxazolo[4,5-c]pyridine-3-carboxamide (8a). Compound **8a** was prepared according to the general procedure D (method A) using **7a** (0.03 g, 0.22 mmol) and 4-phenylbutyl isocyanate (0.045 mL, 0.046 g, 0.26 mmol) in a mixture of DMF/ toluene (3 mL, 2: 1). The crude was purified by column chromatography (Cy/ EtOAc, from 95: 5 to 70: 30) to afford **8a** as white solid (0.015 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (s, 1H), 8.55 (d, *J* = 5.1 Hz, 1H), 7.85 (bs, 1H), 7.31 – 7.21 (m, overlapped with $CDCl_3$ signal, 3H), 7.21 – 7.13 (m, 3H), 3.47 (q, *J* = 6.5 Hz, 2H), 2.68 (t, *J* = 7.1 Hz, 2H), 1.79 - 1.64 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.67, 148.95, 148.06, 146.45, 141.91, 136.75, 136.72, 128.53 (4C), 126.06, 105.75, 40.48, 35.54, 29.11, 28.63. UPLC/MS (*method A*): Rt 2.23 min. MS (ES) $C_{17}H_{17}N_3O_3$ requires 311, found 312 $[M+H]^+$. HRMS $C_{17}H_{18}N_3O_3$ $[M+H]^+$: calculated 312.1348, measured: 312.134, Δppm -2.6.

Synthesis of 2-oxo-*N*-(4-phenylbutyl)oxazolo[5,4-*c*]pyridine-1-carboxamide (8b). Compound **8b** was prepared according to the general procedure D (method A) using **7b** (0.08 g, 0.59 mmol) and 4-phenylbutyl isocyanate (0.11 mL, 0.113 g, 0.65 mmol) in a mixture of DMF/ MeCN (12 mL, 4: 1). The crude was purified by column chromatography

(Cy/ EtOAc, 80: 20) to afford **8b** as white solid (0.107 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.54 (d, *J* = 5.3 Hz, 1H), 8.04 (d, *J* = 5.3 Hz, 1H), 7.91 (bs, 1H), 7.33 – 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.23 – 7.13 (m, 3H), 3.46 (q, *J* = 6.4 Hz, 2H), 2.68 (t, *J* = 7.1 Hz, 2H), 1.80 – 1.61 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.99, 148.79, 146.15, 141.86, 139.86, 130.48, 128.55 (4C), 128.53, 126.10, 110.80, 40.52, 35.54, 29.04, 28.62. UPLC/MS (*method A*): Rt 2.26 min. MS (ES) $C_{17}H_{17}N_3O_3$ requires 311, found 312 [M+H]+. HRMS $C_{17}H_{18}N_3O_3$ $[M+H]^+$: calculated 312.1348, measured: 312.1341, Δppm -2.2.

Synthesis of 2,3-dioxo-*N*-(4-phenylbutyl)indoline-1-carboxamide (8c). Compound **8c** was prepared according to the general procedure D (method A) using **7c** (0.074 g, 0.50 mmol) and 4-phenylbutyl isocyanate (0.097 mL, 0.100 g, 0.55 mmol). The crude was purified by column chromatography (Cy/ EtOAc, 85: 15) to afford **8c** as yellow solid (0.029 g, 21%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (t, *J* = 5.7 Hz, 1H), 8.16 (d, *J* = 8.2 Hz, 1H), 7.75 - 7.64 (m, 2H), 7.31 - 7.23 (m, 3H), 7.23 – 7.13 (m, 3H), 3.39 - 3.24 (m, overlapped with H_2O signal, 2H), 2.62 (t, *J* = 7.3 Hz, 2H), 1.68-1.51 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 180.90, 159.48, 151.08, 148.69, 142.51, 138.04, 128.70 (4C), 126.14, 125.16, 124.81, 119.47, 116.97, 39.70, 35.23, 29.14, 28.66. UPLC/MS (*method A*): Rt 1.41 min. MS (ES) $C_{19}H_{18}N_2O_3$ requires 322, found 323 $[M+H]^+$. HRMS $C_{19}H_{19}N_2O_3$ $[M+H]^+$: calculated 323.1396, measured: 323.1391, Δppm -1.5.

Synthesis of 2-oxo-*N*-(4-phenylbutyl)indoline-1-carboxamide (8d). Compound **8d** was prepared according to the general procedure D (method A) using **7d** (0.066 g, 0.50 mmol) and 4-phenylbutyl isocyanate (0.094 mL, 0.096 g, 0.55 mmol). The crude was purified by column chromatography (Cy/ EtOAc, 90: 10) to afford **8d** as white solid (0.04 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (bs, 1H), 8.25 (d, *J* = 8.2 Hz, 1H), 7.35 – 7.22 (m, overlapped with $CDCl_3$ signal, 4H), 7.21 – 7.11 (m, 4H), 3.71 (s, 2H), 3.42 (q, *J* = 6.7 Hz, 2H), 2.67 (t, *J* = 7.3 Hz, 2H), 1.79 – 1.62 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.17, 152.03, 141.89, 141.69, 128.22, 128.15, 128.11, 125.62, 124.11, 123.61, 122.71, 116.28, 39.52, 36.80, 35.32, 28.96, 28.50. UPLC/MS (*method A*): Rt 2.61 min. MS (ES) $C_{19}H_{20}N_2O_2$ requires 308, found 309 $[M+H]^+$. HRMS $C_{19}H_{21}N_2O_2$ $[M+H]^+$: calculated 309.1603, measured 309.1598, Δppm -1.6.

Synthesis of *tert*-butyl 3-(2,4-dioxothiazolidin-3-yl)-4-oxo-piperidine-1-carboxylate (10). To a solution of **9** (0.782 g, 1.00 mmol, 1.0 eq.) in dry DMF (5 mL) was added TZD (0.141 g, 1.20 mmol, 1.2 eq.) and K_2CO_3 (0.207 g, 1.50 mmol, 1.5 eq.). The reaction was stirred at rt for 2 h, then, diluted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to afford **10** as an orange oil (0.247 g, 79%). ^1H NMR (400 MHz, $CDCl_3$) δ 4.80 – 4.67 (m, 1H), 4.57 – 4.19 (m, 2H), 4.02 (s, 2H), 3.79-3.58 (m, 1H), 3.30-3.10 (m, 1H), 2.68 – 2.47 (m, 2H), 1.49 (s, 9H). UPLC/MS (*method A*): Rt 1.88 min. MS (ES) $C_{13}H_{18}N_2O_5S$ requires 314, found 313$[M-H]^-$.

Synthesis of *tert*-butyl 2-oxo-3,4,6,7-tetrahydrooxazolo[4,5-*c*]pyridine-5-carboxylate (11). Compound **11** was prepared according to the general procedure H using **10** (0.247 g, 0.79 mmol, 1.0 eq.) and *t*BuOK (0.176 g, 1.57 mmol, 2.0 eq.) in dry THF (8 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **11** as yellow oil (0.055 g, 29%). UPLC/MS (*method A*): Rt 1.64 min. MS (ES) $C_{11}H_{16}N_2O_4$ requires 240, found 241 $[M+H]^+$.

Synthesis of *tert*-butyl 2-oxo-3-(4-phenylbutylcarbamoyl)-6,7-dihydro-4*H*-oxazolo[4,5-*c*]pyridine-5-carboxylate (12a). Compound **12a** was prepared according to the general procedure D (method A) using **11** (0.055 g, 0.23 mmol) and 4-phenylbutyl isocyanate

(0.079 mL, 0.081 g, 0.46 mmol, 2.0 eq.) in dry MeCN (1 mL). The crude was purified by column chromatography (Cy/ EtOAc, 90: 10) to afford **12a** as off-white solid (0.070 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (bs, 1H), 7.31-7.23 (m, overlapped signals with $CDCl_3$, 2H), 7.21 – 7.11 (m, 3H), 4.64 – 4.58 (m, 2H), 3.74-3.66 (m, 2H), 3.35 (q, *J* = 6.7 Hz, 2H), 2.64 (q, *J* = 7.6 Hz, 2H), 2.56-2.46 (m, 2H), 1.78 – 1.58 (m, overlapped with H_2O signal, 4H), 1.48 (s, 9H). UPLC/MS (method A): Rt 2.81 min. MS (ES) $C_{22}H_{29}N_3O_5$ requires 415, found 416 $[M+H]^+$.

Synthesis of 2-oxo-*N*-(4-phenylbutyl)-4,5,6,7-tetrahydrooxazolo[4,5-*c*]pyridine-3-carboxamide hydrochloride (12b). Compound **12b** was prepared according to the general procedure E, using compound **12a** (0.065 g, 0.16 mmol). The crude was triturated with Et_2O to afford **12b** as yellow solid (0.030 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.33 (bs, 2H), 7.82 (t, *J* = 5.6 Hz, 1H), 7.31- 7.24 (m, overlapped with $CDCl_3$ signal, 2H), 7.21 – 7.13 (m, 3H), 4.58 – 4.38 (m, 2H), 3.63 – 3.45 (m, 2H), 3.33 (q, *J* = 6.5 Hz, 2H), 2.99 – 2.87 (m, 2H), 2.65 (t, *J* = 7.3 Hz, 2H), 1.74 – 1.53 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.81, 149.01, 141.92, 133.04, 128.54 (4C), 126.04, 114.29, 40.31, 35.53, 29.06, 28.63, 19.24. MS UPLC/MS (*method A*): Rt 1.83 min. MS (ES) $C_{17}H_{21}N_3O_3$ requires 315, found 316 $[M+H]^+$. HRMS $C_{17}H_{22}N_3O_3$ $[M+H]^+$: calculated 316.1661, measured: 316.1661, Δppm 0.0.

Synthesis of 5-methyl-2-oxo-*N*-(4-phenylbutyl)-6,7-dihydro-4*H*-oxazolo[4,5-*c*]pyridine-3-carboxamide hydrochloride (12c). Compound **12c** was prepared according to the general procedure F, using compound **12b** (0.030 g, 0.09 mmol) and 37 % aqueous solution of formaldehyde (0.005 mL, 0.18 mmol), $NaBH(OAc)_3$ (0.381 g, 1.80 mmol), AcOH (0.008 mL, 0.008 g, 0.14 mmol) in dry MeCN (1.0 mL). The crude was dissolved in DCM (1 mL) followed by the addition of HCl (0.68 mL, 2.70 mmol, 4M in 1,4-dioxane). After evaporation of the solvent, the residue was triturated with Et_2O to afford **12c** as white solid (0.026 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.62 (bs, 1H), 7.80 (t, *J* = 5.5 Hz, 1H), 7.31 - 7.25 (m, overlapped with $CDCl_3$ signal, 2H), 7.21 – 7.14 (m, 3H), 4.71 (d, *J* = 16.0 Hz, 1H), 4.14 – 4.01 (m, 1H), 3.75 – 3.60 (m, 1H), 3.54 – 3.39 (m, 1H), 3.34 (p, *J* = 6.3 Hz, 2H), 3.28 – 3.14 (m, 1H), 2.96 (s, 3H), 2.64 (t, *J* = 7.3 Hz, 2H), 1.74 – 1.51 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.71, 149.00, 141.89, 132.64, 128.52 (4C), 126.04, 113.48, 50.56 (2C), 49.25 (2C), 43.26, 40.32, 35.52, 29.03 (2C), 28.60 (2C), 19.49. UPLC/MS (*method A*): Rt 2.13 min. MS (ES) $C_{18}H_{23}N_3O_3$ requires 329, found 330[M+H]$^+$. HRMS $C_{18}H_{24}N_3O_3$ [M+H]$^+$: calculated 330.1818, measured: 330.182, Δppm 0.6.

Synthesis of 5-benzyl-2-oxo-*N*-(4-phenylbutyl)-6,7-dihydro-4*H*-oxazolo[4,5-c]pyridine-3-carboxamide (12d). Compound **12d** was prepared according to the general procedure F, using compound **12b** (0.050 g, 0.16 mmol), benzaldehyde (0.033 mL, 0.32 mmol), $NaBH(OAc)_3$ (0.054 g, 0.26 mmol), AcOH (0.015 mL, 0.015 g, 0.26 mmol) in dry MeCN (2 mL). The crude was purified by column chromatography (Cy/ EtOAc, 85: 15) to afford **12d** as white solid (0.043 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (bs, 1H), 7.41 – 7.23 (overlapped with $CDCl_3$ signal, m, 7H), 7.21 – 7.13 (m, 3H), 3.85 – 3.64 (m, 4H), 3.32 (q, *J* = 6.8 Hz, 2H), 2.89 – 2.76 (m, 2H), 2.64 (t, J = 7.4 Hz, 2H), 2.58 – 2.43 (m, 2H), 1.82 – 1.52 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.72, 142.06, 128.68 (2C), 128.53 (3C), 128.49 (4C), 125.99, 61.29, 48.43 (2C), 40.07, 35.58, 29.18, 28.67, 21.84. UPLC/MS (*method A*): Rt 1.98 min. MS (ES) $C_{24}H_{27}N_3O_3$ requires 405, found 406 [M+H]$^+$. HRMS $C_{24}H_{28}N_3O_3$ [M+H]$^+$: calculated 406.2131, measured: 406.2126, Δppm -1.2.

Synthesis of 5-acetyl-2-oxo-*N*-(4-phenylbutyl)-6,7-dihydro-4*H*-oxazolo[4,5-*c*]pyridine-3-carboxamide (12e). To a solution of **12b** (0.030 g, 0.09 mmol) in dry DCM (0.9 mL) were added Et_3N (0.025 mL, 0.018 g, 0.18 mmol, 2.0 eq.) and acetyl chloride (0.008 g, 0.10 mmol, 1.1 eq.) at 0 °C. The reaction mixture was stirred at rt for 3 h and, then, was diluted with EtOAc, washed with saturated aqueous NH_4Cl solution, brine and dried over Na_2SO_4. After evaporation of the solvent, the crude was triturated with Et_2O to afford **12e** as white solid (0.018 g, 56%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 – 7.85 (m, 1H), 7.31 – 7.24 (m, overlapped with $CDCl_3$ signal, 2H), 7.21 – 7.13 (m, 3H), 4.87 - 4.58 (m, 2H), 3.96 – 3.64 (m, 2H), 3.36 (q, *J* = 6.6 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.61 – 2.49 (m, 2H), 2.17 (s, 3H), 1.76 - 1.58 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 128.52 (4C), 126.04, 43.33, 40.19, 38.26, 35.56, 29.16, 28.64, 22.23, 21.66. UPLC/MS (*method A*): Rt 2.12 min. MS (ES) $C_{19}H_{23}N_3O_4$ requires 357, found 358 $[M+H]^+$.

Synthesis of *tert*-butyl 4-(2,4-dioxothiazolidin-3-yl)-3-hydroxy-piperidine-1-carboxylate (14a). To a solution of **13** (0.220 g, 1.10 mmol, 1.2 eq.) in dry DMF (2 mL) were added TZD (0.100 g, 0.89 mmol, 1 eq.) and magnesium perchlorate (0.040 g, 0.18 mmol, 0.2 eq.). The reaction mixture was stirred at rt for 20 min, then gradually heated to 115°C over 2 h. After 3 h, the reaction was cooled, diluted with EtOAc, washed with H_2O, brine, 15% LiCl in H_2O and dried over Na_2SO_4 After evaporation of the solvent, the crude was purified by column chromatography (Cy/ EtOAc, 60: 40) to afford **14a** as white solid (0.139 g, 50%). ^1H NMR (400 MHz, DMSO-d_6) δ 5.37 (d, *J* = 3.3 Hz, 1H), 4.13 (d, *J* = 4.7 Hz, 2H), 4.11 – 3.86 (m, 4H), 2.88 – 2.61 (m, 1H), 2.06 (qd, *J* = 12.7, 4.7 Hz, 1H), 1.65 – 1.53 (m, 1H), 1.40 (s, 9H). UPLC/MS (*method A*): Rt 1.77 min, MS (ES) $C_{13}H_{20}N_2O_5S$ requires 316, found 315 $[M-H]^-$.

Synthesis of 3-(1-methyl-3-oxo-4-piperidyl)thiazolidine-2,4-dione (15). To a solution of **14a** (0.100 g, 0.32 mmol) in dry DCM (3 mL), Dess-Martin periodinane (0.300 g, 0.70 mmol, 2.2 eq.) was added portionwise, under argon atmosphere. The reaction was stirred at rt for

16 h, then saturated aqueous $NaHCO_3$ solution was added followed by the addition of 10% Na_2SO_3 in H_2O. The mixture was stirred at rt for 30 min, then, the organic phase was separated and the aqueous layer extracted with DCM (3× times). The collected organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude was purified by column chromatography (Cy/ EtOAc, 75: 15) to afford **15** as white solid (0.070 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.80 (dd, *J* = 12.3, 6.6 Hz, 1H), 4.40 (d, *J* = 18.5 Hz, 1H), 4.24-3.91 (m, 3H), 3.29 – 3.49 (m, 1H), 2.63 (qd, *J* = 12.4, 5.1 Hz, 1H), 2.16 – 2.04 (m, 1H), 1.51 – 1.49 (m, 1H), 1.48 (s, 9H). UPLC/MS (*method A*): Rt 1.83 min, MS (ES) $C_{13}H_{18}N_2O_5S$ requires 314, found 315 $[M+H]^+$.

Synthesis of *tert*-butyl 2-oxo-1,4,6,7-tetrahydrooxazolo[5,4-*c*]pyridine-5-carboxylate (16). Compound **16** was prepared according to the general procedure H, using **15** (0.070 g, 0.22 mmol) and *t*BuOK (0.190 g, 0.89 mmol, 4.0 eq.) in dry THF (2 mL). The crude was used in the next step without further purification. UPLC/MS (*method A*): Rt 1.64 min. MS (ES) $C_{11}H_{16}N_2O_4$ requires 240, found 239 $[M-H]^-$.

Synthesis of *tert*-butyl 2-oxo-1-(4-phenylbutylcarbamoyl)-6,7-dihydro-4*H*-oxazolo[5,4-*c*]pyridine-5-carboxylate (17a). Compound **17a** was prepared according to the general procedure D (method A), using **16** (0.052 g, 0.22 mmol) and 4-phenylbutyl isocyanate (0.039 mL, 0.040 g, 0.22 mmol, 1.0 eq.) in dry MeCN (2 mL). The crude was purified by column chromatography (Cy/ EtOAc, 90: 10) to afford **17a** as white solid (0.023 g, 25% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (bs, 1H), 7.31 – 7.24 (m, overlapped signals with $CDCl_3$, 2H), 7.22 – 7.14 (m, 3H), 4.32 – 4.19 (m, 2H), 3.72 – 3.59 (m, 2H), 3.34 (q, *J* = 6.7 Hz, 2H), 2.99 – 2.86 (m, 2H), 2.64 (t, *J* = 7.3 Hz, 2H), 1.75 – 1.59 (m, 4H), 1.48 (s, 9H). UPLC/MS (*method B*): Rt 1.91 min. MS (ES) $C_{22}H_{29}N_3O_5$ requires 415, found 416 $[M+H]^+$.

Synthesis of 2-oxo-*N*-(4-phenylbutyl)-4,5,6,7-tetrahydrooxazolo[5,4-*c*]pyridine-1-carboxamide hydrochloride (17b). Compound **17b** was prepared according to the general procedure E, using **17a** (0.023 g, 0.055 mmol). After evaporation of the solvent, the crude was triturated with Et_2O to obtain **17b** as white solid (0.012 g, 60%). ^1H NMR (400 MHz, DMSO-d_6) δ 9.96 (bs, 2H), 8.05 (bs, 1H), 7.31 – 7.23 (m, 2H), 7.22 – 7.12 (m, 3H), 4.08 - 3.99 (m, 2H), 4.04 (s, 2H), 3.26 (q, *J* = 6.4 Hz, 2H), 3.01 - 2.93 (m, 2H), 2.59 (t, *J* = 6.9 Hz, 2H), 1.66 – 1.44 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.08, 149.11, 141.97, 128.54 (2C), 126.02, 40.28 (2C), 38.16, 36.03, 35.56, 29.09, 28.66. UPLC/MS (*method A*): Rt 1.88 min. MS (ES) $C_{17}H_{21}N_3O_3$ requires 315, found 316 $[M+H]^+$. HRMS $C_{17}H_{22}N_3O_3$ $[M+H]^+$: calculated 316.1661, measured: 316.1669, Δppm 2.5.

Synthesis of 5-(cyclohexen-1-yl)-2-nitro-phenol (19a). Compound **19a** was prepared according to the general procedure A, using 5-bromo-2-nitrophenol (0.218 g, 1.0 mmol), **18a** (0.229 g, 1.10 mmol), $Pd(PPh_3)_4$ (0.058 g, 0.05 mmol) and 2M Na_2CO_3 (1.30 mL, 2.50 mmol) in degassed 1,4-dioxane (20 mL). The crude was purified by column chromatography (Cy) to afford **19a** as colorless oil (0.200 g, 90%). ^1H NMR (400 MHz, $CDCl_3$) δ 10.66 (s, 1H), 8.01 (d, *J* = 9.0 Hz, 1H), 7.09 (d, *J* = 1.9 Hz, 1H), 7.03 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.39 (tt, *J* = 4.0, 1.6 Hz, 1H), 2.43 – 2.34 (m, 2H), 2.31 – 2.22 (m, 2H), 1.85 – 1.74 (m, 2H), 1.73 – 1.62 (m, 2H). UPLC/MS (*method A*): Rt 1.35 min. MS (ES) $C_{12}H_{13}NO_3$ requires 219, found 220 $[M+H]^+$.

Synthesis of 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-nitro-phenol (19b). Compound **19b** was prepared according to the general procedure A, using 5-bromo-2-nitro-phenol (0.218 g, 1.00 mmol), **18b** (0.231 g, 1.10 mmol), $Pd(PPh_3)_4$ (0.058 g, 0.05 mmol) and 2M Na_2CO_3 (1.30 mL, 2.50 mmol) in degassed 1,4-dioxane (20 mL). The crude was purified by column chromatography (Cy/ EtOAc, 85: 15) to afford **19b** as a white powder (0.123 g, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 7.95 – 7.85 (m, 1H), 7.15 – 7.10 (m, 2H), 6.50 – 6.45 (m, 1H), 4.30- 4.20 (m, 2H), 3.82 (t, *J* = 5.4 Hz, 2H), 2.44- 2.35 (m, 2H). UPLC/MS (*method A*): Rt 0.51 min. MS (ES) $C_{11}H_{11}NO_4$ requires 221, found 220 $[M-H]^-$.

Synthesis of *tert*-butyl 4-(3-hydroxy-4-nitro-phenyl)-3,6-dihydro-2*H*-pyridine-1-carboxylate (19c). Compound **19c** was prepared according to the general procedure A, using 5-bromo-2-nitro-phenol (1.60 g, 7.35 mmol), **18c** (2.50 g, 8.09 mmol), $Pd(PPh_3)_4$ (0.424 g, 0.36 mmol) and 2M Na_2CO_3 (9.2 mL, 18.38 mmol) in degassed 1,4-dioxane (15 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **19c** as a white powder (1.88 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 7.91 (d, *J* = 8.7 Hz, 1H), 7.19 – 7.02 (m, 2H), 6.44 – 6.27 (m, 1H), 4.09 – 3.97 (m, 2H), 3.54 (t, *J* = 5.7 Hz, 2H), 2.48 – 2.41 (m, 2H), 1.43 (s, 9H). UPLC/MS (*method A*): Rt 2.54 min. MS (ES) $C_{16}H_{20}N_2O_5$ requires 320, found 319 $[M-H]^-$.

Synthesis of *tert*-butyl 4-(2-fluoro-5-hydroxy-4-nitro-phenyl)-3,6-dihydro-2H-pyridine-1-carboxylate (19d). Compound **19d** was prepared according to the general procedure A, using 5-bromo-4-fluoro-2-nitro-phenol (0.280 g, 1.18 mmol), **18c** (0.402 g, 1.3 mmol), $Pd(PPh_3)_4$ (0.07 g, 0.06 mmol) and 2M Na_2CO_3 (1.48 mL, 2.95 mmol) in degassed 1,4-dioxane (12 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **19d** as yellow solid (0.267 g, 67%). UPLC/MS (*method A*): Rt 2.45 min. MS (ES) $C_{16}H_{19}FN_2O_5$ requires 338, found 339 $[M+H]^+$.

Synthesis of 2-amino-5-cyclohexyl-phenol (20a). Compound **20a** was prepared according to the general procedure B (method B), using **19a** (0.200 g, 0.91 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 – 6.68 (m, 1H), 6.67 – 6.60 (m, 2H), 2.43 – 2.30 (m, 1H), 1.93 – 1.76 (m, 4H), 1.76 – 1.67 (m, 1H), 1.43 – 1.29 (m, 4H), 1.29 – 1.15 (m, 1H). UPLC/MS (*method A*): Rt 0.98 min. MS (ES) $C_{12}H_{17}NO$ requires 191, found 192 $[M+H]^+$.

Synthesis of 2-amino-5-tetrahydropyran-4-yl-phenol (20b). Compound **20b** was prepared according to the general procedure B (method B), using **19b** (0.123 g, 0.56 mmol). UPLC/MS (*method A*): Rt 0.60 min. MS (ES) $C_{11}H_{15}NO_2$ requires 193, found 194 $[M+H]^+$.

Synthesis of *tert*-butyl 4-(4-amino-3-hydroxy-phenyl)piperidine-1-carboxylate (20c). Compound **20c** was prepared according to general procedure B (method B), using **19c** (0.239 g, 0.75 mmol). UPLC/MS (*method A*): Rt 0.98 min. MS (ES) $C_{16}H_{24}N_2O_3$ requires 292, found 293 $[M+H]^+$.

Synthesis of *tert*-butyl 4-(4-amino-2-fluoro-5-hydroxy-phenyl)piperidine-1-carboxylate (20d). Compound **20d** was prepared according to general procedure B (method B), using **19d** (0.265 g, 0.78 mmol). UPLC/MS (*method D*): Rt 1.51 min. MS (ES) $C_{16}H_{23}FN_2O_3$ requires 310, found 311 $[M+H]^+$.

Synthesis of 6-cyclohexyl-3*H*-1,3-benzoxazol-2-one (21a). Compound **21a** was prepared according to the general procedure C, using **20a** (0.174 g, 0.91 mmol) and CDI (0.295 g, 1.82 mmol) in dry MeCN (9 mL). The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (bs, 1H), 7.09 – 7.06 (m, 1H), 7.04 – 6.89 (m, 2H), 2.61 – 2.44 (m, 1H), 1.96 – 1.80 (m, 4H), 1.81 – 1.62 (m, 1H), 1.47 – 1.31 (m, 4H), 1.31 – 1.16 (m, 1H). UPLC/MS (*method A*): Rt 2.38 min. MS (ES) $C_{13}H_{15}NO_2$ requires 217, found 218 $[M+H]^+$.

Synthesis of 6-tetrahydropyran-4-yl-3*H*-1,3-benzoxazol-2-one (21b). Compound **21b** was prepared according to the general procedure C, using **20b** (0.108 g, 0.56 mmol) and CDI (0.136 g, 0.84 mmol) in dry MeCN (6 mL). The crude was purified by column chromatography (Cy/ EtOAc, 70: 30) to afford **21b** as a white powder (0.089 g, 72% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (s, 1H), 7.23 – 7.18 (m, 1H), 7.05– 6.95 (m, 2H), 4.00– 3.85 (m, 2H), 3.45– 3.35 (m, 2H), 2.80– 2.70 (m, 1H), 1.70– 1.60 (m, 4H). UPLC/MS (*method A*): Rt 1.59 min. MS (ES) $C_{12}H_{13}NO_3$ requires 219, found 220 $[M+H]^+$.

Synthesis of *tert*-butyl 4-(2-oxo-3*H*-1,3-benzoxazol-6-yl)piperidine-1-carboxylate (21c). Compound **21c** was prepared according to the general procedure D, using **20c** (0.219 g, 0.75 mmol) and CDI (0.183 g, 1.13 mmol) in dry MeCN (8 mL). The crude was purified by column chromatography (Cy/ EtOAc, 45: 55) to afford **21c** as a white powder (0.195 g, 82% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.49 (bs, 1H), 7.24 – 7.16 (m, 1H), 7.06 – 6.96 (m, 2H), 4.18 – 3.94 (m, 2H), 2.94 – 2.73 (m, 2H), 2.68 (tt, *J* = 11.9, 3.4 Hz, 1H), 1.78 – 1.70 (m, 2H), 1.55 – 1.43 (m, 2H), 1.42 (s, 9H). UPLC/MS (*method A*): Rt 2.16 min. MS (ES) $C_{17}H_{22}N_2O_4$ requires 318, found 317 $[M-H]^-$.

