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# Development and crystallography-aided SAR studies of multifunctional BuChE inhibitors and 5-HT$_6$R antagonists with β-amyloid anti-aggregation properties

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ABSTRACT

The lack of an effective treatment makes Alzheimer's disease a serious healthcare problem and a challenge for medicinal chemists. Herein we report interdisciplinary research on novel multifunctional ligands targeting proteins and processes involved in the development of the disease: BuChE, 5-HT$_6$ receptors and β-amyloid aggregation. Structure-activity relationship analyses supported by crystallography and docking studies led to the identification of a fused-type multifunctional ligand **50**, with remarkable and balanced potencies against BuChE ($IC_{50}$ = 90 nM) and 5-HT$_6$R ($K_i$ = 4.8 nM), and inhibitory activity against Aβ aggregation (53% at 10 μM). In *in vitro* ADME-Tox and *in vivo* pharmacokinetic studies compound **50** showed good stability in the mouse liver microsomes, favourable safety profile and brain permeability with the brain to plasma ratio of 6.79 after *p.o.* administration in mice, thus being a promising candidate for *in vivo* pharmacology studies and a solid foundation for further research on effective anti-AD therapies.


## 1. Introduction

There are nowadays around 35 million people worldwide [1] who have been diagnosed with Alzheimer's disease (AD). The typical course of the disease manifests itself by a decline in memory, language and speech problems, time and space disorientation and personality changes. These symptoms are very often accompanied by a broad range of behavioural and psychological symptoms of dementia (BPSD), such as hyperactivity, psychosis, depression, anxiety and apathy, which significantly worsen the life-quality of patients with AD and their caregivers [2,3].

From the pathophysiological point of view, AD is a neurodegenerative disease characterized by a disruption of neurotransmitter systems, aggregation of amyloid beta (Aβ) and tau proteins, oxidative stress, profound neuroinflammation and many other pathological changes [4–6]. The main neurotransmitter system affected by the disease – the cholinergic system – is targeted by the currently used anti-AD drugs acting as cholinesterase inhibitors (ChEIs): donepezil, rivastigmine and galantamine [7–10]. ChEIs and memantine, antagonist of NMDA receptors and thus modulator of glutamatergic system, constitute current treatment options for AD. The common flaw of these drugs is their limited efficacy; while able to ameliorate several symptoms, they cannot stop the progression of the disease [11,12]. One of the reasons for that may lie in the fact that these drugs were developed based on the "one drug-one target" paradigm. It is generally acknowledged that effective treatment of multifactorial diseases, such as AD, should involve simultaneous action on several targets e.g. by means of multifunctional ligands (multi-target-directed ligands, MTDLs).

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Such ligands, combining several activities in one molecule, lower the risk of potentially dangerous interactions resulting from the concomitant administration of several drugs and are currently the subject of great interest among medicinal chemists [13–16]. This is reflected not only in the number of publications on this topic but also in the statistics of newly registered drugs, according to which from 2015 to 2017, 21% of the 101 new molecular entities approved by the U.S. FDA were multifunctional drugs [17,18].

In the multiplicity of targets used in the development of multifunctional ligands against AD, the cholinergic system and especially cholinesterases are considered to be a clinically verified approach and are extensively used in many research projects. Of the two known cholinesterases – acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), the latter enzyme gained much attention as an interesting target. In AD-affected brains, BuChE concentration is preserved or even rises, while the amount of AChE, the main enzyme responsible for ACh hydrolysis under normal conditions, decreases [19]. The phenomenon of the takeover of ACh hydrolysis by BuChE was shown in studies with AChE and BuChE knockout mice pointing to BuChE as a relevant biological target in the search for anti-AD therapy [20,21]. Although the exact cause of cholinergic dysfunction in AD is not known, a growing body of evidence implies that there are complex reciprocal interactions between cholinergic dysfunction and other pathophysiological features of AD, including Aβ [11]. Recent studies have revealed the association of BuChE with Aβ pathology. Firstly, butyrylcholinesterase was shown by histochemical studies to be present in senile plaques [22,23]. Secondly, in experiments with BuChE-knockout mice researchers observed a reduced Aβ plaque deposition [24] and more recently – a higher resistance to cognitive impairment caused by intracerebroventricular injection of toxic $A\beta_{25-35}$ when compared with wild-type animals [25]. These interactions expand the role of the cholinergic system as a target for AD therapies, as it may not only be involved in the amelioration of symptoms, but also in modifying AD progression [26]. *In vivo* studies of a selective BuChE inhibitor showed improved memory and learning abilities in Morris water maze and passive avoidance tests without producing acute cholinergic adverse effects [27]. These findings support the potential of BuChE inhibition as a viable strategy in the treatment of Alzheimer's disease.

Another potential target for AD therapy is the serotonergic system, especially 5-$HT_4$ and 5-$HT_6$ receptors [28–31]. The spectrum of biological effects caused by the activation of 5-$HT_4$R is very broad and relevant to the pathophysiology of AD, therefore addressing 5-$HT_4$R is a commonly pursued strategy in the search for disease modifying-agents for AD [32–36]. Equally important is the serotonin receptor subtype 6 that is almost exclusively distributed in the brain areas involved in learning and memory processes [37–39] and several studies demonstrated a positive effect of the 5-$HT_6$R antagonists on cognition [40–42]. In particular, the blockade of 5-$HT_6$R was shown to increase acetylcholine levels and reverse cognition deficits induced by the administration of anticholinergic agents [43–45]. This effect is mediated by multiple neurotransmitter systems, including serotonergic, cholinergic, glutamatergic and GABAergic [46]. 5-$HT_6$R antagonists investigated in preclinical studies alleviated affective disorders such as anxiety or depression [47–49], thus expanding the therapeutic potential of such agents in the treatment of BPSD in AD. There are also reports pointing to a disease-modifying potential of the 5-$HT_6$ receptor antagonists related to synaptic remodelling [50] and neuronal hyperexcitability [51], which are perceived as plausible causes of AD. Hence, 5-$HT_6$R antagonism with its pleiotropic and beneficial effects on AD pathologies is believed to offer both symptomatic and causal treatment of AD and this was exemplified by a successful evaluation of 5-$HT_6$R antagonists idalopirdine and intepirdine in Phase II clinical trials. These agents, administered to donepezil-treated patients with mild to moderate AD, were successful in improving their cognitive functions [52,53].

As mentioned earlier, it is generally accepted that the complex pathomechanism of AD requires multi-target therapeutic solutions and the concept of multifunctional ligands responds to these needs perfectly. According to Bolognesi [54], the development of new MTDLs should be based on targets with proved connectivity. This means not only the simple chemical connectivity of different pharmacophores, but also a relevance of the selected targets for the clinical data. The described 5-$HT_6$ receptors and cholinesterases, both involved in cholinergic neurotransmission, are a model example of such "connected" targets. Despite not reaching anticipated results in larger phase III trials of the combination therapy of 5-$HT_6$R antagonist (idalopirdine or intepirdine) and ChE inhibitor donepezil [55,56], which may be attributable to a multitude of factors, the efficacy observed in phase II has opened up the field to research on multifunctional ligands combining 5-$HT_6$R antagonism with cholinesterase inhibition. Previously, we have developed compound **1** [57,58] (Fig. 1) that combines tacrine with 1-(phenylsulfonyl)-4-(piperazin-1-yl)-1*H*-indole and displays well-balanced potency against cholinesterases ($IC_{50\ AChE}$ = 12 nM, $IC_{50\ BuChE}$ = 29 nM), 5-$HT_6$R ($K_i$ = 2 nM), and anti-aggregating activity against Aβ (30% of inhibition at 10 μM) [59]. Based on the preliminary ADMET and *in vivo* results, multifunctional ligand **1** can be regarded as a promising molecule for further development. However, as is the case with many linked-type multifunctional ligands, its physicochemical properties and the resulting drug-likeness were not optimal; it was too large (MW = 621 u), too lipophilic (clogP = 5.26), too flexible (number of rotatable bonds = 11) and had high aromatic ring count (AR = 5). Herein, we report the development of fused multifunctional ligands targeting BuChE and 5-$HT_6$ receptor and amyloid β aggregation with optimized drug-like properties, a promising *in vitro* activity profile and *in vivo* pharmacokinetics.

## 2. Results and discussion

### 2.1. Design

Following the idea of optimizing the physicochemical properties, we have replaced tacrine moiety in compound **1** with a lower molecular weight *N*-benzylamine – donepezil-derived pharmacophore fragment that displays a crucial role in the interaction pattern of donepezil with the active site of AChE [60] – and fused it with 1-(phenylsulfonyl)-4-(piperazin-1-yl)-1*H*-indole (Fig. 1). We have also replaced piperazine with oxyethylene fragment, a modification that has already been successfully applied in the design of 5-$HT_6$R antagonists [61]. Additionally, according to molecular modeling studies (described below), it could also be beneficial in terms of the interactions with the active sites of cholinesterases. These modifications led to the development of compounds **49** and **27** (Fig. 1) with improved physicochemical properties (MW < 500, ClogP <5, AR = 4, number of rotatable bonds = 4 and 7), complying with the Lipinski and Veber rules. In terms of biological activity, both compounds display affinity for 5-$HT_6$R ($K_i$ = 60 nM and 2.6 nM for **49** and **27**, respectively) but only compound **27** potently and selectively inhibited BuChE with an $IC_{50}$ of 473 nM. Therefore, we have selected compound **27** for further optimization that aimed at improving activity against BuChE, while maintaining 5-$HT_6$R affinity.

### 2.2. X-ray crystallography of hBuChE in complex with compound 27

The complex structure of compound **27** with *h*BuChE was solved

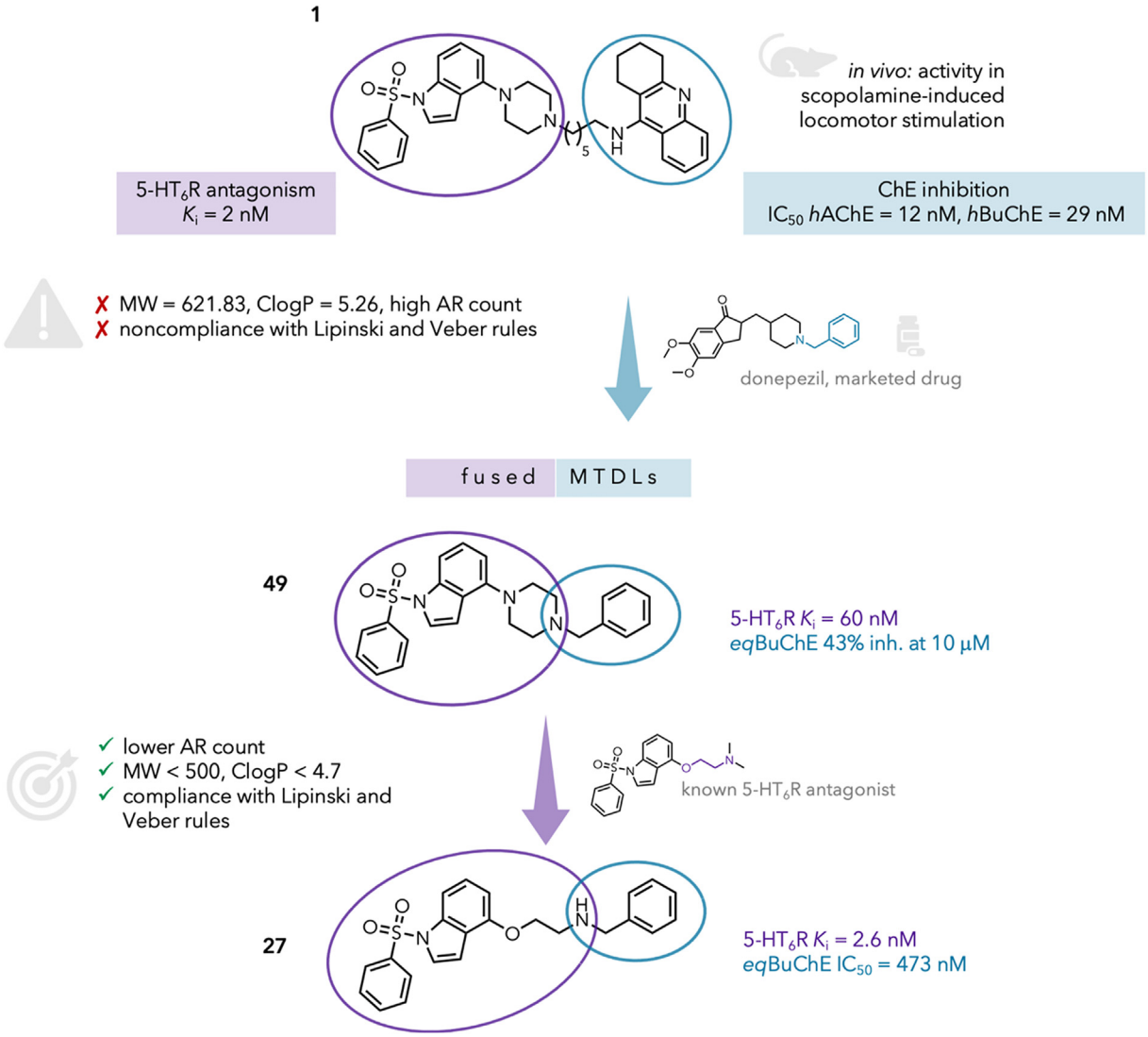



**Fig. 1.** Development of new fused multifunctional ligands **49** and **27**, based on the physicochemical properties-driven optimization of compound **1**.

at 2.0 Å resolution (PDB 7awg, for details see Table S1 in the Supporting Information). After molecular replacement, the most remarkable feature observed was a displacement of the acyl-loop, residues 277–288 (see Fig. S1), except for extra electron density that could account for the ligand, N-glycosylations or water molecules. Leu286Cα atom shifted about 4 Å away from its position in the initial reference model (PDB 1p0i) [62] with a $C\alpha_{model}$, Cα, Cβ angle of 78°. This shift is triggered by the binding of the phenylsulfonyl moiety of **27** (Fig. 2) close to Trp231, which clashes with Leu286 in the initial model (Fig. 2, green loop). The occupancy of **27** is partial and a mixture of acyl-loop positions, original and shifted, exists in the solved structure. Despite our efforts, we were not able to find a crystal with a better occupancy for **27**. The indole of **27** is sandwiched between the displaced acyl-loop and the oxyanion hole (Gly116/Gly117). The benzylamine moiety is stabilized in the choline-binding pocket delimited by residues Trp82, Tyr332, Trp430, Met437 and Tyr440.

Displacement of the acyl-loop in *h*BuChE has only been observed once before, to a lesser extent, in the irreversible complex formed with the aged form of the organophosphate soman [62]. It is probably not energetically favourable due to the presence of Pro285, which restricts the Phi and Psi angles of the main chain due to the cyclic nature of the residue.

Based on docking studies using the obtained crystal structure of **27** with *h*BuChE, we identified additional space around terminal 1-(phenylsulfonyl)-4-(piperazin-1-yl)-1*H*-indole and benzylamine moieties and designed a series of modifications to probe and optimize compound interactions with BuChE. We initially designed *N*-benzylamine fragment modifications that led to **series I** and **II** (Fig. 3A, B). Then we addressed the issue of the acyl-loop displacement in *h*BuChE observed in the crystal structure, by introducing modifications in the 5-$HT_6$R-targeting domain (**series III**–**VIII**, Fig. 3C).

### 2.3. Synthesis

Following the idea of diversifying of the benzylamine fragment, we have developed a synthetic route that would allow an introduction of this fragment in the last step. It involves a multistep transformation of commercially available 4-benzyloxyindole to the appropriate amines, followed by reductive amination reactions with a variety of commercially available aldehydes.

First, 4-benzyloxyindole was reacted with benzenesulfonyl chloride, 1-naphthalenesulfonyl chloride or benzyl bromide in the

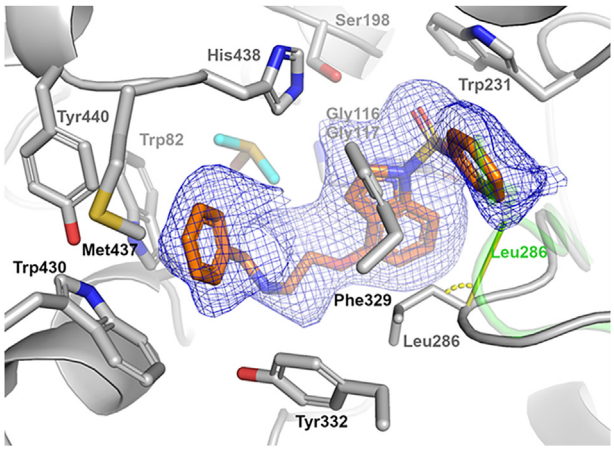



**Fig. 2.** A graphical representation of **27** binding mode into *h*BuChE determined by X-ray crystallography (PDB 7awg). Key residues are presented as sticks with carbon atoms in light grey for *h*BuChE, in orange for **27** and in cyan for DMSO. Oxygen atoms are represented in red, nitrogen atoms in blue and sulfur atoms in yellow. The initial position of the acyl loop (PDB 1p0i model) is represented in green. Distance between Leu286C$\alpha_{model}$ and Leu286C$\alpha$ in the **27** bound-state (acyl-loop alternate position A in 7awg) is represented as a plain yellow lane, about 4 Å long. The angle between Leu286C$\alpha_{model}$, Leu286C$\alpha$ and Leu286C$\beta$ in the **27**-bound state is represented as a dashed yellow arc. A 3-$\sigma$ polder map calculated around **27** is represented in a blue mesh [63].

presence of potassium *tert*-butoxide and 18-crown-6 ether yielding compounds **2**, **3** and **4** respectively (Scheme 1). Debenzylation of the latter in the presence of a catalytic amount of Pd/C in tetrahydrofuran at 45 °C provided phenols **5**, **6** and **7**. These were subsequently transformed into 2-bromoethoxy derivatives **8**, **9** and **10** by refluxing with an excess of 1,2-dibromoethane in ethanol, in the presence of $K_2CO_3$ (Scheme 1).

The obtained primary bromides **8**, **9** and **10** were used in the Gabriel synthesis (Scheme 2). They were reacted with potassium phthalimide in DMF at 50 °C yielding compounds **11**–**13**. Phthalimide was cleaved in a reaction with a 40% aqueous solution of methylamine and 1 M NaOH providing primary amines **14**–**16**. Compound **16** was further reduced to an indoline-based amine **17** in the presence of an excess of sodium cyanoborohydride in trifluoroacetic acid (TFA). The same procedure applied to a reduction of **14** resulted in unacceptably low yield; therefore, the synthetic approach towards **21** was modified. Namely, the indole-based bromide **8** was reduced to its indoline derivative **20** and amine **21** was obtained via the Gabriel synthesis. The indolin-2-one derivative **19** was prepared from **13** in a sequence of reactions comprising chlorination with *N*-chlorosuccinimide in tetrahydrofuran, followed by hydrolysis in an 85% aqueous solution of $H_3PO_4$ and, finally, cleavage with a 40% aqueous solution of methylamine and 1 M NaOH (Scheme 2). Reductive amination was applied to afford the final compounds **27**, **29**–**36**, **38**–**48**, **50**–**52**, **54**, **55**, **57**–**76**. Using this methodology, primary amines **14**–**17**, **19** and **21** were reacted with the appropriate commercially available aldehydes in methanol, in the presence of acetic acid and sodium cyanoborohydride (Scheme 2). To synthesize the final compound **37**, the 2-bromoethoxy derivative **8** was used for 3-(aminomethyl)-*N*,*N*-dimethylaniline alkylation. The reaction was carried out by refluxing the compounds in a suspension of $K_2CO_3$ in acetonitrile.

For the synthesis of series **VIII**, we developed a synthetic route presented in Scheme 3. Subsequent alkylations of 1,3-difluoro-2-nitrobenzene with *tert*-butyl (2-hydroxyethyl)carbamate and benzylamine yielded **23**. These reactions were carried out in tetrahydrofuran with sodium hydride at room temperature and in anhydrous DMSO with potassium carbonate at 70 °C, respectively. Reduction of the compound **23** nitro group using an aqueous solution of sodium dithionite provided **24**. Benzimidazolone derivative **25** was formed in the reaction of **24** with 1,1′-carbonyldiimidazole in dichloromethane. 1 M solution of HCl in ethyl acetate was used to remove Boc-protecting group in **25** to provide amine **26** as a hydrochloride salt. The following reductive amination with the appropriate commercially available aldehydes in methanol with 1,4-diazabicyclo[2.2.2]octane (DABCO) excess and sodium cyanoborohydride (Scheme 3) yielded compounds **77**–**79**.

Additional procedures were applied to obtain compounds **28**, **56**, **49** and **53**. Compound **27** was used to prepare two derivatives: **28** and **56** (Scheme 4A). First, **27** was methylated using an aqueous solution of formaldehyde, acetic acid and sodium cyanoborohydride, yielding **28**. Subsequently, **28** was debenzylated to **56** in Pd-catalyzed hydrogenation. Compound **49** was prepared from 1-(phenylsulfonyl)-4-(piperazin-1-yl)-1*H*-indole [57], by alkylation with benzyl bromide in a suspension of $K_2CO_3$ in acetonitrile (Scheme 4B). Deprotection of the *tert*-butoxycarbonyl protecting group in **54**, carried out in TFA, yielded compound **53** (Scheme 4C).

### *2.4. Cholinesterase (AChE/BuChE) inhibitory potencies, 5-HT$_6$R binding and functional assays and inhibitory activity against A$\beta_{42}$*

Inhibitory activity of the compounds from series **I**–**VIII** against electric eel-derived AChE (*ee*AChE), equine serum-derived and human BuChE (*eq*BuChE or *h*BuChE) was determined using the spectrophotometric method described by Ellman [64]. All compounds were screened at the initial concentration of 10 μM to select those with an inhibitory potency higher than 50% and determine their $IC_{50}$ values. Compounds that displayed less than 50% inhibition against the enzyme were regarded as inactive. Tacrine and donepezil were used as the references in the assay. The affinity of all compounds for the recombinant human 5-$HT_6$ receptor was evaluated in a radioligand binding assay, with methiothepine as a reference [65]. In cell-based functional studies we confirmed the antagonistic properties of the selected derivatives (see Table S6 in the Supporting Information). The inhibitory activity of the synthesized compounds against A$\beta_{42}$ aggregation was evaluated by means of the *in vitro* thioflavin T (ThT)-based fluorometric assay [66]. The principle of this assay relies on the interaction between the benzothiazole dye (ThT) and the cross β-sheet quaternary structure of the amyloid protein. When the dye is free, the intensity of fluorescence is weak, but it increases when the dye binds to β-sheet structures. The assay was performed at a screening concentration of the tested compounds of 10 μM. The results of the *in vitro* biological evaluation have been presented in Tables 1, 2 and 3.

We began SAR analysis by exploring the *N*-benzylamine fragment of compound **27** – a selective *eq*BuChE inhibitor with an $IC_{50}$ value of 473 nM (Table 1). Regarding BuChE inhibitory potency, 3-Me (**29**) and 3,5-di-Cl (**33**) substituents slightly improved the potency ($IC_{50}$ = 401 and 414 nM respectively), while 4-substituted derivatives **32** and **35** impaired it. This points to the importance of steric effects here, which, however, is not unequivocal when we compare e.g. compounds **45** and **46** or **39** and **40**. We have also observed that strongly electron-withdrawing groups (EWG) such as 4-$NO_2$ (**36**) or 3-$CF_3$ (**34**) impair interactions with the enzyme. Molecular modeling studies suggested the probing of 3 and 4 positions of the terminal aromatic ring to augment π-π or cation-π interactions towards aromatic residues at the choline active site. Thus, we introduced the substituents with the following groups: amine (-N(Me)$_2$, compounds **37** and **38**), ether (e.g. -OMe, -di-OMe, -OEt; compounds **39**–**44**), ester (-OCOMe, -COOMe; compounds **45**–**47**) and amide (-NHCOMe; compound **48**). The synthesized derivatives showed potencies against BuChE ranging from 228 nM (**46**) to 3322 nM (**41**), except compound **47** ($IC_{50}$ = 14.3 μM, Table 1),

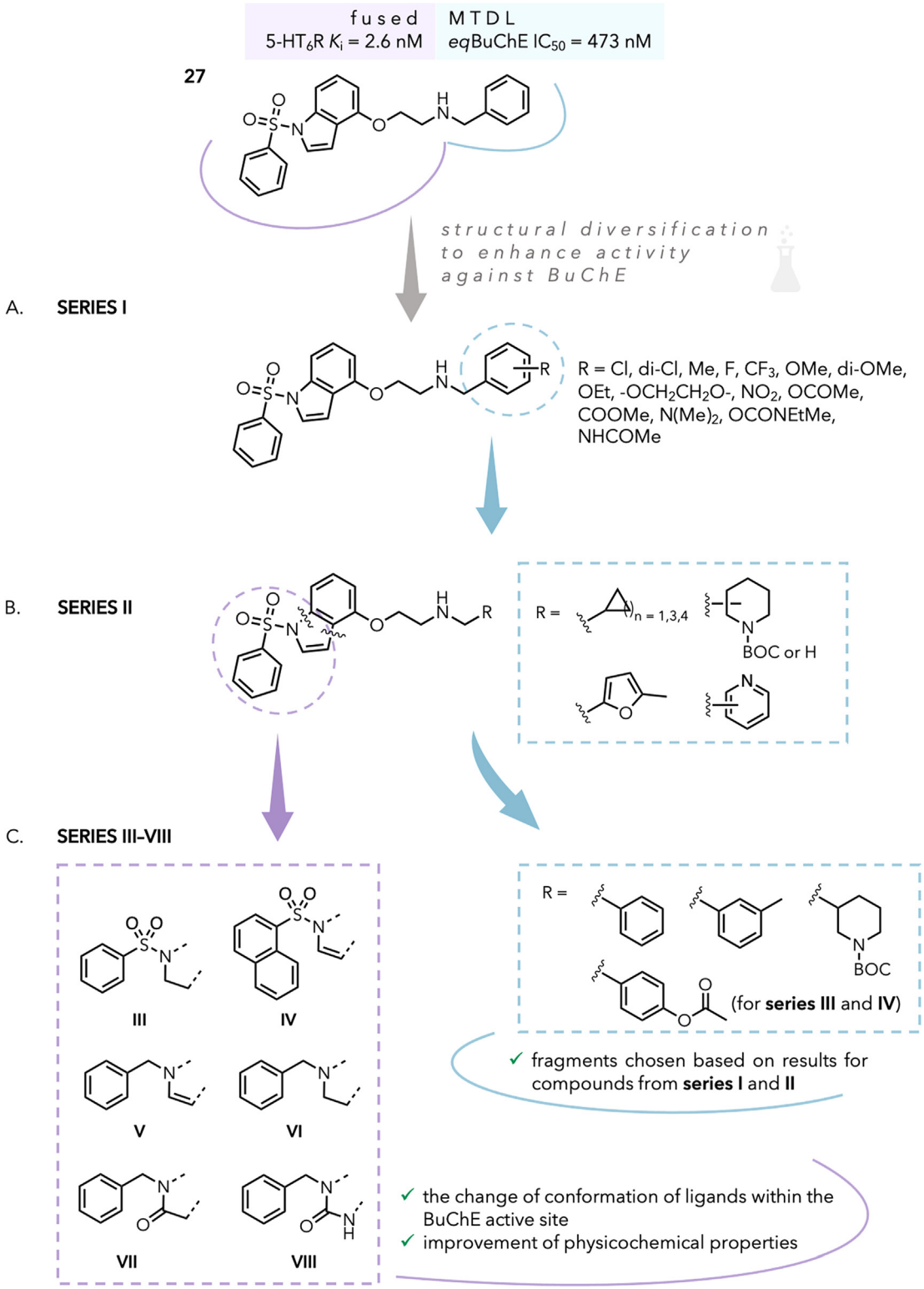
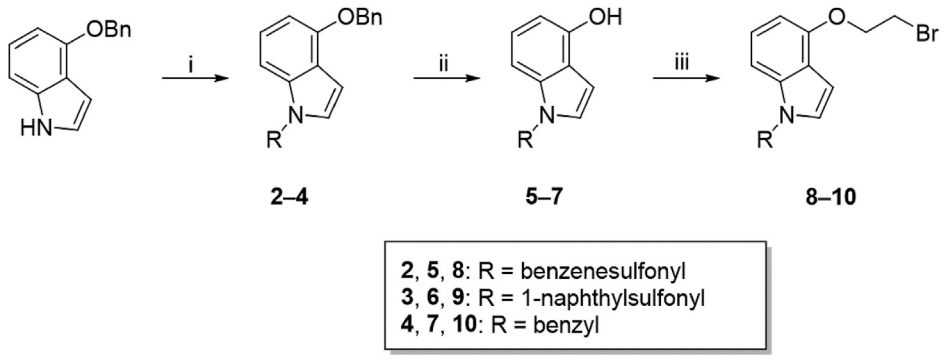


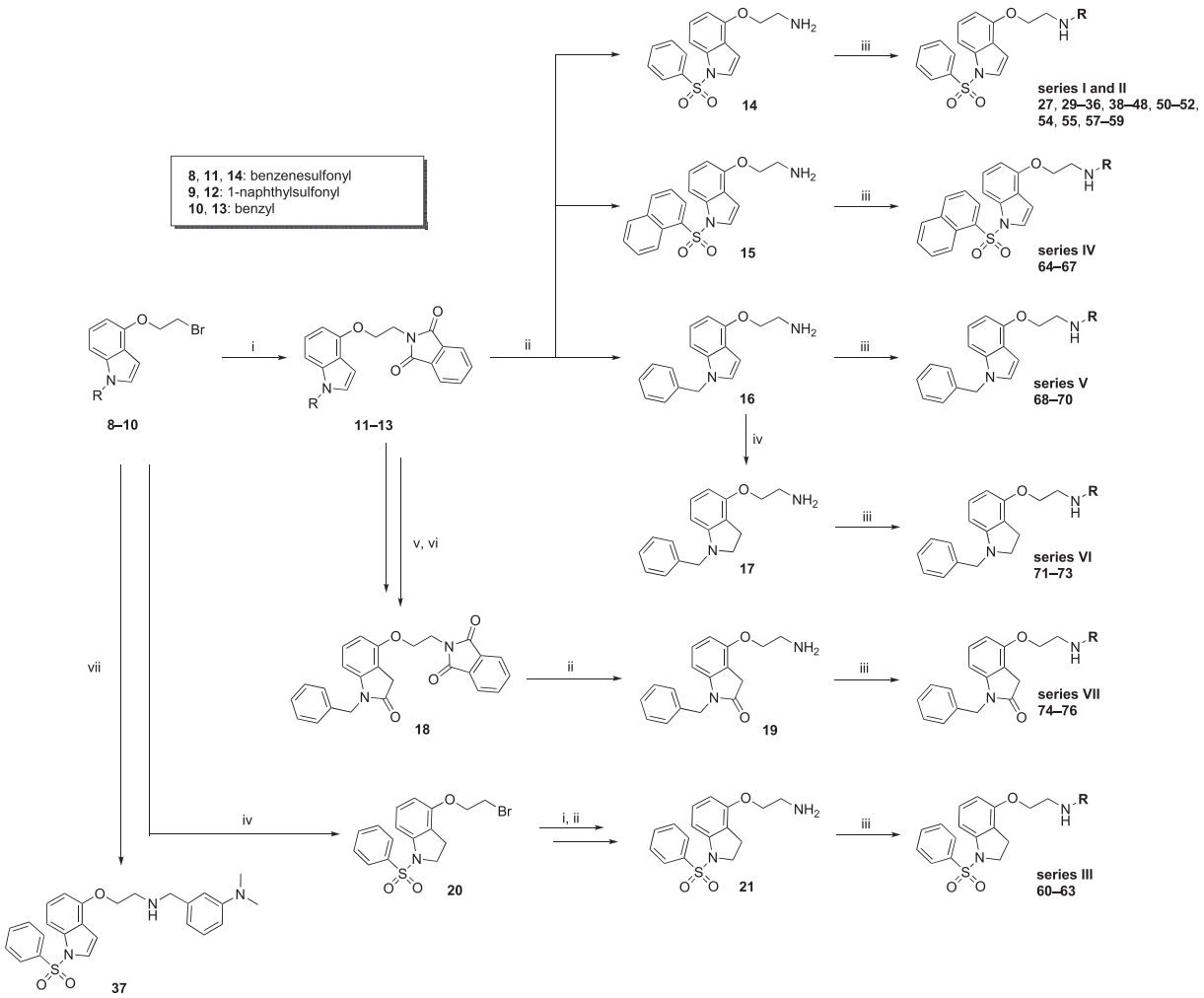

**Fig. 3.** Design of new multifunctional ligands targeting BuChE and the 5-$HT_6$ receptor (series **I**–**VIII**).

which confirms the detrimental effect of EWGs on the potency. When comparing the corresponding ether (**43** and **44**) and ester (**45** and **46**) analogues we observed that the latter display markedly higher activity. Such an effect might be attributed to the presence of additional steric bulk positioning the terminal moiety favourably and the presence of hydrogen-bond acceptor that could interact with Tyr, Trp and His residues in the vicinity.

The described modifications of the aromatic ring within benzylamine moiety did not affect significantly the potency towards 5-$HT_6$ receptor. Affinities of all compounds from **series I**, expressed as $K_i$ are in the range of 0.3—30 nM, whereas $K_i$ value of **27** was 2.6 nM. This corresponds to the molecular modeling studies and the



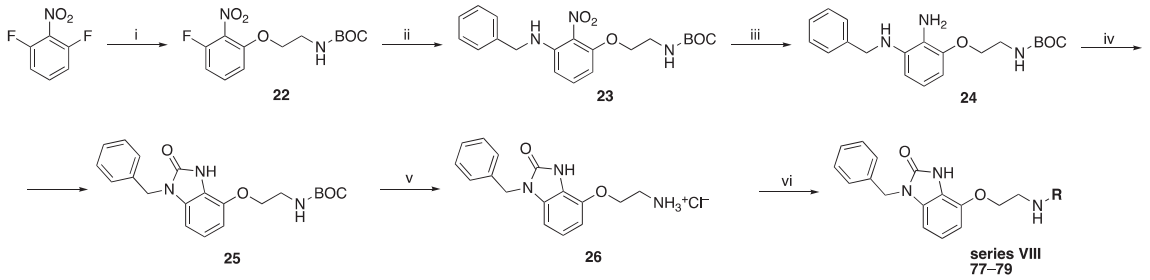

**Scheme 1.** Synthesis of 2-bromoethoxy derivatives **8**, **9** and **10**. Reagents and conditions: (i) benzenesulfonyl chloride (for **2**), 1-naphthalenesulfonyl chloride (for **3**) or benzyl bromide (for **4**), potassium *tert*-butoxide, 18-crown-6 ether, THF, 0 °C–rt, 24 h; (ii) $H_2$, 10% Pd/C, THF, 45 °C, 2 h; (iii) 1,2-dibromoethane, $K_2CO_3$, ethanol, reflux, 48 h.



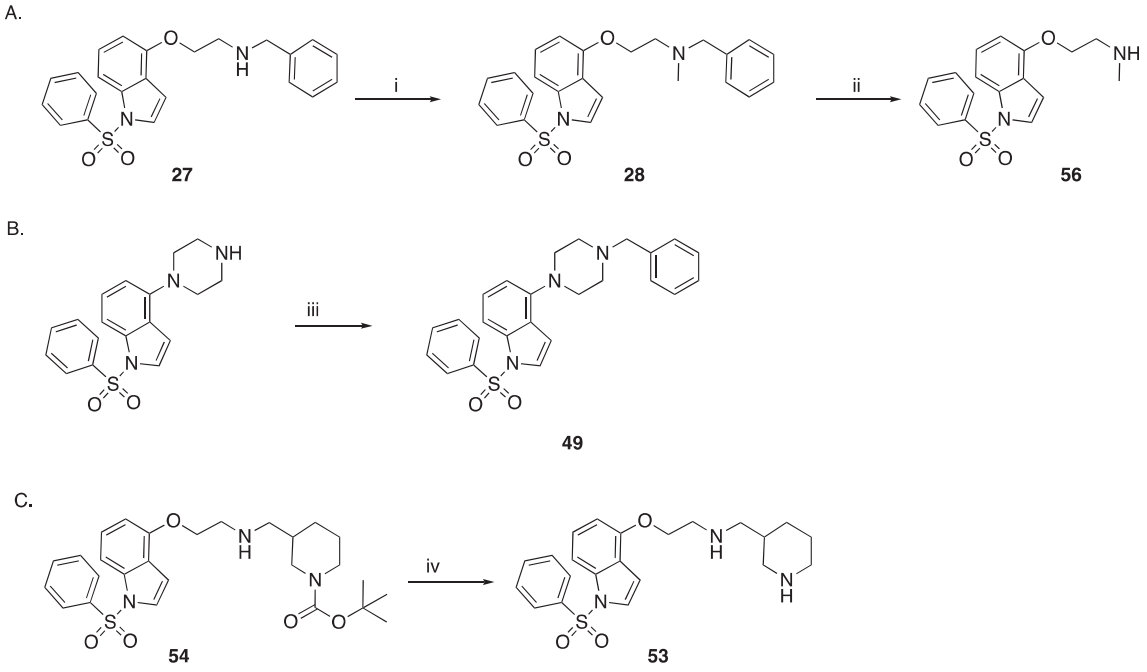

**Scheme 2.** Synthesis of final compounds from **series I**–**VII**. Reagents and conditions: (i) potassium phthalimide, 18-crown-6 ether, DMF, 50 °C, 24 h; (ii) $CH_3NH_2$ (40% aq. sol.), 50 °C, 1 h, then 1 M NaOH, rt, 1 h; (iii) appropriate aldehyde, $CH_3COOH$, $NaBH_3CN$, MeOH, 0 °C–rt, 24 h; (iv) $NaBH_3CN$, TFA, 0 °C–rt, 3 h; (v) *N*-chloro-succinimide, THF, rt, 18 h; (vi) $H_3PO_4$ (85% aq. sol.), 100 °C, 1 h; (vii) 3-(aminomethyl)-*N*,*N*-dimethylaniline, $K_2CO_3$, MeCN, reflux, 18 h.

predicted binding mode of all derivatives from **series I** that reproduce the interaction pattern of **27**. The 1-(phenylsulfonyl)-4-(piperazin-1-yl)-1*H*-indole moiety of compound **27** engages in the obligatory interactions in the orthosteric binding site, characteristic of 5-$HT_6$R ligands (salt bridge with Asp3.32[106] and CH-π stacking with Phe6.52[285]/Phe5.38[188]). The benzyl moiety in the amine fragment of the molecule is capable of retaining π-π stacking with Phe7.35[302], formed by tacrine fragment of **compound 1** in the accessory binding site (Fig. 4).