Synthesis of *tert*-butyl 4-(5-fluoro-2-oxo-3*H*-1,3-benzoxazol-6-yl)piperidine-1-carboxylate (21d). Compound **21d** was prepared according to the general procedure C, using **20d** (0.242 g, 0.78 mmol) and CDI (0.19 g, 1.17 mmol) in dry MeCN (8 mL). The crude was purified by column chromatography (Cy/ EtOAc, 70: 30) to afford **21d** as white solid (0.157 g, 60% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (bs, 1H), 7.29 (d, *J* = 6.0 Hz, 1H), 6.95 (d, *J* = 9.7 Hz, 1H), 4.18 - 3.97 (m, 2H), 2.95 (ddd, *J* = 12.0, 8.7, 3.4 Hz, 1H), 2.90 - 2.67 (m, 2H), 1.74 - 1.63 (m, 2H), 1.54 (qd, *J* = 12.5, 4.1 Hz, 2H), 1.41 (s, 9H). UPLC/MS (*method D*): Rt 2.21 min. MS (ES) $C_{17}H_{21}FN_2O_4$ requires 336, found 337 $[M+H]^+$.

Synthesis of 6-(4-piperidyl)-3*H*-1,3-benzoxazol-2-one;hydrochloric salt (21e). Compound **21e** was prepared according to the general procedure E, using **21c** (0.193 g, 0.61 mmol). The crude was used in the next step without further purification. UPLC/MS (*method A*): Rt 0.91 min. MS (ES) $C_{12}H_{14}N_2O_2$ requires 218, found 219 $[M+H]^+$.

Synthesis of 5-fluoro-6-(4-piperidyl)-3*H*-1,3-benzoxazol-2-one;hydrochloric salt (21f). Compound **21f** was prepared according to the general procedure E, using **21d** (0.157 g, 0.47 mmol). The crude used in the next step without further purification. UPLC/MS (*method D*): Rt 0.37 min. MS (ES) $C_{12}H_{13}FN_2O_2$ requires 236, found 237 $[M+H]^+$.

Synthesis of 6-(1-methyl-4-piperidyl)-3*H*-1,3-benzoxazol-2-one (21g). Compound **21g** was prepared according to the general procedure F, using **21e** (0.155 g, 0.61 mmol), 37 % aqueous solution of formaldehyde (0.03 mL, 1.22 mmol), $NaBH(OAc)_3$ (0.386 g, 1.83 mmol), AcOH (0.07 mL, 0.073 g, 1.22 mmol) in dry MeCN (6 mL). The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.19 – 7.15 (m, 1H), 7.03 – 6.96 (m, 2H), 2.90 – 2.80 (m, 2H), 2.53 – 2.40 (m, overlapped with DMSO signal, 1H), 2.18 (s, 3H), 1.95 (td, J = 11.5, 2.7 Hz, 2H), 1.76 – 1.57 (m, 4H). UPLC/MS (*method A*): Rt 0.91 min. MS (ES) $C_{13}H_{16}N_2O_2$ requires 232, found 233 $[M+H]^+$.

Synthesis of 6-(1-ethyl-4-piperidyl)-3*H*-1,3-benzoxazol-2-one (21h). Compound **21h** was prepared according to the general procedure F, using **21e** (0.254 g, 1.00 mmol), acetaldehyde (0.21 mL, 1.05 mmol, 5 M in THF), $NaBH(OAc)_3$ (0.318 g, 1.6 mmol), AcOH (0.150 g, 2.5 mmol) in dry THF (10 mL). The crude was purified by SCX to afford **21h** as a yellow powder (0.245 g, quant.). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 6.82 (s, 1H), 6.80 (d, *J* = 6.0 Hz, 1H), 6.59 (dd, *J* = 8.5, 2.3 Hz, 1H), 3.04 – 2.97 (m, 4H), 2.52 – 2.43 (m, overlapped with DMSO signal, 5H), 2.35 (q, *J* = 7.2 Hz, 2H), 1.02 (t, *J* = 7.2 Hz, 3H). UPLC/MS (*method A*): Rt 0.98 min. MS (ES) $C_{14}H_{18}N_2O_2$ requires 246, found 247[M+H]$^+$.

Synthesis of 6-(1-isopropyl-4-piperidyl)-3*H*-1,3-benzoxazol-2-one (21i). Compound **21i** was prepared according to the general procedure F, using **21e** (0.254 g, 1.00 mmol), $NaBH(OAc)_3$ (0.318 g, 1.6 mmol), AcOH (0.29 mL, 0.30 g, 5.0 mmol) in acetone (10 mL). The residue was purified by column chromatography (DCM/ MeOH 80: 20) to afford **21i** as a pink powder (0.104 g, 40%). UPLC/MS (*method A*): Rt 0.99 min. MS (ES) $C_{15}H_{20}N_2O_2$ requires 261, found 262 [M+H]$^+$.

Synthesis of 6-(1-isobutyl-4-piperidyl)-3*H*-1,3-benzoxazol-2-one (21j). Compound **21j** was prepared according to the general procedure F, using **21e** (0.254 g, 1.00 mmol), $NaBH(OAc)_3$ (0.318 g, 1.5 mmol), AcOH (0.29 mL, 0.30 g, 5.0 mmol), *iso*-butyraldehyde (0.36 g, 5.0 mmol) in dry MeCN (10 mL). The crude was purified by SCX to afford **21j** as a white solid (0.236 g, 86%). ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 7.16 – 7.13 (m, 1H), 7.02 – 6.93 (m, 2H), 2.95 – 2.85 (m, 2H), 2.53 – 2.43 (m, overlapped with DMSO signal, 1H), 2.05 (d, *J* = 7.4 Hz, 2H), 1.93 (td, *J* = 11.6, 2.5 Hz, 2H), 1.84 – 1.69 (m, 3H), 1.69 – 1.56 (m, 2H), 0.86 (d, *J* = 6.5 Hz, 6H). UPLC/MS (*method A*): Rt 1.23 min. MS (ES) $C_{16}H_{22}N_2O_2$ requires 274, found 275 [M+H]$^+$.

Synthesis of 5-fluoro-6-(1-methyl-4-piperidyl)-3*H*-1,3-benzoxazol-2-one (21k). Compound **21k** was prepared according to the general procedure F, using **21f** (0.123 g, 0.47 mmol) 37 % aqueous solution of formaldehyde (0.03 mL, 0.94 mmol), $NaBH(OAc)_3$ (0.386 g, 1.83 mmol), AcOH (0.054 mL, 0.056 g, 0.94 mmol) in dry MeCN (5 mL). The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 7.24 (d, *J* = 6.03 Hz, 1H), 6.94 (d, *J* = 9.7 Hz, 1H), 2.93 - 2.84 (m, 2H), 2.73 (ddd, *J* = 15.5, 10.1, 4.4 Hz, 1H), 2.22 (s, 3H), 2.02 (td, *J* = 11.3, 3.1 Hz, 2H), 1.79 - 1.60 (m, 4H). UPLC/MS (*method A*): Rt 0.99 min. MS (ES) $C_{13}H_{15}FN_2O_2$ requires 250, found 251 $[M+H]^+$.

Synthesis of 6-cyclohexyl-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22a). Compound **22a** was prepared following the general procedure D (method A), using **21a** (0.169 g, 0.78 mmol) and 4-phenylbutyl isocyanate (0.15 mL, 0.86 mmol) in dry pyridine (8 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **22a** as white solid (0.300 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (t, *J* = 5.4 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 1H), 7.32 - 7.24 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.14 (m, 3H), 7.13 – 7.06 (m, 2H), 3.44 (q, *J* = 6.8 Hz, 2H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.61 – 2.48 (m, 1H), 1.93 - 1.80 (m, 4H), 1.80 – 1.59 (m, 5H), 1.41 (q, *J* = 10.9, 9.8 Hz, 4H), 1.32 - 1.19 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.60, 150.06, 145.57, 142.06, 128.56, 128.52, 126.01, 125.90, 123.62, 115.31, 108.28, 44.64, 40.21, 35.60, 34.78, 29.22, 28.71, 26.91, 26.16. UPLC/MS (*method A*): Rt 2.45 min. MS (ES) $C_{24}H_{28}N_2O_3$ requires 392, found 393 $[M+H]^+$. HRMS $C_{24}H_{29}N_2O_3$ $[M+H]^+$: calculated 393.2178, measured: 393.218, Δppm 0.5.

Synthesis of 2-oxo-*N*-(4-phenylbutyl)-6-tetrahydropyran-4-yl-1,3-benzoxazole-3-carboxamide (22b). Compound **22b** was prepared according to the general procedure D (method A), using **21b** (0.080 g, 0.37 mmol) and 4-phenylbutyl isocyanate (0.07 mL, 0.072 g, 0.41 mmol) in dry MeCN (2 mL). The crude was purified by column chromatography (Cy/ EtOAc, 90: 10) to afford **22b** as a white solid (0.075 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (t, *J* = 5.4 Hz, 1H), 7.97 (d, *J* = 8.2 Hz, 1H), 7.35 – 7.25 (m, overlapped with H_2O signal, 2H), 7.25 – 7.17 (m, 3H), 7.17 – 7.10 (m, 2H), 4.20 – 4.05 (m, 2H), 3.61 – 3.51 (m, 2H), 3.44 (q, *J* = 6.7 Hz, 2H), 2.88 – 2.76 (m, 1H), 2.67 (t, *J* = 7.2 Hz, 2H), 1.88 – 1.68 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.45, 149.96, 143.24, 142.14, 142.03, 128.53, 128.50, 126.01, 123.52, 115.56, 108.24, 68.33, 41.61, 40.23, 35.58, 34.20, 29.19, 28.68. UPLC/MS (*method A*): Rt 2.83 min. MS (ES) $C_{23}H_{26}N_2O_4$ requires 394, found 218 [M-H-CONH$(CH_2)_4$Ph]$^-$. HRMS $C_{23}H_{27}N_2O_4$ $[M+H]^+$: calculated 395.1955, measured: 395.1971, Δppm -4.0.

Synthesis of *tert*-butyl 4-[2-oxo-3-(4-phenylbutylcarbamoyl)-1,3-benzoxazol-6-yl]piperidine-1-carboxylate (22c). Compound **22c** was prepared according to the general procedure D (method A), using **21c** (0.087 g, 0.27 mmol), DMAP (0.037 g, 0.30 mmol), 4-phenylbutyl isocyanate (0.05 mL, 0.053, 0.30 mmol) in dry MeCN (3 mL). The crude was purified by column chromatography (DCM/ MeOH, 90: 10) to afford **22c** as white solid (0.112 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (t, *J* = 5.6 Hz, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.31 – 7.24 (m, overlapped signals with $CDCl_3$, 2H), 7.24 – 7.14 (m, 3H), 7.14 – 7.05 (m, 2H), 4.39 – 4.17 (m, 2H), 3.44 (q, *J* = 6.7 Hz, 2H), 2.80 (t, *J* = 12.3 Hz, 2H), 2.74 – 2.62 (m, 3H), 1.89 – 1.78 (m, 2H), 1.78 – 1.54 (m, overlapped with H_2O signal, 6H), 1.48 (s, 9H). UPLC/MS (*method B*): Rt 2.42 min. MS (ES) $C_{28}H_{35}N_3O_5$ requires 493, found 494 $[M+H]^+$.

Synthesis of 2-oxo-*N*-(4-phenylbutyl)-6-(4-piperidyl)-1,3-benzoxazole-3-carboxamide hydrochloride (22d). Compound **22d** was prepared according to the general procedure E**,** using **22c** (0.112 g, 0.23 mmol). The crude was triturated with Et_2O to afford **22d** as white solid (0.085 g, 86%). ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 9.00 (bs, 2H), 8.11 (t, *J* = 5.8 Hz, 1H), 7.83 (d, *J* = 8.3 Hz, 1H), 7.32 – 7.23 (m, 3H), 7.23 – 7.08 (m, 4H), 3.40 - 3.28 (m, overlapped with H_2O signal, 3H), 3.05 – 2.82 (m, 3H), 2.61 (t, *J* = 7.2 Hz, 2H), 2.02 – 1.77 (m, 4H), 1.68 – 1.49 (m, 4H). ^{13}C NMR (101 MHz, DMSO-*d$_6$*) δ 152.25, 149.26, 142.00, 141.70, 141.21, 128.27, 128.21, 126.64, 125.65, 122.52, 114.48, 108.08, 43.36, 38.67, 34.73, 29.38, 28.59, 28.12. UPLC/MS (*method A*): Rt 2.40 min. MS (ES) $C_{23}H_{27}N_3O_3$ requires 393, found 394 $[M+H]^+$. HRMS $C_{23}H_{28}N_3O_3$ $[M+H]^+$: calculated 394.213, measured: 394.2131, Δppm -0.3.

Synthesis of 6-(1-methyl-4-piperidyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22e). Compound **22e** was prepared according to the general procedure D, using **21g** (0.142 g, 0.61 mmol), and 4-phenylbutyl isocyanate (0.11 mL, 0.118 g, 0.67 mmol) in dry MeCN (3 mL). The crude was purified by column chromatography (DCM/ MeOH, 70: 30) to afford **22e** as a white solid (0.181 g, 73% over three steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (t, J = 5.8 Hz, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.32 (d, J = 1.5 Hz, 1H), 7.30 – 7.24 (m, 2H), 7.23 – 7.13 (m, 4H), 3.38 – 3.25 (m, overlapped with H_2O signal, 2H), 2.85 (d, J = 11.4 Hz, 2H), 2.61 (t, J = 7.3 Hz, 2H), 2.54 – 2.46 (m, overlapped with DMSO signal, 1H), 2.19 (s, 3H), 1.95 (dd, J = 11.4 , 2.8 Hz, 2H), 1.77 – 1.49 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.40, 149.98, 143.59, 142.05, 140.24, 128.55, 128.51, 126.30, 126.01, 123.69, 115.50, 108.32, 56.28, 46.43, 42.04, 40.23, 35.60, 33.65, 29.21, 28.69. UPLC/MS (*method A*): Rt 2.21 min. MS (ES) $C_{24}H_{29}N_3O_3$ requires 407, found 408 $[M+H]^+$. HRMS $C_{24}H_{30}N_3O_3$ $[M+H]^+$: calculated 408.2287, measured: 408.2291, Δppm 1.0.

Synthesis of 6-(1-ethyl-4-piperidyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22f). Compound **22f** was prepared according to the general procedure D (method A), using **21h** (0.100 g, 0.41 mmol) and 4-phenylbutyl isocyanate (0.08 mL, 0.079 g, 0.45 mmol). The crude was purified by column chromatography (DCM/ MeOH, 70: 30) to afford **22f** as yellow solid (0.105 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (t, J = 5.7 Hz, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.32 – 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.21 – 7.15 (m, 3H), 7.15 – 7.10 (m, 2H), 3.44 (q, J = 6.6 Hz, 2H), 3.09 (d, J = 12.0 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 2.61 – 2.51 (m, 1H), 2.47 (q, J = 7.2 Hz, 2H), 2.04 (td, J = 11.7, 2.9 Hz, 2H), 1.92

– 1.76 (m, 4H), 1.76 – 1.60 (m, 4H), 1.13 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.49, 149.97, 143.81, 142.09, 142.04, 128.54 (2C), 128.50, 126.00, 123.69, 115.44, 108.30, 53.89, 52.74, 42.81, 40.21, 35.59, 33.73, 29.20, 28.69, 12.25. UPLC/MS (*method A*): Rt 2.22 min, MS (ES) $C_{25}H_{31}N_3O_3$ requires 421, found 422 [M+H]+, 245 [M-CONH(CH_2)$_4$Ph)]$^-$. HRMS $C_{25}H_{32}N_3O_3$ $[M+H]^+$: calculated 422.2444, measured 422.2449, Δppm 1.2.

Synthesis of 6-(1-isopropyl-4-piperidyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22g). Compound **22g** was prepared according to the general procedure D (method A), using **21i** (0.100 g, 0.38 mmol) and 4-phenylbutyl isocyanate (0.074 g, 0.42 mmol) in dry MeCN (2 mL). The crude was purified by column chromatography (DCM/ MeOH, 70: 30) to afford **22g** as white solid (0.113 g, 68%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (t, *J* = 5.7 Hz, 1H), 7.94 (d, *J* = 8.84 Hz, 1H), 7.31 – 7.24 (m, overlapped with $CDCl_3$ signal, 2H), 7.21 – 7.15 (m, 3H), 7.15 – 7.10 (m, 2H), 3.44 (q, *J* = 6.59 Hz, 2H), 3.09 – 2.96 (m, 2H), 2.85 – 2.72 (m, 1H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.53 (tt, *J* = 12.1, 4.0 Hz, 1H), 2.32 – 2.20 (m, 2H), 1.92 – 1.61 (m, 8H), 1.09 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.50, 149.98, 144.01, 142.08, 128.53 (2C), 128.50, 126.15, 126.00, 123.70, 115.40, 108.32, 54.85, 49.44, 43.11, 40.21, 35.59, 34.12, 29.20, 28.69, 18.55. UPLC/MS (*method A*): Rt 2.25 min, MS (ES) $C_{26}H_{33}N_3O_3$ requires 435, found 436 $[M+H]^+$. HRMS $C_{26}H_{34}N_3O_3$ $[M+H]^+$: calculated 436.2603, measured 436.26, Δppm 0.6.

Synthesis of 6-(1-isobutyl-4-piperidyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22h). Compound **22h** was prepared according to the general procedure D (method A), using **21j** (0.10 g, 0.36 mmol) and 4-phenylbutyl isocyanate (0.07 mL, 0.07 g, 0.4 mmol). The residue was purified by column chromatography (Cy/ EtOAc, 75: 25) to afford **22h** as a white powder (0.115 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (t, *J* = 5.7 Hz, 1H), 7.94 (d, *J* = 8.86 Hz, 1H), 7.32 – 7.24 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.15 (m, 3H), 7.15 – 7.07 (m, 2H), 3.44 (q, *J* = 6.5 Hz, 2H), 3.08 – 2.92 (m, 2H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.60 – 2.46 (m, 1H), 2.19 – 2.07 (m, 2H), 2.07 – 1.93 (m, 2H), 1.90 – 1.50 (m, 9H), 0.92 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.54, 150.01, 142.66, 142.09, 128.55 (2C), 128.52, 126.01, 123.70, 115.40, 108.35, 67.38, 54.75, 42.87, 40.23, 35.60, 33.83, 29.22, 28.71, 25.79, 21.21 (2C). UPLC/MS (*method A*): Rt 2.43 min, MS (ES) $C_{27}H_{35}N_3O_3$ requires 449, found 450 $[M+H]^+$. HRMS $C_{27}H_{36}N_3O_3$ $[M+H]^+$: calculated 450.2756, measured 450.2757, Δppm -0.2.

Synthesis of 6-(1-acetyl-4-piperidyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22i). To a solution of **22d** (0.184 g, 0.47 mmol, 1.0 eq.) in dry THF (5 mL) was added Et_3N (0.10 g, 0.98 mmol, 2.0 eq.) dropwise at 0°C, followed by the addition of AcCl (0.039 g, 0.49 mmol, 1.05 eq.). The reaction mixture was stirred at rt for 4h and, then, diluted with EtOAc, washed with saturated aqueous NH_4Cl solution, brine and dried over $NaSO_4$. After evaporation of the solvent, the crude was purified by column chromatography (DCM/ MeOH, 90: 10) to afford **22i** as a white powder (0.181 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (t, *J* = 5.6 Hz, 1H), 7.97 (d, *J* = 8.2 Hz, 1H), 7.31 – 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.21 – 7.14 (m, 3H), 7.12 – 7.05 (m, 2H), 4.90 – 4.70 (m, 1H), 4.05 – 3.84 (m, 1H), 3.43 (q, *J* = 6.6 Hz, 2H), 3.28 – 3.07 (m, 1H), 2.79 (tt, *J* = 12.1, 3.6 Hz, 1H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.65 – 2.55 (m,1H), 2.13 (s, 3H), 1.99 – 1.83 (m, 2H), 1.80 – 1.49 (m, overlapped with H_2O signal, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.02, 149.90, 144.12, 142.54, 142.02, 128.53 (2C), 128.45, 126.56, 126.02, 123.53, 115.66, 108.24, 47.01, 42.78, 42.17, 40.24, 35.58, 29.19, 28.69, 21.64. UPLC/MS (*method A*): Rt 2.48 min, MS (ES)

$C_{25}H_{29}N_3O_4$ requires 435, found 436 $[M+H]^+$. HRMS $C_{25}H_{30}N_3O_4$ $[M+H]^+$: calculated 436.2244, measured 436.2236, Δppm 1.8.

Synthesis of *N*-(2-benzyloxyethyl)-6-(1-methyl-4-piperidyl)-2-oxo-1,3-benzoxazole-3-carboxamide (22j). Compound **22j** was prepared according to the general procedure D (method C), using **21g** (0.080 g, 0.34 mmol), 2-(benzyloxy)-1-ethanamine (0.056 g, 0.37 mmol) in dry MeCN (3 mL). The crude was purified by column chromatography (DCM/ MeOH, 94: 6) to afford **22j** as a white solid (0.033 g, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (bs, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.40 – 7.30 (m, 4H), 7.29 – 7.23 (m, overlapped with $CDCl_3$ signal, 1H), 7.16 – 7.08 (m, 2H), 4.57 (s, 2H), 3.71 – 3.60 (m, 4H), 3.06 – 2.94 (m, 2H), 2.59 – 2.48 (m, 1H), 2.35 (s, 3H), 2.09 (td, *J* = 11.4, 3.5 Hz, 2H), 1.92 – 1.74 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.27, 150.07, 143.59, 128.61 (2C), 127.93 (2C), 127.90, 126.26, 123.63, 115.42, 108.33, 73.39, 68.32, 56.29 46.46, 42.05, 40.32, 33.68. UPLC/MS (*method A*): Rt 1.92 min, MS (ES) $C_{23}H_{27}N_3O_4$ requires 409, found 410 $[M+H]^+$. HRMS $C_{23}H_{28}N_3O_4$ $[M+H]^+$: calculated 410.208, measured 410.2087, Δppm 1.7.

Synthesis of 6-(1-methyl-4-piperidyl)-2-oxo-*N*-pentyl-1,3-benzoxazole-3-carboxamide (22k). Compound **22k** was prepared according to the general procedure D (method A), using **21g** (0.050 g, 0.22 mmol) and pentyl isocyanate (0.031 mL, 0.027g, 0.24 mmol) in dry MeCN (1 mL). The crude was purified by column chromatography (DCM/ MeOH, 95: 5) to afford **22k** as a white solid (0.039 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (t, *J* = 5.8 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.16 – 7.07 (m, 2H), 3.41 (q, *J* = 7.0 Hz, 2H), 3.08 – 2.92 (m, 2H), 2.62 – 2.45 (m, 1H), 2.35 (s, 3H), 2.09 (td, *J* = 11.3, 3.8 Hz, 2H), 1.91 – 1.74 (m, 4H), 1.69 – 1.57 (m, 2H), 1.44 – 1.29 (m, 4H), 0.98 – 0.85 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.50, 149.96, 143.55, 126.32, 123.68, 115.50, 108.30, 56.28, 46.44, 42.03, 40.40, 33.66, 29.27, 29.11, 22.45, 14.09. UPLC/MS (*method A*): Rt 2.00 min, MS (ES) $C_{19}H_{27}N_3O_3$ requires 345, found 346 $[M+H]^+$. HRMS $C_{19}H_{28}N_3O_3$ $[M+H]^+$: calculated 346.2131, measured 346.2116, Δppm. -4.3.

Synthesis of *N*-(2-ethoxyethyl)-6-(1-methyl-4-piperidyl)-2-oxo-1,3-benzoxazole-3-carboxamide (22l). Compound **22l** was prepared according to the general procedure D (method C), using **21g** (0.050 g, 0.22 mmol) and 2-ethoxyethylamine (0.021 g, 0.24 mmol) in dry MeCN (2 mL). The crude was purified by column chromatography (DCM/ MeOH, 92: 8) to afford **22l** as a white solid (0.030 g, 39%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29 (bs, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 7.14 – 7.08 (m, 2H), 3.63 – 3.59 (m, 4H), 3.54 (q, *J* = 7.0 Hz, 2H), 3.04 – 2.94 (m, 2H), 2.59 – 2.47 (m, 1H), 2.33 (s, 3H), 2.07 (td, *J* = 11.5, 3.3 Hz, 2H), 1.90 – 1.72 (m, 4H), 1.23 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.26, 150.06, 143.10, 142.13, 126.36, 123.63, 115.46, 108.35, 68.64, 66.77, 56.11, 46.13, 41.78, 40.33, 33.24, 15.23. UPLC/MS (*method A*): Rt 1.57 min, MS (ES) $C_{18}H_{25}N_3O_4$ requires 347, found 348 $[M+H]^+$. HRMS $C_{18}H_{26}N_3O_4$ $[M+H]^+$: calculated 348.1923, measured 348.1921, Δppm. 0.6.

Synthesis of *N*-isobutyl-6-(1-methyl-4-piperidyl)-2-oxo-1,3-benzoxazole-3-carboxamide (22m). Compound **22m** was prepared according to the general procedure D (method B), using **21g** (0.404 g, 1.74 mmol) and isobutylamine (0.382 g, 5.22 mmol) in dry DCM (20 mL). The crude was purified by column chromatography (DCM/ MeOH, 92: 8) to afford **22m** as a white solid (0.259 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (t, *J* = 5.9 Hz, 1H), 7.94 (d, *J* = 8.9 Hz, 1H), 7.16 – 7.05 (m, 2H), 3.24 (t, *J* = 6.4 Hz, 2H), 3.05 – 2.93 (m, 2H), 2.59 – 2.45 (m, 1H), 2.33 (s, 3H), 2.07 (td, *J* = 11.4, 3.4 Hz, 2H), 1.97 – 1.86 (m, 1H), 1.86 – 1.72 (m, 4H), 0.96 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.51, 150.06, 143.58, 142.08, 126.29, 123.65, 115.47, 108.27, 56.27, 47.66, 46.45, 42.03, 33.68, 28.58, 20.14.

UPLC/MS (*method A*): Rt 1.82 min, MS (ES) $C_{18}H_{25}N_3O_3$ requires 331, found 332 $[M+H]^+$. HRMS $C_{18}H_{26}N_3O_3$ $[M+H]^+$: calculated 332.1974, measured 332.1969, Δppm. -1.5.

Synthesis of 6-(1-methyl-4-piperidyl)-2-oxo-*N*-*sec*-butyl-1,3-benzoxazole-3-carboxamide (22n). Compound **22n** was prepared according to the general procedure D (method C), using **21g** (0.08g, 0.34 mmol) and *sec*-butylamine (0.027g, 0.37 mmol) in dry MeCN (1 mL). The crude was purified by column chromatography (DCM/ MeOH, 95: 5) to afford **22n** as white solid (0.029 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.14 - 7.08 (m, 2H), 4.07 – 3.83 (m, 1H), 3.07 - 2.94 (m, 2H), 2.60 – 2.46 (m, 1H), 2.35 (s, 3H), 2.09 (td, *J* = 11.4, 3.3 Hz, 2H), 1.89 - 1.75 (m, 4H), 1.61 (p, *J* = 7.3, 6.9 Hz, 2H), 1.26 (d, *J* = 6.6 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.92, 154.18, 149.39, 143.50, 126.36, 123.65, 115.51, 108.27, 56.28, 48.18, 46.45, 42.02, 33.67, 29.63, 20.49, 10.41. UPLC/MS (*method A*): Rt 1.83 min. MS (ES) $C_{18}H_{25}N_3O_3$ requires 331, found 332 $[M+H]^+$. HRMS $C_{18}H_{26}N_3O_3$ $[M+H]^+$: calculated 332.1974, measured: 332.1967, Δppm -2.1.