We did not observe a significant improvement in potency against BuChE caused by modifications of the phenyl ring, therefore we tested the importance of this fragment by its removal and



**Scheme 3.** Synthesis of compounds from **series VIII**. Reagents and conditions: (i) *tert*-butyl (2-hydroxyethyl)carbamate, NaH, THF, 0 °C–rt, 1–3 h; (ii) benzylamine, $K_2CO_3$, DMSO (anh), 70 °C, 18–24 h; (iii) $Na_2S_2O_4$, EtOH, $H_2O$, reflux, 15 min; (iv) CDI, DCM, rt, 12–24 h; (v) 1 M HCl in EtOAc, rt, 24 h; (v) appropriate aldehyde, DABCO, $NaBH_3CN$, MeOH, THF 0 °C–rt, 24 h.



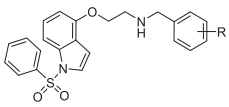
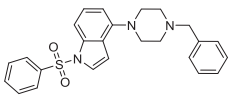
**Scheme 4.** Reagents and conditions: (i) 37% $HCHO_{(aq)}$, $CH_3COOH$, $NaBH_3CN$, MeOH, 0 °C–rt, 24 h; (ii) HCOOH, 10% Pd/C, ethanol, rt, 5 h; (iii) benzyl bromide, $K_2CO_3$, MeCN, rt, 18 h; (iv) TFA, DCM, rt, 1 h.

replacements to optimize the contacts at the choline binding site (**series II**, Table 2). Replacement with $sp^3$ carbon-containing cyclohexyl and cyclopentyl rings resulted in the increase in activity against BuChE (**50**, $IC_{50}$ = 90 nM, **51**, $IC_{50}$ = 269 nM), but further reduction of the ring to cyclopropyl decreased it (**52**, $IC_{50}$ = 2222 nM). It did not affect the affinity of the obtained derivatives for 5-$HT_6$R, their $K_i$ values remained very close to that of **27** ($K_i$ = 4.8, 8.5, 2.7 nM for **50**, **51** and **52** respectively). These modifications changed not only the geometry of this fragment of the molecule but also the $pK_a$ values of the amine group (calculated $pK_a$ = 9.90 for **50** vs. 8.92 for compound **27**). The protonation of this atom is important for the binding with both biological targets (crucial interactions with Asp3.32[106] in 5-$HT_6$R, and choline mimicry in ChE), which was demonstrated by the introduction of piperidine in place of cyclohexyl ring in compound **53**. It shifted the predicted protonation site from the nitrogen atom of oxyethylene linker to that of the piperidine ring and resulted in a drop of activity against BuChE ($IC_{50}$ = 2263 nM) and 5-$HT_6$R ($K_i$ = 75 nM). The potency was recovered in BOC-substituted piperidine derivatives restricting the movement and abolishing the charged centre in **54** and **55**, although in terms of BuChE inhibition much higher activity was determined for 3-substituted derivative **54** ($IC_{50}$ = 125 nM vs. 4824 nM for **55**).

It is worth mentioning that compound **54** shows very similar potencies against *eq*BuChE and *h*BuChE. The removal of a terminal phenyl ring, as in compound **56**, led to the loss of activity against BuChE, leaving it at 42% inhibition of the enzyme at 10 μM. Similarly, bioisosteric replacements by 2-pyridine (**57**) or 5-methylfuran-2-yl (**59**) led to a one order of magnitude drop in activity ($IC_{50}$ = 1294, 2997, 473 nM for **57**, **59** and **27** respectively). Noteworthy, they markedly improved the physicochemical properties of ligands (CNS MPO for **27, 57, 58, 59** equal 4.1, 5.3, 5.2, 4.9 respectively, for further details regarding the calculated physicochemical properties see Tables S7, S8 and S9 in the Supporting Information). At the same time, we observed that the binding of the compounds with the 5-$HT_6$ receptor is tolerant of modifications of the ChE-targeting fragment, consistently we observed improved, subnanomolar affinities for compounds **41**, **42**, **48** and **57** ($K_i$ = 0.5, 0.5, 0.3 and 0.4 nM respectively).

In terms of Aβ inhibitory activity, within 25 compounds that we classified as active (above 20% of inhibition), the inhibitory potency was ranging from 20% for compound 64–79% for compounds **70** and **73** (Tables 1, 2 and 3). While this is quite a structurally diversified group of compounds and results, attempts to determine the

**Table 1**
Affinity for 5-$HT_6$R, inhibition of *eq*BuChE, *h*BuChE and *ee*AChE and *in vitro* inhibitory activity against $A\beta_{42}$ by compounds from **series I** and compound **49**.

| Comp. | R | 5-$HT_6$R | | *eq*BuChE | *h*BuChE | *ee*AChE | Inhibition of $A\beta_{42}$ aggregation [%][c] |
|---|---|---|---|---|---|---|---|
| | | $K_i$[a] [nM] | $K_b$[a] [nM] | % or $IC_{50}$[b] | | | |
|  | | | | | | | |
| **27** | H | 2.6 ± 0.1 | 2.3 ± 0.1 | 473.3 ± 17.2 | 1611 ± 36 | 21% | 45.9 ± 7.3 |
| **28** | *N*–$CH_3$/R = H | 27.0 ± 3.0 | – | 1422 ± 62 | – | 2% | n.i.[d] |
| **29** | 3-Me | 15.0 ± 1.6 | 33.0 ± 1.2 | 401.3 ± 6.4 | 2822 ± 41 | 10% | 33.3 ± 14.2 |
| **30** | 2-Cl | 30.0 ± 3.7 | 50.0 ± 1.7 | 880.5 ± 25.1 | 10530 ± 182 | 3% | n.i.[d] |
| **31** | 3-Cl | 5.0 ± 0.2 | – | 580 ± 8.1 | 11790 ± 527 | 22% | n.d.[e] |
| **32** | 4-Cl | 16.0 ± 1.2 | – | 1323 ± 28 | – | 2% | n.i.[d] |
| **33** | 3,5-diCl | 11.0 ± 1.3 | 37.0 ± 0.9 | 414 ± 8.2 | – | 0% | n.i.[d] |
| **34** | 3-$CF_3$ | 4.0 ± 0.9 | – | 2205 ± 63 | – | 4% | n.i.[d] |
| **35** | 4-F | 6.0 ± 1.0 | – | 985.1 ± 17.8 | – | 9% | 40.5 ± 14.7 |
| **36** | 4-$NO_2$ | 22.0 ± 2.6 | – | 18330 ± 500 | – | 0% | 35.1 ± 5.6 |
| **37** | 3-*N*-$(Me)_2$ | 6.0 ± 0.4 | – | 893.8 ± 26.1 | – | 28% | n.i.[d] |
| **38** | 4-*N*-$(Me)_2$ | 3.5 ± 0.4 | – | 3081 ± 129 | – | 19% | n.i.[d] |
| **39** | 3-OMe | 3.6 ± 0.5 | – | 1661 ± 48 | – | 10% | 27.6 ± 16.8 |
| **40** | 4-OMe | 1.4 ± 0.2 | – | 1326 ± 44 | – | 5% | 54.2 ± 7.8 |
| **41** | 3,4-di-OMe | 0.5 ± 0.1 | – | 3322 ± 113 | – | 6% | 36.8 ± 3.8 |
| **42** |  | 0.5 ± 0.06 | – | 2165 ± 87 | – | 7% | n.i.[d] |
| **43** | 3-OEt | 3.0 ± 0.2 | – | 2412 ± 72 | – | 12% | n.i.[d] |
| **44** | 4-OEt | 13.0 ± 1.0 | – | 3297 ± 103 | – | 10% | n.i.[d] |
| **45** | 3-OCOMe | 6.5 ± 0.6 | 2.6 ± 0.3 | 474.2 ± 10.9 | 5620 ± 218 | 0% | n.i.[d] |
| **46** | 4-OCOMe | 11.0 ± 0.6 | 11.0 ± 0.7 | 227.9 ± 6.5 | 1920 ± 60 | 0% | n.i.[d] |
| **47** | 4-COOMe | 13.0 ± 2.0 | – | 14300 ± 300 | – | 0% | n.d.[e] |
| **48** | 4-NHCOMe | 0.3 ± 0.05 | – | 3800 ± 354 | – | 4% | n.i.[d] |
|  | | | | | | | |
| **49** | – | 60.0 ± 4.8 | – | 43% | – | 0% | n.i.[d] |
| **Donepezil** | | – | – | 1830 ± 40 | – | 11.0 ± 0.2 | – |
| **Tacrine** | | – | – | 2.0 ± 0.1 | 33.6 ± 0.4 | 23.0 ± 0.4 | – |
| **Methiothepine** | | 0.30 ± 0.03 | – | – | – | – | – |
| **SB258585** | | – | 0.36 ± 0.03 | – | – | – | – |

[a] $K_i$ and $K_b$ values are expressed as the mean ± standard error of the mean (SEM) of at least three experiments. [b] % – a percentage of enzyme inhibition at screening concentration of 10 μM; $IC_{50}$ values are expressed as the mean ± standard error of the mean (SEM) of at least three experiments; BuChE from equine serum or human recombinant BuChE, AChE from electric eel. [c] Inhibition percentage at 10 μM compound and 1.5 μM $A\beta_{42}$. Inhibition percentage means ± SD for at least two independent experiments. [d] No inhibition (percentage lower than 20%). [e] Not determined. Statistical analysis: one-way ANOVA, followed by *post-hoc* Bonferroni *t*-test (SigmaPlot v 12.0), compared to control experiment ($A\beta_{42}$ + DMSO); $p < 0.05$.

structure-activity or the physicochemical properties-activity relationship did not lead to unambiguous conclusions. Further structural studies would be required to elucidate the nature of factors affecting the inhibitory activity against $A\beta_{42}$.

### 2.5. X-ray crystallography of hBuChE in complex with compound 54

In parallel, we determined the crystallographic structure of *h*BuChE in complex with **54** (PDB 7awh, Fig. 5B) at 2.3 Å resolution (Table S1). Compound **54** adopts a binding mode similar to **27** (Fig. 5A). In accordance with the above reported SAR study for series II, the flexibility afforded by the piperidine ring allows the extra BOC substituent to orient in the choline pocket, making an additional interaction between *tert*-butyl group and Trp82. Noteworthy, both enantiomers could be modeled in the electron density, demonstrating no clear enantioselectivity.

In further modifications, we focused on the 5-$HT_6$R-aiming moiety assuming it will give more space for the optimization of physicochemical properties, but also an exploration of the displacement of acyl loop observed in the crystal structure of the *h*BuChE complex with compound **27**. We have replaced 1-(phenylsulfonyl)-1*H*-indole with 1-(phenylsulfonyl)indoline (series **III**), 1-(naphthalen-1-ylsulfonyl)-1*H*-indole (series **IV**), 1-benzyl-1*H*-indole (series **V**), 1-benzylindoline (series **VI**), 1-benzylindolin-2-one (series **VII**) and 1-benzyl-1,3-dihydro-2*H*-benzo[*d*]imidazole-2-one (series **VIII**) (Table 3). We observed a pronounced impact of 5-$HT_6$-aiming fragments on both BuChE inhibitory activity and 5-$HT_6$R affinity. Sulphonyl-containing derivatives (series **III** and **IV**) display improved affinities for 5-$HT_6$R ($K_i$ = 3–70 nM) when compared to 1-benzylindolin-2-one and 1-benzyl-1,3-dihydro-2*H*-benzo[*d*]imidazole-2-one derivatives ($K_i$ = 55–207 nM) or 1-benzyl-1*H*-indole and 1-benzylindoline ($pK_i$ = 232–1469 nM). These results are consistent with molecular modeling studies, indicating an important role of a hydrogen bond formed by S=O or C=O with Asn6.55[288] (Fig. 4). The lack of such interactions, as exemplified in compound **71** (Fig. 6), affects the conformation of the compound and precludes it from forming aromatic interactions in the accessory cavity.

Considering BuChE inhibitory activity, bulkier 1-(naphthalen-1-

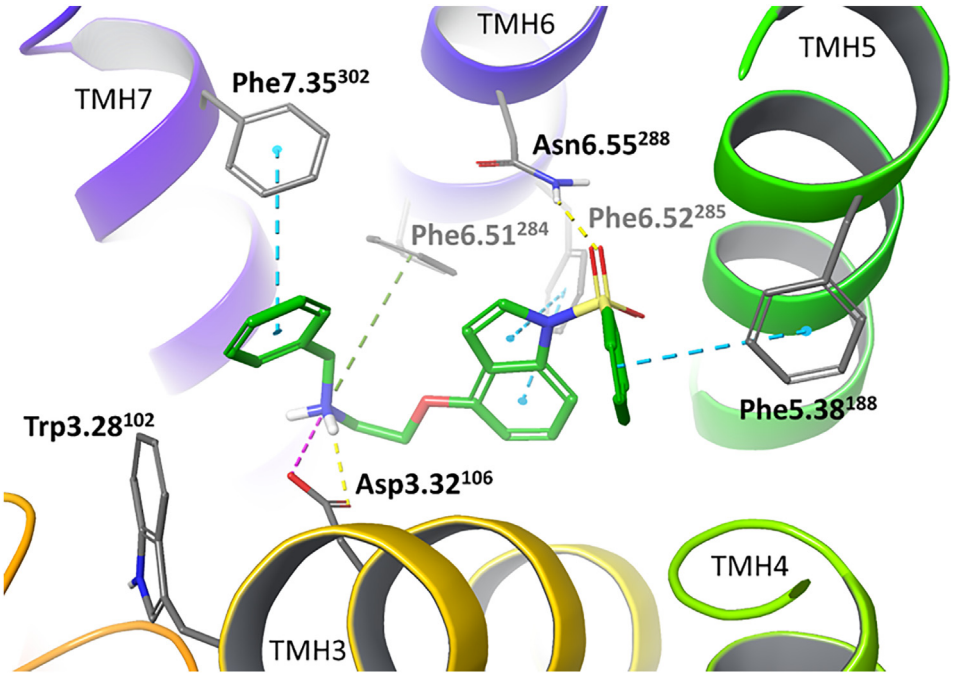



**Fig. 4.** The predicted binding mode of **27** within 5-$HT_6$R. The compound retained all the interactions in the orthosteric binding site characteristic of 5-$HT_6$R ligands – with Asp3.32[106] (salt bridge/charge-assisted hydrogen bond), Phe6.52[285] and Phe5.38[188] (CH-π stackings), and a hydrogen bond with Asn6.55[288]. The reduced aromatic moiety (relative to tacrine) preserved the CH-π stacking with Phe7.35[302] in the accessory binding site. Amino acid residues engaged in ligand binding (within 4 Å from the ligand atoms) are represented as thick sticks. The extracellular loop 2 (ECL2) was hidden for clarity. TMH – transmembrane helix.

ylsulfonyl)-1*H*-indole improves potency, which is particularly evident in compound **66** with a 1-BOC-piperidin-3-ylmethanamine fragment ($IC_{50}$ = 6 nM). At the same time, the difference between potency against horse serum-derived and human-derived enzymes increased, which confirms the presence of a steric hindrance observed in the crystal structure and an observed difference in the cavity size between human and equine enzymes. Expectedly, we observed that indole derivatives are more potent than their reduced counterparts due to their reduced flexibility (**27**, **29** and **54** vs. **60**, **61** and **62** respectively, as well as **68**, **69** and **70** vs. **71**, **72** and **73**). The introduction of a carbonyl group, as in the 1-benzyl-1,3-dihydro-2*H*-benzo[*d*]imidazole-2-one and 1-benzylindolin-2-one derivatives (**74**–**79**), led to a drop in activity, apparently due to a shift in the predicted binding mode (the vicinity of the His438, Phe329 and Ser198 cavity interface). Within series **III**–**VIII**, the most potent BuChE inhibitors were compounds containing a 1-BOC-piperidin-3-ylmethanamine fragment, and compound **66** was also the most potent ligand among all compounds described in this paper.

### 2.6. X-ray crystallography of hBuChE in complex with compound 70

To complete the SAR studies, we determined the crystallographic structure at 2.3 Å of *h*BuChE in complex with **70** (PDB 7awi, Table S1), one of the most potent inhibitors of both *eq*BuChE ($IC_{50}$ = 11 nM) and *h*BuChE ($IC_{50}$ = 115 nM) presented herein. The binding mode of **70** into *h*BuChE remains similar to those previously determined for **27** and **54**, with the additional BOC substituent interaction with Trp82 (Fig. 5C). Similarly to **54**, both enantiomers of **70** could be modeled in the electron density, demonstrating no clear enatioselectivity.

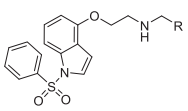
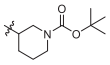
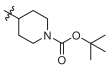
### 2.7. Kinetics of eqBuChE inhibition

We have tested the kinetics of *eq*BuChE inhibition by the selected compounds **27** and **50**, and based on the analysis of Lineweaver-Burk and Cornish-Bowden plots we have identified them as reversible, non-competitive inhibitors (see Figs. S2 and S3 in the Supporting Information). Carbamates, with anti-AD marketed drug rivastigmine among them, represent a pseudo-irreversible mode inhibition resulting from the carbamoylation of a serine residue in the catalytic site of ChE. Therefore, we have performed additional experiments to verify the inhibition mode of the *N*-BOC carbamates **54**, **66**, **73** and **70**. Using reversibility 100-fold dilution assay, performed according to the protocol described by Kosak et al. [67], we have shown that compounds **54**, **66**, **73** and **70**, despite the carbamate group, are reversible enzyme inhibitors (see Fig. S4 in the Supporting Information). Moreover, the determined crystallographic structures of **54** and **70** complexes into *h*BuChE (Fig. 5B and C) are in accordance with the absence of carbamate reactivity as the catalytic serine Oγ lies respectively 5.6 and 5.7 Å away from the carboxylic carbon atom of the BOC substituent.