Synthesis of *N*-[4-(4-fluorophenyl)butyl]-6-(1-methyl-4-piperidyl)-2-oxo-1,3-benzoxazole-3-carboxamide (22o). Compound **22o** was prepared according to the general procedure D (method C), using **21g** (0.085g, 0.51 mmol) and 4-fluoro-benzenebutanamine, (0.085g, 0.51 mmol) in dry MeCN (2 mL). The crude was purified by column chromatography (DCM/ MeOH, 87: 13) to afford **22o** as white solid (0.042 g, 29%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (t, *J* = 5.5 Hz, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.19 – 7.06 (m, 4H), 7.01 – 6.89 (m, 2H), 3.43 (q, *J* = 6.5 Hz, 2H), 3.06 - 2.95 (m, 2H), 2.63 (t, *J* = 7.0 Hz, 2H), 2.59 - 2.47 (m, 1H), 2.34 (s, 3H), 2.08 (td, *J* = 11.4, 3.2 Hz, 2H), 1.90 - 1.76 (m, 4H), 1.74 – 1.56 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.41 (d, $J_{C\text{-}F}$ = 243.5 Hz), 153.45, 149.96, 143.29, 142.08, 137.61 (d, J_{C-F} = 3.2 Hz), 129.82 (d, J_{C-F} = 7.7 Hz), 126.30, 123.70, 115.48, 115.22 (d, J_{C-F} = 21.0 Hz), 108.33, 56.14, 46.21, 41.84, 40.14, 34.75, 33.36, 29.10, 28.81. UPLC/MS (*method A*): Rt 2.20 min. MS (ES) $C_{24}H_{28}FN_3O_3$ requires 425, found 426 $[M+H]^+$. HRMS $C_{24}H_{29}FN_3O_3$ $[M+H]^+$: calculated 426.2193, measured: 426.2188, Δppm -1.2.

Synthesis of 5-fluoro-6-(1-methyl-4-piperidyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22p). Compound **22p** was prepared according to the general procedure D (method A), using **21k** (0.117 g, 0.47 mmol) and 4-phenylbutyl isocyanate (0.088 mL, 0.091 g, 0.52 mmol) in dry MeCN (5 mL). The crude was purified by column chromatography (DCM/ MeOH, 92: 8) to afford **22p** as white solid (0.099 g, 50% over three steps). ^1H NMR (600 MHz, $CDCl_3$) δ 7.98 (t, *J* = 5.3 Hz, 1H), 7.77 (d, *J* = 9.9 Hz, 1H), 7.31 - 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.14 (m, 3H), 7.11 (d, *J* = 5.8 Hz, 1H), 3.43 (q, *J* = 6.6 Hz, 2H), 3.05-2.97 (m, 2H), 2.95 – 2.84 (m, 1H), 2.66 (t, *J* = 7.4 Hz, 2H), 2.35 (s, 3H), 2.17 (td, *J* = 11.5, 2.9 Hz, 2H), 1.89 – 1.78 (m, 4H), 1.75 – 1.58 (m, overlapped with H_2O signal, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.38 (d, J_{C-F} = 241.2 Hz), 153.43, 149.58, 141.98, 138.08 (d, J_{C-F} = 2.2 Hz), 129.37 (d, *J* = 17.7 Hz), 128.53, 128.51, 126.42 (d, *J* = 14.5 Hz), 126.02, 108.44 (d, J_{C-F} = 5.6 Hz), 103.97 (d, J_{C-F} = 33.4 Hz), 56.08, 46.27, 40.26, 35.56, 34.29, 32.05, 29.13, 28.66. UPLC/MS (*method A*): Rt 2.20 min. MS (ES) $C_{24}H_{28}FN_3O_3$ requires 425, found 426 $[M+H]^+$. HRMS $C_{24}H_{29}FN_3O_3$ $[M+H]^+$: calculated 426.2193, measured: 426.2191, Δppm -0.5.

Synthesis of (6-oxo-2,3-dihydro-1*H*-pyridin-4-yl) trifluoromethanesulfonate (29a). To a solution of **28** (0.400 g, 3.54 mmol, 1.0 eq.) in dry THF (30 mL) were added at 0°C under stirring Et_3N (0.716 g, 7.08 mmol, 2.0 eq.) and *N,N*-bis(trifluoromethylsulfonyl)aniline (1.388 g, 4.24 mmol, 1.2 eq.) dissolved in dry THF (6 mL). The reaction mixture was slowly warmed to rt and stirred for 16 h. The mixture was diluted with EtOAc, washed with a saturated aqueous NH_4Cl solution and dried over Na_2SO_4. After evaporation of the solvent, the crude was purified by column chromatography (DCM/ MeOH, 94: 6) to afford **29a** as a white solid (0.640 g, 74%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.79 (s, 1H), 5.95 – 5.93 (m, 1H), 3.37 (td, *J* = 7.1, 2.7 Hz, 2H), 2.72 (td, *J* = 7.2, 1.4 Hz, 2H). UPLC/MS (*method A*): Rt 1.47 min, MS (ES) $C_6H_6F_3NO_4S$ requires 245, found 246 $[M+H]^+$.

Synthesis of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydro-1*H*-pyridin-6-one (29b). To a stirred solution of compound **29a** (0.100 g, 0.41 mmol, 1.0 eq.) in degassed dioxane (4 mL), were added ($[B_2(pin)_2]$) (0.124 g, 0.49 mmol, 1.2 eq.), Pd(dppf)Cl_2 (0.058 g, 0.08 mmol, 0.2 eq.) and KOAc (0.080 g, 0.82 mmol, 2.0 eq.). The reaction mixture was stirred at 70 °C for 90 min and, then, cooled to rt and used directly in the next step. UPLC/MS (*method A*): Rt 0.44 min, MS (ES) $C_{11}H_{18}BNO_3$ requires 223, found 141 $[M-(CH_3)_2CC(CH_3)_2]^+$.

Synthesis of 4-(3-benzyloxy-4-nitro-phenyl)-2,5-dihydro-1*H*-pyridin-6-one (30a). Compound **30a** was prepared according to the general procedure A, using **29b** (0.091 g, 0.41 mmol), 2-benzyloxy-4-bromo-1-nitro-benzene (0.138 g, 0.45 mmol), $Pd(PPh_3)_4$ (0.023 g, 0.02 mmol) and 2M Na_2CO_3 (0.51 mL, 1.025 mmol) in degassed 1,4-dioxane (10 mL). The crude was purified by column chromatography (DCM/ MeOH, 90: 10) to afford **30a** as a brown powder (0.124 g, 93% over two steps). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.93 (d, *J* = 8.5 Hz, 1H), 7.61 (d, *J* = 1.7 Hz, 1H), 7.60 (bs, 1H), 7.50 – 7.47 (m, 2H), 7.46 – 7.40 (m, 2H), 7.39 – 7.33 (m, 2H), 6.34 (q, *J* = 1.5 Hz, 1H), 5.42 (s, 2H), 3.39 (td, *J* = 7.0, 2.6 Hz, 2H), 2.74 (td, *J* = 7.0, 1.5 Hz, 2H). UPLC/MS (*method A*): Rt 1.80 min, MS (ES) $C_{18}H_{16}N_2O_4$ requires 324, found 325 $[M+H]^+$.

Synthesis of 4-(3-benzyloxy-4-nitro-phenyl)-1-methyl-2,3-dihydropyridin-6-one (30b). To a stirred solution of **30a** (0.124 g, 0.38 mmol, 1.0 eq.) in dry THF (4 mL) was added NaH (0.018 g, 60% in mineral oil, 0.46 mmol, 1.2 eq.) at 0°C under stirring. After 30 min, CH_3I (0.047 mL, 0.76 mmol, 2.0 eq.) was added and the mixture was slowly warmed to rt. After 5 h, saturated aqueous NH_4Cl solution was added and the mixture extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. The crude was purified by column chromatography (DCM/ EtOAc, 70: 30) to afford **30b** as a brown solid (0.058 g, 45%). ^1H NMR (400 MHz, DMSO-d_6) δ 7.94 (d, J = 8.5 Hz, 1H), 7.63 (d, J = 1.8 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.46 – 7.39 (m, 2H), 7.40 – 7.32 (m, 2H), 6.41 (t, J = 1.4 Hz, 1H), 5.42 (s, 2H), 3.53 (t, J = 7.1 Hz, 2H), 2.92 (s, 3H), 2.83 (td, J = 7.2, 1.4 Hz, 2H). UPLC/MS (*method A*): Rt 1.89 min, MS (ES) $C_{19}H_{18}N_2O_4$ requires 338, found 339 $[M+H]^+$.

Synthesis of 4-(4-amino-3-hydroxy-phenyl)-1-methyl-piperidin-2-one (31). Compound **31** was prepared according to general procedure B (method B), using **30b** (0.056 g, 0.16 mmol). UPLC/MS (*method A*): Rt 1.05 min, MS (ES) $C_{12}H_{16}N_2O_2$ requires 220, found 221 $[M+H]^+$.

Synthesis of 6-(1-methyl-2-oxo-4-piperidyl)-3H-1,3-benzoxazol-2-one (21l). Compound **21l** was prepared according to the general procedure C, using **31** (0.035 g, 0.16 mmol) and CDI (0.039 g, 0.24 mmol) in dry MeCN (2 mL). The crude was purified by column chromatography (DCM/ MeOH, 95: 5) to afford **21l** as a white powder (0.028 g, 70% over two steps). ^1H NMR (400 MHz, DMSO-d_6) δ 7.26 – 7.23 (m, 1H), 7.08 – 6.96 (m, 2H), 3.45 – 3.23 (m, 2H), 3.14 – 3.03 (m, 1H), 2.85 (s, 3H), 2.49 – 2.28 (m, 2H), 2.06 – 1.76 (m, 2H). UPLC/MS (*method A*): Rt 1.17 min, MS (ES) $C_{13}H_{14}N_2O_3$ requires 246, found 247 $[M+H]^+$.

Synthesis of 6-(1-methyl-2-oxo-4-piperidyl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22q). Compound **22q** was prepared according to the general procedure D (method A), using **21l** (0.025 g, 0.10 mmol), 4-phenylbutyl isocyanate (0.019 mL, 0.019 g, 0.11 mmol) in dry MeCN (1 mL). The crude was purified by column chromatography (DCM/ EtOAc, 70: 30) to afford **22q** as a white solid (0.038 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 7.95 (m, 2H), 7.33 – 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.13 (m, 3H), 7.13 – 7.05 (m, 2H), 3.51 – 3.38 (m, 3H), 3.38 – 3.28 (m, 1H), 3.21 – 3.08 (m, 1H), 2.99 (s, 3H), 2.79 – 2.68 (m, 1H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.46 (dd, *J* = 17.3, 11.1 Hz, 1H), 2.19 – 2.08 (m, 1H), 2.07 – 1.91 (m, 1H), 1.80 – 1.67 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.96, 149.85, 142.26, 142.01, 140.73, 128.53 (2C), 126.87, 126.02, 123.31, 115.86, 108.10, 100.13, 49.07, 40.27, 39.61, 38.89, 35.59, 34.65, 30.45, 29.19, 28.68. UPLC/MS (*method A*): Rt 2.09 min, MS (ES) $C_{24}H_{27}N_3O_4$ requires 421, found 422 $[M+H]^+$. HRMS $C_{24}H_{28}N_3O_4$ $[M+H]^+$: calculated 422.208, measured 422.2074, Δppm -1.4.

Synthesis of 6-[1-(2,2-difluoroethyl)-4-hydroxy-4-piperidyl]-3*H*-1,3-benzoxazol-2-one (43). Compound **43** was prepared according to the general procedure I, using 6-bromo-3*H*-1,3-benzoxazol-2-one (0.150 g, 0.7 mmol), **42a** (0.171 g, 1.05 mmol), MeMgBr (0.35 mL, 0.125 g, 1.05 mmol, 3M in Et_2O), *n*-BuLi (0.336 mL, 0.84 mmol, 2.5M in hexanes) in dry THF (7 mL). The crude was purified by column chromatography (DCM/ MeOH, 98: 4) to afford **43** as a white solid (0.063 g, 30%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.37 (d, *J* = 1.4 Hz, 1H), 7.26 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 6.14 (tt, *J* = 55.9, 4.3 Hz, 1H), 4.88 (bs, 1H), 2.82 – 2.68 (m, 4H), 2.63 (td, *J* = 11.6, 2.4 Hz, 2H), 1.93 (td, *J* = 12.8, 4.7 Hz, 2H), 1.57 (dd, *J* = 13.8, 2.5 Hz, 2H). UPLC/MS (*method A*): Rt 1.11 min, MS (ES) $C_{14}H_{16}F_2N_2O_3$ requires 298, found 299 $[M+H]^+$.

Synthesis of 6-[1-(2,2-difluoroethyl)-3,6-dihydro-2*H*-pyridin-4-yl]-3H-1,3-benzoxazol-2-one (44). Compound **44** was prepared according to general procedure L, using **43** (0.033 g, 0.11 mmol). The crude was purified by SCX to afford **44** as pale brown solid (0.031 g, quant.). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.57 (bs, 1H), 7.38 (d, *J* = 1.6 Hz, 1H), 7.22 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.18 (tt, *J* = 55.8, 4.3 Hz, 1H), 6.10 (td, *J* = 3.5, 1.7 Hz, 1H), 3.27 – 3.19 (m, 2H), 2.92 – 2.74 (m, 4H), 2.49 – 2.41 (m, 2H). UPLC/MS (*method A*): Rt 1.56 min, MS (ES) $C_{14}H_{14}F_2N_2O_2$ requires 280, found 281 [M+H]$^+$.

Synthesis of 6-[1-(2,2-difluoroethyl)-4-piperidyl]-3*H*-1,3-benzoxazol-2-one (21m). Compound **21m** was prepared according to the general procedure B (method B), using **44** (0.028 g, 0.1 mmol). The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.46 (bs, 1H), 7.20 (d, *J* = 1.4 Hz, 1H), 7.08 – 6.95 (m, 2H), 6.14 (tt, *J* = 55.8, 4.4 Hz, 1H), 3.04 – 2.95 (m, 2H), 2.75 (td, *J* = 15.7, 4.4 Hz, 2H), 2.26 (td, *J* = 11.6, 2.8 Hz, 2H), 1.80 – 1.56 (m, 4H). UPLC/MS (*method A*): Rt 1.50 min, MS (ES) $C_{14}H_{16}F_2N_2O_2$ requires 282, found 283 [M+H]$^+$.

Synthesis of 6-[1-(2,2-difluoroethyl)-4-piperidyl]-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (22r). Compound **22r** was prepared according to the general procedure D (method A), using **21m** (0.028 g, 0.1 mmol) and 4-phenylbutyl isocyanate (0.019 mL, 0.019 g, 0.11 mmol) in dry MeCN (2.0 mL). The crude was purified by column chromatography (DCM/ EtOAc, 90: 10) to afford **22r** as a white solid (0.033 g, 73% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (t, *J* = 5.7 Hz, 1H), 7.95 (d, *J* = 8.3 Hz, 1H), 7.32 – 7.24 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.15 (m, 3H), 7.15 – 7.08 (m, 2H), 5.92 (tt, J = 56.0, 4.3 Hz, 1H), 3.44 (q, *J* = 6.6 Hz, 2H), 3.07 (d, *J* = 11.6 Hz, 2H), 2.79 (td, *J* = 15.0, 4.3 Hz, 2H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.61 – 2.49 (m, 1H), 2.34 (td, *J* = 11.3, 3.5 Hz, 2H), 1.91 – 1.78 (m, 4H), 1.78 – 1.66 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.47, 150.61, 149.97, 143.39, 142.04, 128.54 (2C), 128.49, 126.02, 123.62, 115.52 (2C), 108.28, 60.57 (t, J_{C-F} = 24.9 Hz), 55.07, 42.14, 40.24, 35.59, 33.63, 29.20, 28.69. UPLC/MS (*method A*): Rt 1.82 min, MS (ES) $C_{25}H_{29}F_2N_3O_3$ requires 457, found 458 $[M+H]^+$. HRMS $C_{25}H_{30}F_2N_3O_3$ $[M+H]^+$: calculated 458.2255, measured 458.2258, Δppm 0.7.

Synthesis of 2-nitro-5-(1-piperidyl)phenol (49a). Compound **49a** was prepared according to the general procedure G, using 5-fluoro-2-nitro-phenol (0.150 g, 0.95 mmol), **48a** (0.265 g, 1.43 mmol) and DIPEA (0.33 mL, 0.25 g, 1.90 mmol) in dry MeCN (8 mL) under MW irradiation. Saturated aqueous NH_4Cl solution was added and the aqueous phase was extracted with DCM. The organic layers were collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.31 (s, 1H), 7.93 (d, J = 9.7 Hz, 1H), 6.45 (dd, J = 9.7, 2.7 Hz, 1H), 6.31 (d, J = 2.7 Hz, 1H), 3.47 (d, J = 5.8 Hz, 4H), 1.70 (s, 6H). UPLC/MS (*method A*): Rt 2.45 min. MS (ES) $C_{11}H_{14}N_2O_3$ requires 222, found 223 $[M+H]^+$.

Synthesis of 5-(1,1-dioxo-1,4-thiazinan-4-yl)-2-nitro-phenol (49c). Compound **49c** was prepared according to the general procedure G, using 5-fluoro-2-nitrophenol (0.470 g, 3.00 mmol), **48c** (0.811 g, 6.00 mmol), DIPEA (1.05 mL, 0.775 g, 6.00 mmol) in MeCN (15 mL), heating at reflux for 16 h. The crude was purified by column chromatography (EtOAc) to afford **49c** as a yellow powder (0.33 g, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (bs, 1H), 7.91 (d, J = 9.6 Hz, 1H), 6.73 (dd, J = 9.6, 2.8 Hz, 1H), 6.59 (d, J = 2.8 Hz, 1H), 4.02 - 3.94 (m, 4H), 3.20- 3.14 (m, 4H). UPLC/MS (*method A*): Rt 1.49 min. MS (ES) $C_{10}H_{12}N_2O_5S$ requires 272, found 273 $[M+H]^+$.

Synthesis of *tert*-butyl 4-(3-hydroxy-4-nitro-phenyl)piperazine-1-carboxylate (49d). Compound **49d** was prepared according to the general procedure G, using 5-fluoro-2-nitrophenol (2.00 g, 12.73 mmol), **48d** (3.56 g, 19.09 mmol) and DIPEA (2.47 g, 19.1 mmol) in MeCN (25 mL), heating at reflux for 16 h. The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (bs, 1H), 7.88 (d, *J* = 9.7 Hz, 1H), 6.64 (dd, *J* = 9.7, 2.7 Hz, 1H), 6.42 (d, J = 2.7 Hz, 1H), 3.56 – 3.40 (m, 8H), 1.42 (s, 9H). UPLC/MS (*method A*): Rt 2.36 min, MS (ES) $C_{15}H_{21}N_3O_5$ requires 323, found 324 $[M+H]^+$.

Synthesis of 2-amino-5-(1-piperidyl)phenol (49e). Compound **49e** was prepared according to the general procedure B (method A), using **49a** (0.130 g, 0.59 mmol). After evaporation of the solvent, the crude was used in the next step without purification. UPLC/MS (*method A*): Rt 0.94 min. MS (ES) $C_{11}H_{16}N_2O$ requires 192, found 193 $[M+H]^+$.

Synthesis of 2-amino-5-morpholino-phenol (49f). Compound **49f** was prepared according to the general procedure B (method A), using the commercially available **49b** (0.224 g, 1.0 mmol). After evaporation of the solvent, the crude was used in the next step without purification. UPLC/MS (*method A*): Rt 1.18 min. MS (ES) $C_{10}H_{14}N_2O_2$ requires 194, found 195 $[M+H]^+$.

Synthesis of 2-amino-5-(1,1-dioxo-1,4-thiazinan-4-yl)phenol (49g). Compound **49g** was prepared according to the general procedure B (method A), using **49c** (0.272 g, 1.00 mmol). After evaporation of the solvent, the crude was used in the next step without purification. UPLC/MS (*method A*): Rt 0.52 min. MS (ES) $C_{10}H_{14}N_2O_3S$ requires 242, found 243 $[M+H]^+$.

Synthesis of *tert*-butyl 4-(4-amino-3-hydroxy-phenyl)piperazine-1-carboxylate (49h). Compound **49h** was prepared according to the general procedure B (method B), using **49d**

(4.11 g, 12.73 mmol). After evaporation of the solvent, the crude was used in the next step without purification. UPLC/MS (*method A*): Rt min. MS (ES) $C_{15}H_{23}N_3O_3$ requires 293, found 294 $[M+H]^+$.

Synthesis of 6-(1-piperidyl)-3*H*-1,3-benzoxazol-2-one (50a). Compound **50a** was prepared according to the general procedure C, using **49e** (0.110 g, 0.58 mmol) and CDI (0.141 g, 0.87 mmol) in dry MeCN (6 mL). The pink solid was triturated with Et_2O to afford **50a** as a pinkish solid (0.165 g, 80% over three steps). ^1H NMR (400 MHz, DMSO-d_6) δ 11.26 (bs, 1H), 6.93 (d, *J* = 2.2 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.70 (dd, J = 2.3, 8.5 Hz, 1H), 3.08 – 2.93 (m, 4H), 1.61 (p, J = 5.7 Hz, 4H), 1.50 (p, J = 5.7 Hz, 2H). UPLC/MS (*method A*): Rt 2.38 min. MS (ES) $C_{12}H_{14}N_2O_2$ requires 218, found 219 $[M+H]^+$.

Synthesis of 6-morpholino-3*H*-1,3-benzoxazol-2-one (50b). Compound **50b** was prepared according to the general procedure C, using **49f** (0.194 g, 1.00 mmol) and CDI (0.243 g, 1.50 mmol) in dry MeCN (10 mL). The crude was purified by column chromatography (Cy/ EtOAc, 70: 30) to afford **50b** as a pink powder (0.132 g, 60%, over two steps). ^1H NMR (400 MHz, DMSO-d_6) δ 11.31 (bs, 1H), 6.98 (d, *J* = 2.3 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.72 (dd, *J* = 8.6, 2.3 Hz, 1H), 3.81 – 3.65 (m, 4H), 3.10 – 2.93 (m, 4H). UPLC/MS (*method A*): Rt 1.32 min. MS (ES) $C_{11}H_{12}N_2O_3$ requires 220, found 221 $[M+H]^+$.

Synthesis of 6-(1,1-dioxo-1,4-thiazinan-4-yl)-3*H*-1,3-benzoxazol-2-one (50c). Compound **50c** was prepared according to the general procedure C, using **49g** (0.242 g, 1.0 mmol) and CDI (0.162 g, 1.0 mmol) in dry MeCN (10 mL). The crude was purified by column chromatography (Cy/ EtOAc 70: 30) to afford **50c** as yellow solid (0.120 g, 45% over two steps). ^1H NMR (400 MHz, DMSO-d_6) δ 6.94 (d, *J* = 2.4 Hz, 1H), 6.87 (d, *J* = 8.4Hz, 1H), 6.72

(dd, *J* = 8.4, 2.4 Hz, 1H), 3.70 - 3.65 (m, 4H), 3.17-3.12 (m, 4H). UPLC/MS (*method A*): Rt 1.15 min. MS (ES) $C_{11}H_{12}N_2O_4S$ requires 268, found 267 $[M-H]^-$.

Synthesis of *tert*-butyl 4-(2-oxo-3*H*-1,3-benzoxazol-6-yl)piperazine-1-carboxylate (50d). Compound **50d** was prepared according to the general procedure C, using **49h** (3.73 g, 12.73 mmol) and CDI (3.096 g, 19.09 mmol) in dry MeCN (25 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **50d** as a pink powder (3.046 g, 75% over three steps). ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 11.34 (bs, 1H), 7.00 (d, *J* = 2.2 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.74 (dd, *J* = 8.5, 2.3 Hz, 1H), 3.50 – 3.40 (m, 4H), 3.05 – 2.96 (m, 4H), 1.42 (s, 9H). UPLC/MS (*method A*): Rt 2.14 min, MS (ES) $C_{16}H_{21}N_3O_4$ requires 319, found 320 $[M+H]^+$.

Synthesis of 6-piperazin-1-yl-3*H*-1,3-benzoxazol-2-one hydrochloride (50e). Compound **50e** was prepared according to the general procedure E, using **50d** (1.50 g, 4.70 mmol). The reaction mixture was concentrated under reduce pressure to afford **50e** as grey solid (1.198 g, quant.). ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 11.41 (bs, 1H), 8.80 (bs, 2H), 7.05 (d, *J* = 2.2 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 6.77 (dd, *J* = 8.5, 2.3 Hz, 1H), 3.31 - 3.18 (m, 8H). UPLC/MS (*method A*): Rt 0.55 min. MS (ES) $C_{11}H_{13}N_3O_2$ requires 219, found 220 $[M+H]^+$.

Synthesis of 6-(4-methylpiperazin-1-yl)-3*H*-1,3-benzoxazol-2-one (50f). Compound **50f** was prepared according to the general procedure F, using **50e** (0.388 g, 1.52 mmol), 37 % aqueous solution of formaldehyde (0.17 mL, 6.08 mmol) and $NaBH(OAc)_3$ (0.21 g, 1.0 mmol), AcOH (0.096 mL, 0.101 g, 1.68 mmol) in dry MeCN (5 mL). The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 – 6.90 (m, 2H), 6.73 – 6.69 (m, 1H), 3.09 – 3.02 (m, 4H), 2.47 – 2.41 (m, 4H) 2.22 (s, 3H). UPLC/MS (*method A*): Rt 0.85 min. MS (ES) $C_{12}H_{15}N_3O_2$ requires 233, found 234 $[M+H]^+$.

Synthesis of 6-(4-ethylpiperazin-1-yl)-3*H*-1,3-benzoxazol-2-one (50g). Compound **50g** was prepared according to the general procedure F, using **50e** (0.171 g, 0.67 mmol), $NaBH(OAc)_3$ (0.21 g, 1.0 mmol), AcOH (0.096 mL, 0.101 g, 1.68 mmol), acetaldehyde (0.14 mL, 0.70 mmol, 5 M in THF), in dry THF (7 mL). The crude was purified by SCX to afford **50g** as a white solid (0.150 g, 90%). UPLC/MS (*method A*): Rt 0.88 min. MS (ES) $C_{13}H_{17}N_3O_2$ requires 247, found 248 $[M+H]^+$.

Synthesis of 6-(4-isopropylpiperazin-1-yl)-3*H*-1,3-benzoxazol-2-one (50h). Compound **50h** was prepared according to the general procedure F, using **50e** (0.256 g, 1.00 mmol), $NaBH(OAc)_3$ (0.636 g, 3.0 mmol), AcOH (0.286 mL, 0.300 g, 5.00 mmol) in acetone (10 mL). The crude was purified by SCX to afford **50h** as a white solid (0.158 g, 60%). ^1H NMR (400 MHz, DMSO-d_6) δ 11.40 (bs, 1H), 7.10 – 7.03 (m, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.78 (dd, J = 8.5, 2.2 Hz, 1H), 3.94 – 2.79 (m, overlapped with H_2O signal, 9H), 1.25 (s, 6H). UPLC/MS (*method A*): Rt 0.99 min, MS (ES) $C_{14}H_{19}N_3O_2$ requires 261, found 262 $[M+H]^+$.

Synthesis of 6-(4-isobutylpiperazin-1-yl)-3*H*-1,3-benzoxazol-2-one (50i). Compound **50i** was prepared according to the general procedure F, using **50e** (0.256 g, 1.0 mmol), *iso*-butyraldehyde (0.361 g, 5.0 mmol), $NaBH(OAc)_3$ (0.318 g, 1.50 mmol), AcOH (0.286 mL, 0.300 g, 5.0 mmol) in dry MeCN (10 mL). The crude was purified by SCX to afford **50i** as violet solid (0.220 g, 80%). ^1H NMR (400 MHz, DMSO-d_6) δ 6.88 – 6.80 (m, 2H), 6.63 (dd, J = 8.5, 2.3 Hz, 1H), 3.09 – 2.83 (m, 4H), 2.47 – 2.39 (m, 4H), 2.07 (d, J = 7.4 Hz, 2H), 1.78 (dt, J = 13.6, 6.8 Hz, 1H), 0.87 (d, J = 6.6 Hz, 6H). UPLC/MS (*method A*): Rt 1.21 min. MS (ES) $C_{15}H_{21}N_3O_2$ requires 275, found 276 $[M+H]^+$.