### 2.8. A comparative study of molecular dynamics in equine and human BuChE

The binding mode of **27**, **54** and **70** in *h*BuChE and the observed displacement of the acyl-loop led us to study the dynamics of both equine and human BuChE. We ran three independent molecular simulations for each enzyme, calculated the rmsd of the mainchain atoms position and converted them into B-factors. *Eq*BuChE displays more flexibility of the acyl-loop (residues 284–287) than *h*BuChE (Fig. 7). This can be explained by the presence of Pro285 in *h*BuChE that greatly restricts the allowed Phi and Psi angles, compared to Leu285 in *eq*BChE. This flexibility discrepancy could

**Table 2**
Affinity for 5-$HT_6$R, inhibition of *eq*BuChE, *h*BuChE and *ee*AChE and *in vitro* inhibitory activity against $A\beta_{42}$ by compounds from series II.

| Comp. | R | 5-$HT_6$R | | *eq*BuChE | *h*BuChE | *ee*AChE | Inhibition of $A\beta_{42}$ aggregation [%][c] |
|---|---|---|---|---|---|---|---|
| | | $K_i^a$ [nM] | $K_b^a$ [nM] | % or $IC_{50}^b$ | | | |
|  | | | | | | | |
| **50** |  | 4.8 ± 0.3 | 81.0 ± 2.0 | 89.9 ± 2.3 | 551.1 ± 20.5 | 13% | 53.5 ± 5.5 |
| **51** |  | 8.5 ± 0.2 | 9.0 ± 0.3 | 268.8 ± 4.8 | 1358 ± 48 | 28% | n.i.[d] |
| **52** |  | 2.7 ± 0.1 | – | 2222 ± 71 | – | 7% | n.i.[d] |
| **53** |  | 75.0 ± 2.5 | – | 2263 ± 53 | – | 0% | 27.3 ± 1.2 |
| **54** |  | 26.0 ± 1.1 | 54.0 ± 1.9 | 125.1 ± 3.0 | 206 ± 6 | 15% | 59.2 ± 12.7 |
| **55** |  | 7.0 ± 0.6 | – | 4824 ± 231 | – | 22% | 69.7 ± 8.8 |
| **56** | H | 1.6 ± 0.05 | – | 42% | – | 1% | n.i.[d] |
| **57** |  | 0.4 ± 0.05 | – | 1294 ± 29 | – | 5% | n.i.[d] |
| **58** |  | 3.0 ± 0.4 | – | 37% | – | 0% | n.i.[d] |
| **59** |  | 3.0 ± 0.1 | – | 2997 ± 173 | – | 10% | n.i.[d] |

[a] $K_i$ and $K_b$ values are expressed as the mean ± standard error of the mean (SEM) of at least three experiments. [b] % – a percentage of enzyme inhibition at screening concentration of 10 μM; $IC_{50}$ values are expressed as the mean ± standard error of the mean (SEM) of at least three experiments; BuChE from equine serum or human recombinant BuChE, AChE from electric eel. [c] Inhibition percentage at 10 μM compound and 1.5 μM $A\beta_{42}$. Inhibition percentage means ± SD for at least two independent experiments; [d] No inhibition (percentage lower than 20%). Statistical analysis: one-way ANOVA, followed by *post-hoc* Bonferroni *t*-test (SigmaPlot v 12.0), compared to control experiment ($A\beta_{42}$ + DMSO); $p < 0.05$.

explain the compounds' inhibition discrepancies observed between equine and human BuChE (Tables 1, 2 and 3).

### 2.9. In vitro ADME-Tox evaluation

To ensure that novel ligands have the perspective for further development, determination of their *in vitro* activity towards targets of interest was accompanied by studies of ADME-Tox properties. Before proceeding to *in vivo* tests, we performed *in vitro* evaluation of metabolic stability and potential toxicity for the selected multifunctional ligands **27**, **50** and **54**. Their stability was tested by incubation with mouse liver microsomes (MLMs) for 120 min. In these conditions ca. 58% of compounds **27** and **50** remained unchanged. As their main metabolite, we have identified the primary amine **14**, which was formed in the reaction of oxidative deamination (Table 4, for the detailed analysis, see Table S2 and Fig. S5–S12 in the Supporting Information). Carbamate derivative - compound **54** was not detected in the assay mixture after 2 h of incubation, proving to be highly unstable.

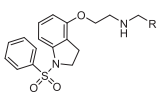
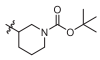
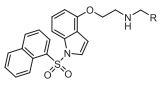
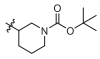
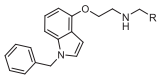
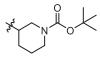
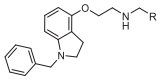
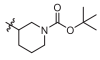
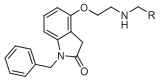
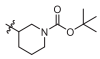
To assess the cytotoxicity of compounds, we performed cell viability assays starting with the MTS assay on HepG2 (human hepatocellular carcinoma) cells (Table 4; Fig. S13 in the Supporting Information). Compounds **27**, **50** and **54** were tested at 0.1, 1, 10 and 100 μM, and cytostatic drug doxorubicin (DX) and the mitochondrial toxin carbonyl cyanide 3-chlorophenyl-hydrazone (CCCP) were used as reference compounds. While compound **27** proved to be non-cytotoxic up to the concentration of 10 μM, **50** and **54** did not affect HepG2 cells viability up to 1 μM. Next, we assessed the cytotoxicity of compound **50** in the PrestoBlue assay against HepG2, BV-2 (mouse microglial) and N2a (mouse neuroblastoma) cell lines and determined the $IC_{50}$ values which are equal to 22, 24 and 25 μM respectively and are at least 240-fold higher than *eq*BuChE $IC_{50}$ value (Table 4; Tables S3, S4 and S5 in the Supporting Information).

Based on the obtained results we conclude that compound **50** combines a favourable profile of activity towards 5-$HT_6$R, *eq*BuChE and Aβ aggregation with satisfying metabolic stability and low cytotoxicity, pronounced at much higher concentrations than those required for interaction with 5-$HT_6$R and *eq*BuChE. Therefore, we selected compound **50** for the evaluation of the pharmacokinetic profile in mice.

### 2.10. Pharmacokinetic profile of compound ***50*** in vivo

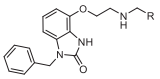
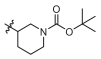
We investigated a pharmacokinetic profile of **50** after intravenous (*i.v.*) and oral (*p.o.*) administration to mice at a dose of 3 mg/kg and 30 mg/kg respectively. The concentration of the target compound in plasma and brain was determined using LC/ESI-MS/MS system. The plots of mean plasma and brain concentrations versus time profile for **50** after *i.v.* and *p.o.* administration are depicted in Fig. 8. The pharmacokinetic parameters calculated by non-compartmental approach are given in Table 5. Compound **50** was eliminated moderately slowly from mice bodies with a half-life of ca. 5 h. The volume of distribution (ca. 24 L/kg) indicates that this molecule is distributed throughout the total body water. After *p.o.*

**Table 3**
Affinity for 5-$HT_6$R, inhibition of *eq*BuChE, *h*BuChE and *ee*AChE and *in vitro* inhibitory activity against $A\beta_{42}$ by compounds from series III–VIII.

| Comp. | R | 5-$HT_6$R | | *eq*BuChE | *h*BuChE | *ee*AChE | Inhibition of $A\beta_{42}$ aggregation [%][c] |
|---|---|---|---|---|---|---|---|
| | | $K_i^a$ [nM] | $K_b^a$ [nM] | % or $IC_{50}^b$ | | | |
| **series III** | |  | | | | | |
| **60** |  | 3.0 ± 0.2 | 60.0 ± 2.6 | 1319 ± 37 | – | 27% | n.i.[d] |
| **61** |  | 12.0 ± 0.7 | – | 2304 ± 51 | – | 18% | n.i.[d] |
| **62** |  | 70.0 ± 7.0 | 459 ± 15 | 201.0 ± 4.3 | 1360 ± 49 | 24% | n.i.[d] |
| **63** |  | 70.0 ± 1.3 | – | 659.9 ± 12.0 | – | 28% | n.i.[d] |
| **series IV** | |  | | | | | |
| **64** |  | 9.0 ± 0.6 | 37.0 ± 1.6 | 401.3 ± 11.4 | – | 0% | 20.5 ± 2.9 |
| **65** |  | 12.0 ± 0.5 | – | 147.9 ± 3.2 | – | 9% | 23.6 ± 5.0 |
| **66** |  | 6.0 ± 0.2 | 189 ± 13 | 5.8 ± 0.1 | 1529 ± 44 | 31% | n.i.[d] |
| **67** |  | 24.0 ± 1.5 | – | 261.2 ± 6.2 | – | 24% | n.i.[d] |
| **series V** | |  | | | | | |
| **68** |  | 232.0 ± 45.0 | – | 625.6 ± 17.5 | – | 29% | 71.3 ± 4.1 |
| **69** |  | 293.0 ± 8.3 | – | 957.5 ± 42.1 | – | 11% | n.d.[e] |
| **70** |  | 369.0 ± 46.0 | – | 11.0 ± 0.2 | 115.2 ± 4.1 | 4% | 79.4 ± 7.7 |
| **series VI** | |  | | | | | |
| **71** |  | 389.0 ± 12.2 | – | 997.1 ± 21.4 | – | 18% | 72.5 ± 7.5 |
| **72** |  | 1469.0 ± 152.0 | – | 1263 ± 33 | – | 28% | 72.6 ± 6.3 |
| **73** |  | 1075.0 ± 20.2 | – | 25.7 ± 1.1 | 174.8 ± 6.2 | 2% | 79.7 ± 7.7 |
| **series VII** | |  | | | | | |
| **74** |  | 55.0 ± 6.0 | – | 4431 ± 103 | – | 1714 ± 72 | 42.4 ± 10.2 |
| **75** |  | 53.0 ± 1.5 | – | 1847 ± 56 | – | 1277 ± 34 | 58.5 ± 11.1 |
| **76** |  | 146.0 ± 9.5 | – | 1085 ± 23 | – | 37% | 65.2 ± 0.6 |

(*continued on next page*)

**Table 3** (*continued*)

| Comp. | R | 5-HT$_6$R | | *eq*BuChE | *h*BuChE | *ee*AChE | Inhibition of A$\beta_{42}$ aggregation [%][c] |
|---|---|---|---|---|---|---|---|
| | | $K_i$[a] [nM] | $K_b$[a] [nM] | % or $IC_{50}$[b] | | | |
| **series VIII** |  | | | | | | |
| **77** |  | 144.0 ± 8.0 | – | 1844 ± 37 | – | 1080 ± 41 | 32.9 ± 7.8 |
| **78** |  | 207.0 ± 5.4 | – | 1501 ± 30 | – | 1796 ± 49 | 64.3 ± 6.6 |
| **79** |  | 155.0 ± 4.4 | – | 466.6 ± 13.3 | – | 20% | 43.9 ± 12.3 |

[a] $K_i$ and $K_b$ values are expressed as the mean ± standard error of the mean (SEM) of at least three experiments. [b] % – percentage of enzyme inhibition at screening concentrations of 10 μM; $IC_{50}$ values are expressed as the mean ± standard error of the mean (SEM) of at least three experiments; BuChE from equine serum or human recombinant BuChE, AChE from electric eel. [c] Inhibition percentage at 10 μM compound and 1.5 μM A$\beta_{42}$. Inhibition percentage means ± SD for at least two independent experiments. [d] No inhibition (percentage lower than 20%). [e] Not determined. Statistical analysis: one-way ANOVA, followed by *post-hoc* Bonferroni *t*-test (SigmaPlot v 12.0), compared to control experiment (A$\beta_{42}$ + DMSO); $p < 0.05$.

administration, **50** achieves maximum concentration in the blood within 60 min. The absolute bioavailability of **50** after *p.o.* administration equals 28.7%. The compound penetrates the blood-brain barrier well with the brain to plasma ratio of 3.33 and 6.79 after *i.v.* and *p.o.* administration, respectively. Considering the results, we could conclude that the molecule has a desirable pharmacokinetic profile to be characterized as compound of choice to study *in vivo* pharmacology.

## 3. Conclusions

There is no doubt that extensive research on novel therapeutics against Alzheimer's disease must be undertaken to address the issue of the ever-increasing number of patients and the lack of effective therapy of this fatal condition. The strategy of multifunctional ligands offers a unique possibility of designing compounds that can exert enhanced biological effect by interacting with several disease-relevant targets and at the same time have indisputable advantages over concomitant administration of several active substances. In the present paper we describe novel multifunctional ligands targeting 5-HT$_6$ receptors, BuChE and amyloid β peptide aggregation, whose structure evolved from first-in-class tacrine-based ligands combining activities on these targets, described previously by our group. Clinical trials of the combination therapy of the 5-HT$_6$R antagonist and the ChE inhibitor have paved the way for the search for MTDLs with the same mode of action, and our previous studies have proven the connectivity of both targets. In this project, following the idea of optimizing the physicochemical properties, we fused *N*-benzylamine – donepezil-derived pharmacophore fragment – with 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl) oxy)ethan-1-amine and obtained compound **27** with favourable biological activity (5-HT$_6$R $K_i$ = 2.6 nM and BuChE $IC_{50}$ of 473 nM) and optimized physicochemical properties (MW < 500, ClogP <5, AR = 4, number of rotatable bonds = 4 and 7, compliance with Lipinski and Veber rules). To better understand the binding mode of **27** within BuChE, we determined the crystal structure of *h*BuChE in complex with compound **27** in which we observed a remarkable feature consisting in the displacement of the acyl-loop in the enzyme. Next, to probe and optimize compound **27** interactions with BuChE, we performed a series of modifications at the terminal 1-(phenylsulfonyl)-4-(piperazin-1-yl)-1*H*-indole and benzylamine moieties. The results of these studies combined with comprehensive crystallography experiments led to the determination of detailed structure-activity relationships that provide a solid base for further research in the field. Additionally, we have shown that *eq*BuChE, routinely used as a substitute for the more expensive *h*BuChE, is not always adequate for the development of BuChE inhibitors in the context of AD. Based on crystallography supplemented by molecular dynamic studies, we have determined the key differences within both enzymes leading to discrepancies in the potencies.

Among the compounds presented herein, we have selected the cyclohexylmethanamine derivative **50**, with remarkable and balanced potencies against BuChE ($IC_{50}$ = 90 nM) and 5-HT$_6$R ($K_i$ = 4.8 nM), and inhibitory activity against Aβ aggregation (53% at 10 μM). Compound **50** was characterized by good stability in the mouse liver microsomes and a favourable safety profile ($IC_{50}$ on HepG2, BV-2 and N2a cell lines at least 400 times higher than BuChE $IC_{50}$ and 5-HT$_6$R p$K_i$ values). Data from the pharmacokinetic experiments in mice have revealed that compound **50** permeates the blood-brain barrier well with the brain to plasma ratio of 3.33 and 6.79 after *i.v.* and *p.o.* administration respectively, which makes this compound a promising candidate for *in vivo* pharmacology studies.

To sum up, the research described herein has allowed us to determine the structure-activity relationship within the obtained groups of multifunctional ligands, and identify new, interesting scaffolds and compounds which may be considered a solid foundation for further research aiming at the development of effective anti-AD therapy.

## 4. Methods

### 4.1. Chemistry

#### 4.1.1. General chemistry information

All reagents were purchased from commercial suppliers and were used without further purification unless stated otherwise. Tetrahydrofuran (THF) and dichloromethane (DCM) were distilled under argon immediately before use. The drying agent used for THF was sodium/benzophenone ketyl, and for DCM, calcium hydride. Reactions were monitored by thin-layer chromatography carried out on aluminium sheets precoated with silica gel 60 $F_{254}$ (Merck). Compounds were visualized with UV light and by suitable visualization reagents (solution of ninhydrin). Compounds were purified with flash chromatography on IsoleraTM Spectra (Biotage) with silica gel 60 (63–200 μm; Merck) as a stationary phase. The UPLC-MS analyses were done on UPLC-MS/MS system comprising Waters

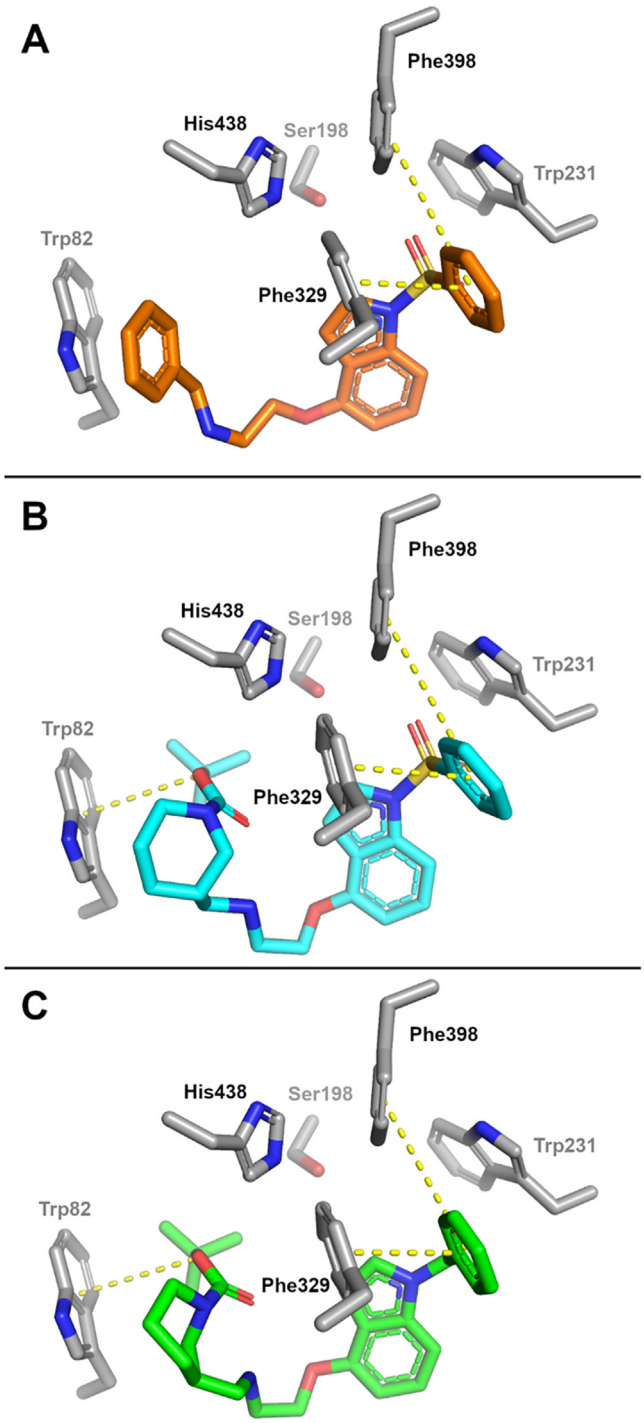



**Fig. 5.** Comparison of **27**, **54** and **70** binding mode into *h*BuChE based on X-ray crystallographic structures. A: binding mode of **27**, B: binding mode of **54**, C: binding mode of **70**. Ligands and residues are represented as sticks, with nitrogen atoms in blue, oxygen atoms in red, sulfur atoms in yellow and carbon atoms in grey. Carbon atoms for **27**, **54** and **70** are respectively represented in orange, cyan and green.

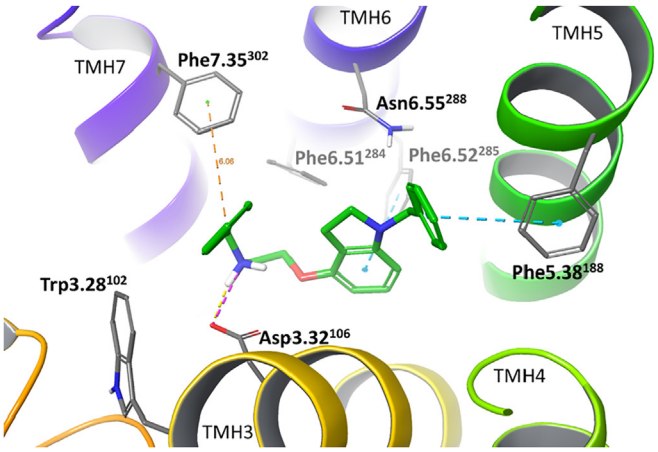



**Fig. 6.** The predicted binding mode of **71** within 5-$HT_6$R. The 1-benzylindoline core was unable to interact with Asn6.55[288], and caused undesirable conformation of the compound, which did not form CH-π stacking with Phe7.35[302]. The distance measured between the centroids of Phe7.35[302] and the nearby phenyl ring of **71** was 6.06 Å (orange dashed line). Amino acid residues engaged in ligand binding (within 4 Å from the ligand atoms) are represented as thick sticks. ECL2 was hidden for clarity. TMH – transmembrane helix.

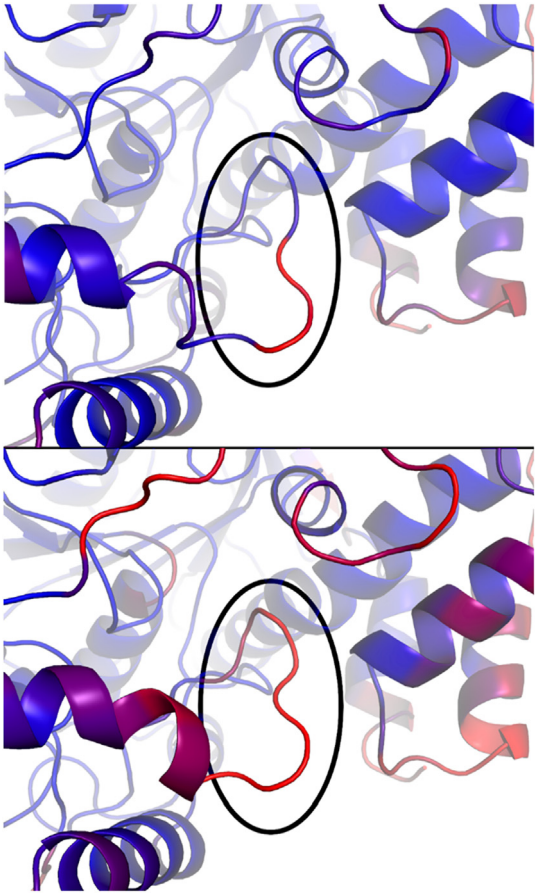


**Fig. 7.** Acyl-loop flexibility comparison in equine and human BuChE. The rmsd of the mainchain atoms position during 10 ns molecular simulation of human (upper panel) or equine BuChE (lower panel) are colored as B-factor fluctuations, from lower (blue) to higher (red) values. The region corresponding to the acyl-loop (residues 280–287) is circled.

ACQUITY UPLC (Waters Corporation, Milford, MA, USA) coupled with Waters TQD mass spectrometer (electrospray ionization mode ESI with tandem quadrupole). Chromatographic separations were carried out using the ACQUITY UPLC BEH (bridged ethyl hybrid) C18 column: 2.1 × 100 mm and 1.7 μm particle size. The column was maintained at 40 °C and eluted under gradient conditions using 95%–0% of eluent A over 10 min, at a flow rate of 0.3 mL/min. Eluent

**Table 4**
ADME-Tox parameters of compounds **27**, **54** and **50** determined by *in vitro* methods.

| | **27** | **50** | **54** |
|---|---|---|---|
| Metabolic stability[a] | | | |
| % of remaining compound[b]<br>main metabolic pathway | 57%<br>oxidative deamination | 59%<br>double hydroxylation and double bond reduction | 0%<br>oxidative deamination |
| Cell viability/toxicity | | | |
| HepG2 cells dose causing cytotoxic effect [μM][c] | 100 | 10 | 10 |
| HepG2 cells $IC_{50}$ [μM][d] | – | 21.6 ± 1.3[e] | – |
| BV-2 cells $IC_{50}$ [μM][d] | – | 23.9 ± 1.6[e] | – |
| N2a cells $IC_{50}$ [μM][d] | – | 25.0 ± 1.0[e] | – |

[a] assay on mouse liver microsomes.
[b] reference compound: verapamil.
[c] MTS assay.
[d] PrestoBlue assay.
[e] values are expressed as the mean ± standard error of the mean (SEM) of at least three experiments.

A: 0.1% solution of formic acid in water (v/v); eluent B: 0.1% solution of formic acid in acetonitrile (v/v). A total of 10 μL of each sample was injected and chromatograms were recorded using Waters eλ PDA detector. The spectra were analysed in the range of 200–700 nm with 1.2 nm resolution and at a sampling rate of 20 points/s. The UPLC/MS purity of all the test compounds was determined to be ≥ 95% and is given for each compound in the following description. $^1$H NMR and $^{13}$C NMR spectra were recorded on Varian Mercury 300 MHz (Varian, Inc., Palo Alto, CA) or Jeol 500 MHz (Jeol Inc., Peabody, MA). The chemical shifts are reported in ppm and were referenced to the residual solvent signals ($CDCl_3$ $^1$H: 7.26 ppm, $^{13}$C: 77.16 ppm; $CD_3OD$ $^1$H: 3.31 ppm, $^{13}$C: 49.00 ppm, DMSO-$d_6$ $^1$H: 2.50 ppm, $^{13}$C: 39.52 ppm), coupling constants are reported in hertz (Hz). HRMS analyses were performed on MALDI-TOF/TOF mass spectrometer UltrafleXtreme from Bruker Daltonics (Bremen, Germany) with α-cyano-4-hydroxycinnamic acid (CHCA) MALDI matrix after standard dried droplet preparation on ground steel target plate.

*4.1.2. Previously reported compounds*

4-(Benzyloxy)-1-(phenylsulfonyl)-1*H*-indole (2) [68], 1-benzyl-4-(benzyloxy)-1*H*-indole (4) [69], 1-(phenylsulfonyl)-1*H*-indol-4-ol (5) [70], 1-benzyl-1*H*-indol-4-ol (7) [71], 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) [72], *tert*-butyl (2-(3-fluoro-2-nitrophenoxy)ethyl)carbamate (22) [73], *N*-benzyl-2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (27) [72], *N*-(3-methoxybenzyl)-2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (39) [72], 4-(4-benzylpiperazin-1-yl)-1-(phenylsulfonyl)-1*H*-indole (49) [74], *N*-methyl-2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (56) [72].

*4.1.3. General procedure for the synthesis of compounds 2, 3 and 4 (GP1)*

To an ice-bath solution of potassium *tert*-butoxide (1.2 equiv) and 18-crown-6 ether (0.2 equiv) in THF, 4-(benzyloxy)-1*H*-indole (1.0 equiv) dissolved in THF was added dropwise; after 30 min, appropriate chloride or bromide (1.3–1.5 equiv) was added and the mixture was stirred at ambient temperature for 18 h. After that time, the solvent was removed in vacuo, the residue was dissolved in DCM, washed with water and brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by crystallization or flash chromatography according to methods described below.

*4.1.3.1. 4-(Benzyloxy)-1-(phenylsulfonyl)-1H-indole (2).* Following GP1, compound 2 was prepared using 4-(benzyloxy)-1*H*-indole (5.00 g, 22.4 mmol), benzenesulfonyl chloride (4.31 mL, 33.6 mmol), potassium *tert*-butoxide (3.02 g, 26.9 mmol), 18-crown-6 ether (1.18 g, 4.48 mmol) in 215 mL anhydrous THF. Purification: crystallization (chloroform/methanol). Yield: 7.14 g (81%), white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.85–7.91 (m, 2H), 7.62 (d, $J$ = 8.5 Hz, 1H), 7.50–7.56 (m, 1H), 7.48 (d, $J$ = 3.6 Hz, 1H), 7.29–7.47 (m, 7H), 7.22 (t, $J$ = 8.2 Hz, 1H), 6.84 (dd, $J$ = 0.8, 3.9 Hz, 1H), 6.72 (d, $J$ = 7.9 Hz, 1H), 5.15 (s, 2H). Formula: $C_{21}H_{17}NO_3S$; MS: *m/z* 364 ($M + H^+$).

*4.1.3.2. 4-(Benzyloxy)-1-(naphthalen-1-ylsulfonyl)-1H-indole (3).* Following GP1, compound 3 was prepared using 4-(benzyloxy)-1*H*-indole (893 mg, 4.00 mmol), 1-naphthalenesulfonyl chloride (1179 mg, 5.20 mmol, 1.3 equiv), potassium *tert*-butoxide (539 mg, 4.80 mmol), 18-crown-6 ether (211 mg, 0.80 mmol) in 40 mL anhydrous THF. Purification: flash chromatography (0–100% EtOAc in petroleum ether). Yield: 1297 mg (78%), white solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.71 (d, $J$ = 8.6 Hz, 1H), 8.11 (dd, $J$ = 1.2, 7.5 Hz, 1H), 8.04 (d, $J$ = 8.6 Hz, 1H), 7.87 (d, $J$ = 8.0 Hz, 1H), 7.71 (d, $J$ = 3.4 Hz, 1H), 7.61–7.68 (m, 1H), 7.53–7.58 (m, 1H), 7.48–7.52 (m, 1H), 7.43 (d, $J$ = 8.6 Hz, 3H), 7.36–7.40 (m, 2H), 7.30–7.35 (m, 1H), 7.14 (t, $J$ = 8.3 Hz, 1H), 6.86 (d, $J$ = 4.6 Hz, 1H), 6.68 (d, $J$ = 8.0 Hz, 1H), 5.14 (s, 2H). Formula: $C_{25}H_{19}NO_3S$.

*4.1.3.3. 1-Benzyl-4-(benzyloxy)-1H-indole (4).* Following GP1, compound 4 was prepared using 4-(benzyloxy)-1*H*-indole (2.46 g, 11.0 mmol), benzyl bromide (1.96 mL, 16.5 mmol, 1.5 equiv), potassium *tert*-butoxide (1.48 g, 13.2 mmol), 18-crown-6 ether (582 mg, 2.20 mmol) in 110 mL anhydrous THF. Purification: crystallization (EtOAc). Yield: 3.107 g (90%), white solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.51–7.54 (m, 2H), 7.39–7.43 (m, 2H), 7.24–7.36 (m, 4H), 7.10–7.13 (m, 2H), 7.08 (t, $J$ = 8.0 Hz, 1H), 7.05 (d, $J$ = 3.4 Hz, 1H), 6.93 (d, $J$ = 8.0 Hz, 1H), 6.74 (d, $J$ = 2.3 Hz, 1H), 6.60 (d, $J$ = 8.0 Hz, 1H), 5.31 (s, 2H), 5.25 (s, 2H). Formula: $C_{22}H_{19}NO$.

*4.1.4. General procedure for the synthesis of compounds 5, 6 and 7 (GP2)*

To the adequate substrate (2, 3 or 4) dissolved in THF 10% Pd/C (35–38% w/w) was added and the resulting mixture was hydrogenated at 45 °C for 3 h. Then, the solution was filtered through Celite® and concentrated in vacuo. The resulting solid was used in further steps without purification.

*4.1.4.1. 1-(Phenylsulfonyl)-1H-indol-4-ol (5).* Following GP2, compound 5 was prepared using 4-(benzyloxy)-1-(phenylsulfonyl)-1*H*-indole (2) (7.137 g, 19.6 mmol), 10% Pd/C (2600 mg, 36% w/w) in 150 mL THF. Yield: 5.357 g (100%), white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.85–7.90 (m, 2H), 7.39–7.60 (m, 5H), 7.14 (t, $J$ = 8.1 Hz,

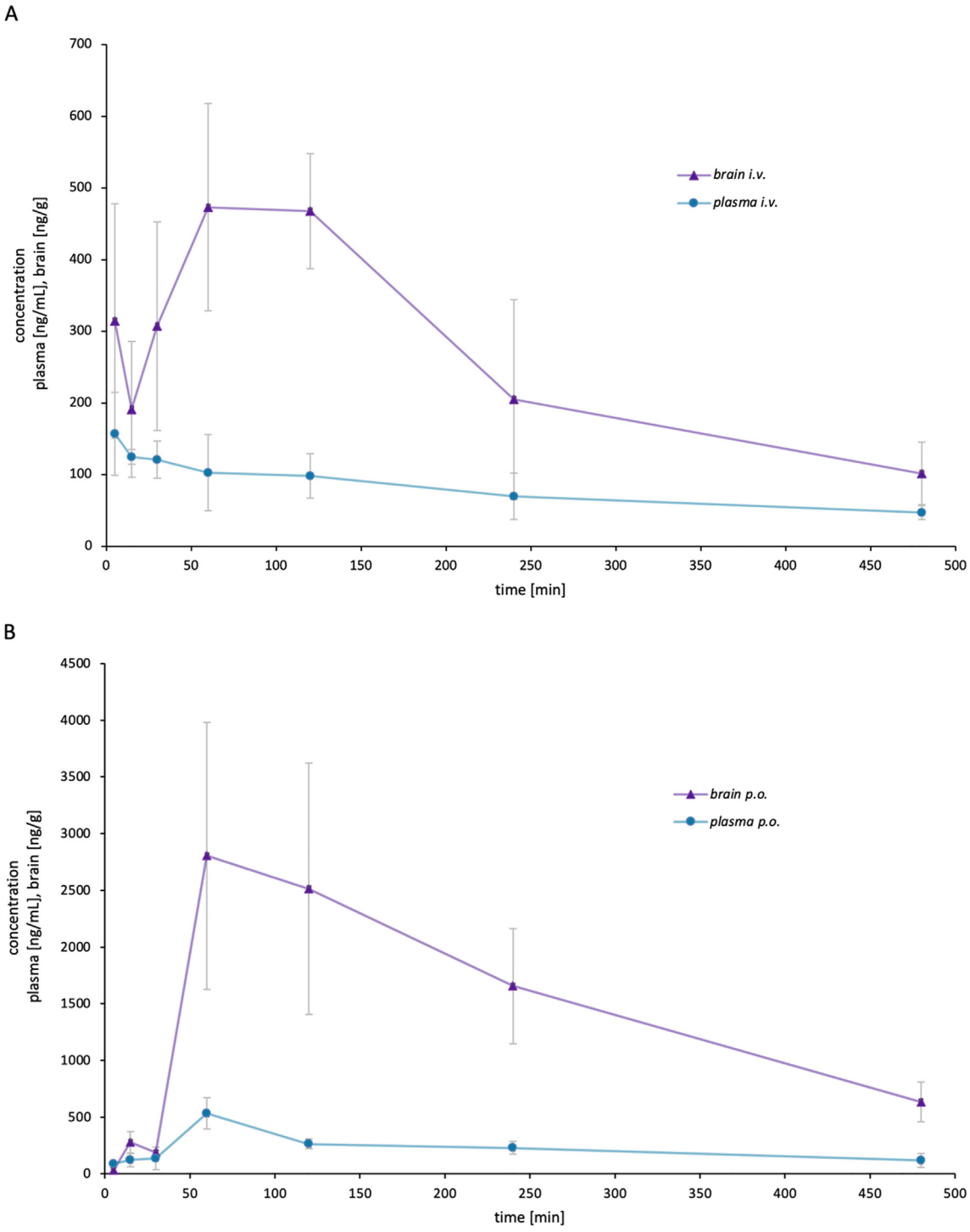



**Fig. 8.** Concentration-time profiles for compound **50** in plasma and brain after: A. intravenous administration to mice at a dose of 3 mg/kg, B. intragastric administration to mice at a dose of 30 mg/kg. 6 animals were used for each time point. The results are presented as means ± SEM.

1H), 6.76 (dd, $J = 0.8$, 3.6 Hz, 1H), 6.62 (dd, $J = 0.8$, 7.9 Hz, 1H), 5.47 (s, 1H). Formula: $C_{14}H_{11}NO_3S$; MS: *m/z* 274 ($M + H^+$).

*4.1.4.2. 1-(Naphthalen-1-ylsulfonyl)-1H-indol-4-ol (6).* Following GP2, compound 6 was prepared using 4-(benzyloxy)-1-(naphthalen-1-ylsulfonyl)-1*H*-indole (3) (1.282 g, 3.10 mmol), 10% Pd/C (490 mg, 38% w/w) in 40 mL THF. Yield: 0.447 g (45%), white solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.68 (d, $J = 8.6$ Hz, 1H), 8.08–8.15 (m, 1H), 8.03 (d, $J = 8.0$ Hz, 1H), 7.86 (d, $J = 8.0$ Hz, 1H), 7.71 (d, $J = 4.0$ Hz, 1H), 7.59–7.66 (m, 1H), 7.52–7.58 (m, 1H), 7.49 (t, $J = 7.7$ Hz, 1H), 7.38 (d, $J = 8.0$ Hz, 1H), 7.06 (t, $J = 8.3$ Hz, 1H), 6.75 (d, $J = 4.0$ Hz, 1H), 6.56 (d, $J = 8.0$ Hz, 1H), 5.04 (br. s., 1H). Formula: $C_{18}H_{13}NO_3S$.

*4.1.4.3. 1-Benzyl-1H-indol-4-ol (7).* Following GP2, compound 7 was prepared using 1-benzyl-4-(benzyloxy)-1*H*-indole (4) (3.107 g,

**Table 5**
The pharmacokinetic parameters for compound **50** calculated by non-compartmental approach.

| PK parameter [unit] | plasma | | brain | |
|---|---|---|---|---|
| | I.V. | P.O. | I.V. | P.O. |
| $C_0$ or $C_{max}$ [ng/mL][a] | 176.1 | 532.5 | 403.5 | 2805 |
| $t_{max}$ [min] | – | 60 | – | 60 |
| $t_{0.5}$ [min] | 332 | 298 | 178 | 180 |
| $AUC_{0\rightarrow t}$ [ng · min/mL][b] [ng · min/g][c] | 37637 | 108176 | 125194 | 734469 |
| MRT [min] | 191 | 194.5 | 170 | 194.6 |
| $V_{ss}$ [L/kg] | 23.9 | – | – | – |
| Cl [mL/min/kg] | 49.8 | – | – | – |
| F [%] | – | 28.7 | – | – |
| $V_{ss}$/F [L/kg] | – | 81.4 | – | – |
| Cl/F [mL/min/kg] | – | 189 | – | – |

[a] $C_0$ for *i.v.*, $C_{max}$ for *p.o.*
[b] concentration in plasma.
[c] concentration in the brain.

9.91 mmol), 10% Pd/C (1100 mg, 35% w/w) in 50 mL THF. Yield: 2.212 g (99%), white solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.23–7.32 (m, 3H), 7.09–7.12 (m, 2H), 7.04–7.06 (m, 1H), 7.02 (d, $J$ = 8.0 Hz, 1H), 6.88 (d, $J$ = 8.6 Hz, 1H), 6.62 (d, $J$ = 4.0 Hz, 1H), 6.52 (d, $J$ = 7.5 Hz, 1H), 5.73 (br. s., 1H), 5.29 (s, 2H). Formula: $C_{14}H_{13}NO$; MS: $m/z$ 224 ($M + H^+$).