Synthesis of 2-oxo-*N*-(4-phenylbutyl)-6-piperidin-1-ium-1-yl-1,3-benzoxazole-3-carboxamide hydrochloride (23a). Compound **23a** was prepared according to the general procedure D (method A), using **50a** (0.055 g, 0.25 mmol) and 4-phenylbutyl isocyanate (0.047 mL, 0.049 g, 0.86 mmol) in a mixture of toluene/DMF (3 mL, 9:1). The crude was purified by column chromatography (Cy) (0.029 g, 30%). The free base of **23a** was dissolved in DCM (0.7 mL, 0.1M) followed by the addition of HCl (0.55 mL, 0.08 g, 2.14 mmol, 4M in 1,4-dioxane). After evaporation of the solvent, the residue was triturated with Et_2O to afford **23a** as white solid (0.026 g, 86%). ^1H NMR (400 MHz, DMSO-*d6*) δ 8.10 (t, *J* = 5.8 Hz, 1H), 8.06 – 7.83 (m, 2H), 7.82 – 7.46 (m, 1H), 7.32 – 7.24 (m, 2H), 7.23 – 7.13 (m, 3H), 4.63 (bs, 1H), 3.58 – 3.40 (m, 4H), 3.34 (q, *J* = 6.4 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 2.22 – 1.79 (m 4H), 1.78 – 1.51 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.59, 149.20, 141.91, 141.89, 139.51, 129.29, 128.52, 128.50, 126.05, 118.19, 116.86, 105.34, 58.14, 40.39, 35.52, 29.06, 28.63, 23.14, 21.80. UPLC/MS (*method A*): Rt 2.33 min. MS (ES) $C_{23}H_{27}N_3O_3$ requires 393, found 394 $[M+H]^+$. HRMS $C_{23}H_{28}N_3O_3$ $[M+H]^+$: calculated 394.2131, measured: 394.2122, Δppm -2.3.

Synthesis of 6-morpholino-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23b). Compound **23b** was prepared according to the general procedure D (method A), using **50b** (0.130 g, 0.6 mmol) and 4-phenylbutyl isocyanate (0.157 g, 0.9 mmol) in dry MeCN (6 mL). The crude was purified by column chromatography (Cy/ EtOAc, 90: 10) to afford **23b** as a white powder (0.142 g, 60%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (t, *J* = 5.7 Hz, 1H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.32 – 7.24 (m, overlapped with $CDCl_3$, 2H), 7.20 – 7.07 (m, 3H), 6.90 – 6.78 (m, 2H), 3.95– 3.82 (m, 4H), 3.44 (q, *J* = 6.6 Hz, 2H), 3.20 – 3.10 (m, 4H), 2.67 (t, *J* = 7.2 Hz, 2H), 1.78 – 1.60 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.48, 150.00, 142.91, 142.05, 128.54, 128.51, 126.01, 115.97, 112.77, 98.66, 66.76, 50.27, 40.22, 35.60, 29.22, 28.70. UPLC/MS (*method A*): Rt 2.63 min. MS (ES) $C_{22}H_{25}N_3O_4$ requires 395, found

396 [M+H]$^+$. HRMS $C_{22}H_{26}N_3O_4$ [M+H]$^+$: calculated 396.1923, measured 396.1925, Δppm 0.5.

Synthesis of 6-(1,1-dioxo-1,4-thiazinan-4-yl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23c). Compound **23c** was prepared according to the general procedure D (method A), using **50c** (0.100 g, 0.37 mmol) and 4-phenylbutyl isocyanate (0.20 g, 1.12 mmol) in dry MeCN (4 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **23c** as a white powder (0.050 g, 20%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.06 (t, *J* = 5.8 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.32 – 7.24 (m, 2H), 7.24 – 7.12 (m, 4H), 6.94 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.87 – 3.67 (m, 4H), 3.42 – 3.26 (m, 2H), 3.21 – 3.06 (m, 4H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.73 – 1.43 (m, 4H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 152.82, 149.82, 145.77, 143.21, 142.51, 128.76, 128.70, 126.14, 121.16, 115.39, 112.38, 99.29, 50.27, 47.85, 39.90, 35.22, 29.10, 28.63. UPLC/MS (*method A*): Rt 2.49 min. MS (ES) $C_{22}H_{25}N_3O_5S$ requires 443, found 267 [M-CO(CH_2)$_4$Ph]$^-$. HRMS $C_{22}H_{26}N_3$O5S [M+H]$^+$: calculated 444.1593, measured 444.1588, Δppm -1.1.

Synthesis of *tert*-butyl 4-[2-oxo-3-(4-phenylbutylcarbamoyl)-1,3-benzoxazol-6-yl]piperazine-1-carboxylate (23d). Compound **23d** was prepared according to the general procedure D (method A), using **50d** (0.10 g, 0.31 mmol), 4-phenylbutyl isocyanate (0.060 g, 0.34 mmol), DMAP (0.042 g, 0.34 mmol) in dry MeCN (3 mL). The crude was purified by column chromatography (DCM/ MeOH, 97: 3) to afford **23d** as a white solid (0.130 g, 85%). UPLC/MS (*method B*): Rt 2.21 min, MS (ES) $C_{27}H_{34}N_4O_5$ requires 494, found 495 [M+H]$^+$.

Synthesis of 2-oxo-*N*-(4-phenylbutyl)-6-piperazin-1-yl-1,3-benzoxazole-3-carboxamide hydrochloride (23e). Compound **23e** was prepared according to the general procedure E, using **23d** (0.120 g, 0.24 mmol). The reaction mixture was concentrated under reduced pressure to afford **23e** as a white solid (0.103 g, quant.). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.53 (bs, 2H), 8.06 (t, *J* = 5.8 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.33 – 7.24 (m, 2H), 7.24 – 7.11 (m, 4H), 6.90 (dd, *J* = 8.9, 2.4 Hz, 1H), 3.44 – 3.35 (m, 4H), 3.32 (q, *J* = 6.4 Hz, 2H), 3.27 – 3.15 (m, 4H), 2.62 (t, *J* = 7.2 Hz, 2H), 1.76 – 1.49 (m, 4H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 152.35, 149.33, 147.49, 142.50, 142.02, 128.28, 128.22, 125.66, 121.16, 114.71, 112.07, 98.98, 45.99, 42.35, 39.42, 34.74, 28.62, 28.14. UPLC/MS (*method A*): Rt 2.12 min, MS (ES) $C_{22}H_{26}N_4O_3$ requires 394, found 395 $[M+H]^+$. HRMS $C_{22}H_{27}N_4O_3$ $[M+H]^+$: calculated 395.2083, measured 395.2086, Δppm 1.4.

Synthesis of 6-(4-methylpiperazin-1-yl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23f). Compound **23f** was prepared according to the general procedure D (method A), using **50f** (0.060 g, 0.26 mmol), 4-phenylbutyl isocyanate (0.088 mL, 0.090 g, 0.51 mmol) in dry MeCN (3 mL). The crude was purified by column chromatography (DCM/ MeOH, 98: 2) to afford **23f** as a white powder (0.080 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (t, *J* = 5.8 Hz, 1H), 7.93 – 7.85 (m, 1H), 7.33 – 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.14 (m, 3H), 6.86 – 6.77 (m, 2H), 3.43 (q, *J* = 6.4 Hz, 2H), 3.37 – 3.18 (m, 4H), 2.83 – 2.70 (m, 4H), 2.67 (t, *J* = 7.2 Hz, 2H) 2.47 (s, 3H), 1.80 – 1.61 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.52, 150.03, 142.90, 142.06, 128.55, 128.52, 126.01, 115.93, 113.26, 99.01, 54.79, 49.32, 45.66, 40.21, 35.60, 29.23, 28.71. UPLC/MS (*method A*): Rt 2.23 min. MS (ES) $C_{23}H_{28}N_4O_3$ requires 408, found 409 $[M+H]^+$. HRMS $C_{23}H_{29}N_4O_3$ $[M+H]^+$: calculated 409.224, measured 409.224, Δppm 0.0.

Synthesis of 6-(4-ethylpiperazin-1-yl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23g). Compound **23g** was prepared according to the general procedure **D** (method **A**), using **50g** (0.100 g, 0.40 mmol) and 4-phenylbutyl isocyanate (0.077 g, 0.44

mmol) in dry MeCN (4 mL). The crude was purified by column chromatography (DCM/ MeOH, 95: 5) to afford **23g** as a yellow solid (0.109 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (t, *J* = 5.8 Hz, 1H), 7.87 (d, *J* = 9.5 Hz, 1H), 7.32– 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.14 (m, 3H), 6.83 – 6.77 (m, 2H), 3.43 (q, *J* = 6.6 Hz, 2H), 3.24 – 3.16 (m, 4H), 2.66 (t, *J* = 7.3 Hz, 2H), 2.64 – 2.59 (m, 4H), 2.49 (q, *J* = 7.2 Hz, 2H), 1.78 – 1.60 (m, overlapped with H_2O signal, 4H), 1.14 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.57, 150.07, 149.47, 142.92, 142.06, 128.54, 128.50, 125.99, 120.71, 115.82, 112.81, 98.52, 52.79, 52.44, 49.78, 40.18, 35.59, 29.23, 28.70, 12.10. UPLC/MS (*method A*): Rt 2.24 min, MS (ES) $C_{24}H_{30}N_4O_3$ requires 422, found 423 $[M+H]^+$. HRMS $C_{24}H_{31}N_4O_3$ $[M+H]^+$: calculated 423.2396, measured 423.2397, Δppm 0.2.

Synthesis of 6-(4-isopropylpiperazin-1-yl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23h). Compound **23h** was prepared according to the general procedure D (method A), using **50h** (0.100 g, 0.38 mmol), 4-phenylbutyl isocyanate (0.074 g, 0.42 mmol) in dry MeCN (4 mL). The crude was purified by column chromatography (DCM/ MeOH, 95:5) to afford **23h** as a pink solid (0.050 g, 30%). ^{1}H NMR (400 MHz, , $CDCl_3$) δ 8.00 (t, *J* = 5.7 Hz, 1H), 7.87 (d, *J* = 9.5 Hz, 1H), 7.32 – 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.14 (m, 3H), 6.83 – 6.77 (m, 2H), 3.43 (q, *J* = 6.7 Hz, 2H), 3.27 – 3.11 (m, 4H), 2.79 – 2.69 (m, 5H), 2.67 (t, *J* =7.3 Hz, 2H), 1.80 – 1.60 (m, 4H), 1.10 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, , $CDCl_3$) δ 153.56, 150.07, 149.43, 142.90, 142.06, 128.53, 128.49, 125.99, 120.77, 115.82, 112.89, 98.57, 54.86, 49.96, 48.65, 40.17, 35.59, 29.22, 28.69, 18.59. UPLC/MS (*method A*): Rt 2.31 min, MS (ES) $C_{25}H_{32}N_4O_3$ requires 436, found 437 $[M+H]^+$. HRMS $C_{25}H_{33}N_4O_3$ $[M+H]^+$: calculated 437.2553, measured 437.2557, Δppm 0.9.

Synthesis of 6-(4-isobutylpiperazin-1-yl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23i). Compound **23i** was prepared according to the general procedure D (method A), using **50i** (0.100 g, 0.36 mmol) and 4-phenylbutyl isocyanate (0.07 mL, 0.07 g, 0.4 mmol) in dry MeCN (4 mL). The crude was purified by column chromatography (DCM/ MeOH, 95: 5) to afford **23i** as white solid (0.116 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (t, *J* = 5.7 Hz, 1H), 7.87 (d, *J* = 9.5 Hz, 1H), 7.32 – 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.22 – 7.14 (m, 3H), 6.83 – 6.77 (m, 2H), 3.43 (q, *J* = 6.7 Hz, 2H), 3.26 - 3.10 (m, 4H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.61 - 2.46 (m, 4H), 2.22 – 2.06 (m, 2H), 1.90 – 1.78 (m, 1H), 1.77 - 1.62 (m, 4H), 0.93 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.11, 149.62, 142.93, 142.08, 128.55, 128.51, 126.00, 115.79, 112.76, 98.45, 66.91, 53.51, 49.79, 40.19, 35.60, 29.24, 28.71, 25.56, 21.06. UPLC/MS (*method B*): Rt 2.08 min. MS (ES) $C_{26}H_{34}N_4O_3$ requires 450, found 451 $[M+H]^+$. HRMS $C_{26}H_{35}N_4O_3$ $[M+H]^+$: calculated 451.2719, measured: 451.2716, Δppm 1.6.

Synthesis of 4-(3-benzyloxy-4-nitro-phenyl)-1-methyl-piperazin-2-one (52a). Compound **52a** was prepared according to the general procedure **G**, using 2-benzyloxy-4-fluoro-1-nitro-benzene (0.490 g, 2.00 mmol), **51a** (0.300 g, 2.00 mmol) and Et_3N (0.55 mL, 0.405 g, 4.00 mmol) in dry MeCN (20 mL) heating at reflux for 16 h. The crude was purified by column chromatography (EtOAc) to afford **52a** as a yellow solid (0.350 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 9.3 Hz, 1H), 7.51 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.33 (dd, *J* = 8.4, 6.3 Hz, 1H), 6.38 (dd, *J* = 9.3, 2.6 Hz, 1H), 6.29 (d, *J* = 2.5 Hz, 1H), 5.23 (s, 2H), 3.98 (s, 2H), 3.61 (dd, *J* = 6.5, 4.2 Hz, 2H), 3.50 (dd, *J* = 6.5, 4.2 Hz, 2H), 3.07 (s, 3H). UPLC/MS (*method A*): Rt 1.98 min, MS (ES) $C_{18}H_{19}N_3O_4$ requires 341, found 342 $[M+H]^+$.

Synthesis of 1-(3-benzyloxy-4-nitro-phenyl)-4-methyl-piperazin-2-one (52b). To a solution of 2-benzyloxy-4-bromo-1-nitro-benzene (1.00 g, 3.25 mmol, 1.0 eq.) in degassed 1,4-dioxane (26 mL) were added **51b** (0.410 g, 3.6 mmol, 1.1 eq.), K_3PO_4 (1.38 g, 6.50 mmol, 2.0 eq.) and *N,N'*-dimethylethylenediamine (0.06g, 0.07 mL, 0.65mmol, 0.2 eq.). The reaction mixture was degassed for another 15 min and, then, copper (I) iodide (0.06 g, 0.33 mmol, 0.1 eq.) was added. The reaction was refluxed for 24 h and, then, diluted with EtOAc and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure and the crude was purified by column chromatography (EtOAc) to afford **52b** as orange solid (0.554 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 8.8 Hz, 1H), 7.46 (d, J = 7.3 Hz, 2H), 7.43 – 7.36 (m, 2H), 7.36 – 7.30 (m, 2H), 6.97 (dd, J = 8.8, 2.1 Hz, 1H), 5.22 (s, 2H), 3.72 (t, J = 5.1 Hz, 2H), 3.33 (s, 2H), 2.83 (s, 2H), 2.43 (s, 3H). UPLC/MS (*method A*): Rt 1.74 min. MS (ES) $C_{18}H_{19}N_3O_4$ requires 341, found 342 $[M+H]^+$.

Synthesis of 4-(4-amino-3-hydroxy-phenyl)-1-methyl-piperazin-2-one (49i). Compound **49i** was prepared according to the general procedure B (method A), using **52a** (0.341 g, 1.00 mmol). UPLC/MS (*method A*): Rt 0.89 min. MS (ES) $C_{11}H_{15}N_3O_2$ requires 221, found 222 $[M+H]^+$.

Synthesis of 1-(4-amino-3-hydroxy-phenyl)-4-methyl-piperazin-2-one (49j). Compound **49j** was prepared according to the general procedure B (method A), using **52b** (0.50 g, 1.46 mmol). UPLC/MS (*method C*): Rt 1.42 min. MS (ES) $C_{11}H_{15}N_3O_2$ requires 221, found 222 $[M+H]^+$.

Synthesis of 6-(4-methyl-3-oxo-piperazin-1-yl)-3*H*-1,3-benzoxazol-2-one (50j). Compound **50j** was prepared according to the general procedure C, using **49i** (0.221 g, 1.00 mmol) and CDI (0.162 g, 1.00 mmol) in dry MeCN (10 mL). The crude was purified by column chromatography (DCM/ MeOH, 90: 10) to afford **50j** as violet solid (0.197 g, 80% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (d, *J* = 8.5 Hz, 1H), 6.82 (d, *J* = 2.3 Hz, 1H), 6.67 (dd, *J* = 2.3, 8.5 Hz, 1H), 3.81 (s, 2H), 3.48 (dd, *J* = 3.4, 5.8 Hz, 2H), 3.42 (dd, *J* = 4.0, 6.2 Hz, 2H), 3.04 (s, 3H). UPLC/MS (*method A*): Rt 1.16 min. MS (ES) $C_{12}H_{13}N_3O_3$ requires 247, found 248 $[M+H]^+$.

Synthesis of 6-(4-methyl-2-oxo-piperazin-1-yl)-3*H*-1,3-benzoxazol-2-one (50k). Compound **50k** was prepared according to the general procedure C, using **49j** (0.323 g, 1.46 mmol) and CDI (0.240 g, 1.46 mmol) in dry MeCN (15 mL). The crude was purified by column chromatography (DCM/ MeOH, 80: 20) to afford **50k** as orange oil (0.270 g, 70% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73-7.65 (m, 1H), 7.03-6.86 (m, 2H), 3.76 – 3.66 (m, 2H), 3.29 (s, 2H), 2.87 – 2.68 (m, 2H), 2.42 (s, 4H). UPLC/MS (*method A*): Rt 1.16 min. MS (ES) $C_{12}H_{13}N_3O_3$ requires 247, found 248 $[M+H]^+$.

Synthesis of 6-(4-methyl-3-oxo-piperazin-1-yl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23j). Compound **23j** was prepared according to the general procedure D (method A), using **50j** (0.170 g, 0.69 mmol) and 4-phenylbutyl isocyanate (0.13 mL, 0.130 g, 0.76 mmol) in dry MeCN (7 mL). The crude was purified by column chromatography (DCM/ MeOH, 90: 10) to afford **23j** as white solid (0.060 g, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (t, *J* = 5.3 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.32 - 7.23 (m, overlapped with $CDCl_3$ signal, 2H), 7.21 – 7.14 (m, 3H), 6.82 - 6.73 (m, 2H), 3.85 (s, 2H), 3.54 – 3.46 (m, 4H), 3.44 (q, *J* = 6.7 Hz, 2H), 3.05 (s, 3H), 2.67 (t, *J* = 7.1 Hz, 2H), 1.77 – 1.62 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 128.54 (2C), 126.05, 120.78, 115.97, 107.97, 47.76, 46.79, 40.34, 35.58, 35.35, 29.15, 28.67. UPLC/MS (*method A*): Rt 2.32 min. MS (ES) $C_{23}H_{26}N_4O_4$ requires 422, found 423 $[M+H]^+$. HRMS $C_{23}H_{27}N_4O_4$ $[M+H]^+$: calculated 423.2032, measured: 423.202, Δppm -2.8.

Synthesis of 6-(4-methyl-2-oxo-piperazin-1-yl)-2-oxo-*N*-(4-phenylbutyl)-1,3-benzoxazole-3-carboxamide (23k). Compound **23k** was prepared according to the general procedure D (method A), using **50k** (0.288 g, 1.17 mmol) and 4-phenylbutyl isocyanate (0.22 mL, 0.225 g, 1.28 mmol) in dry MeCN (8 mL). The crude was purified by column chromatography (DCM/ MeOH, 90: 10) to afford **23k** as white solid (0.049 g, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 8.6 Hz, 1H), 7.98 (t, *J* = 5.5 Hz, 1H), 7.31 – 7.26 (m, overlapped with $CDCl_3$ signal, 3H), 7.24 – 7.13 (m, 4H), 3.76 (s, 2H), 3.44 (q, *J* = 6.7 Hz, 2H), 3.33 (s, 2H), 2.93 – 2.80 (m, 2H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.45 (s, 3H), 1.79 – 1.63 (m, 4H).^{13}C NMR (101 MHz, $CDCl_3$) δ 153.26, 149.71, 142.03, 141.92, 128.56, 128.55, 126.92, 126.05, 122.56, 116.07, 109.05, 59.54, 52.08, 50.30, 45.11, 40.32, 35.60, 29.18, 28.70. UPLC/MS (*method A*): Rt 1.94 min. MS (ES) $C_{23}H_{26}N_4O_4$ requires 422, found 423 $[M+H]^+$. HRMS $C_{23}H_{27}N_4O_4$ $[M+H]^+$: calculated 423.2032, measured: 423.2026, Δppm -1.4.

Synthesis of *tert*-butyl 4-(4-hydroxy-3-nitro-phenyl)-3,6-dihydro-2*H*-pyridine-1-carboxylate (32a). Compound **32a** was prepared according to the general procedure A, using 4-bromo-2-nitrophenol (0.500 g, 2.29 mmol), **18c** (0.92 g, 2.98 mmol), $PdCl_2(PPh_3)_4$ (0.016 g, 0.023 mmol) and 2M Na_2CO_3 (2.87 mL, 5.73 mmol) in degassed 1,4-dioxane (25 mL). The crude was purified by column chromatography (heptane/ EtOAc, 90: 10) to afford **32a** as yellow oil (0.700 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.54 (s, 1H), 8.07 (d, *J* = 2.2 Hz, 1H), 7.65 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.07 (s, 1H), 4.09 (d, *J* = 2.8 Hz, 2H), 3.65 (t, *J* = 5.7 Hz, 2H), 3.53 (t, *J* = 5.8 Hz, 2H), 1.47 (s, 9H). UPLC/MS (*method B*): Rt 1.48 min. MS (ES) $C_{16}H_{20}N_2O_5$ requires 320, found 319 $[M-H]^-$.

Synthesis of *tert*-butyl 4-(3-amino-4-hydroxy-phenyl)piperidine-1-carboxylate (32b). Compound **32b** was prepared according to the general procedure B (method A), using **32a** (0.078 g, 0.24 mmol). UPLC/MS (*method A*): Rt 1.51 min. MS (ES) $C_{16}H_{24}N_2O_3$ requires 292, found 291 $[M-H]^-$.

Synthesis of *tert*-butyl 4-(2-oxo-3*H*-1,3-benzoxazol-5-yl)piperidine-1-carboxylate (33). Compound **33** was prepared according to the general procedure C, using **32b** (0.070 g, 0.24 mmol) and CDI (0.058 g, 0.36 mmol) in dry MeCN (2.5 mL). The crude was purified by column chromatography (Cy/ EtOAc, 70: 30) to afford **33** as white solid (0.046 g, 60% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.55 (bs, 1H), 7.22 – 7.14 (m, 1H), 6.99 – 6.90 (m, 2H), 4.07 (d, *J* = 13.0 Hz, 2H), 2.98 – 2.56 (m, 3H), 1.84 – 1.65 (m, 2H), 1.54 – 1.44 (m, 2H), 1.42 (s, 9H). UPLC/MS (*method A*): Rt 2.20 min. MS (ES) $C_{17}H_{22}N_2O_4$ requires 318, found 319 $[M+H]^+$.

Synthesis of *tert*-butyl 4-[3-(isobutylcarbamoyl)-2-oxo-1,3-benzoxazol-5-yl]piperidine-1-carboxylate (24a). Compound **24a** was prepared according to the general procedure D (method B), using **33** (0.200 g, 0.63 mmol), *iso*-butylamine (0.094 mL, 0.069 g, 0.94 mmol) and Et_3N (0.44 mL, 0.318 g, 3.14 mmol) in dry DCM (7 mL). The crude was purified by column chromatography (Cy/ EtOAc, 90: 10) to afford **24a** as white solid (0.186 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (t, *J* = 5.8 Hz, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 7.08 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.27 (d, *J* = 13.2 Hz, 2H), 3.28 (dd, *J* = 6.8, 5.9 Hz, 2H), 2.89-2.65 (m, 3H), 2.02 – 1.76 (m, 3H), 1.72 – 1.59 (m, 2H), 1.50 (s, 9H), 1.02 (d, *J* = 6.7 Hz, 6H). UPLC/MS (*method B*): Rt 2.07 min. MS (ES) $C_{22}H_{31}N_3O_5$ requires 417, found 418 $[M+H]^+$.

Synthesis of *N*-isobutyl-2-oxo-5-(4-piperidyl)-1,3-benzoxazole-3-carboxamide hydrochloride (24b). Compound **24b** was prepared according to the general procedure E, using **24a** (0.160 g, 0.38 mmol). The crude was triturated with Et_2O to afford **24b** as white solid (0.128 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (bs, 1H), 8.96 (bs, 1H), 8.14 (t, *J* = 5.9 Hz, 1H), 7.83 (d, *J* = 1.8 Hz, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.13 (dd, *J* = 8.4, 1.9 Hz, 1H), 3.37 (s, 1H), 3.17 (t, *J* = 6.4 Hz, 2H), 3.08 – 2.81 (m, 3H), 2.00 – 1.77 (m, 5H), 0.92 (d, *J* = 6.7 Hz, 6H). UPLC/MS (*method A*): Rt 1.78 min. MS (ES) $C_{17}H_{23}N_3O_3$ requires 317, found 318 $[M+H]^+$.

Synthesis of N-isobutyl-5-(1-methyl-4-piperidyl)-2-oxo-1,3-benzoxazole-3-carboxamide (24c). Compound **24c** was prepared according to the general procedure F, using **24b** (0.088 g, 0.25 mmol), 37 % aqueous solution of formaldehyde (0.038 mL, 1.25 mmol), $NaBH(OAc)_3$ (0.106 g, 0.5 mmol), AcOH (0.03 mL, 0.024 g, 0.4 mmol) in dry MeCN (3 mL). The crude was triturated with Et_2O to afford **24c** as white solid (0.07 g, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (t, *J* = 5.1 Hz, 1H), 7.80 (s, 1H), 7.33 (d, *J* = 8.3 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 3.15 – 3.07 (t, *J* = 6.4 Hz, 2H), 2.94 – 2.79 (m, 2H), 2.59 – 2.43 (m, overlapped with DMSO signal, 1H), 2.20 (s, 3H), 2.06 – 1.91 (m, 2H), 1.91 – 1.79 (m, 1H), 1.79 – 1.54 (m, 4H), 0.92 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.56, 149.50, 142.88, 139.85, 128.14, 122.36, 112.74, 109.60, 55.68, 46.79, 46.12, 41.27, 33.32, 27.90, 19.77. UPLC/MS (*method A*): Rt 1.81 min. MS (ES) $C_{18}H_{25}N_3O_3$ requires 331, found 332 $[M+H]^+$. HRMS $C_{18}H_{26}N_3O_3$ $[M+H]^+$: calculated 332.1974, measured: 332.1964, Δppm -3.0.

Synthesis of *tert*-butyl 4-hydroxy-4-(2-oxo-3*H*-1,3-benzoxazol-7-yl)piperidine-1-carboxylate (45). Compound **45** was prepared according to the general procedure I, using **42b** (0.793 g, 3.98 mmol), 7-bromo-3*H*-1,3-benzoxazol-2-one (0.500 g, 2.34 mmol), MeMgBr (1.17 mL, 0.418 g, 3.51 mmol, 3M in Et_2O), *n*-BuLi (1.12 mL, 2.81 mmol, 2.5M in hexanes) in dry THF (25 mL). The crude was purified by column chromatography (Cy/ EtOAc, 35: 65) to afford **45** as a white solid (0.345 g, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (bs, 1H), 7.26 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.12 (t, *J* = 7.9 Hz, 1H), 6.97 (dd, *J* = 7.7, 1.3 Hz, 1H), 5.34 (s, 1H), 3.97 – 3.78 (m, 2H), 3.25 – 2.99 (m, 2H), 2.09 (td, *J* = 13.1, 4.7 Hz, 2H), 1.63 – 1.52 (m, 2H), 1.43 (s, 9H). UPLC/MS (*method A*): Rt 1.76 min. MS (ES) $C_{17}H_{22}N_2O_5$ requires 334, found 335 $[M+H]^+$.

Synthesis of 7-(1,2,3,6-tetrahydropyridin-4-yl)-3*H*-1,3-benzoxazol-2-one (46a). Compound **46a** was prepared according to the general procedure L, using **45** (0.345 g, 1.03 mmol). The crude was purified by SCX to afford **46a** as brown solid (quant.). UPLC/MS (*method A*): Rt 0.95 min. MS (ES) $C_{12}H_{12}N_2O_2$ requires 216, found 217 $[M+H]^+$.

Synthesis of 7-(1-methyl-3,6-dihydro-2*H*-pyridin-4-yl)-3*H*-1,3-benzoxazol-2-one (46b). Compound **46b** was prepared according to the general procedure F, using **46a** (0.108 g, 0.5 mmol), $NaBH(OAc)_3$ (0.318 g, 1.50 mmol), AcOH (0.03 mL, 0.030 g, 0.50 mmol), 37 % aqueous solution of formaldehyde (0.080 mL, 2.5 mmol) in dry MeCN (5 mL). The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.87 (d, *J* = 7.76 Hz, 1H), 6.80 – 6.74 (m, 2H), 6.36 (t, *J* = 3.49 Hz, 1H), 3.07 – 3.00 (m, 2H), 2.58 – 2.47 (m, overlapped with DMSO signal, 4H), 2.27 (s, 3H). UPLC/MS (*method A*): Rt 0.98 min. MS (ES) $C_{13}H_{14}N_2O_2$ requires 230, found 231 $[M+H]^+$.

Synthesis of 7-(1-methyl-4-piperidyl)-3*H*-1,3-benzoxazol-2-one (47). Compound **47** was prepared according to the general procedure B (method B), using **46b** (0.115 g, 0.50 mmol). The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.12 – 7.06 (m, 1H), 6.94 (t, *J* = 7.4, 1.2 Hz, 2H), 2.98 (d, *J* = 11.8, 3.4 Hz, 2H), 2.82 – 2.69 (m, 1H), 2.31 (s, 3H), 2.25 – 2.11 (m, 2H), 1.88 – 1.72 (m, 4H). UPLC/MS (*method A*): Rt 0.98 min, MS (ES) $C_{13}H_{16}N_2O_2$ requires 232, found 233 $[M+H]^+$.

Synthesis of *N*-isobutyl-7-(1-methyl-4-piperidyl)-2-oxo-1,3-benzoxazole-3-carboxamide (25). Compound **25** was prepared according to the general procedure D (method B), using **47** (0.116 g, 0.5 mmol), *iso*-butylamine (0.014 g, 0.19 mmol), and Et_3N (0.15 mL, 0.111 g, 1.10 mmol) in dry DCM (7 mL). The crude was purified by column chromatography (DCM/ MeOH, 92: 8) to afford **25** as a pink solid (0.050 g, 30% over three steps). ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (t, *J* = 5.0 Hz, 1H), 7.91 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 7.11 (dd, *J* = 8.1, 1.3 Hz, 1H), 3.30 – 3.21 (m, 2H), 3.07 – 2.96 (m, 2H), 2.96 – 2.82 (m, 1H), 2.35 (s, 3H), 2.13 (td, *J* = 11.5, 3.2 Hz, 2H), 2.03 – 1.81 (m, 5H), 0.99 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.43, 150.10, 139.67, 128.76, 127.90, 125.28, 122.48, 113.52, 56.18, 47.71, 46.50, 35.66, 31.80, 28.61, 20.18. UPLC/MS (*method A*): Rt 1.79 min, MS (ES) $C_{18}H_{25}N_3O_3$ requires 331, found 332 $[M+H]^+$. HRMS $C_{18}H_{26}N_3O_3$ $[M+H]^+$: calculated 332.1974, measured 332.1967, Δppm -2.1.

Synthesis of 2-(3-benzyloxy-4-nitro-phenyl)pyridine (35). Compound **35** was prepared according to the general procedure A, using 2-benzyloxy-4-bromo-1-nitro-benzene (0.309 g, 1.00 mmol), **34** (0.174 g, 1.10 mmol), Pd(dppf)Cl_2 (0.146 g, 0.2 mmol), KOAc (0.196 g, 2 mmol) and 2M Na_2CO_3 (1.30 mL, 2.50 mmol) in degassed 1,4-dioxane (15 mL). The crude was purified by column chromatography (Cy/ EtOAc, 80: 20) to afford **35** as a yellow solid (0.121 g, 40%). ^1H NMR (400 MHz, DMSO-d_6) δ 8.75 (ddd, *J* = 4.8, 1.7, 0.9, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 1.6 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.98 (td, *J* = 7.8, 1.8 Hz, 1H), 7.85 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.54 – 7.32 (m, 6H), 5.45 (s, 2H). UPLC/MS (*method A*): Rt 2.47 min, MS (ES) $C_{18}H_{14}N_2O_3$ requires 306, found 307 $[M+H]^+$.

Synthesis of 2-amino-5-(2-piperidyl)phenol (36). Compound **36** was prepared according to the general procedure B (method B), using **35** (0.520 g, 1.69 mmol). UPLC/MS (*method A*): Rt 0.93 min. MS (ES) $C_{11}H_{16}N_2O$ requires 192, found 193 $[M+H]^+$.

Synthesis of 6-(2-piperidyl)-3H-1,3-benzoxazol-2-one (37a). Compound **37a** was prepared according to the general procedure C, using **36** (0.390 g, 1.69 mmol) and CDI (0.274 g, 1.69 mmol) in dry MeCN (17 mL). The crude was used in the next step without further purification. UPLC/MS (*method A*): Rt 1.05 min. MS (ES) $C_{12}H_{14}N_2O_2$ requires 218, found 219 $[M+H]^+$.

Synthesis of 6-(1-methyl-2-piperidyl)-3H-1,3-benzoxazol-2-one (37b). Compound **37b** was prepared according to general procedure F, using **37a** (0.368 g, 1.69 mmol), 37 % aqueous solution of formaldehyde (0.09 mL, 3.38 mmol), $NaBH(OAc)_3$ (1.075 g, 5.07 mmol), AcOH (0.15 mL, 0.162 g, 2.70 mmol) in dry MeCN (9 mL). The crude was purified by SCX to afford **37b** as white solid (0.169 g, 43% over three steps). ^1H NMR (400 MHz, DMSO-*d₆*) δ 7.64 (bs, 1H), 7.14 (d, *J* = 1.2 Hz, 1H), 7.06 – 6.95 (m, 2H), 3.01 – 2.85 (m, 1H), 2.74 (dd, *J* = 10.8, 2.7 Hz, 1H), 2.01 (td, *J* = 11.6, 3.2 Hz, 1H), 1.88 (s, 3H), 1.78 – 1.66 (m, 1H), 1.67 – 1.50 (m, 3H), 1.50 – 1.36 (m, 1H), 1.37 – 1.22 (m, 1H). UPLC/MS (*method A*): Rt 1.04 min. MS (ES) $C_{13}H_{16}N_2O_2$ requires 232, found 233 $[M+H]^+$.

Synthesis of (±)-*N*-isobutyl-6-(1-methyl-2-piperidyl)-2-oxo-1,3-benzoxazole-3-carboxamide (26). Compound **26** was prepared according to the general procedure D (method B), using **37b** (0.170 g, 0.73 mmol) and *iso*-butylamine (0.160 g, 2.19 mmol) in dry DCM (10 mL). The crude was purified by column chromatography (DCM/ MeOH, 92: 8) to afford **26** as a white solid (0.069 g, 29%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (t, *J* = 5.3 Hz, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.30 – 7.23 (m, overlapped with $CDCl_3$ signal, 1H), 7.20 (dd, *J*

= 8.3, 1.6 Hz, 1H), 3.25 (dd, *J* = 6.8, 5.8 Hz, 2H), 3.09 – 3.00 (m, 1H), 2.95 (dd, *J* = 11.1, 2.8 Hz, 1H), 2.80 (dd, *J* = 11.1, 2.8 Hz, 1H), 2.18 – 2.05 (m, 1H), 1.98 (s, 3H), 1.96 – 1.85 (m, 1H), 1.85 – 1.76 (m, 1H), 1.76 – 1.65 (m, 2H), 1.61 – 1.47 (m, 1H), 1.42 – 1.28 (m, 1H), 0.98 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.56, 150.07, 142.34, 142.16, 126.95, 124.20, 115.37, 108.82, 70.79, 57.48, 47.68, 44.59, 36.37, 28.60, 26.12, 24.94, 20.15. UPLC/MS (*method A*): Rt 1.88 min, MS (ES) $C_{18}H_{25}N_3O_3$ requires 331, found 332 $[M+H]^+$. HRMS $C_{18}H_{26}N_3O_3$ $[M+H]^+$: calculated 332.1971, measured 332.1974, Δppm -0.9.

Synthesis of *tert*-butyl 5-(3-hydroxy-4-nitro-phenyl)-3,4-dihydro-2*H*-pyridine-1-carboxylate (39). Compound **39** was prepared according to the general procedure A, using **38** (0.834 g, 2.7 mmol), 5-bromo2-nitrophenol (0.530 g, 2.43 mmol), $Pd(PPh_3)_4$ (0.156 g, 0.135 mmol) and 2M Na_2CO_3 (3.04 mL, 6.075 mmol) in degassed 1,4-dioxane (27 mL). The crude was purified by column chromatography (Cy/ EtOAc, 90: 10) to afford **39** as yellow solid (0.460 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.79 (s, 1H), 7.99 (d, *J* = 8.8 Hz, 1H), 7.82 - 7.48 (m, 1H), 7.11 - 6.94 (m, 2H), 3.62 (s, 2H), 2.42 (t, *J* = 6.0 Hz, 2H), 1.98 (p, *J* = 6.1 Hz, 2H), 1.54 (s, 9H). UPLC/MS (*method B*): Rt 1.86 min. MS (ES) $C_{16}H_{20}N_2O_5$ requires 320, found 321 $[M+H]^+$.

Synthesis of *tert*-butyl 3-(4-amino-3-hydroxy-phenyl)piperidine-1-carboxylate (40). Compound **40** was prepared according to the general procedure B (method A), using **39** (0.450 g, 1.41 mmol). UPLC/MS (*method B*): Rt 0.73 min. MS (ES) $C_{16}H_{24}N_2O_3$ requires 292, found 291 $[M-H]^-$.

Synthesis of *tert*-butyl 3-(2-oxo-3*H*-1,3-benzoxazol-6-yl)piperidine-1-carboxylate (49a). Compound **49a** was prepared according to the general procedure C, using **40** (0.412 g, 1.41 mmol) and CDI (0.229 g, 1.41 mmol) in dry MeCN (14 mL). The crude was purified by column chromatography (Cy/ EtOAc, 70: 30) to afford **41a** as brown oil (0.403 g, 90% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (bs, 1H), 7.07 - 6.88 (m, 3H), 4.20 – 4.06 (m, 2H), 2.80 – 2.43 (m, 3H), 2.03-1.93 (m, 1H), 1.72 (dd, *J* = 3.2, 6.4 Hz, 1H), 1.66 – 1.52 (m, 2H), 1.44 (s, 9H). UPLC/MS (*method A*): Rt 2.19 min. MS (ES) $C_{17}H_{22}N_2O_4$ requires 318, found 319 $[M+H]^+$.

Synthesis of 6-(3-piperidyl)-3H-1,3-benzoxazol-2-one hydrochloride (41b). Compound **41b** was prepared according to the general procedure E, using **41a** (0.185 g, 0.58 mmol). The crude was used in the next step without further purification. UPLC/MS (*method A*): Rt 0.99 min. MS (ES) $C_{12}H_{14}N_2O_2$ requires 218, found 219 $[M+H]^+$.

Synthesis of 6-(1-methyl-3-piperidyl)-3H-1,3-benzoxazol-2-one (41c). Compound **41** was prepared according to general procedure F, using **41b** (0.147 g, 0.58 mmol), 37 % aqueous solution of formaldehyde (0.03 mL, 1.16 mmol), $NaBH(OAc)_3$ (0.370 g, 1.74 mmol), AcOH (0.07 mL, 0.070 g, 1.16 mmol) in dry MeCN (3 mL). The crude was purified by SCX to afford **41c** as white solid (0.094 g, 70% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (s, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.95 (d, *J* = 8.1 Hz, 1H), 3.01-2.81 (m, 3H), 2.31 (s, 3H), 2.03 - 1.87 (m, 3H), 1.87 – 1.67 (m, 2H), 1.39 (qd, *J* = 12.6, 4.2 Hz, 1H). UPLC/MS (*method A*) : Rt 1.81 min. MS (ES) $C_{13}H_{16}N_2O_2$ requires 232, found 233 $[M+H]^+$.

Synthesis of (±)-*N*-isobutyl-6-(1-methyl-3-piperidyl)-2-oxo-1,3-benzoxazole-3-carboxamide (27). Compound **27** was prepared according to the general procedure D (method B), using **41c** (0.088 g, 0.28 mmol) and *iso*-butylamine (0.06 mL, 0.06 g, 0.84 mmol) in dry DCM (4 mL). The crude was purified by column chromatography (DCM/ MeOH, 90: 10) to afford **27** as white solid (0.08 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (bs, 1H), 7.96 (d, *J* = 8.9 Hz, 1H), 7.17 – 7.08 (m, 2H), 3.25 (d, *J* = 6.59, 2H), 3.03 – 2.87 (m, 3H), 2.34 (s, 3H), 2.13 – 1.68 (m, 6H), 1.41 (qd, *J* = 12.1, 5.5 Hz, 1H), 0.99 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.50, 150.05, 142.02, 141.71, 126.47, 123.99, 115.48, 108.69, 63.12, 55.85, 47.69, 46.54, 42.82, 31.21, 28.60, 25.64, 20.16. UPLC/MS (*method A*): Rt 1.87 min. MS (ES) $C_{18}H_{25}N_3O_3$ requires 331, found 332 $[M+H]^+$. HRMS $C_{18}H_{26}N_3O_3$ $[M+H]^+$: calculated 332.1974, measured 332.1972, Δppm -0.9.

IN VITRO PHARMACOLOGICAL ASSAY

*In vitro h*AC fluorescent assay.

Cell culture conditions and preparation of *h*AC- enriched lysate. HEK293 cells stably expressing *h*AC were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS, 1% glutamine, 1 mM sodium pyruvate and 500 µg/mL G418. Cells were harvested and pellets were stored at -80°C until lysosomal-enriched lysate preparation. Cells were suspended in 20 mM Tris HCl (pH 7.5) with 0.32 M sucrose, sonicated and centrifuged at 800 × g for 30 min at 4°C. Supernatants were then centrifuged at 12,000 x g for 30 min at 4°C. Pellets were re-suspended in PBS (pH 7.4) and subjected to three freeze-thaw cycles at -80°C. The suspension was finally centrifuged at 105,000 × g for 1 h at 4°C and protein concentration was measured in the supernatant with bicinchinonic acid based protein assay. This *h*AC-enriched preparation allowed us to further optimize the enzymatic assay and to use small amounts of lysate (2 µg/well) at 5 µM substrate (Rbm14-12) around its K_M (K_M = 5.0 µM).

Fluorogenic *h*AC Assay. The assay was performed in Optiplate 96-wells black plates, with each reaction well containing a mixture of 25 mM NaOAc buffer (pH 4.5) and a fixed amount of protein (2 µg) in a volume of 85 µL. After 10 min of pre-incubation with test compounds (diluted 20x from DMSO stock solutions at different concentrations), the fluorogenic probe was added (diluted 40 x from EtOH stock solution, final concentration 5 μM). After 3 h incubation at 37°C, reactions were stopped with 50 μL of MeOH and 100 μL of a 2.5 mg/mL $NaIO_4$ fresh solution in 100 mM Glycine/NaOH buffer (pH 10.6). The plates were further incubated for 2 h at 37°C in the dark and fluorescence intensities were measured at excitation/emission wavelengths of 355/460 nm. Negative control samples consisted of the same incubation mixture in the absence of protein-enriched extracts. Data were plotted as a function of compound concentrations. IC_{50} values were calculated by nonlinear regression analysis using GraphPad Prism 5 (GraphPad Software Inc., CA, USA) applying a standard slope curve fitting. The reported IC_{50} values are the mean of at least three independent experiments, performed in three technical replicates.

KINETIC STUDIES.

Michaelis-Menten analysis. Assay conditions for the kinetic studies were the same as those described for the fluorogenic *h*AC assay. Enzyme-enriched lysate (2 μg) was incubated with the following concentrations of substrate Rbm14-12: 0.25 μM, 0.5 μM, 1 μM, 2.5 μM, 5 μM, 10 μM, 12.5 μM, 15 μM. Compound **22m** was used at a final concentration of 100 nM and 400 nM. Initial velocities (V_0) were determined and automatically fitted to Michaelis-Menten equation to obtain kinetic parameters (K_M and V_{max}). The graph is representative of two independent experiments, each performed in three technical replicates. Graphs and data analysis were performed using GraphPad Prism 5 software (GraphPad Software Inc., CA, USA).

Determination of kinetic parameter k_i/K_I. *h*AC activity was measured as a function of reaction time in the presence of different concentrations of **22m**. The apparent inactivation rate constant of *h*AC (k_{obs}) was analyzed by non-linear square fitting each data set to the pseudo first order rate equation: $Y=vi*(1-exp(-k_{obs}*t))/k_{obs}$. Replot of calculated k_{obs} vs [**22m**] was made and the kinetic parameter k_i/K_I was calculated by non-linear square fitting data to the equation: $Y=k_i*I/(K_I+I)$. The graphs are representative of two independent experiments, each performed in two technical replicates.

***In vitro h*ASM assay.** *h*ASM activity measurement was conducted using the fluorogenic substrate 6-hexadecanoylamino-4-methylumbelliferylphosphorylcholine (HMU-PC, Toronto Research Chemicals) at 0.5 μM and 1.3 nM purified human full-length ASM enzyme (purified in house) in a buffer containing 50 mM citrate, 150 mM NaCl, 5 mM $ZnCl_2$, 0.43 mM triton X-100 at pH 4.7 in a final volume of 50 μL. The reaction mixtures were incubated for 45 min at rt and stopped by the addition of 150 μL of 1 M glycine at pH 12.5. The formation of the fluorescent product was monitored by a plate reader at excitation/emission wavelengths of 385/450 nm. The average *h*ASM activity was calculated from two independent experiments, each performed in two technical replicates.

***In vitro h*GCase assay.** *h*GCase activity measurement was conducted using the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide hydrate (4-MUG, Merck) at 1 mM and 5 nM purified human full-length GCase enzyme and 50 nM its natural activator SapC (both GCase and SapC were purified in house) in a buffer containing 50 mM citric acid, 174 mM K_2HPO_4, 15 μM phosphatidylserine, and 0.32 mM triton X-100 at pH 4.7, in a final volume of

50 μL. The reaction mixtures were incubated for 15 min at rt and stopped by the addition of 150 μL of 1 M glycine at pH 12.5. The cleavage of 4-MUG was monitored by a plate reader at excitation/emission wavelengths of 365/440 nm. The average *h*GCase activity was calculated from two independent experiments, each performed in two technical replicates.

***In vitro h*NAAA fluorescent assay.**

Cell culture and preparation of *h*NAAA-enriched lysate. HEK-293 cells stably transfected with the *h*NAAA coding sequence cloned from a human spleen cDNA library (catalog no. 639124, Clontech, Mountain View, CA, USA) were used as enzyme source. Cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS, 1% glutamine, 1 mM sodium pyruvate and 500 μg/mL G418. Cells were harvested and pellets were stored at -80°C until lysosomal-enriched lysate preparation. Cells were suspended in 20 mM Tris HCl (pH 7.4) with 0.32 M sucrose, sonicated and centrifuged at 800 × g for 30 min at 4°C. Supernatants were then ultracentrifuged at 12,000 × g for 30 min at 4°C. Pellets were re-suspended in PBS buffer (pH 7.4) and subjected to three freeze-thaw cycles at -80°C. The suspension was finally ultracentrifuged at 105,000 × g for 1h at 4°C, supernatants were collected, protein concentration was measured and samples aliquoted and stored at –80°C until use.

Fluorogenic *h*NAAA Assay. The assay was run in 96-well microplates (Black OptiPlate™-96 F; PerkinElmer, Massachusetts, USA), in a total reaction volume of 200 μL. *h*NAAA protein preparation (4.0 μg) was pre-incubated for 30 min with various concentrations of test compounds or vehicle control (DMSO 5%) in 100 mM citrate/phosphate buffer (pH 4.5) containing 3.0 mM DTT, 0,1% NP40 0,1%, 0,05% BSA, 150 mM NaCl. N-(4-methyl-2-oxo-chromen-7-yl)-hexadecanamide (PAMCA) was used as a substrate (2.0 μM) and the reaction carried for 50 min at 37°C. Fluorescence was measured with EnVision 2014 Multilabel Reader (PerkinElmer, Massachusetts, USA) using an excitation wavelength of 355 nm and emission 460 nm. IC50 values were calculated by non-linear regression analysis of log[concentration]/inhibition curves using GraphPad Prism 5 (GraphPad Software Inc., CA, USA) applying a standard slope curve fitting. The reported IC_{50} values are the mean of at least three independent experiments, performed in three technical replicates.

***In vitro h*FAAH fluorescent assay.**

Cell culture and preparation of *h*FAAH-enriched lysate. *h*FAAH was obtained from a HEK-293 FAAH-1 overexpressing stable cell line. Cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS, 1% penicillin/streptomycin, 1% glutamine, 1 mM sodium pyruvate and 500 μg /mL G418. Cells were harvested and pellets were stored at -80°C until membrane-enriched lysate preparation. Cell pellet was re-suspended in 20 mM Tris-HCl pH 7.4, 0.32 M sucrose, sonicated and centrifuged at 1000 x g, 10 min, 4°C. The collected supernatant was centrifuged at 12,000 x g for 10 min at 4°C and the supernatants were further centrifuged at 100,000 x g for 1 h at 4°C. Membrane pellets were re-suspended in PBS, protein concentration was measured and samples aliquoted and stored at -80°C until use.

Fluorogenic *h*FAAH Assay. The fluorescent assay to measure FAAH activity was performed in 96 wells black plates (Black OptiPlate™-96 F; PerkinElmer, Massachusetts, USA): 2.5 μg of *h*FAAH membrane preparation were pre-incubated for 50 min at 37 °C, in 190 μL of assay buffer (50 mM TrisHCl pH 7.4, 0.05% Fatty acid-free BSA), with 5 μL of inhibitor or 5 μL DMSO to measure FAAH total activity. The background (no activity) samples were prepared using 190 μL of assay buffer without *h*FAAH and 5 μL of DMSO. The reaction was then started by the addition of 5 μL of substrate (AMC Arachidonyl Amide, A6855, Merck) dissolved in DMSO and used at a final concentration of 800 nM. The reaction was carried out for 45 min at 37 °C and fluorescence was measured with EnVision 2014 Multilabel Reader (PerkinElmer, Massachusetts, USA) (excitation wavelength 355 nm / emission wavelength 460 nm). The concentration causing half-maximal inhibition (IC_{50}) was determined by nonlinear regression analysis of the Log[concentration]/ response curves generated with mean replicate values using a four-parameter Hill equation curve fitting with GraphPad Prism 5 (GraphPad Software Inc., CA, USA). The reported IC_{50} values are the mean of at least three independent experiments, performed in three technical replicates.

***In vitro h*MAGL colorimetric assay.** The colorimetric assay to measure *h*MAGL activity was performed using assay kit provided by Cayman Scientific (item. 705192), according to the manufacturer's instructions. Briefly, *in vitro* activity was measured in 96-well plates and DMSO was used as solvent. 10 μL of DMSO (100% initial activity wells: 100% IA) or compounds at two concentrations (1 and 10 μM) were preincubated for 5 min at rt with 150 μL of diluted assay buffer (10 mM Tris-HCl, pH 7.2, containing 1 mM EDTA) containing *h*MAGL. In blank wells, 160 μL of the diluted assay buffer and 10 μL of DMSO were added. The reactions were initiated by adding 10 μL of MAGL substrate to all the wells and plates were incubated for 10 min at rt. Absorbance values were measured at 405 nm and percent inhibition was calculated by following method: 100 – (Inhibitor/100% IA) x 100. The reported percentages of inhibition are the mean of at least three independent experiments, performed in three technical replicates.

CELL CULTURE AND TREATMENTS.

SH-SY5Y cells were purchased from Sigma Aldrich (Italy) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum at 37 °C and 5% CO_2. Drugs were dissolved in DMSO (10 mM) and diluted in cell culture medium with reduced serum (1%) for cell treatments.

*h*AC LC-MS-based activity assay.

*h*AC activity measurement was performed as previously described.[36-37] Total lysates from cells were diluted in assay buffer (100 mM sodium phosphate, 0.1% Nonidet P-40, 150 mM NaCl, 3 mM DTT, 100 mM sodium citrate, pH 4.5). Reactions were started by the addition of 50 µM N-lauroyl ceramide (Nu-Chek Prep, Elysian, MN) and carried on for 1 h at 37 °C. Reactions were stopped by addition of a mixture of $CHCl_3$/MeOH (2:1) containing 1 nmol 11-lauroleic acid (NuChek Prep). The organic phases were collected, dried under nitrogen and analyzed by UPLC/MS (Acquity, Waters) in the negative-ion mode monitoring the reaction product (lauric acid, m/z: 199) using 11-lauroleic acid as internal standard. Lipids were eluted on an Acquity UPLC BEH C18 column (50mm length, 2.1 mm ID, 1.7 µm pore size, Waters) column at 0.5 mL-min-1 for 1.5 min with a gradient of MeCN and H_2O, both containing 0.25% acetic acid and 5 mM ammonium acetate (70% to 100% MeCN in 0.5 min, 100% MeCN for 0.5 min, 70% MeCN for 0.4 min). The column temperature was 40 °C.

Electrospray ionization (ESI) was in the negative mode, capillary voltage was 1 kV and cone voltage was 50 V. N_2 was used as drying gas at a flow rate of 500 L/h and at a temperature of 400 °C. The $[M-H]^-$ ion was monitored in the selected-ion monitoring mode (m/z values: lauric acid 199, 11-lauroleic acid 197.35). Calibration curves were generated with authentic lauric acid (Nu Check Prep).

Lipid extraction and ceramide analysis.

Lipid extraction and sphingolipid measurements were performed as previously described.[36-37] Lipids were extracted with a $CHCl_3$/MeOH mixture (2:1, 3 mL) containing internal standards. The organic phase was collected, dried under nitrogen, and dissolved in $CHCl_3$/MeOH (1:3) for LC-MS analyses. Ceramides and sphingosine were analyzed by LC-MS/MS, using a Waters Acquity UPLC coupled to a Waters Xevo TQMS and interfaced with ESI ion source. Separation was performed on a Waters Acquity BEH C18 1.7 µm column (2.1 × 50 mm) at 60 °C. A linear gradient of 0.1% formic acid in MeCN/isopropyl alcohol (20:80) as solvent B in 0.1% formic acid in MeCN/ H_2O (20:80) as solvent A and was applied at a flow rate of 0.4 mL/min. Detection of sphingolipids was performed in positive ion mode. Capillary voltage was 3.5 kV and cone voltage was 25 V. The source temperature and desolvation temperatures were set at 120 °C and 600 °C respectively. Desolvation gas and cone gas (N_2) flow were 800 and 20 L/h, respectively. Ceramides were identified by comparison of their LC retention times and MS/MS fragmentation patterns with those of authentic standards (Avanti Polar Lipids). Multiple Reaction Monitoring (MRM) ion chromatograms were used to quantify myristoyl ceramide (C14:0, m/z: 492.5 > 264.3), palmitoyl ceramide (C16:0, m/z 520.3 > 264.3), stearoyl ceramide (C18:0 m/z: 548.3 >

264.3), lignoceroyl ceramide (C24:0 m/z: 632.3 > 264.3), nervonoyl ceramide (C24:1 m/z: 630.3 > 264.3) and using lauroyl ceramide standard (m/z: 464.5 >264.3). Detection and analysis were controlled by Waters MassLynx software version 4.1. Sphingosine was identified by comparison of its LC retention times and MS2 fragmentation patterns with those of authentic standards (Avanti Polar Lipids). Extracted ion chromatograms were used to quantify sphingosine standard (d18:1, m/z: 300.5 > 282.5). Detection and analysis were controlled by Waters MassLynx software version 4.1. Calibration curves were prepared for every experiment.

Statistics.

GraphPad Prism software (GraphPad Software, Inc., USA) was used for statistical analysis. Data were analyzed using the Student t-test or 1-way ANOVA followed by Bonferroni post hoc test for multiple comparisons. Differences between groups were considered statistically significant at values of $p < 0.05$. Results are expressed as mean ± S.E.M.