#### 4.1.5. General procedure for the synthesis of compounds 8, 9 and 10 (GP3)

The mixture of compound 5, 6 or 7 (1 equiv), 1,2-dibromoethane (5 equiv) and $K_2CO_3$ (3 equiv) in ethanol was stirred at 80 °C for 48 h. After that time, the mixture was filtered, concentrated in vacuo and purified according to methods described below.

*4.1.5.1. 4-(2-Bromoethoxy)-1-(phenylsulfonyl)-1H-indole (8).* Following GP3, compound 8 was prepared using 1-(phenylsulfonyl)-1*H*-indol-4-ol (5) (5.439 g, 19.9 mmol), 1,2-dibromoethane (8.574 mL, 99.5 mmol), $K_2CO_3$ (8.251 g, 59.7 mmol) in 150 mL EtOH. Purification: flash chromatography (0–50% EtOAc in petroleum ether). Yield: 4.762 g (63%), colourless oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.84–7.88 (m, 2H), 7.63 (d, $J$ = 8.6 Hz, 1H), 7.50–7.54 (m, 1H), 7.48 (d, $J$ = 4.0 Hz, 1H), 7.39–7.44 (m, 2H), 7.21 (t, $J$ = 8.0 Hz, 1H), 6.79–6.82 (m, 1H), 6.62 (d, $J$ = 8.0 Hz, 1H), 4.36 (t, $J$ = 6.3 Hz, 2H), 3.63–3.68 (m, 2H). Formula: $C_{16}H_{14}BrNO_3S$; MS: $m/z$ 380 ($M + H^+$).

*4.1.5.2. 4-(2-Bromoethoxy)-1-(naphthalen-1-ylsulfonyl)-1H-indole (9).* Following GP3, compound 9 was prepared using 1-(naphthalen-1-ylsulfonyl)-1*H*-indol-4-ol (6) (447 mg, 1.38 mmol), 1,2-dibromoethane (595 μL, 6.90 mmol), $K_2CO_3$ (572 mg, 4.14 mmol) in 10 mL EtOH. The crude product was used further without purification. Yield: 447 mg (75%), white solid. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.60 (d, $J$ = 8.6 Hz, 1H), 8.40 (d, $J$ = 7.5 Hz, 1H), 8.32 (d, $J$ = 8.0 Hz, 1H), 8.09 (d, $J$ = 8.0 Hz, 1H), 8.03 (d, $J$ = 4.0 Hz, 1H), 7.73 (t, $J$ = 7.7 Hz, 2H), 7.62–7.68 (m, 1H), 7.34 (d, $J$ = 8.0 Hz, 1H), 7.18 (t, $J$ = 8.3 Hz, 1H), 6.73–6.80 (m, 2H), 4.37 (t, $J$ = 5.2 Hz, 2H), 3.80 (t, $J$ = 5.2 Hz, 2H). Formula: $C_{20}H_{16}BrNO_3S$.

*4.1.5.3. 1-Benzyl-4-(2-bromoethoxy)-1H-indole (10).* Following GP3, compound 10 was prepared using 1-benzyl-1*H*-indol-4-ol (7) (4.263 g, 19.10 mmol), 1,2-dibromoethane (8.229 mL, 95.50 mmol), $K_2CO_3$ (7.919 g, 57.30 mmol) in 140 mL EtOH. Purification: flash chromatography (10% EtOAc in petroleum ether). Yield: 2.638 g (42%), colourless oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.22–7.33 (m, 3H), 7.01–7.13 (m, 4H), 6.90–6.97 (m, 1H), 6.66–6.72 (m, 1H), 6.52 (d, $J$ = 7.5 Hz, 1H), 5.30 (s, 2H), 4.41–4.48 (m, 2H), 3.68–3.76 (m, 2H). Formula: $C_{17}H_{16}BrNO$; MS: $m/z$ 331 ($M + H^+$).

#### 4.1.6. General procedure for the synthesis of compounds 11, 12, 13 (GP4)

A mixture of an appropriate primary bromide (8, 9 or 10) (1 equiv), potassium phthalimide salt (1.06 equiv) and 18-crown-6 ether (0.03 equiv) in DMF was stirred at 50 °C for 24 h. After that time, the solvent was removed in vacuo, the residue was dissolved in DCM, washed with water and brine, dried over $Na_2SO_4$ and concentrated in vacuo. A crude product was purified by crystallization or flash chromatography according to the methods described below.

*4.1.6.1. 2-(2-((1-(Phenylsulfonyl)-1H-indol-4-yl)oxy)ethyl)isoindoline-1,3-dione (11).* Following GP4, compound 11 was prepared using 4-(2-bromoethoxy)-1-(phenylsulfonyl)-1*H*-indole (8) (3.705 g, 9.74 mmol), potassium phthalimide salt (1.912 g 10.32 mmol), 18-crown-6 ether (77 mg, 6.29 mmol) in 60 mL DMF. Purification: crystallization (EtOAc). Yield: 3.686 g (85%), white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.80–7.86 (m, 4H), 7.68–7.72 (m, 2H), 7.56 (d, $J$ = 8.5 Hz, 1H), 7.46–7.52 (m, 1H), 7.35–7.43 (m, 3H), 7.17 (t, $J$ = 8.1 Hz, 1H), 6.72 (dd, $J$ = 0.8, 3.9 Hz, 1H), 6.60 (d, $J$ = 7.7 Hz, 1H), 4.27 (t, $J$ = 5.1 Hz, 2H), 4.15 (t, $J$ = 5.4 Hz, 2H). Formula: $C_{24}H_{18}N_2O_5S$; MS: $m/z$ 447 ($M + H^+$).

*4.1.6.2. 2-(2-((1-(Naphthalen-1-ylsulfonyl)-1H-indol-4-yl)oxy)ethyl) isoindoline-1,3-dione (12).* Following GP4, compound 12 was prepared using 4-(2-bromoethoxy)-1-(naphthalen-1-ylsulfonyl)-1*H*-indole (9) (412 mg, 0.96 mmol), potassium phthalimide salt (189 mg, 1.02 mmol), 18-crown-6 ether (8 mg, 0.03 mmol) in 8 mL DMF. Purification: flash chromatography (30–60% EtOAc in petroleum ether). Yield: 304 mg (63%), white solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.67 (dd, $J$ = 1.2, 8.6 Hz, 1H), 7.97–8.05 (m, 2H), 7.81–7.87 (m, 3H), 7.70 (dd, $J$ = 3.2, 5.4 Hz, 2H), 7.64 (d, $J$ = 3.4 Hz, 1H), 7.61 (ddd, $J$ = 1.2, 7.0, 8.5 Hz, 1H), 7.53 (ddd, $J$ = 1.2, 6.9, 8.0 Hz, 1H), 7.44–7.48 (m, 1H), 7.38 (d, $J$ = 8.6 Hz, 1H), 7.10 (t, $J$ = 8.3 Hz, 1H), 6.74–6.76 (m, 1H), 6.56 (d, $J$ = 8.0 Hz, 1H), 4.25–4.29 (m, 2H), 4.13–4.18 (m, 2H). Formula: $C_{28}H_{20}N_2O_5S$; MS: $m/z$ 497 ($M + H^+$).

*4.1.6.3. 2-(2-((1-Benzyl-1H-indol-4-yl)oxy)ethyl)isoindoline-1,3-dione (13).* Following GP4, compound 13 was prepared using 1-benzyl-4-(2-bromoethoxy)-1*H*-indole (10) (1341 mg, 4.06 mmol), potassium phthalimide salt (796 mg, 4.30 mmol), 18-crown-6 ether (32 mg, 0.12 mmol) in 30 mL DMF. Purification: crystallization (EtOAc). Yield: 1083 mg (67%), white solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.83–7.89 (m, 2H), 7.66–7.72 (m, 2H), 7.19–7.29 (m, 3H), 7.04 (dd, $J$ = 5.4, 7.2 Hz, 3H), 6.99 (d, $J$ = 2.9 Hz, 1H), 6.87 (d, $J$ = 8.0 Hz, 1H), 6.59–6.61 (m, 1H), 6.48 (d, $J$ = 7.5 Hz, 1H), 5.25 (s, 2H), 4.33–4.38 (m, 2H), 4.19–4.23 (m, 2H). Formula: $C_{25}H_{20}N_2O_3$; MS: $m/z$ 397 ($M + H^+$).

#### 4.1.7. General procedure for the synthesis of primary amines 14, 15, 16 and 19 (GP5)

A mixture of phthalimide derivative (11, 12, 13, 18) and 40% aqueous solution of $CH_3NH_2$ (10 ml for 1 mmol of a compound) was stirred at 50 °C for 2.5 h. After that time, the mixture was cooled to ambient temperature, 1 M $NaOH_{(aq)}$ was added (10 ml for 1 mmol of a compound) and the mixture was stirred for 1 h. Next, the mixture was extracted with DCM, combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography according to the methods described below or used in further steps without purification.

*4.1.7.1. 2-((1-(Phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (14).* Following GP5, compound 14 was prepared using 2-(2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethyl)isoindoline-1,3-dione (11) (3.686 g, 8.26 mmol), 40% aqueous solution of $CH_3NH_2$ (80 mL), 1 M $NaOH_{(aq)}$ (80 mL). The product was used without further purification. Yield: 2.613 g (100%), brownish solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.80–7.87 (m, 2H), 7.57–7.62 (m, 1H), 7.33–7.51 (m, 4H), 7.17 (t, $J$ = 7.9 Hz, 1H), 6.82 (d, $J$ = 3.5 Hz, 1H), 6.56 (d, $J$ = 8.2 Hz, 1H), 5.39 (br. s., 3H, $-NH_3^+$), 4.05 (t, $J$ = 5.0 Hz, 2H), 3.14 (t, $J$ = 5.0 Hz, 2H). Formula: $C_{16}H_{16}N_2O_3S$; MS: *m/z* 317 ($M + H^+$).

*4.1.7.2. 2-((1-(Naphthalen-1-ylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (15).* Following GP5, compound 15 was prepared using 2-(2-((1-(naphthalen-1-ylsulfonyl)-1*H*-indol-4-yl)oxy)ethyl)isoindoline-1,3-dione (12) (304 mg, 0.61 mmol), 40% aqueous solution of $CH_3NH_2$ (6 mL), 1 M $NaOH_{(aq)}$ (6 mL). Purification: flash chromatography (1% $NH_{3(aq)}$ in MeOH/DCM, 10:90). Yield: 187 mg (84%), yellow oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.68 (d, $J$ = 8.6 Hz, 1H), 8.02 (t, $J$ = 7.7 Hz, 2H), 7.85 (d, $J$ = 8.0 Hz, 1H), 7.66 (d, $J$ = 3.4 Hz, 1H), 7.58–7.64 (m, 1H), 7.51–7.56 (m, 1H), 7.44–7.49 (m, 1H), 7.39 (d, $J$ = 8.6 Hz, 1H), 7.12 (t, $J$ = 8.0 Hz, 1H), 6.76 (d, $J$ = 4.0 Hz, 1H), 6.64 (d, $J$ = 8.0 Hz, 1H), 4.29 (t, $J$ = 6.3 Hz, 2H), 3.64 (t, $J$ = 6.3 Hz, 2H), $-NH_2$ signal not detected. Formula: $C_{20}H_{18}N_2O_3S$; MS: *m/z* 367 ($M + H^+$).

*4.1.7.3. 2-((1-Benzyl-1H-indol-4-yl)oxy)ethan-1-amine (16).* Following GP5, compound 16 was prepared using 2-(2-((1-benzyl-1*H*-indol-4-yl)oxy)ethyl)isoindoline-1,3-dione (13) (848 mg, 2.16 mmol), 40% aqueous solution of $CH_3NH_2$ (22 mL), 1 M $NaOH_{(aq)}$ (22 mL). The product was used without further purification. Yield: 520 mg (90%), yellow oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.22–7.31 (m, 3H), 7.02–7.11 (m, 4H), 6.91 (d, $J$ = 8.0 Hz, 1H), 6.67 (d, $J$ = 4.0 Hz, 1H), 6.52 (d, $J$ = 8.0 Hz, 1H), 5.29 (s, 2H), 4.15 (t, $J$ = 5.2 Hz, 2H), 3.16 (t, $J$ = 5.2 Hz, 2H), 1.78 (br. s., 2H). Formula: $C_{17}H_{18}N_2O$.

*4.1.8. General procedure for the synthesis of compounds 17, 20 (GP6)*

$NaBH_3CN$ (4.5 equiv) was added portionwise, under argon, to ice cold TFA. The mixture was stirred for 15 min and the indole derivative (1 equiv) was added portionwise. The mixture was allowed to attain rt. and stirred for 1.5 h. After this time, second portion of $NaBH_3CN$ (4.5 equiv) was added, and the mixture was stirred for 1.5 h. Next, the pH of the mixture was adjusted carefully to ca. 8 with saturated $NaOH_{(aq)}$ and the resulting solution was extracted with DCM. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography according to the methods described below.

*4.1.8.1. 2-((1-Benzylindolin-4-yl)oxy)ethan-1-amine (17).* Following GP6, compound 17 was prepared using 2-((1-benzyl-1*H*-indol-4-yl)oxy)ethan-1-amine (16) (520 mg, 1.95 mmol), $NaBH_3CN$ (1.103 g, 17.55 mmol) in 6 mL TFA. Purification: column chromatography (DCM/EtOAc/petroleum ether/10% $NH_{3(aq)}$ in MeOH, 78:10:6:6). Yield: 382 (73%), pale-yellow oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.29–7.39 (m, 4H), 7.17–7.23 (m, 1H), 7.00 (t, $J$ = 8.0 Hz, 1H), 6.19–6.26 (m, 2 H), 4.65 (br. s., 2H), 4.24 (s, 2H), 3.35 (t, $J$ = 8.6 Hz, 2H), 3.13–3.24 (m, 2H), 2.92 (t, $J$ = 8.6 Hz, 2H), 1.81 (br. s., 2H). Formula: $C_{17}H_{20}N_2O$; MS: *m/z* 269 ($M + H^+$).

*4.1.8.2. 4-(2-Bromoethoxy)-1-(phenylsulfonyl)indoline (20).* Following GP6, compound 20 was prepared using 4-(2-bromoethoxy)-1-(phenylsulfonyl)-1*H*-indole (8) (608 mg, 1.60 mmol), $NaBH_3CN$ (905 mg, 14.40 mmol) in 24 mL TFA. Purification: flash chromatography (15–30% EtOAc in petroleum ether). Yield: 611 (99%), colourless oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.79–7.83 (m, 2H), 7.49–7.60 (m, 1H), 7.41–7.48 (m, 2H), 7.32 (d, $J$ = 8.0 Hz, 1H), 7.12–7.19 (m, 1H), 6.48 (d, $J$ = 8.6 Hz, 1H), 4.25 (t, $J$ = 6.0 Hz, 2H), 3.94 (t, $J$ = 8.6 Hz, 2H), 3.57 (t, $J$ = 6.0 Hz, 2H), 2.87 (t, $J$ = 8.6 Hz, 2H). Formula: $C_{16}H_{16}BrNO_3S$; MS: *m/z* 384.

*4.1.9. Synthesis of 2-((1-(phenylsulfonyl)indolin-4-yl)oxy)ethan-1-amine (21)*

Compound 21 was prepared stepwise. *Step 1.* According to GP4 using 4-(2-bromoethoxy)-1-(phenylsulfonyl)indoline (20) (581 mg, 1.52 mmol), potassium phthalimide salt (298 mg, 1.61 mmol), 18-crown-6 ether (13 mg, 0.15 mmol) in 10 mL DMF. Purification: flash chromatography (35% EtOAc in petroleum ether). Yield: 472 mg (70%), white solid.$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.79–7.84 (m, 2H), 7.76 (dd, $J$ = 1.4, 8.3 Hz, 2H), 7.70 (dd, $J$ = 2.9, 5.2 Hz, 2H), 7.48–7.53 (m, 1H), 7.37–7.43 (m, 2H), 7.22–7.26 (m, 1H), 7.09 (t, $J$ = 8.3 Hz, 1H), 6.45 (d, $J$ = 8.0 Hz, 1H), 4.15 (t, $J$ = 5.7 Hz, 2H), 4.05 (t, $J$ = 5.7 Hz, 2H), 3.86 (t, $J$ = 8.5 Hz, 2H), 2.73 (t, $J$ = 8.6 Hz, 2H). Formula: $C_{24}H_{20}N_2O_5S$. *Step 2*. According to GP5 using the product from the previous step (472 mg, 1.05 mmol), 40% aqueous solution of $CH_3NH_2$ (10 mL), 1 M $NaOH_{(aq)}$ (10 mL). The product was used without further purification. Yield: 293 mg (88%). Formula: $C_{16}H_{18}N_2O_3S$; MS: *m/z* 319 ($M + H^+$).

*4.1.10. Synthesis of 2-(2-((1-benzyl-2-oxoindolin-4-yl)oxy)ethyl) isoindoline-1,3-dione (18)*

Compound 18 was prepared stepwise. *Step 1.* To a solution of 2-(2-((1-benzyl-1*H*-indol-4-yl)oxy)ethyl)isoindoline-1,3-dione (13) (235 mg, 0.59 mmol) in 4 ml of anhydrous THF, *N*-chlorosuccinimide (79 mg, 0.59 mmol) was added in two portions over 1 h. The reaction was stirred overnight and then the solvent was removed in vacuo. The resulting residue was used in the next step without purification. *Step 2*. A mixture of the product from step 1 was mixed with 85% $H_3PO_4$ (2.5 mL) in 5 mL of 2-methoxyethanol and heated at 100 °C for 1 h. Next, after cooling the reaction mixture to ambient temperature, the pH of the mixture was adjusted carefully to ca. 9–10 with a saturated $NaOH_{(aq)}$ and the resulting solution was extracted with DCM. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The product did not require any additional purification procedures. Yield: 189 mg (78%, overall two steps), white solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.82–7.88 (m, 2H), 7.68–7.74 (m, 2H), 7.19–7.31 (m, 5H), 7.06 (t, $J$ = 8.0 Hz, 1H), 6.52 (d, $J$ = 8.0 Hz, 1H), 6.34 (d, $J$ = 8.0 Hz, 1H), 4.84 (s, 2H), 4.21–4.28 (m, 2H), 4.10 (t, $J$ = 5.7 Hz, 2H), 3.44 (s, 2H). Formula: $C_{25}H_{20}N_2O_4$; MS: *m/z* 413 ($M + H^+$).

*4.1.11. Synthesis of 4-(2-aminoethoxy)-1-benzylindolin-2-one (19)*

Compound 19 was prepared according to GP5 using 2-(2-((1-benzyl-2-oxoindolin-4-yl)oxy)ethyl)isoindoline-1,3-dione (18) (189 mg, 0.46 mmol), 40% aqueous solution of $CH_3NH_2$ (9 mL), 1 M $NaOH_{(aq)}$ (5 mL). The product was used without further purification. Yield: 98 mg (76%), colourless oil. Formula: $C_{17}H_{18}N_2O_2$ MS: *m/z* 283 ($M + H^+$).