EC_{50} determination in primary fibroblast cells from Krabbe's disease patients. Cells were plated in a 6-well plate. After 24 h, cells were treated with **22m** at different concentrations for 2 h. Next, cells were washed with PBS and cell pellets were washed, collected, and stored at -80°C. Finally, cell pellets were lysed and *h*AC activity in cell lysates was analyzed using the same biochemical fluorogenic assay as described for compound IC_{50} determination. Using this methodology the EC_{50} was determined to be 0.41±0.1 μM. EC_{50} value is a mean of two independent experiments, each performed in two technical replicates.

IN VITRO PHYSICOCHEMICAL AND METABOLIC STABILITY ASSAY

Aqueous kinetic solubility assay.

The aqueous kinetic solubility was determined from a 10 mM MeCN stock solution of test compound in Phosphate Buffered Saline (PBS) at pH 7.4. The study was performed by incubation of an aliquot of 10 mM MeCN stock solution in PBS (pH 7.4) at a target concentration of 250 μM. The incubation was carried out under shaking at 25°C for 1 h followed by centrifugation at 21,100 x g for 30 min. The supernatant was analyzed by UPLC/MS for the quantification of dissolved compound (in μM) by UV at a specific wavelength (215 nm). The aqueous kinetic solubility (in μM) was calculated by dividing the peak area of the dissolved test compound (supernatant) by the peak area of the test compound in the reference (250μM in MeCN) and further multiplied by the target concentration and dilution factor. The UPLC/MS analyses were performed on a Waters ACQUITY UPLC/MS system consisting of a single quadrupole detector (SQD) Mass Spectrometer (MS) equipped with an Electrospray Ionization (ESI) interface and a Photodiode Array Detector (PDA). The PDA range was 210-400 nm. ESI in positive mode was used in the mass scan range 100-650 Da. The analyses were run on an ACQUITY UPLC BEH C18 column (50x2.1 mm ID, particle size 1.7 μm) with a VanGuard BEH C18 pre-column (5x2.1 mm ID, particle size 1.7 μm), using 10 mM NH_4OAc in H_2O at pH 5 adjusted with AcOH (A) and 10 mM NH_4OAc in MeCN-H_2O (95:5) at pH 5 (B) as mobile phase. Values are reported as mean values of ≥2 experiments performed.

Chemical stability assay.

Chemical stability of selected compounds was evaluated under physiological pH conditions (0.01 M phosphate-buffered saline, pH 7.4) for up to 8 h. The buffer was added with 10% of MeCN. Stock solutions of each compound (10 mM) were freshly prepared in MeCN. Each compound was incubated at a final concentration of 1 μM in pre-heated buffer (37°C). The sample solutions were divided into aliquots in glass vials (preheated at 37°C) for each time point. The samples were maintained at 37°C in the UPLC/MS autosampler during the study (no shaking). A reference solution of each compound (final concentration: 1 μM) in preheated MeCN was prepared from the stock solutions and maintained at 37 °C in the UPLC/MS autosampler during the study. For each time point, the samples were analyzed directly by LC-MS without any further sample preparation. The samples were analyzed by integrating the corresponding MRM peak areas. The relative compound concentration was calculated by dividing the peak area at each time point by the peak area at t = 0 min. The reference solution was analyzed at the beginning (t = 0 min.) and at the end of the study (t =8 h). The apparent half-life (t½) of the disappearance of compound was calculated using the best fitting equation by GraphPad Prism (GraphPad Software, Inc., USA). The analyses were performed on a Waters ACQUITY UPLC/MS TQD system consisting of a triple quadrupole detector (TQD) MS equipped with an ESI interface and a photodiode array detector. The analyses were run on an ACQUITY UPLC BEH C18 1.7 μm 2.1 x 50 mm column with a VanGuard BEH C18 1.7 μm pre-column at 40°C. For each compound the appropriate mobile phase was chosen. ESI was applied in positive mode. Values are the mean of at least two independent experiments, performed in two technical replicates.

In vitro plasma stability study.

Freshly prepared 10 mM MeCN stock solution of test compound was diluted 50-fold with DMSO-H_2O (1:1) and incubated at 37˚C for 2 h with mouse plasma added 5% DMSO (pre-heated at 37˚C for 10 min). The final concentration was 2 μM. At each time point (0, 5, 15, 30, 60, 120 min), 50 μL of incubation mixture was diluted with 200 μL of cold MeCN spiked with 200 nM of internal standard, followed by centrifugation at 3,300 x g for 20 min. The supernatant was further diluted with H_2O (1:1) for analysis. The concentration of test compound was quantified by LC/MS-MS on a Waters ACQUITY UPLC/MS TQD system consisting of a TQD MS equipped with an ESI interface. The analyses were run on an ACQUITY UPLC BEH C18 (50 x 2.1 mm ID, particle size 1.7 μm) with a VanGuard BEH C18 pre-column (5 x 2.1 mm ID, particle size 1.7 μm) at 40°C. For each compound the appropriate mobile phase was chosen. ESI was applied in positive mode. The response factors, calculated on the basis of the internal standard peak area, were plotted over time. When possible, response vs. time profiles were fitted with Prism (GraphPad Software, Inc., USA) to estimate compounds t½ in plasma. Values are the mean of at least two independent experiments, performed in two technical replicates.

In vitro microsomal stability study.

Freshly prepared 10 mM MeCN stock solution of test compound was pre-incubated at 37˚C for 15min with mouse liver microsomes added 0.1M Tris-HCl buffer (pH 7.4). The final concentration was 4.6 μM. After pre-incubation, the cofactors (NADPH, G6P, G6PDH and $MgCl_2$ pre-dissolved in 0.1M Tris-HCl) were added to the incubation mixture and the incubation was continued at 37˚C for 1 h. At each time point (0, 5, 15, 30, 60 min), 30 μL of incubation mixture was diluted with 200 μL of cold MeCN spiked with 200 nM of internal standard, followed by centrifugation at 3,300 x g for 15 min. The supernatant was further diluted with H_2O (1:1) for analysis. The concentration of test compound was quantified by LC/MS-MS on a Waters ACQUITY UPLC/MS TQD system consisting of a TQD MS equipped with an ESI interface. The analyses were run on an ACQUITY UPLC BEH C18 (50 x 2.1 mm ID, particle size 1.7 μm) with a VanGuard BEH C18 pre-column (5 x 2.1 mm ID, particle size 1.7 μm) at 40°C. For each compound the appropriate mobile phase was chosen. ESI was applied in positive mode. The percentage of test compound remaining at each time point relative to t=0 was calculated. The t½ were determined by a one-phase decay equation using a non-linear regression of compound concentration versus time. Values are the mean of at least two independent experiments, performed in two technical replicates.

***In vitro* mouse plasma and mouse brain tissue protein binding.** Studies were performed by the DMPK Group at Shanghai ChemPartner Co., Ltd using equilibrium dialysis method. Values are the mean of two technical replicates.

ANIMAL MODELS

***In vivo* pharmacokinetic study.** Male CD1 mice (22-24 g, 6 weeks old, SLAC Laboratory Animal Co. LTD) were group-housed in ventilated cages and had free access to water and food. They were maintained under a 24 h light/dark cycle at controlled temperature and relative humidity. All efforts were made to minimize animal suffering and to use the minimal number of animals required to produce reliable results. All procedures were performed in accordance with the Ethical Guidelines on the protection of animals used for scientific purposes at the DMPK Group at Shanghai ChemPartner Co., Ltd. **22m** was administrated intravenously (i.v.) at 3 mg kg^{-1} (vehicle: 100% saline at 0.6 mg/mL) via tail vein injection (N=18) and via oral administration (p.o.) at 10 mg kg^{-1} (vehicle: 100% saline at 2.0 mg/mL) by oral gavage (N=18). *Sample collection*. Samples were collected at 0.25, 0.5, 1, 4, 8, 24 h. Animals were sacrificed 24 h after **22m** administration; plasma, brain and CSF samples were collected and stored at -80°C. *Blood collection*: the animal was restrained manually and approximately 150 μL blood/time point was collected into K_2EDTA tube via retro orbital puncture under anesthesia with isoflurane. Blood sample was put on ice and centrifuged to obtain plasma sample (2,000 x g, 5 min under 4 °C) within 15 min, then acidified following 100 μL plasma +1.0 μL of formic acid. An aliquot of 20 μL sample (pre-treatment with 1% formic acid) was added with 200 μL IS (propranolol, 40 ng/mL) in MeCN. The mixture was vortexed for 5min and centrifuged at 6,000 rpm for 10 min. The 0.5 μL mixture was injected into LC-MS/MS. *Brain collection:* brain was removed and immediately homogenized immediately for 2 min with 3 volumes (v/w) of homogenizing solution (PBS: formic acid = 100: 1) and then stored the tubes under -70°C until analysis. An aliquot of 20 μL sample was added with 200 μL MeCN which contains IS (propranolol, 40 ng/mL) for protein precipitation. The mixture was vortexed for 5 min and centrifuged at 6,000 rpm for 10 min. The 0.5 μL mixture was injected into LC-MS/MS. *CSF collection:* a mid-line incision was made on the neck. The muscle under the skin was cut to expose the cisterna magna. The CSF was collected by capillary. An aliquot of 3 μL sample was added with 90 μL IS (propranolol, 40 ng/mL) in MeCN: H_2O = 2: 1 (added 1% formic acid). The mixture was vortexed for 5 min and centrifuged at 6,000 rpm for 10 min. The 1.5 μL mixture was injected into LC-MS/MS. **22m** sample levels were monitored on LC-MS/MS-

19 (API5500, Qtriple) system, using the calibration curve and propanol as internal standard. Chromatography was carried out on waters BEH C18 column (2.1x 50 mm, 1.7 µm) at 60°C, setting a flow rate of 0.60 mL/min. Mobile phases were: A = H_2O - 0.025% formic acid – 1 mM NH_4OAc and B = MeOH- 0.025% formic acid – 1 mM NH_4OAc. After the initial 0.20 min at 10% of mobile phase B, the percentage of mobile phase B increased at 70% at 0.50 min, reaching steadily 90% in the range of 0.80 – 1.30 min. Then, the system returned to the initial conditions in a single step until 1.80 min. The following parent (m/z) / daughter (m/z) transitions were monitored: **22m**: m/z = 332.20/333.20 Da; propanol (I.S.): m/z: 260.30/116.10 Da.

Maximum Tolerated Dose (MTD) study. MTD study was conducted on male C57BL/6 mice (15-19 g, 5 weeks old, SLAC Laboratory Animal Co. LTD). Animals were injected via intraperitoneal (i.p.) injection with single administration at 20 mg/kg (N = 18, vehicle: 100% saline at 0.6 mg/mL) and multiple administrations for a duration of 4 days at 20 mg/kg (N = 18, day 1), 40 mg/kg (N = 18, day 2), 80 mg/kg (N = 18, day 3), 120 mg/kg (N = 18, day 4). Clinical observations/ samples of plasma, CSF and brain were collected at 0.25, 0.5, 1, 4, 8, 24 h. All procedures were performed in accordance with the Ethical Guidelines on the protection of animals used for scientific purposes at the DMPK Group at Shanghai ChemPartner Co., Ltd.

***In vivo* mouse model studies.** 4L;C* mice (C57BL/6J/129SvEV) were randomly assigned to 3 treatment groups (N = 4-8 with mixed males and females) and dosed once a day i.p. with 90 mg/kg or 30 mg/kg of **22m** or vehicle. Treatment started at 5 days of age for a duration of 14 days. The application volume was set to 10 µL per g body weight and dosage was adjusted accordingly. Animals of all groups were sacrificed 1 h after the last dose and the brain tissues and plasma were collected. The left brain containing cortex, cerebella, thalamus and brainstem was analyzed for SphL levels by MS. Data were analyzed using the Student t-test. The right brain containing cortex, cerebella, thalamus and brainstem and plasma was analyzed for drug levels of **22m**. All mice were housed under pathogen-free conditions in the animal facility and animal experiment was performed according to IACUC approved protocol (2018-0056) at Cincinnati Children's Hospital Research Foundation. Wild Type (WT) (GALC+/+) and Twitcher (Twi) (GALC−/−) mice were genotyped by PCR as previously described.[58] Twi mice were randomly assigned to treatment 3 groups (N= 3 males + N=3 females for each group) and dosed once a day i.p. with 90 mg/kg or 30 mg/kg of **22m** or vehicle for a duration of 20 days. Treatment started at 10 days of age. The application volume was set to 10 μL per g body weight and dosage was adjusted accordingly. Two groups (N= 3 males + N=3 females for each group) of WT controls treated with vehicle or high dose (90 mg/kg) of **22m** were also included in the study. Animals of all groups were sacrificed 1 h after the last dose and the brain tissues and plasma were collected. The left brain containing cortex, cerebella, thalamus and brainstem was analyzed for SphL levels by MS. Data were analyzed using the Student t-test. The right brain containing cortex, cerebella, thalamus and brainstem and plasma was analyzed for drug levels of **22m**. All animal work in this study was performed in accordance with approved animal protocols from the Animal Care and Use committee at the University of Illinois at Chicago.

ABBREVIATION USED

AC, acid ceramidase; Cer, ceramide; GALC, β-galactosyl-ceramidase; GalCer, galactosylceramide; GalSph, galactosylsphingosine; GD, Gaucher's disease; GCase, β-glucocerebrosidase; GluCer, glucosylceramide; GluSph, glucosylsphingosine; LSDs, lysosomal storage diseases; KD, Krabbe's disease; SphLs, Sphingolipids; Sph1P, sphingosine 1-phosphate; Twi, Twitcher; AcOH, glacial acetic acid; MeCN, acetonitrile; NH_4OAc, ammonium acetate; NH_4Cl, ammonium chloride; *n*-BuLi, *n*-butyllithium; CDI, 1′-carbonyldiimidazole; $CHCl_3$, chloroform; Cy, cyclohexane; Celite, diatomaceous earth; Et_2O, diethyl ether; DIPEA, *N,N*-diisopropylethylamine; ($Pd(dppf)Cl_2$), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II); EtOH, ethanol; EtOAc, ethyl acetate; eq., equivalent; HCl, hydrochloric acid; LiCl, lithium chloride; MeOH, methanol; MeMgBr, methylmagnesium bromide; Pd/C, palladium on carbon; $[B_2(pin)_2]$, bis(pinacolato)diboron; KOAc potassium acetate; K_2CO_3, potassium carbonate; K_3PO_4, potassium phosphate tribasic; Rt, retention time; SiO_2, silica gel; NaOAc, sodium acetate; $NaHCO_3$, sodium bicarbonate; Na_2CO_3, sodium carbonate; Na_2SO_4, sodium sulfate; $NaBH(OAc)_3$ sodium triacetoxyborohydride; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); Dess-Martin periodinane, 1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1H)-one; Et_3N, trimethylamine; Boc_2O, di-tert-butyl dicarbonate; TZD, 2,4-thiazolidinedione; *p*-TsOH, *p*-toluenesulfonic acid monohydrate; H_2O, water.

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Table of Contents Graphic:

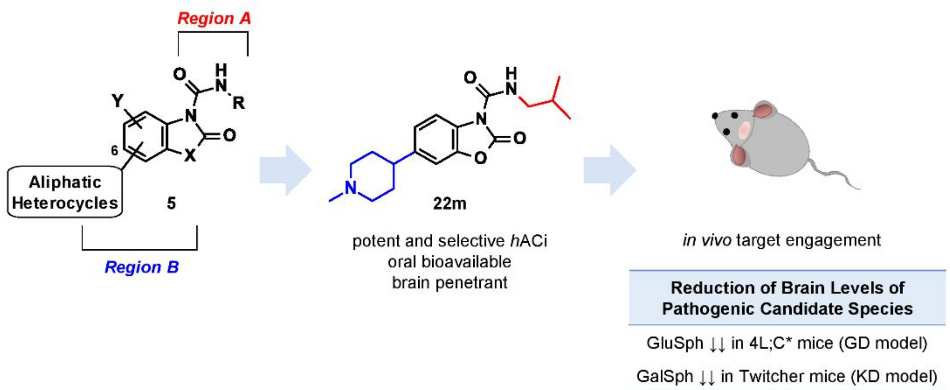


Figure 1. Some potential applications of AC inhibition therapy.

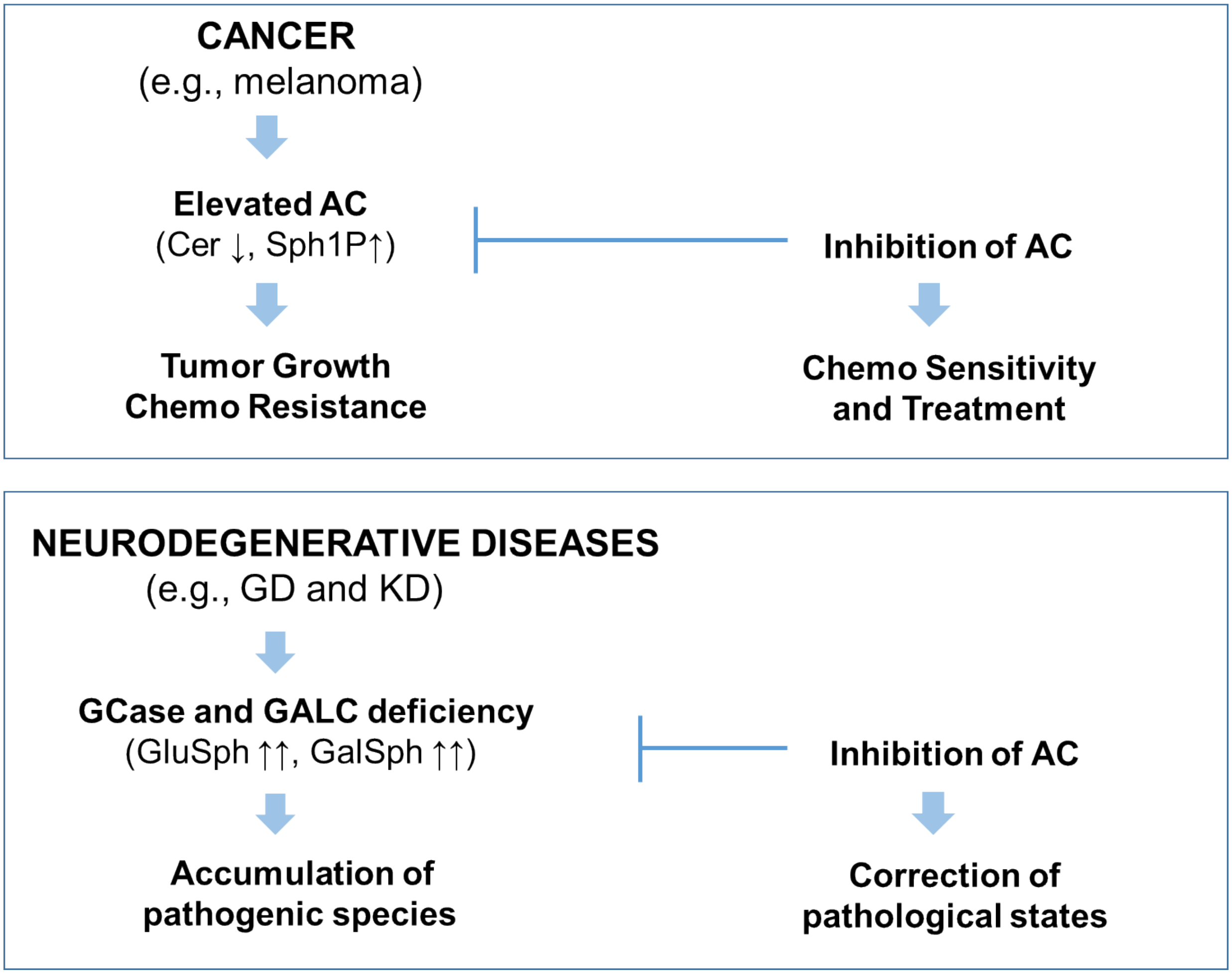


Figure 2. Structures of representative known AC inhibitors (**1-4**) (top) and general structure of the benzoxazolone carboxamide series **5** explored in this study (bottom).

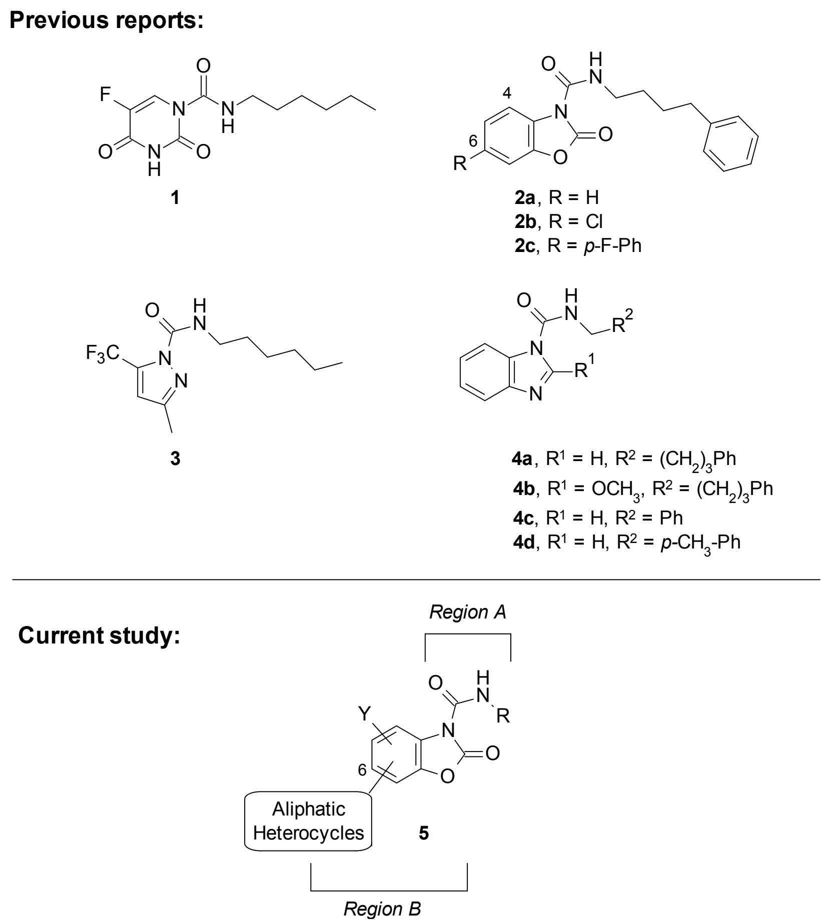
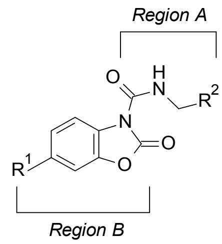
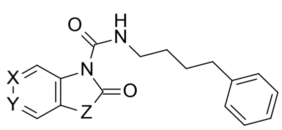


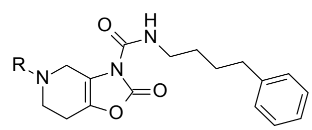
Figure 3. Inhibitory potencies (IC_{50} in μM) of compounds **2d-g**, **8a-d**, **12b-e** and **17b** on the activity of *h*AC expressed in HEK-293 cells.



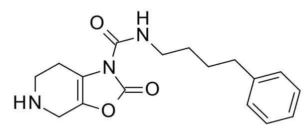
	*h*AC, IC_{50} (μM)
2a, R^1 = H, R^2 = $(CH_2)_3Ph$	0.064 [Ref.39]
2b, R^1 = Cl, R^2 = $(CH_2)_3Ph$	0.033 [Ref.40]
2c, R^1 = *p*-F-Ph, R^2 = $(CH_2)_3Ph$	0.079 [Ref.39]
2d, R^1 = *p*-F-Ph, R^2 = $(CH_2)OCH_2Ph$	4.70
2e, R^1 = *p*-F-Ph, R^2 = $(CH_2)_3CH_3$	0.060
2f, R^1 = *p*-F-Ph, R^2 = $(CH_2)OCH_2CH_3$	2.30
2g, R^1 = *p*-F-Ph, R^2 = $(CH_2)_2OCH_3$	2.60



	*h*AC, IC_{50} (μM)
8a, X= N, Y = CH, Z = O	0.006
8b, X= CH, Y = N, Z = O	0.003
8c, X= CH, Y = CH, Z = CO	not active up to 10μM
8d, X= CH, Y = CH, Z = CH_2	not active up to 10μM



	*h*AC, IC_{50} (μM)
12b, R = H	0.247
12c, R = Me	0.516
12d, R = Bn	0.436
12e, R = MeCO	0.293



	*h*AC, IC_{50} (μM)
17b	0.340

Figure 4. (A) Concentration- response curve for inhibition of *h*AC activity by **22m**; (B) Michaelis–Menten analysis of the reaction of *h*AC in the presence of vehicle (DMSO 1%, ●) or **22m** (100 nM, ▲; 400 nM, ■). Rbm 14-12: fluorogenic substrate of *h*AC; (C) Time-dependent inhibition of *h*AC by **22m** (two independent experiments, each performed in two technical replicates); (D) Determination of kinetic parameter k_i/K_I of **22m** (two independent experiments, each performed in two technical replicates).

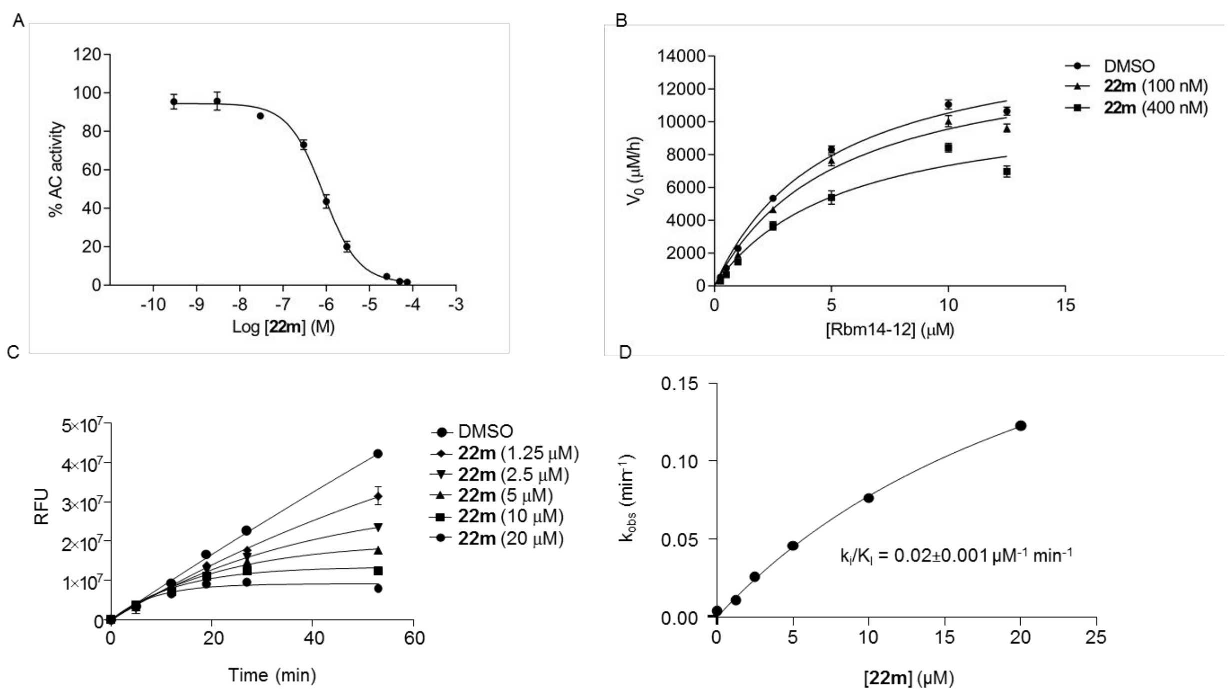


Figure 5: Effects of **22m** in SH-SY5Y cells after a 3 h incubation. Concentration dependence of the effects on AC activity (A) and sphingolipid levels (B-F). GraphPad Prism software (GraphPad Software, Inc., USA) was used for statistical analysis. Data were analyzed using the Student t-test or 1-way ANOVA followed by Bonferroni post hoc test for multiple comparisons. Differences between groups were considered statistically significant at values of $p < 0.05$. Values are expressed as means ± S.E.M of at least six determinations. Experiments were repeated twice with similar results.

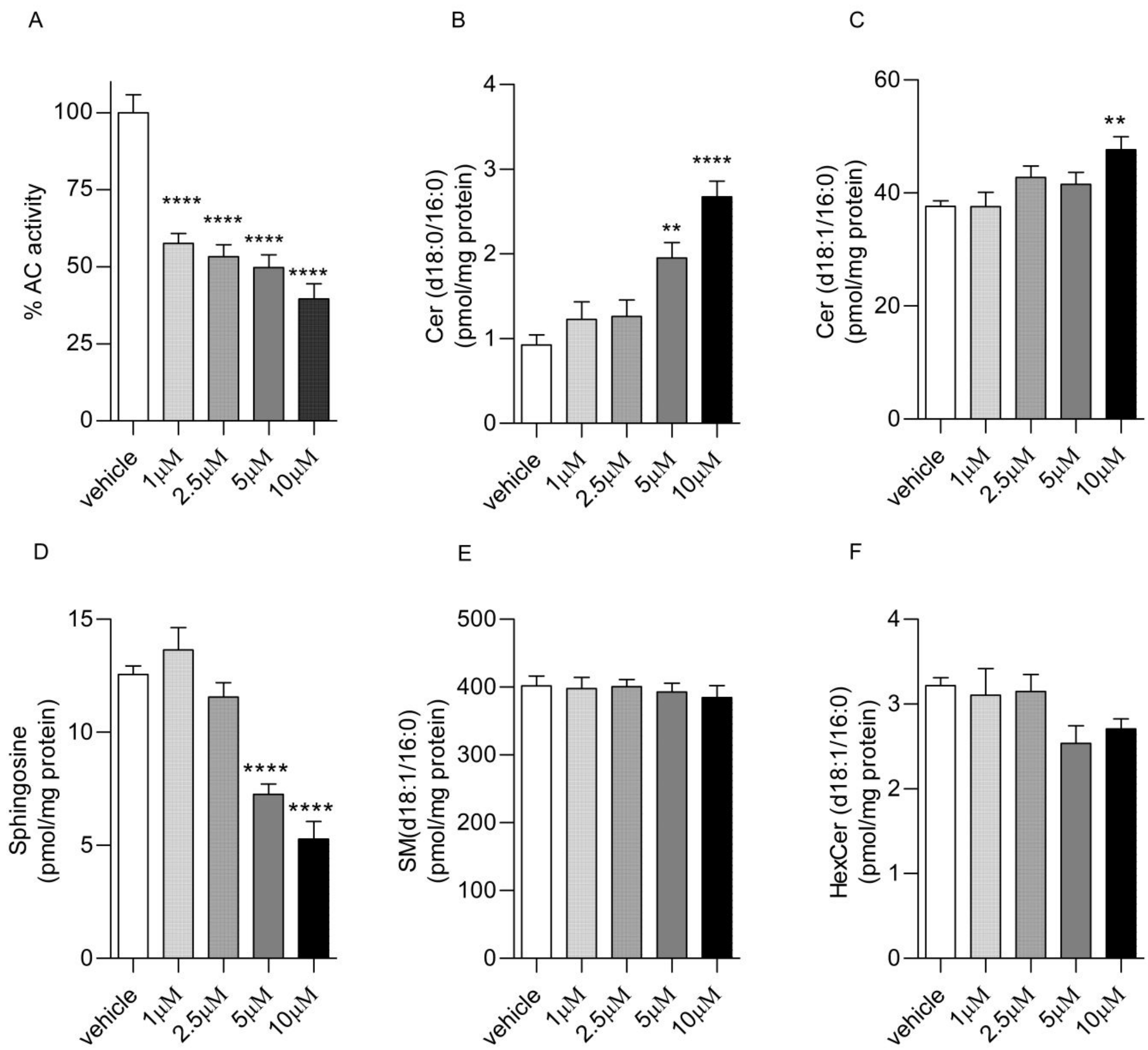


Figure 6: Time-course of the effects of **22m** (10 μM) in SH-SY5Y cells on AC activity (A) and sphingolipid levels (B-F). GraphPad Prism software (GraphPad Software, Inc., USA) was used for statistical analysis. Data were analyzed using the Student t-test or 1-way ANOVA followed by Bonferroni post hoc test for multiple comparisons. Differences between groups were considered statistically significant at values of $p < 0.05$. Values are expressed as means ± S.E.M of at least six determinations. Experiments were repeated twice with similar results.

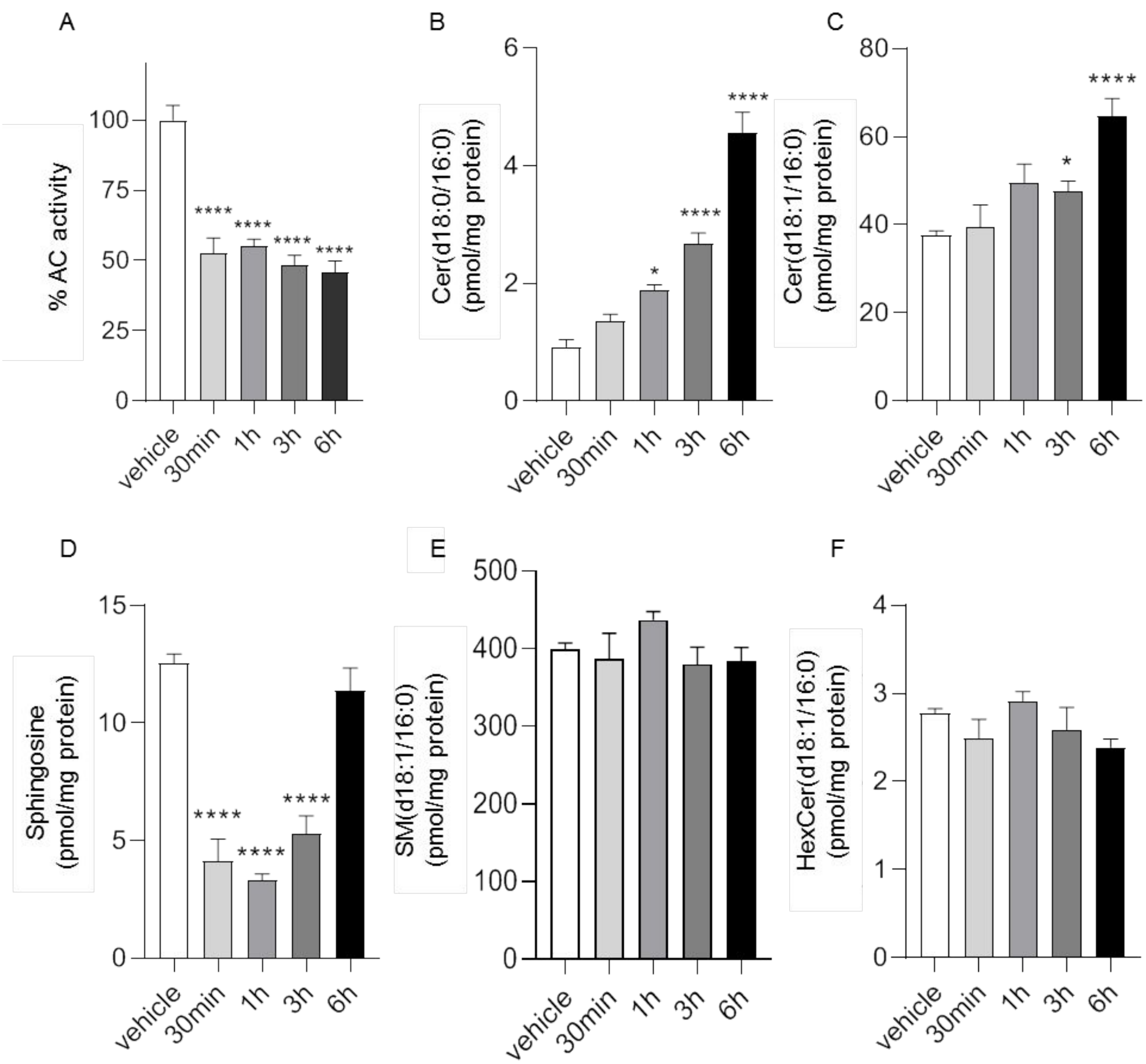


Figure 7. Dose response reduction of brain levels of GluSph (d18:1) after intraperitoneal injection of **22m** at 30 and 90 mg Kg^{-1} in 4L;C* mice (N = 4-8 with mixed males and females for each group).

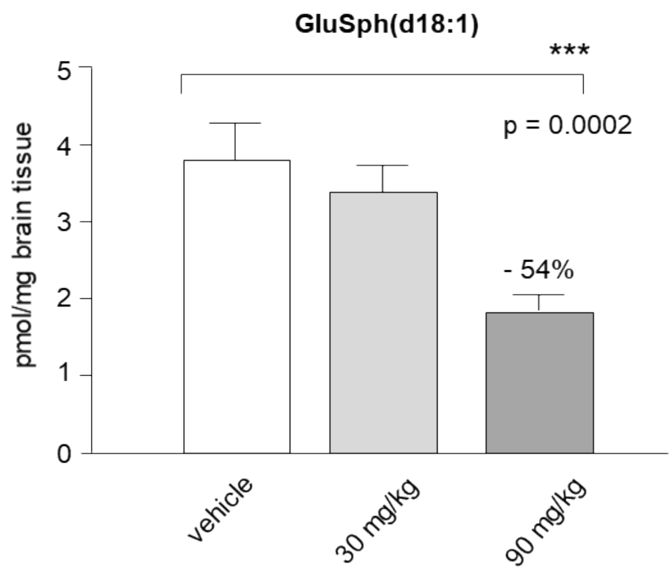


Figure 8. Dose response reduction of brain levels of GalSph (d18:1) after intraperitoneal injection of **22m** at 30 and 90 mg Kg^{-1} in Twitcher (Twi) mice (N= 3 males + N=3 females for each group).

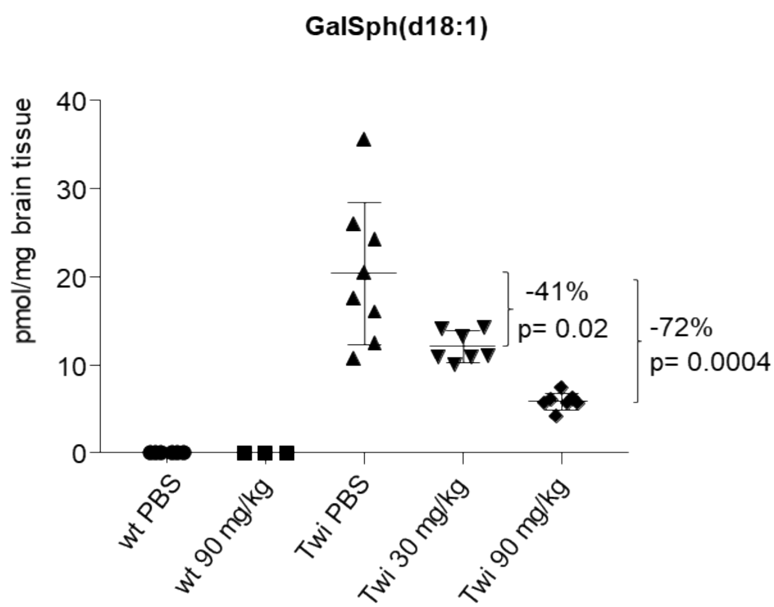
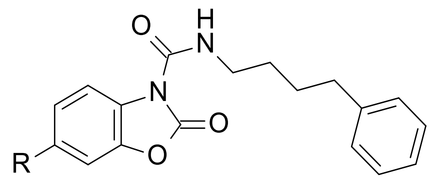
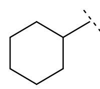
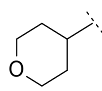
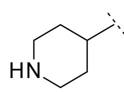
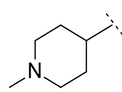
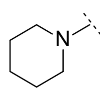
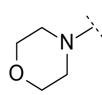
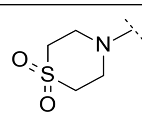
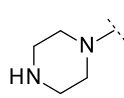
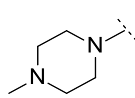


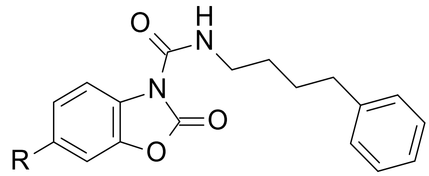
Table 1. Inhibitory potencies of compounds **22a-b**, **22d-e**, **23a-c** and **23e-f** on the activity of *h*AC.

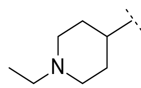
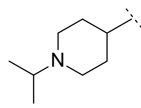
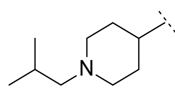
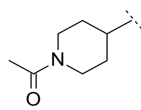
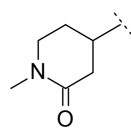
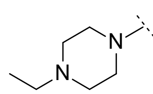
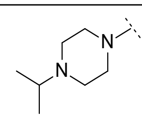
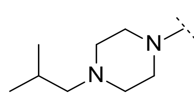
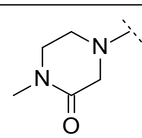
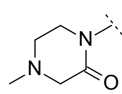


Compound	R	*h*AC IC_{50} (µM)±SD[a]
22a		0.089±0.054
22b		0.068±0.022
22d		0.134±0.014
22e		0.129±0.008
23a		0.080±0.017
23b		0.192±0.087
23c		0.160±0.014
23e		0.363±0.180
23f		0.116±0.069

[a]IC_{50} values are the mean of at least three independent experiments, performed in three technical replicates.

Table 2. Inhibitory potencies of piperidines **22f-i**, **22q** and piperazines **23g-k** on the activity of *h*AC.



Compound	R	*h*AC IC_{50} (μM)±SD[a]
22f		0.092±0.043
22g		0.094±0.042
22h		0.120±0.030
22i		0.064±0.029
22q		0.105±0.055
23g		0.056±0.006
23h		0.211±0.065
23i		0.127±0.037
23j		0.092±0.026
23k		0.052±0.040

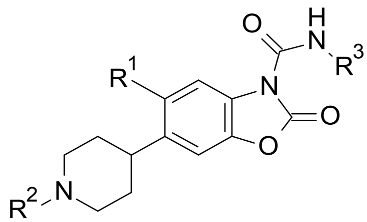
[a]IC_{50} values are the mean of at least three independent experiments, performed in three technical replicates.

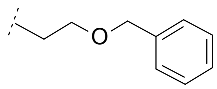
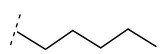
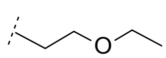
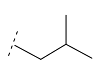
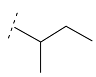
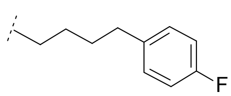
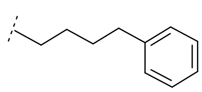
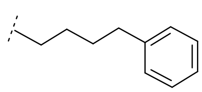
Table 3. Aqueous kinetic solubility and in vitro metabolism of some selected compounds in the piperidine **22d-i**, **22q** and piperazine **23e-g**, **23j-k** series.

Compound	Solubility (μM)[a] (in PBS, pH 7.4)	*m*-plasma[b] $t_{1/2}$ (min)	*m*-LM[c] $t_{1/2}$ (min) [% at 60 min]
Piperidine series			
22d	150	60	>60 [70%]
22e	120	50	40
22f	198	50	60
22g	50	40	60
22h	<1	60	60
22i	<1	30	60
22q	20	36	35
Piperazine series			
23e	20	30	45
23f	<1	<5	<5
23g	<1	<5	<5
23j	<1	20	30
23k	20	20	<5

[a]Aqueous kinetic solubility in phosphate buffered saline (PBS); [b]mouse plasma; [c]mouse liver microsomes. [a, b, c]Values are the mean of at least two independent experiments, performed in two technical replicates.

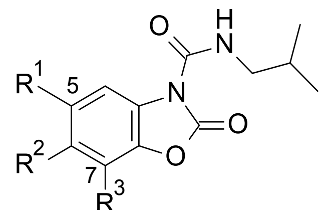
Table 4. Inhibitory potencies of piperidines **22j-p** and **22r** on the activity of *h*AC and aqueous kinetic solubility and in vitro metabolism of some selected compounds.

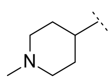
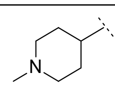
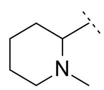
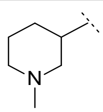


Compound	R^1	R^2	R^3	*h*AC IC_{50} (μM)±SD[a]	Solubility (μM) (PBS pH 7.4)[b]	*m*-plasma[c] $t_{1/2}$ (min)	*m*-LM[d] $t_{1/2}$ (min) [% at 60 min]
22j	H	CH_3		0.215±0.013	73	40	30
22k	H	CH_3		0.396±0.075	240	75	>60 [60%]
22l	H	CH_3		1.049±0.312	-	-	-
22m	H	CH_3		0.166±0.089	240	80	>60 [80%]
22n	H	CH_3		2.095±0.019	-	-	-
22o	H	CH_3		0.094±0.030	22	20	30
22p	F	CH_3		0.024±0.002	70	20	>60[70%]
22r	H	CHF_2CH_2		0.095±0.030	<1	50	20

[a]IC_{50} values are the mean of at least three independent experiments, performed in three technical replicates; [b]aqueous kinetic solubility in phosphate buffered saline (PBS); [c]mouse plasma; [d]mouse liver microsomes. [b,c,d]Values are the mean of at least two independent experiments, performed in two technical replicates.

Table 5. Inhibitory potencies of compounds **24c** and **25-27** on *h*AC and aqueous kinetic solubility and in vitro metabolism.



Compound	**R¹**	**R²**	**R³**	***h*AC IC_{50} (μM)±SD[a]**	**Solubility (μM) (PBS pH 7.4)[b]**	***m*-plasma[c] $t_{1/2}$ (min)**	***m*-LM[d] $t_{1/2}$ (min) [% at 60 min]**
24c		H	H	0.295±0.060	60	30	40
25	H	H		0.535±0.073	230	40	30
26	H		H	1.01±0.432	190	30	15
27	H		H	1.75±0.705	>250	50	>60[70%]

[a]IC_{50} values are the mean of at least three independent experiments, performed in three technical replicates; [b]aqueous kinetic solubility in phosphate buffered saline (PBS); [c]mouse plasma; [d]mouse liver microsomes. [b,c,d]Values are the mean of at least two independent experiments, performed in two technical replicates.

Table 6. Pharmacokinetic properties of **22m** after intravenous (A, 3 mg kg^{-1}, N = 18) and oral administration (B, 10 mg kg^{-1}, N = 18) in male CD1 mice.

A

Parameters (3mpk, i.v.)	plasma	brain	CSF
t_{max} (h)	-	0.250	0.250
C_{max} (ng/mL)	-	6443	71.6
$t_{1/2}$ (h)	1.26	1.01	0.661
Cl (L/h/Kg)	14.1	-	-
Vd_{ss} (L/Kg)	12.5	-	-
AUC (h x ng/mL)	212	10128	77.8

B

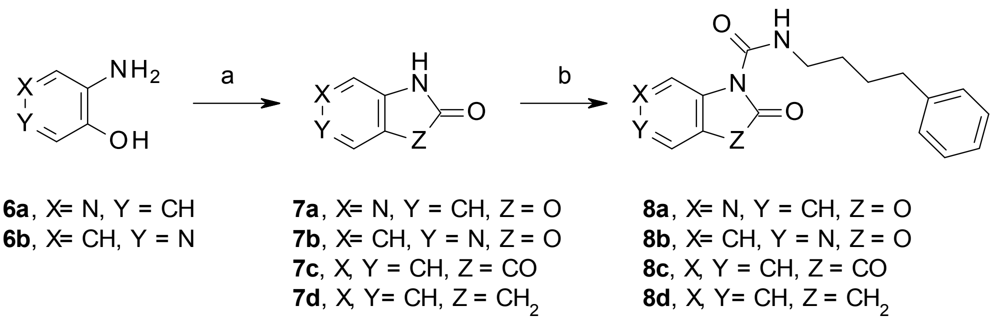
Parameters (10mpk, p.o.)	plasma	brain	CSF
t_{max} (h)	0.5	1.00	1.00
C_{max} (ng/mL)	216	6900	52.2
$t_{1/2}$ (h)	1.03	1.18	1.23
AUC (h x ng/mL)	412	14648	119
F (%)	58.3	-	-

Table 7. Plasma and brain concentrations of **22m** in 4L; C* and Twitcher mice.

EC_{50} (μM)[a]	Fp, u (%)[b]	Fb, u (%)[c]	Mouse Model	Dose (mg Kg^{-1})	Cp (μM)[d]	Cp,u (μM)[e]	Cb (μM)[f]	Cb,u (μM)[g]
0.410±0.100	13.8	0.70	4L;C*[h]	90	16.81	2.32	373.34	2.61
				30	3.08	0.42	110.12	0.77
			Twitcher[i]	90	3.85	0.52	307.92	2.15
				30	0.85	0.11	114.50	0.80

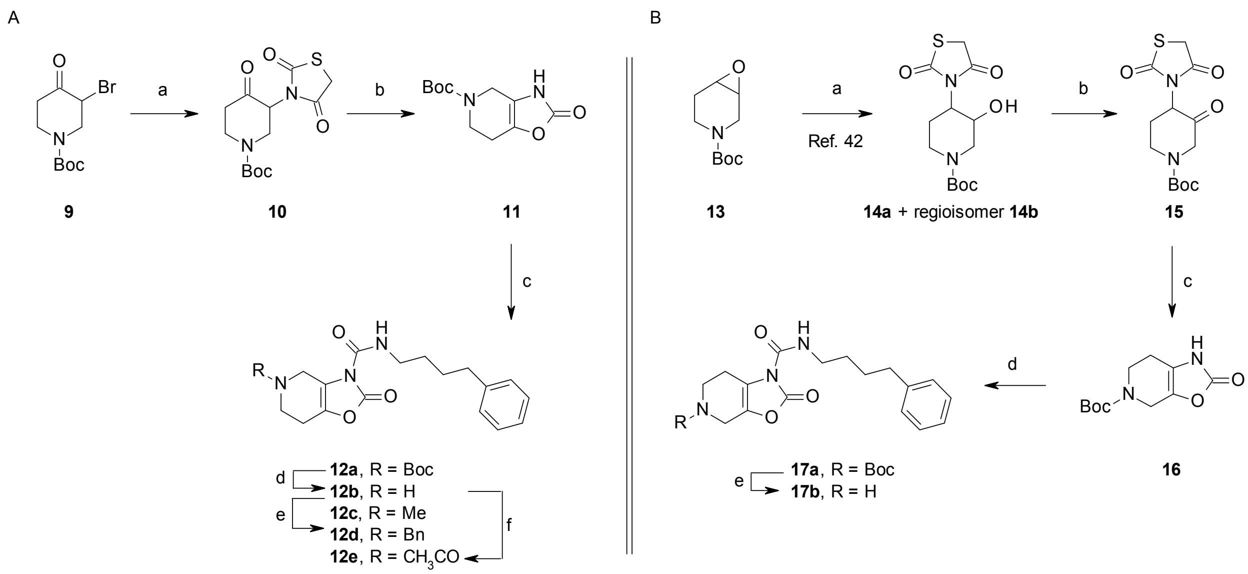
[a]EC_{50} value as a mean of two independent experiments, each performed in two technical replicates. Primary fibroblast cells from Krabbe's disease patients were incubated with **22m** for 2 h at different concentrations; [b]Fp, u: plasma fraction unbounded; [c]Fb, u: brain fraction unbounded; [b, c]Values are the mean of two technical replicates; [d]Cp: plasma concentration; [e]Cp, u: plasma unbounded concentration; [f]Cb: brain concentration; [g]Cb, u: brain unbounded concentration; [h]4L;C* mice were sacrified 1 h after the last doses (day 14) and the compound **22m** levels were measured in plasma and brain (N = 4-8 with mixed males and females); [i]Twitcher mice were sacrificed 1 h after the last doses (day 20) and the compound **22m** levels were measured in plasma and brain (N= 3 males + N=3 females for each group).

Scheme 1. Synthesis of **8a-d.**[a]



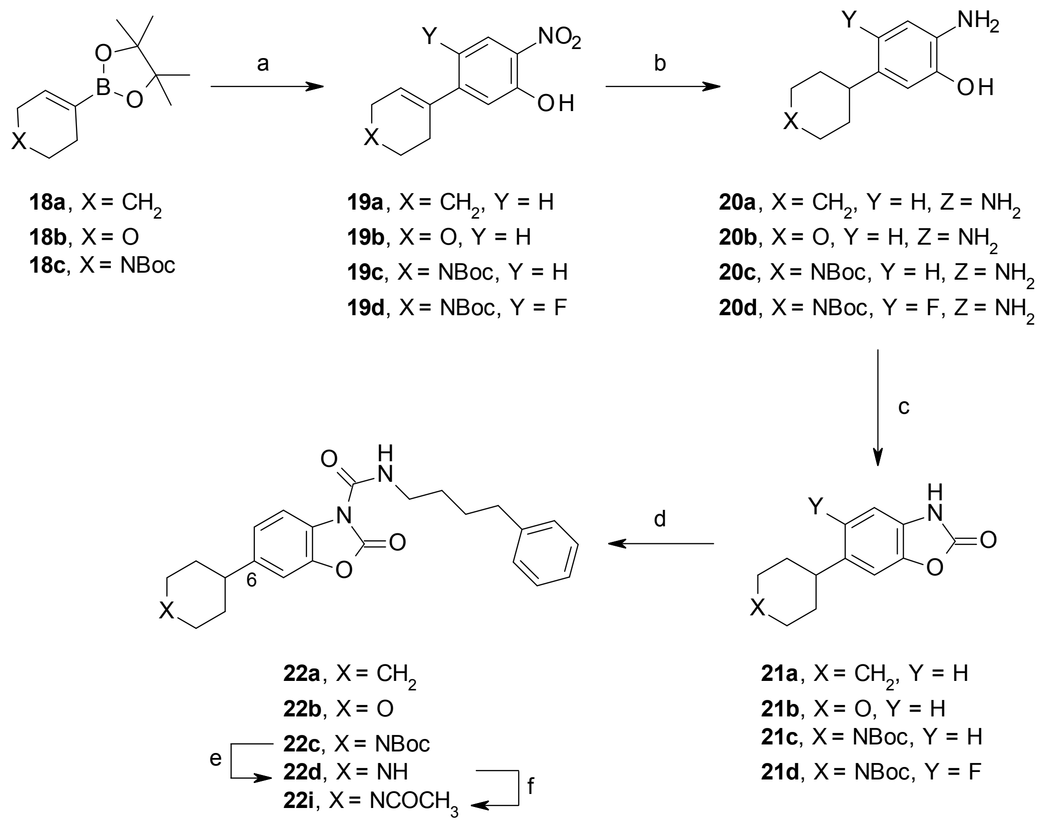
[a]Reagents and conditions: (a) CDI, MeCN, rt, 2h; (b) 4-phenylbutyl isocyanate, DMAP, toluene/DMF, rt, 2h (20- 60% over two steps for **8a-b**); 4-phenylbutyl isocyanate, Et_3N, MeCN, rt, 2 h (20- 26% for **8c-d**).

Scheme 2. Synthesis of fused bicyclic piperidine-oxazolone derivatives **12a-e** and **17a-b.**[a]



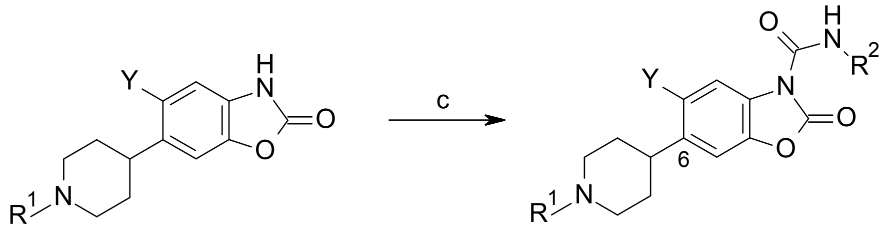
[a]Reagents and conditions: for the synthesis of **12a-e**: (a) thiazolidinedione, K_2CO_3, DMF, rt, 2 h (85%); (b) *t*BuOK, THF, rt, 30 min (60%); (c) 4-phenylbutyl isocyanate, DMAP, MeCN, rt, 16 h (68%); (d) 4M HCl, dioxane, rt, 1 h (60%); (e) HCHO (for **12c**) or PhCHO (for **12d**), $NaBH(OAc)_3$, AcOH, MeCN, rt, 3 h (56 - 90%); (f) AcCl, Et_3N, DCM, rt, 3 h (62%). For the synthesis of **17a-b**: (a) TZD, $Mg(ClO_4)_2$, DMF, 115°C, 5 h (50%); (b) Dess Martin reagent, DCM, 0°C to rt, 12 h (70%); (c) *t*BuOK, THF, rt, 30 min; (d) 4-phenylbutyl isocyanate, DMAP, MeCN, rt, 30 min (25% over two steps); (e) 4M HCl, dioxane, rt, 1 h (60%).