*4.1.12. Synthesis of tert-butyl (2-(3-fluoro-2-nitrophenoxy)ethyl) carbamate (22)*

To an ice-cooled solution of *tert*-butyl-2-hydroxyethyl carbamate (4557 mg, 28.30 mmol) in THF (35 mL), NaH (680 mg, 28.30 mmol, 60% dispersion in mineral oil) was added. The resulting mixture was stirred at room temperature for 15 min, and next added portionwise to an ice-cooled solution of 2,6-difluoronitrobenzene (5000 mg, 31.45 mmol) in THF (10 mL). Stirring was continued at room temperature until the *tert*-butyl-2-hydroxyethyl carbamate was consumed (1–3 h). Then, the mixture was cooled to 0 °C, quenched with a saturated aqueous

solution of ammonium chloride and extracted with EtOAc. The combined organics were dried over magnesium sulfate, filtered and concentrated in vacuo. The obtained crude product was purified by flash chromatography (n-hexane/EtOAc, 6/4, v/v) to yield the pure product in 56% yield, as a crystallizing oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.39 (dt, $J$ = 5.9, 8.5 Hz, 1H), 6.89–6.78 (m, 2H), 4.97 (br s, 1H), 4.15 (t, $J$ = 5.3 Hz, 2H), 3.52 (q, $J$ = 5.3 Hz, 2H), 1.44 (s, 9H). $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$ 122.1 (s, 1F). Formula: $C_{13}H_{17}FN_2O_5$. MS: *m/z* 201 (M − BOC + $H^+$).

#### 4.1.13. Synthesis of tert-butyl (2-(3-(benzylamino)-2-nitrophenoxy) ethyl)carbamate (23)

To a solution of 22 (2660 mg, 8.86 mmol) in dry DMSO (40 mL), $K_2CO_3$ (2450 mg, 17.73 mmol) and benzylamine (1423 mg, 13.30 mmol) was added. The mixture was stirred at 70 °C for 18–24 h, then cooled to room temperature, poured into cold solution of brine (185 mL) and diluted with water (185 mL). The obtained precipitate was filtered off, washed with water, dried on air and crystalized from EtOH (99.9%, ~10 mL) affording crude product as an orange solid. The obtained solid was purified by flash chromatography (n-hexane/DCM/MeOH, 5/4.5/0.5, v/v/v) to yield the pure product in 56% yield, as a yellowish solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.33 (m, 5H), 7.17 (t, $J$ = 8.2 Hz, 1H), 6.64 (br s, 1H), 6.35 (d, $J$ = 7.6 Hz, 1H), 6.24 (d, $J$ = 7.6 Hz, 1H), 5.18 (br s, 1H), 4.43 (d, $J$ = 5.3 Hz, 2H), 4.08 (t, $J$ = 5.0 Hz, 2H), 3.57–3.49 (m, 2H), 1.44 (s, 9H). Formula: $C_{20}H_{25}N_3O_5$. MS: *m/z* 288 (M − BOC group+$H^+$).

#### 4.1.14. Synthesis of tert-butyl (2-(2-amino-3-(benzylamino) phenoxy)ethyl) carbamate (24)

To a solution of 23 (3771 mg, 9.73 mmol) in EtOH (155 mL) heated to 80 °C, a freshly prepared solution of sodium dithionite (7624 mg, 43.80 mmol) in water (50 mL) was added within 1 min. The reaction mixture was refluxed for 15 min (until substrate was consumed). Then it was cooled to the room temperature and saturated, aqueous solution of $Na_2CO_3$ was added to alkaline pH. Subsequently EtOH was removed in vacuo and the obtained mixture was extracted with EtOAc. Combined organic phases were washed with water, brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The obtained crude yellowish oil was used immediately in the next step without further purification.

#### 4.1.15. Synthesis of tert-butyl (2-((1-benzyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazole-4-yl)oxy)ethyl)carbamate (25)

To a solution of 24 (4119 mg, 11.53 mmol) in DCM (100 mL), 1,1′-carbonyldiimidazole (2429 mg, 14.98 mmol) was added. The reaction mixture was stirred at room temperature until substrate consumption (12–24 h). Then, it was quenched with water and extracted with DCM. The combined organics were dried over magnesium sulfate, filtered and concentrated in vacuo. The obtain crude product was purified by flash chromatography (n-hexane/DCM/MeOH/$NH_{3(aq)}$, 3/6.5/0.5/0.02, v/v/v/v) to yield the pure product in 67% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 11.11 (br s, 1H), 7.37–7.23 (m, 5 H), 6.97–6.87 (m, 1H), 6.60 (d, $J$ = 8.2 Hz, 1 H), 6.54–6.58 (m, 1 H), 6.05 (br. s., 1H), 5.12 (s, 2H), 4.11 (t, $J$ = 4.7 Hz, 2H), 3.56 (q, $J$ = 5.3 Hz, 2H), 1.39 (s, 9H). Formula: $C_{21}H_{25}N_3O_4$. MS: *m/z* 384 [M + $H^+$].

#### 4.1.16. Synthesis of 2-((1-benzyl-2-oxo-2,3-dihydro-1H-benzo[d] imidazole-4-yl)oxy)ethan-1-aminium chloride (26)

The obtained 25 (2930 mg, 7.65 mmol) was mixed with 1.0 M solution of HCl in EtOAc (140 mL), and stirred at room temperature for 24 h. Then, the mixture was filtered giving pure product as a hydrochloride salt (pale pink powder). The compound was used in the next step without further purification. Yield: 2,04 g (94%).$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.11 (s, 1H), 8.31 (br s, 3H), 7.36–7.19 (m, 5H), 6.94–6.82 (m, 1H), 6.69 (dd, $J$ = 3.8, 7.9 Hz, 2H), 4.96 (s, 2H), 4.21 (t, $J$ = 4.7 Hz, 2H), 3.22 (d, $J$ = 4.7 Hz, 2H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 154.5, 142.4, 137.7, 131.3, 129.0 (2C), 127.8, 127.7, 121.6, 119.6, 117.8, 105.8, 102.8, 65.0, 43.9, 39.0. Formula: $C_{16}H_{18}ClN_3O_2$. MS: *m/z* 284 [M + $H^+$].

#### 4.1.17. General procedure for the synthesis of final compounds 27, 29, 30–36, 38–48, 50–52, 54–55, 57–76 (GP7)

To an adequate amine (1.0–1.3 equiv) dissolved in MeOH or a 1:1 mixture of MeOH and THF, aldehyde (1.0 equiv) and glacial acetic acid (catalytic amounts) were added. After 1 h of stirring at ambient temperature, the reaction mixture was cooled on an ice bath and $NaBH_3CN$ or $NaBH_4$ (3.0–4.0 equiv) was added. The reaction was stirred 18 h at room temperature then, the solvents were removed in vacuo, the residue was treated with water and extracted with DCM. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by methods described below.

*4.1.17.1. N-Benzyl-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (27).* Following GP7, compound 27 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (70 mg, 0.22 mmol, 1.3 equiv), benzaldehyde (17 μL, 0.17 mmol), glacial acetic acid (20 μL), $NaBH_3CN$ (41 mg, 0.66 mmol, 3.9 equiv) in 3 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 39 mg (56%), yellowish oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83–7.91 (m, 2H), 7.61 (d, $J$ = 8.2 Hz, 1H), 7.18–7.56 (m, 10H), 6.76–6.80 (m, 1H), 6.65 (d, $J$ = 8.2 Hz, 1H), 4.18 (t, $J$ = 5.3 Hz, 2H), 3.89 (s, 2H), 3.07 (t, $J$ = 5.3 Hz, 2H), 1.85 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.3, 140.1, 138.2, 136.2, 133.7, 129.2, 128.4, 128.0, 127.0, 126.7, 125.6, 124.8, 121.2, 106.6, 106.3, 104.5, 67.7, 53.7, 48.0. Formula: $C_{23}H_{22}N_2O_3S$. HRMS: *m/z* found [M + $H^+$] 407.1481, $C_{23}H_{22}N_2O_3S$ requires 407.1429.

*4.1.17.2. N-(3-Methylbenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl) oxy)ethan-1-amine (29).* Following GP7, compound 29 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (56 mg, 0.18 mmol, 1.1 equiv), 3-methylbenzaldehyde (19 μL, 0.16 mmol, 1 equiv), glacial acetic acid (10 μL), $NaBH_4$ (17 mg, 0.45 mmol, 2.8 equiv) in 2 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 33 mg (50%), colourless oil. Purity 96% (UPLC/MS). $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$ 7.87 (d, $J$ = 7.0 Hz, 2H), 7.61 (d, $J$ = 8.2 Hz, 1H), 7.36–7.56 (m, 4H), 7.02–7.25 (m, 5H), 6.78 (d, $J$ = 3.5 Hz, 1H), 6.65 (d, $J$ = 7.6 Hz, 1H), 4.19 (t, $J$ = 5.3 Hz, 2H), 3.85 (s, 2H), 3.07 (t, $J$ = 5.3 Hz, 2H), 2.33 (s, 3H), 1.87 (br. s., 1H).$^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.3, 139.9, 138.2, 138.1, 136.2, 133.8, 129.2, 128.9, 128.4, 127.8, 126.7, 125.7, 125.1, 124.8, 106.7, 106.3, 104.6, 67.7, 53.7, 48.0, 21.4. Formula: $C_{24}H_{24}N_2O_3S$; MS: *m/z* 422 (M + $H^+$).

*4.1.17.3. N-(2-Chlorobenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl) oxy)ethan-1-amine (30).* Following GP7, compound 30 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (58 mg, 0.18 mmol, 1.3 equiv), 2-chlorobenzaldehyde (16 μL, 0.14 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (26 mg, 0.42 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (3% MeOH in DCM). Yield: 51 mg (83%), colourless oil. Purity 98% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83–7.90 (m, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.33–7.56 (m, 6H), 7.16–7.24 (m, 3H), 6.75–6.80 (m, 1H), 6.64 (d, $J$ = 7.6 Hz, 1H), 4.19 (t, $J$ = 5.3 Hz, 2H), 3.97 (s, 2H), 3.06 (t, $J$ = 5.0 Hz, 2H), 1.90 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.3, 138.2, 137.4, 136.2, 133.8, 133.7, 130.1, 129.6, 129.2, 128.4, 126.9, 126.7, 125.7, 124.8, 121.2, 106.6, 106.3, 104.5, 67.7, 51.3, 48.0. Formula: $C_{23}H_{21}ClN_2O_3S$.

*4.1.17.4. N-(3-Chlorobenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl) oxy)ethan-1-amine (31).* Following GP7, compound 31 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (90 mg, 0.28 mmol, 1.3 equiv), 3-chlorobenzaldehyde (24.5 μL, 0.22 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (54 mg, 0.86 mmol, 3.9 equiv) in 3 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 26 mg (28%), colourless oil. Purity 95% (UPLC/MS). $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$ 7.83–7.90 (m, 2H), 7.61 (d, $J$ = 8.2 Hz, 1H), 7.32–7.56 (m, 5H), 7.16–7.28 (m, 4H), 6.78 (d, $J$ = 3.5 Hz, 1H), 6.65 (d, $J$ = 7.6 Hz, 1H), 4.18 (t, $J$ = 5.3 Hz, 2H), 3.86 (s, 2H), 3.05 (t, $J$ = 5.3 Hz, 2H), 1.78–2.01 (m, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.2, 142.2, 138.2, 136.2, 134.3, 133.8, 129.7, 129.2, 128.1, 127.2, 126.7, 126.1, 125.7, 124.8, 121.2, 106.7, 106.2, 104.5, 67.7, 53.0, 47.9. Formula: $C_{23}H_{21}ClN_2O_3S$; MS: *m/z* 441 ($M + H^+$).

*4.1.17.5. N-(4-Chlorobenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl) oxy)ethan-1-amine (32).* Following GP7, compound 32 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (70 mg, 0.22 mmol, 1.3 equiv), 4-chlorobenzaldehyde (24 μL, 0.17 mmol), glacial acetic acid (20 μL), $NaBH_3CN$ (41 mg, 0.66 mmol, 3.9 equiv) in 3 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 30 mg (40%), yellowish oil. Purity 98% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.90 (m, 2H), 7.61 (d, $J$ = 8.2 Hz, 1H), 7.38–7.56 (m, 4H), 7.25–7.31 (m, 4H), 7.21 (t, $J$ = 8.2 Hz, 1H), 6.77 (d, $J$ = 3.5 Hz, 1H), 6.64 (d, $J$ = 8.2 Hz, 1H), 4.17 (t, $J$ = 5.0 Hz, 2H), 3.85 (s, 2H), 3.04 (t, $J$ = 5.3 Hz, 2H), 2.10 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.2, 138.4, 138.2, 136.2, 133.8, 132.7, 129.4, 129.2, 128.5, 126.7, 125.7, 124.8, 121.2, 106.7, 106.2, 104.5, 67.6, 52.9, 47.9. Formula: $C_{23}H_{21}ClN_2O_3S$; MS: *m/z* 441 ($M + H^+$).

*4.1.17.6. N-(3,5-Dichlorobenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (33).* Following GP7, compound 33 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (90 mg, 0.28 mmol, 1.3 equiv), 3,5-dichlorobenzaldehyde (38 mg, 0.22 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (54 mg, 0.86 mmol, 3.9 equiv) in 3 mL MeOH. Purification: flash chromatography (0–7% MeOH in DCM). Yield: 55 mg (56%), colourless oil. Purity 95% (UPLC/MS). $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$ 7.83–7.91 (m, 2H), 7.61 (d, $J$ = 8.2 Hz, 1H), 7.38–7.56 (m, 4H), 7.17–7.29 (m, 4H), 6.78 (d, $J$ = 3.5 Hz, 1H), 6.65 (d, $J$ = 7.6 Hz, 1H), 4.17 (t, $J$ = 5.3 Hz, 2H), 3.84 (s, 2H), 3.03 (t, $J$ = 5.3 Hz, 2H), 1.65 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.2, 143.8, 138.2, 136.2, 134.9, 133.8, 129.2, 127.1, 126.7, 126.4, 125.7, 124.9, 121.2, 106.8, 106.2, 104.5, 67.6, 52.6, 48.0. Formula: $C_{23}H_{20}Cl_2N_2O_3S$; MS: *m/z* 476 ($M + H^+$).

*4.1.17.7. 2-((1-(Phenylsulfonyl)-1H-indol-4-yl)oxy)-N-(3-(trifluoromethyl)benzyl)ethan-1-amine (34).* Following GP7, compound 34 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy) ethan-1-amine (14) (90 mg, 0.28 mmol, 1 equiv), 3-(trifluoromethyl)benzaldehyde (37.5 μL, 0.28 mmol, 1 equiv), glacial acetic acid (10 μL), $NaBH_4$ (27 mg, 0.7 mmol, 2.5 equiv) in 3 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 37 mg (28%), yellowish oil. Purity 96% (UPLC/MS). $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$ 7.83–7.90 (m, 2H), 7.37–7.65 (m, 9H), 7.22 (t, $J$ = 8.2 Hz, 1H), 6.77 (d, $J$ = 2.9 Hz, 1H), 6.65 (d, $J$ = 7.6 Hz, 1H), 4.19 (t, $J$ = 5.3 Hz, 2H), 3.94 (s, 2H), 3.06 (t, $J$ = 5.3 Hz, 2H), 1.80 (s, 1H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta$ 152.2, 141.2, 138.2, 136.2, 133.8, 131.4, 130.9, 130.5, 129.2, 128.8, 126.7, 125.7, 124.8, 124.7 (m), 123.9 (dd, $J$ = 8.1, 3.5 Hz), 121.2, 106.7, 106.2, 104.5, 67.7, 53.1, 48.1. Formula: $C_{24}H_{21}F_3N_2O_3S$; MS: *m/z* 475 ($M + H^+$).

*4.1.17.8. N-(4-Fluorobenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl) oxy)ethan-1-amine (35).* Following GP7, compound 35 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (58 mg, 0.18 mmol, 1.3 equiv), 4-fluorobenzaldehyde (15 μL, 0.14 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (26 mg, 0.42 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (3% MeOH in DCM). Yield: 44 mg (74%), colourless oil. Purity 97% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83–7.90 (m, 2H), 7.61 (d, $J$ = 8.8 Hz, 1H), 7.38–7.56 (m, 4H), 7.25–7.33 (m, 2H), 7.21 (t, $J$ = 8.2 Hz, 1H), 6.94–7.04 (m, 2H), 6.76 (d, $J$ = 2.9 Hz, 1H), 6.64 (d, $J$ = 7.6 Hz, 1H), 4.18 (t, $J$ = 5.0 Hz, 2H), 3.84 (s, 2H), 3.05 (t, $J$ = 5.3 Hz, 2H), 1.79 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ ppm 160.31, 152.27, 138.17, 136.17, 135.79 (d, $J$ = 3.5 Hz), 133.80, 129.60 (d, $J$ = 8.1 Hz), 129.24, 126.74, 125.68, 124.81, 121.20, 115.34, 115.06, 106.47 (d, $J$ = 32.3 Hz), 104.53, 67.68, 52.93, 47.96. Formula: $C_{23}H_{21}FN_2O_3S$.

*4.1.17.9. N-(4-Nitrobenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl) oxy)ethan-1-amine (36).* Following GP7, compound 36 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (58 mg, 0.18 mmol, 1.3 equiv), 4-nitrobenzaldehyde (21 mg, 0.14 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (26 mg, 0.42 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (3% MeOH in DCM). Yield: 40 mg (63%), orange oil. Purity 96% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.13–8.21 (m, 2H), 7.83–7.90 (m, 2H), 7.61 (d, $J$ = 8.2 Hz, 1H), 7.38–7.57 (m, 6H), 7.22 (t, $J$ = 8.2 Hz, 1H), 6.76 (d, $J$ = 4.7 Hz, 1H), 6.65 (d, $J$ = 8.2 Hz, 1H), 4.19 (t, $J$ = 5.0 Hz, 2H), 3.99 (s, 2H), 3.06 (t, $J$ = 5.0 Hz, 2H), 1.82 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.2, 148.0, 138.1, 136.2, 133.8, 129.2, 128.6, 126.9, 126.8, 125.7, 124.9, 123.7, 123.6, 121.2, 106.8, 106.1, 104.5, 67.6, 52.9, 48.1. Formula: $C_{23}H_{21}N_3O_5S$.

*4.1.17.10. N,N-Dimethyl-4-(((2-((1-(phenylsulfonyl)-1H-indol-4-yl) oxy)ethyl)amino)-methyl)aniline (38).* Following GP7, compound 38 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy) ethan-1-amine (14) (57 mg, 0.18 mmol, 1 equiv), 4-(dimethylamino)benzaldehyde (27 mg, 0.18 mmol, 1 equiv), glacial acetic acid (15 μL), $NaBH_3CN$ (16 mg, 0.25 mmol, 1.4 equiv) in 3 mL of MeOH and THF mixture (1:1). Purification: flash chromatography (2–10% MeOH in DCM). Yield: 27 mg (33%), pale-orange oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.91 (m, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.37–7.55 (m, 4H), 7.15–7.25 (m, 3H), 6.78 (d, $J$ = 3.5 Hz, 1H), 6.71 (d, $J$ = 8.8 Hz, 2H), 6.64 (d, $J$ = 7.6 Hz, 1H), 4.17 (t, $J$ = 5.3 Hz, 2H), 3.79 (s, 2H), 3.05 (t, $J$ = 5.3 Hz, 2H), 2.93 (s, 6H), 2.04 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.4, 149.9, 138.2, 136.2, 133.8, 129.2, 129.1, 127.8, 126.7, 125.7, 124.8, 121.3, 112.7, 106.6, 106.4, 104.6, 67.7, 53.2, 47.8, 40.7. Formula: $C_{25}H_{27}N_3O_3S$; MS: *m/z* 450 ($M + H^+$).