Scheme 3. Synthesis of C(6)- substituted benzoxazolone carboxamides **22a-d** and **22i**.[a]



[a]Reagents and conditions: (a) 5-bromo-2-nitrophenol (for **19a-c**), 5-bromo-4-fluoro-2-nitrophenol (for **19d**), $Pd(PPh_3)_4$, 2M Na_2CO_3, dioxane, reflux, 18 h (56 - 90%); (b) H-Cube, Pd/C, EtOAc, rt 1-2 h; (c) CDI, MeCN, 60°C, 2 h (60 - 84% over two steps); (d) 4-phenylbutyl isocyanate, DMAP, MeCN, rt, 16 h (50 - 98%); (e) 4M HCl, dioxane, rt, 3 h (86%); (f) AcCl, Et_3N, THF, rt, 4 h (90%).

Scheme 4. Synthesis of C(6)- substituted benzoxazolone carboxamides **22e-h**, **22j-p.**[a]



21c, R^1 = Boc, Y = H
21d, R^1= Boc, Y = F

a

21e, R^1= H, Y = H
21f, R^1 = H, Y = F

b

21g, R^1 = Me, Y = H
21h, R^1 = Et, Y = H
21i, R^1 = *iso*-propyl, Y = H
21j, R^1 = *iso*-butyl, Y = H

21k, R^1 = Me, Y = F

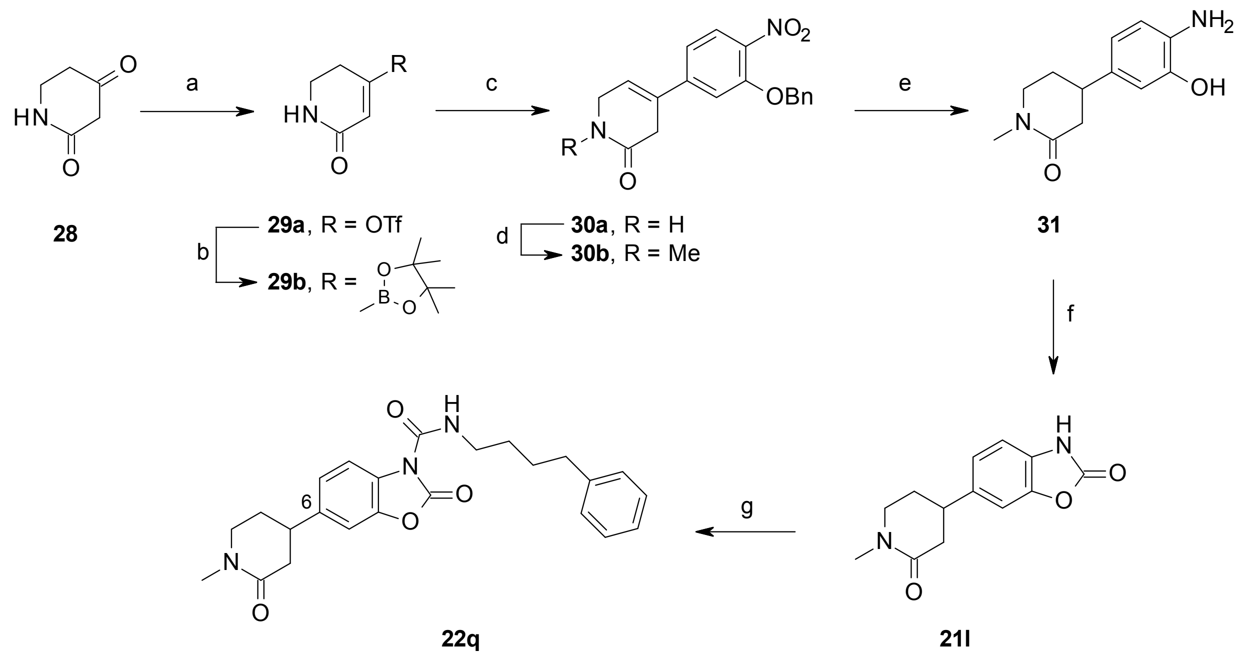
22e, R^1 = Me, Y = H, R^2 = $(CH_2)_4Ph$
22f, R^1 = Et, Y = H, R^2 = $(CH_2)_4Ph$
22g, R^1 = *iso*-propyl, Y = H, R^2 = $(CH_2)_4Ph$
22h, R^1 = *iso*-butyl, Y = H, R^2 = $(CH_2)_4Ph$

22j, R^1 = Me, Y = H, R^2 = $(CH_2)_2OCH_2Ph$
22k, R^1 = Me, Y = H, R^2 = $(CH_2)_4CH_3$
22l, R^1 = Me, Y = H, R^2 = $(CH_2)_2OCH_2CH_3$
22m, R^1 = Me,Y = H, R^2 = *iso*-butyl
22n, R^1 = Me, Y = H, R^2 = *sec*-butyl
22o, R^1= Me, Y = H, R^2 = $(CH_2)_4$-(*p*-F-Ph)

22p, R^1 = Me, Y = F, R^2 = $(CH_2)_4Ph$

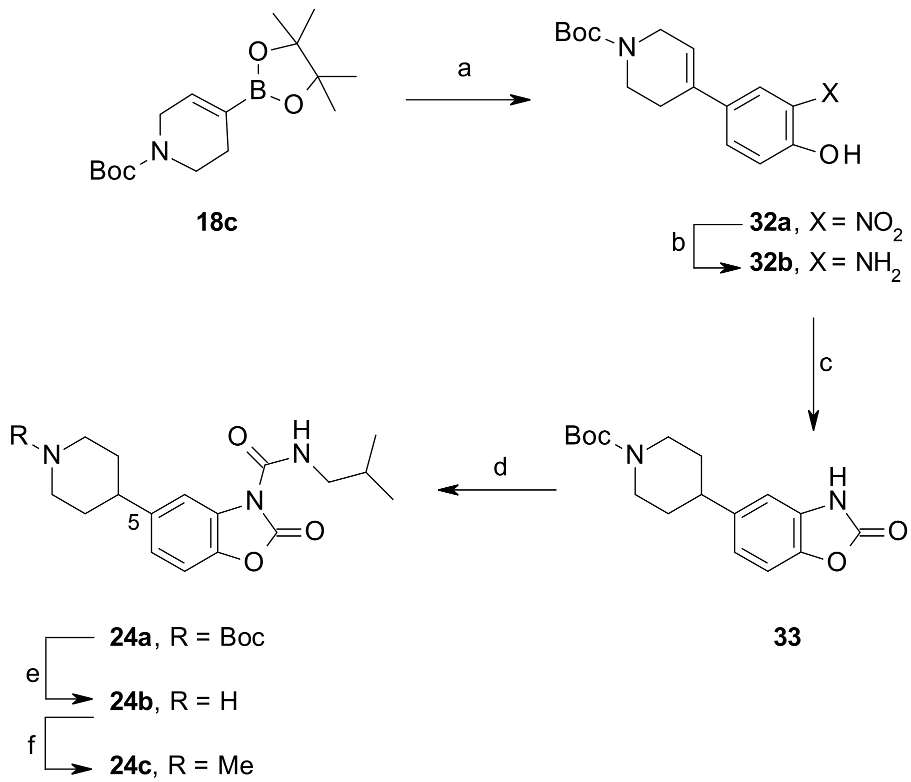
[a]Reagents and conditions: (a) 4M HCl, dioxane, rt, 3 h; (b) RCHO, AcOH, $NaBH(OAc)_3$, DCE, THF or MeCN, rt, 1-3 h (40% over two steps for **21i**, quant. for 21h); (c) RNCO, DMAP, MeCN, rt, 2 - 16 h (50 – 73% for **22e-h**, **22k** and **22p**); or RNH_2, triphosgene, Et_3N, DCM, 0 °C -rt, 2 h (45% for **22m**), or RNH_2, Boc_2O, DMAP, MeCN, rt, 1 h (24 - 40% for **22j**, **l**, **22n-o**).

Scheme 5. Synthesis of **22q**.[a]



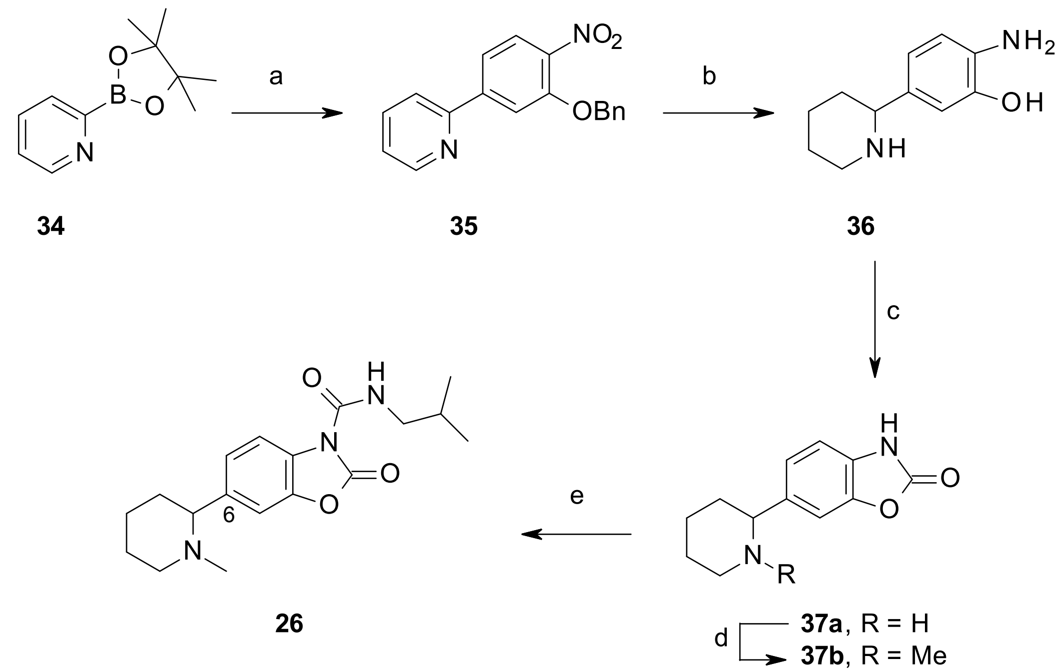
[a]Reagents and conditions: (a) N,N-Bis(trifluoromethanesulfonyl)aniline, Et_3N, THF, 0°C, 16 h (74%); (b) $Pd(dppf)Cl_2$, $[B_2(pin)_2]$, KOAc, dioxane, 70°C, 3 h; (c) 2-(benzyloxy)-4-bromo-1-nitrobenzene, Na_2CO_3 2M, 70°C, 1 h (93% over two steps) (d) NaH, MeI, THF, 0°C, 20 h (45%); (e) H_2, 10% Pd/C, EtOH, rt, 1 h; (f) CDI, MeCN, rt, 1 h (70% over two steps); (g) 4-phenylbutyl isocyanate, DMAP, pyridine, rt, 16 h (90%).

Scheme 6. Synthesis of **24c.**[a]



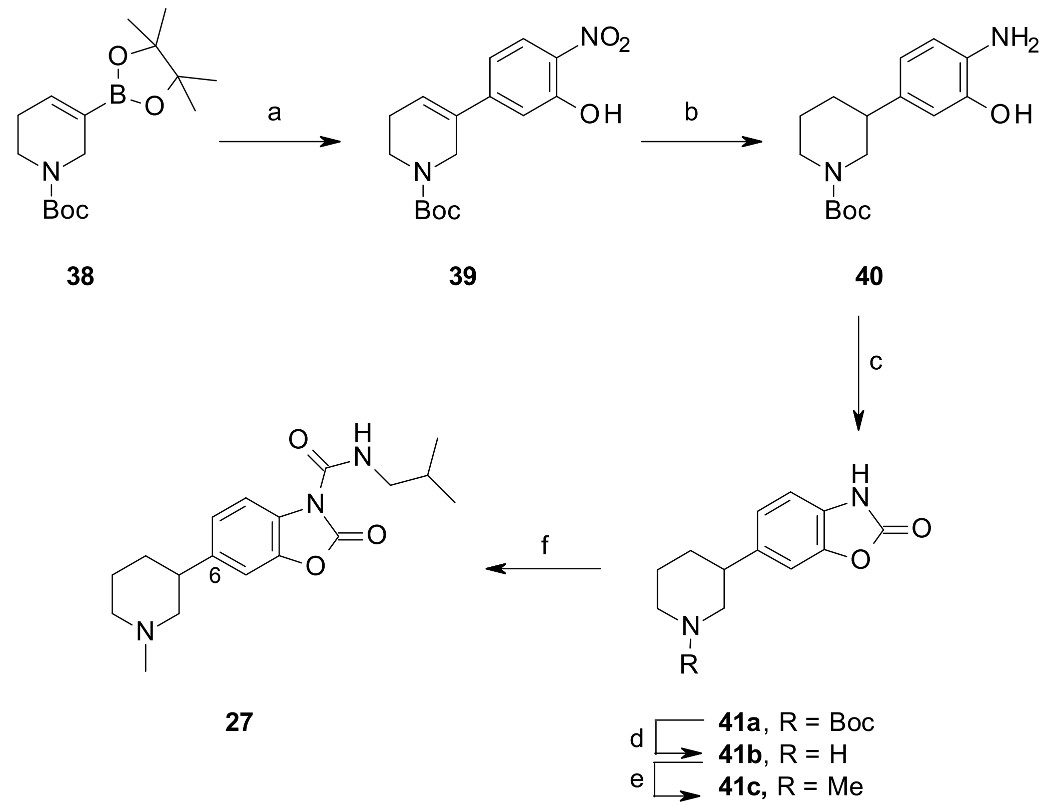
[a]Reagents and conditions: (a) 4-bromo-2-nitro-phenol, $PdCl_2(PPh_3)_2$, 2M Na_2CO_3, dioxane, reflux, 2 h (95%); (b) 10 % Pd/C, cyclohexene, MeOH, reflux, 4 h; (c) CDI, MeCN, rt 3 h (60% over two steps); (d) *iso*-butylamine, triphosgene, Et_3N, DCM (70%); (e) 4M HCl, dioxane (95%); (f) HCHO, $NaBH(OAc)_3$, AcOH, MeCN, rt, 2 h (83%).

Scheme 7. Synthesis of **26.**[a]



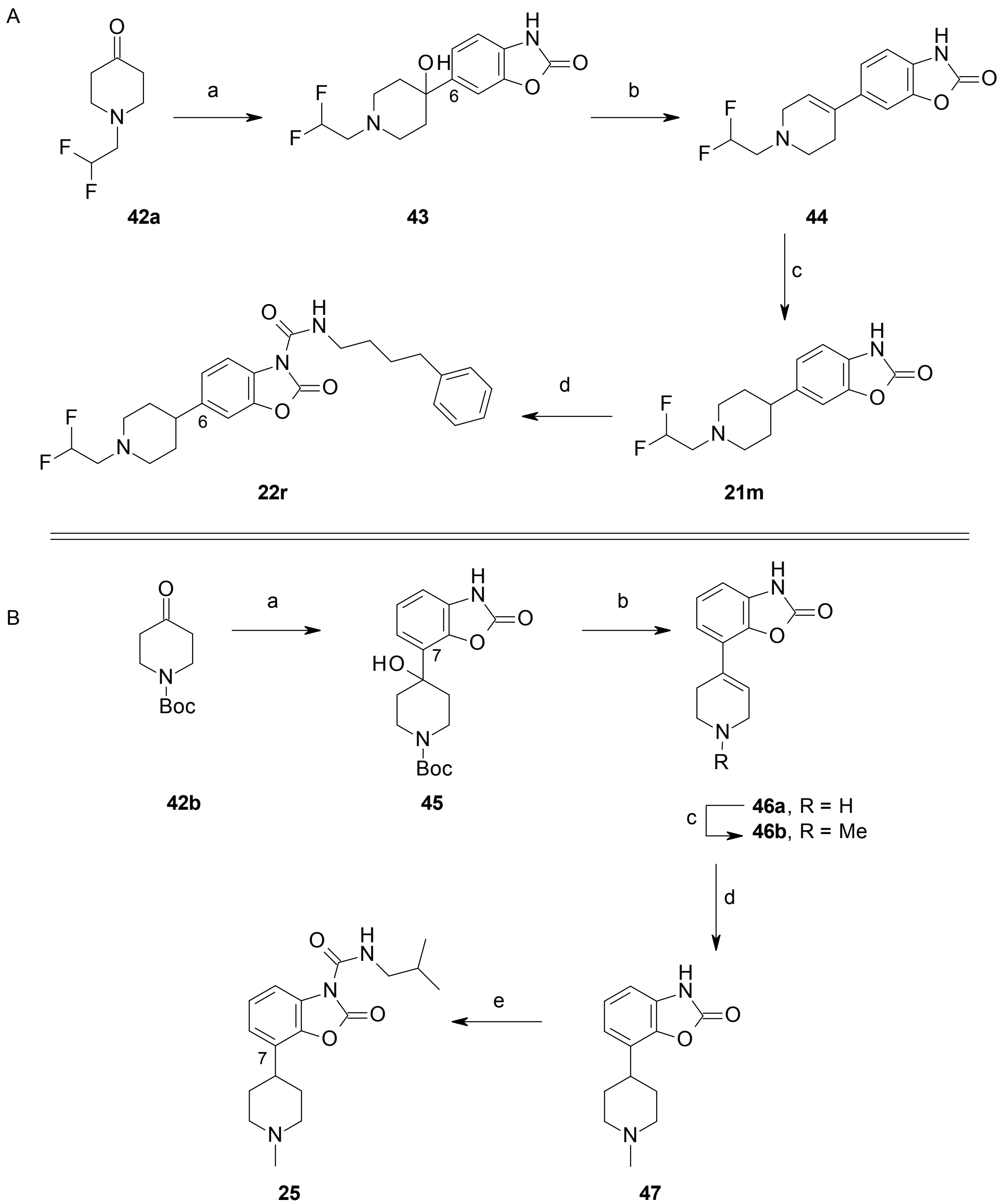
[a]Reagents and conditions: (a) 2-benzyloxy-4-bromo-1-nitro-benzene, Pd(dppf)Cl_2, Na_2CO_3, dioxane, reflux, 16 h (40%); (b) cyclohexene, Pd/C, MeOH, 70°C, 2 h; (c) CDI, MeCN, rt, 16 h; (d) HCHO, NaBH$(OAc)_3$, AcOH, MeCN, rt, 2 h (43% over three steps); (e) *iso*-butylamine, triphosgene, Et_3N, DCM, rt, 4 h (30%).

Scheme 8. Synthesis of **27**.[a]



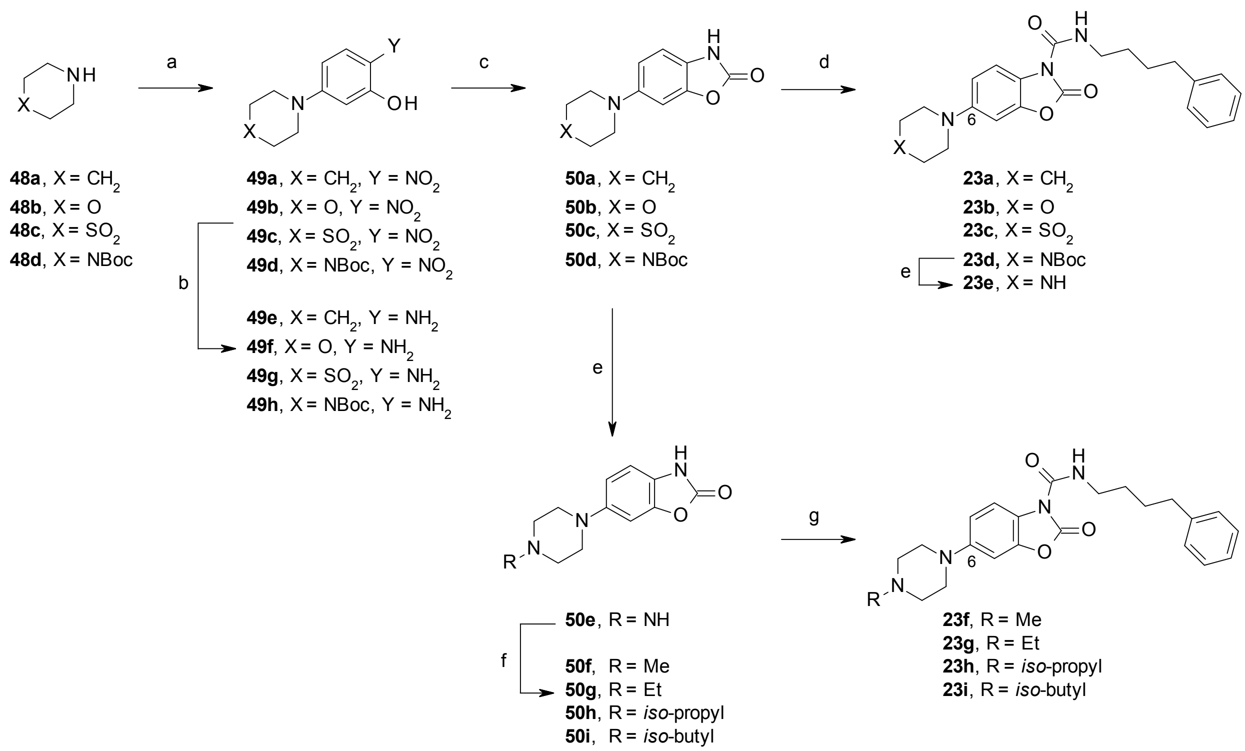
[a]Reagents and conditions: (a) 5-bromo-2-nitro-phenol, Pd(dppf)Cl_2, Na_2CO_3, dioxane, reflux, 2 h (53%); (b) 10% Pd/C, cyclohexene, EtOH, 65°C, 4 h; (c) CDI, MeCN, rt, 1 h (90% over two steps) (d) 4M HCl, dioxane, rt, 30 minutes; (e) HCHO, NaBH$(OAc)_3$, AcOH, MeCN, rt, 30 min (70% over two steps); (f) *iso*-butylamine, triphosgene, Et_3N, DCM, rt, 3 h (70%).

Scheme 9. Synthesis of **22r** and **25**.[a]



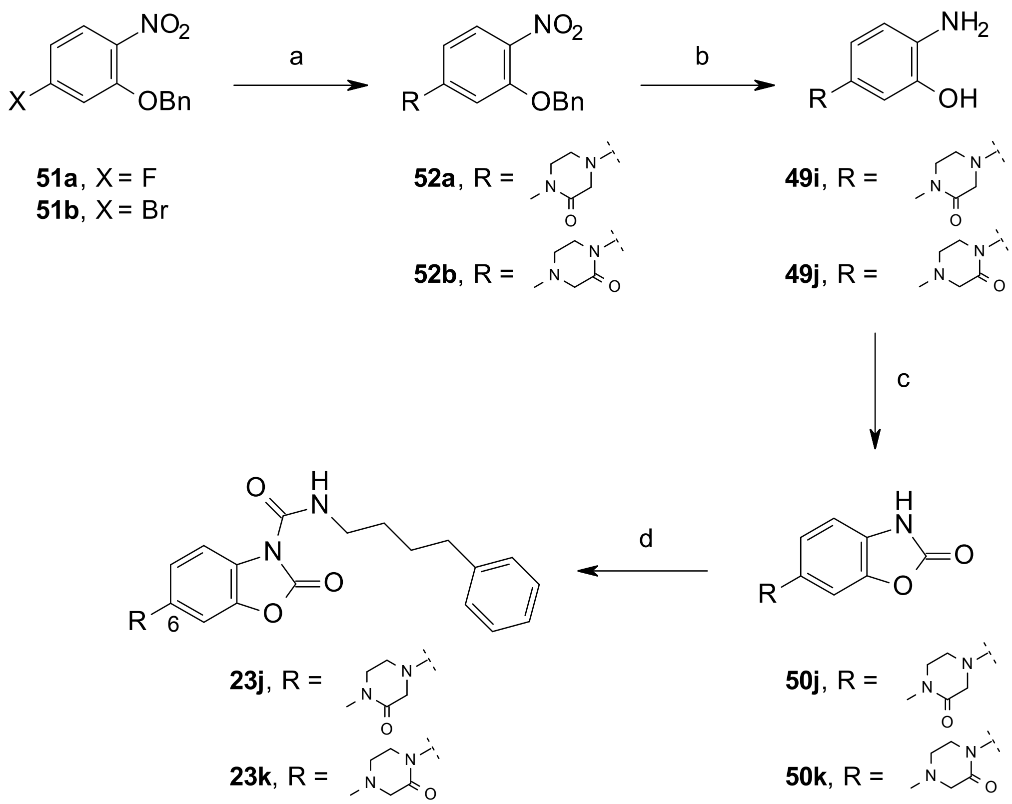
[a]Reagents and conditions: for the synthesis of **22r**: (a) 6-bromo-3*H*-1,3-benzoxazol-2-one, MeMgBr, n-BuLi, THF, -78°C, 2 h (30%); (b) *p*-TsOH, toluene, reflux, 1 h (quant.); (c) H_2, 10% Pd/C, MeOH, 60°C, 2 h; (d) 4-phenylbutyl isocyanate, DMAP, pyridine (73% over two steps). For the synthesis of **25**: (a) 7-bromo-3*H*-1,3-benzoxazol-2-one, MeMgBr, *n*-BuLi, THF, -78°C 1.5 h (44%); (b) *p*-TsOH, toluene, 90°C, 3 h (quant.); (c) HCHO, $NaBH(OAc)_3$, MeCN, rt, 16 h; (d) H_2, Pd/C, MeOH, 40°C, 2 h; (e) *iso*-butylamine, triphosgene, Et_3N, DCM (30% over three steps).

Scheme 10. Synthesis of C(6)- substituted benzoxazolone carboxamides **23a-i.**[a]



[a]Reagents and conditions: (a) 5-fluoro-2-nitrophenol, DIPEA, MeCN, 60 - 80°C, 15 h (40% for **49c**); (b) 10% Pd/C, cyclohexene, MeOH, reflux, 2-16 h; (c) CDI, MeCN, rt (or 50°C for **49g**), 2 h (75 – 80% over three steps for **50a** and **50d**; 45 – 60% over two steps for **50b**,**c**); d) 4-phenylbutyl isocyanate, DMAP, MeCN, rt, 16 h (20 - 85%); (e) 4M HCl, dioxane, rt, 3 h (quant.). (f) RCHO, AcOH, NaBH(OAc)$_3$, DCE, THF or MeCN, rt, 2 h (60 - 90%); (g) 4-phenylbutyl isocyanate, DMAP, MeCN, rt, 16 h (30 - 75%).

Scheme 11. Synthesis of **23j-k.**[a]



[a]Reagents and conditions: (a) 1-methylpiperazin-2-one (for **52a**), Et_3N, MeCN, 80 °C, 16 h (60%); 4-methylpiperazin-2-one (for **52b**), CuI, K_3PO_4, N,N-dimethyl-1,2-ethanediamine, dioxane, rt, 24 h (50%); (b) 10% Pd/C, cyclohexene, EtOH, 65°C, 16 h; (c) CDI, MeCN, rt, 1 h (70 - 80% over two steps); (d) 4-phenylbutyl isocyanate, DMAP, MeCN, rt, 16 h (10-20%).

ASSOCIATED CONTENT

Supporting Information

V_{max} and K_M determinations; concentration- response curve of **22m** in primary fibroblast cells of Krabbe's patients; ^{1}H NMR and ^{13}C NMR spectra of the final compounds; retention times and UPLC analytical methods of the final compounds; UPLC traces of the final compounds; a csv file containing molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.org.

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