*4.1.17.11. N-(3-Methoxybenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (39).* Following GP7, compound 39 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (80 mg, 0.25 mmol, 1.3 equiv), 3-methoxybenzaldehyde (23 μL, 0.19 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (36 mg, 0.58 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 38 mg (45%), yellow oil. Purity 97% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.87 (d, $J$ = 7.0 Hz, 2H), 7.61 (d, $J$ = 8.2 Hz, 1H), 7.38–7.55 (m, 4H), 7.18–7.28 (m, 2H), 6.89–6.95 (m, 2H), 6.75–6.83 (m, 2H), 6.65 (d, $J$ = 7.6 Hz, 1H), 4.18 (t, $J$ = 5.3 Hz, 2H), 3.86 (s, 2H), 3.79 (s, 3H), 3.06 (t, $J$ = 5.3 Hz, 2H), NH signal not detected. $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 159.8, 152.3, 141.8, 138.2, 136.2, 133.8, 129.4, 129.2, 126.7, 125.7, 124.8, 121.2, 120.3, 113.5, 112.5, 106.6, 106.3, 104.5, 67.7, 55.2, 53.6, 48.0. Formula: $C_{24}H_{24}N_2O_4S$; MS: *m/z* 437 ($M + H^+$).

*4.1.17.12. N-(4-Methoxybenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (40).* Following GP7, compound 40 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (70 mg, 0.22 mmol, 1.3 equiv), 4-methoxybenzaldehyde (20 μL, 0.17 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (41 mg, 0.66 mmol, 3.9 equiv) in 3 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 51 mg (69%), yellow oil. Purity 98% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.90 (m, 2H), 7.59 (d, $J$ = 8.2 Hz, 1H), 7.38–7.55 (m, 4H), 7.15–7.31 (m, 3H), 6.82–6.90 (m, 2H), 6.79 (d, $J$ = 3.5 Hz, 1H), 6.63 (d, $J$ = 7.6 Hz, 1H), 4.18 (t, $J$ = 5.3 Hz, 2H), 3.83 (s, 2H), 3.79 (s, 3H), 3.05 (t, $J$ = 5.3 Hz, 2H), –NH– signal not detected. $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 175.2, 158.9, 152.2, 138.2, 136.2, 133.8, 129.5, 129.2, 126.7, 125.6, 124.8, 121.2, 113.9, 106.7, 106.3, 104.6, 67.3, 55.3, 52.8, 47.6. Formula: $C_{24}H_{24}N_2O_4S$; MS: *m/z* 437 ($M + H^+$).

*4.1.17.13. N-(3,4-Dimethoxybenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (41).* Following GP7, compound 41 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (80 mg, 0.25 mmol, 1.3 equiv), 3,4-dimethoxybenzaldehyde (32 mg, 0.19 mmol, 1 equiv), glacial acetic acid (30 μL), $NaBH_3CN$ (36 mg, 0.58 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 53 mg (60%), yellowish oil. Purity 97% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83–7.89 (m, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.37–7.54 (m, 4H), 7.21 (t, $J$ = 8.2 Hz, 1H), 6.79–6.93 (m, 3H), 6.76 (d, $J$ = 3.5 Hz, 1H), 6.64 (d, $J$ = 8.2 Hz, 1H), 4.18 (t, $J$ = 5.0 Hz, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.82 (s, 2H), 3.06 (t, $J$ = 5.0 Hz, 2H), 2.18 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.3, 149.0, 148.1, 138.2, 136.2, 133.8, 132.6, 129.2, 126.7, 125.7, 124.8, 121.2, 120.2, 111.4, 111.1, 106.7, 106.3, 104.6, 67.6, 55.9, 55.8, 53.4, 47.9. Formula: $C_{25}H_{26}N_2O_5S$; MS: *m/z* 467 ($M + H^+$).

*4.1.17.14. N-((2,3-Dihydrobenzo[b] [1,4]dioxin-6-yl)methyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (42).* Following GP7, compound 42 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (57 mg, 0.18 mmol, 1 equiv), 2,3-dihydrobenzo[*b*] [1,4]dioxine-6-carbaldehyde (29 mg, 0.18 mmol, 1 equiv), glacial acetic acid (15 μL), $NaBH_3CN$ (34 mg, 0.54 mmol, 3 equiv) in 3 mL of MeOH and THF mixture (1:1). Purification: flash chromatography (3–5% MeOH in DCM). Yield: 37 mg (44%), yellowish oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.90 (m, 2H), 7.59 (d, $J$ = 8.2 Hz, 1H), 7.37–7.56 (m, 4H), 7.20 (t, $J$ = 8.2 Hz, 1H), 6.75–6.88 (m, 4H), 6.64 (d, $J$ = 8.2 Hz, 1H), 4.24 (s, 4H), 4.16 (t, $J$ = 5.0 Hz, 2H), 3.77 (s, 2H), 3.04 (t, $J$ = 5.3 Hz, 2H), 1.86 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.4, 143.5, 142.6, 138.3, 136.3, 133.9, 133.6, 129.3, 126.8, 125.8, 124.9, 121.3, 121.1, 117.2, 117.0, 106.7, 106.5, 104.6, 67.9, 64.5, 64.4, 53.2, 48.0. Formula: $C_{25}H_{24}N_2O_5S$; MS: *m/z* 465 ($M + H^+$).

*4.1.17.15. N-(3-Ethoxybenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (43).* Following GP7, compound 43 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (80 mg, 0.25 mmol, 1.3 equiv), 3-ethoxybenzaldehyde (27 μL, 0.19 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (36 mg, 0.58 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 32 mg (37%), yellowish oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83–7.90 (m, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.38–7.56 (m, 4H), 7.17–7.27 (m, 2H), 6.87–6.94 (m, 2H), 6.75–6.82 (m, 2H), 6.65 (d, $J$ = 7.6 Hz, 1H), 4.17 (t, $J$ = 5.3 Hz, 2H), 4.02 (q, $J$ = 6.6 Hz, 2H), 3.85 (s, 2H), 3.06 (t, $J$ = 5.3 Hz, 2H), 1.80 (br. s., 1H), 1.40 (t, $J$ = 6.7 Hz, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 159.2, 152.3, 141.7, 138.2, 136.2, 133.8, 129.4, 129.2, 126.7, 125.7, 124.8, 121.2, 120.2, 114.1, 113.0, 106.6, 106.4, 104.5, 67.7, 63.3, 53.6, 48.0, 14.9. Formula: $C_{25}H_{26}N_2O_4S$; MS: *m/z* 451($M + H^+$).

*4.1.17.16. N-(4-Ethoxybenzyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (44).* Following GP7, compound 44 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (100 mg, 0.32 mmol, 1.3 equiv), 4-ethoxybenzoate (33 μL, 0.24 mmol, 1 equiv), glacial acetic acid (20 μL), $NaBH_3CN$ (45 mg, 0.72 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 40 mg (37%), colourless oil. Purity 98% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.91 (br. s., 2H), 8.33 (br. s., 1H), 7.76–7.86 (m, 2H), 7.57 (d, $J$ = 8.2 Hz, 1H), 7.41–7.46 (m, 2H), 7.31–7.39 (m, 2H), 7.22 (d, $J$ = 8.2 Hz, 2H), 7.12 (t, $J$ = 7.9 Hz, 1H), 6.74–6.82 (m, 3H), 6.44 (d, $J$ = 7.6 Hz, 1H), 4.10 (br. s., 2H), 3.85–3.96 (m, 4H), 3.10 (br. s., 2H), 1.34 (t, $J$ = 7.0 Hz, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.5, 159.5, 151.2, 138.0, 136.1, 133.8, 131.2, 129.2, 126.7, 125.6, 125.0, 123.4, 121.1, 114.8, 107.2, 106.3, 104.4, 63.7, 63.4, 50.5, 44.8, 14.7. Formula: $C_{25}H_{26}N_2O_4S$; MS: *m/z* 451 ($M + H^+$).

*4.1.17.17. 3-(((2-((1-(Phenylsulfonyl)-1H-indol-4-yl)oxy)ethyl)amino)methyl)phenyl acetate (45).* Following GP7, compound 45 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (80 mg, 0.25 mmol, 1.3 equiv), 3-acetoxybenzaldehyde (31 mg, 0.19 mmol, 1 equiv), glacial acetic acid (30 μL), $NaBH_3CN$ (36 mg, 0.58 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 34 mg (40%), colourless oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.90 (m, 2H), 7.60 (d, $J$ = 8.8 Hz, 1H), 7.37–7.54 (m, 4H), 7.28–7.35 (m, 1H), 7.15–7.27 (m, 2H), 7.10 (d, $J$ = 1.8 Hz, 1H), 6.94–7.01 (m, 1H), 6.75–6.81 (m, 1H), 6.64 (d, $J$ = 8.2 Hz, 1H), 4.17 (t, $J$ = 5.3 Hz, 2H), 3.88 (s, 2H), 3.05 (t, $J$ = 5.0 Hz, 2H), 2.28 (s, 3H), 2.06 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 169.5, 152.3, 150.8, 141.9, 138.2, 136.2, 133.8, 129.4, 129.2, 126.7, 125.7, 125.4, 124.8, 121.2, 121.1, 120.2, 106.7, 106.3, 104.6, 67.7, 53.1, 48.0, 21.1. Formula: $C_{25}H_{24}N_2O_5S$; MS: *m/z* 465 ($M + H^+$).

*4.1.17.18. 4-(((2-((1-(Phenylsulfonyl)-1H-indol-4-yl)oxy)ethyl)amino)methyl)phenyl acetate (46).* Following GP7, compound 46 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (80 mg, 0.25 mmol, 1.3 equiv), 4-acetoxybenzaldehyde (27 mg, 0.19 mmol, 1 equiv), glacial acetic acid (30 μL), $NaBH_3CN$ (36 mg, 0.58 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 40 mg (45%), colourless oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.90 (m, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.31–7.53 (m, 6H), 7.17–7.25 (m, 1H), 7.00–7.08 (m, 2H), 6.75–6.79 (m, 1H), 6.60–6.67 (m, 1H), 4.17 (t, $J$ = 5.0 Hz, 2H), 3.86 (s, 2H), 3.05 (t, $J$ = 5.3 Hz, 2H), 2.29 (s, 3H), 1.84 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 169.3, 167.3, 151.4, 150.8, 138.1, 136.1, 133.8, 131.2, 130.5, 129.2, 126.7, 125.6, 125.1, 122.1, 121.1, 107.2, 106.2, 104.5, 64.6, 50.9, 45.8, 21.1. Formula: $C_{25}H_{24}N_2O_5S$; MS: *m/z* 465 ($M + H^+$).

*4.1.17.19. Methyl 4-(((2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethyl)amino)methyl)-benzoate (47).* Following GP7, compound 47 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (80 mg, 0.25 mmol, 1.3 equiv), methyl 4-formylbenzoate (31 mg, 0.19 mmol, 1 equiv), glacial acetic acid (30 μL), $NaBH_3CN$ (36 mg, 0.58 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (0–10% MeOH in DCM). Yield: 30 mg (34%), yellowish oil. Purity 96% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.99 (d, $J$ = 8.2 Hz, 2H), 7.86 (d, $J$ = 7.0 Hz, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.45–7.51 (m, 2H), 7.37–7.44 (m, 4H), 7.21 (t, $J$ = 8.2 Hz, 1H), 6.76 (d, $J$ = 2.9 Hz, 1H), 6.64 (d, $J$ = 8.2 Hz, 1H), 4.17 (t, $J$ = 5.3 Hz, 2H), 3.92 (s, 2H), 3.90 (s, 3H), 3.05 (t, $J$ = 5.0 Hz, 2H), 2.01 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.0, 152.3, 145.5, 136.2,

133.8, 129.8, 129.2, 128.9, 127.9, 126.7, 126.4, 125.7, 124.8, 121.2, 106.7, 106.2, 104.6, 67.7, 53.3, 52.0, 48.1. Formula: $C_{25}H_{24}N_2O_5S$; MS: *m/z* 465 ($M + H^+$).

*4.1.17.20. N-(4-(((2-((1-(Phenylsulfonyl)-1H-indol-4-yl)oxy)ethyl) amino)methyl)phenyl)-acetamide (48).* Following GP7, compound 48 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy) ethan-1-amine (14) (57 mg, 0.18 mmol, 1 equiv), 4-acetamidobenzaldehyde (29 mg, 0.18 mmol, 1 equiv), glacial acetic acid (15 μL), $NaBH_3CN$ (16 mg, 0.25 mmol, 1.4 equiv) in 3 mL of MeOH and THF mixture (1:1). Purification: flash chromatography (0–5% MeOH in DCM). Yield: 41 mg (49%), yellowish oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.88 (m, 2H), 7.58 (d, $J$ = 8.2 Hz, 1H), 7.36–7.55 (m, 7H), 7.23–7.29 (m, 2H), 7.19 (t, $J$ = 8.2 Hz, 1H), 6.75 (d, $J$ = 3.5 Hz, 1H), 6.63 (d, $J$ = 7.6 Hz, 1H), 4.16 (t, $J$ = 5.3 Hz, 2H), 3.81 (s, 2H), 3.02 (t, $J$ = 5.3 Hz, 2H), 2.14 (s, 3H), 1.96 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 168.4, 152.3, 138.1, 136.9, 136.2, 135.9, 133.8, 129.2, 128.7, 126.7, 125.7, 124.8, 121.2, 120.0, 106.6, 106.3, 104.6, 67.7, 53.1, 47.9, 24.5. Formula: $C_{25}H_{25}N_3O_4S$; MS: *m/z* 464 ($M + H^+$).

*4.1.17.21. N-(Cyclohexylmethyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (50).* Following GP7, compound 50 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (57 mg, 0.18 mmol, 1.3 equiv), cyclohexanecarboxaldehyde (17 μL, 0.14 mmol, 1 equiv), glacial acetic acid (3.6 μL), $NaBH_3CN$ (34 mg, 0.54 mmol, 3.9 equiv) in 2 mL MeOH. Purification: flash chromatography (5% MeOH in DCM). Yield: 38 mg (51%), colourless oil. Purity 98% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.85 (d, $J$ = 8.0 Hz, 2H), 7.59 (d, $J$ = 8.0 Hz, 1H), 7.48–7.53 (m, 1H), 7.46 (d, $J$ = 4.0 Hz, 1H), 7.41 (t, $J$ = 8.0 Hz, 2H), 7.20 (t, $J$ = 8.0 Hz, 1H), 6.76 (d, $J$ = 3.4 Hz, 1H), 6.64 (d, $J$ = 8.0 Hz, 1H), 4.15 (t, $J$ = 5.2 Hz, 2H), 3.01 (t, $J$ = 5.2 Hz, 2H), 2.51 (d, $J$ = 6.3 Hz, 2H), 1.82 (br. s., 1H), 1.61–1.77 (m, 5H), 1.41–1.51 (m, 1H), 1.08–1.27 (m, 3H), 0.84–0.95 (m, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.3, 138.2, 136.1, 133.7, 129.2, 126.7, 125.6, 124.7, 121.2, 106.6, 106.3, 104.5, 67.5, 56.5, 48.7, 37.8, 31.3, 26.6, 26.0. Formula: $C_{23}H_{28}N_2O_3S$; MS: *m/z* 413 ($M + H^+$).

*4.1.17.22. N-(Cyclopentylmethyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (51).* Following GP7, compound 51 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (57 mg, 0.18 mmol, 1.3 equiv), cyclopentanecarboaldehyde (15 μL, 0.14 mmol, 1 equiv), glacial acetic acid (3.6 μL), $NaBH_3CN$ (26 mg, 0.42 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (5% MeOH in DCM). Yield: 33 mg (59%), colourless oil. Purity 100% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.84 (d, $J$ = 7.5 Hz, 2H), 7.59 (d, $J$ = 8.0 Hz, 1H), 7.44–7.52 (m, 2H), 7.36–7.43 (m, 2H), 7.19 (t, $J$ = 8.3 Hz, 1H), 6.79 (d, $J$ = 3.4 Hz, 1H), 6.61 (d, $J$ = 8.0 Hz, 1H), 4.17 (t, $J$ = 5.2 Hz, 2H), 3.51 (br. s., 1H), 3.08 (t, $J$ = 5.4 Hz, 2H), 2.67 (d, $J$ = 6.9 Hz, 2H), 2.06 (spt, $J$ = 7.7 Hz, 1H), 1.77 (dt, $J$ = 6.9, 11.7 Hz, 2H), 1.44–1.61 (m, 4H), 1.07–1.20 (m, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.2, 138.2, 136.3, 133.9, 129.3, 126.8, 125.8, 125.0, 121.3, 106.9, 106.4, 104.7, 66.7, 55.1, 48.5, 39.4, 30.9, 25.3. Formula: $C_{22}H_{26}N_2O_3S$; MS: *m/z* 399 ($M + H^+$).

*4.1.17.23. N-(Cyclopropylmethyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (52).* Following GP7, compound 52 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (57 mg, 0.18 mmol, 1.3 equiv), cyclopropanecarboxaldehyde (10.6 μL, 0.14 mmol, 1 equiv), glacial acetic acid (3.6 μL), $NaBH_3CN$ (26 mg, 0.42 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (7% MeOH in DCM). Yield: 21 mg (40%), yellow oil. Purity 100% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.84 (d, $J$ = 7.5 Hz, 2H), 7.59 (d, $J$ = 8.6 Hz, 1H), 7.44–7.52 (m, 2H), 7.36–7.43 (m, 2H), 7.14–7.22 (m, 1H), 6.83 (d, $J$ = 3.4 Hz, 1H), 6.61 (d, $J$ = 8.0 Hz, 1H), 4.18 (t, $J$ = 5.2 Hz, 2H), 3.50 (br. s., 1H), 3.11 (t, $J$ = 5.2 Hz, 2H), 2.60 (d, $J$ = 6.9 Hz, 2H), 0.98 (dtd, $J$ = 2.9, 7.4, 12.1 Hz, 1H), 0.42–0.52 (m, 2H), 0.13 (q, $J$ = 4.8 Hz, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.1, 138.2, 136.3, 133.9, 129.3, 126.8, 125.8, 124.9, 121.3, 106.9, 106.5, 104.6, 66.8, 54.4, 47.9, 10.4, 3.7. Formula: $C_{20}H_{22}N_2O_3S$; MS: *m/z* 371 ($M + H^+$).

*4.1.17.24. Tert-butyl 3-(((2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy) ethyl)amino)methyl)piperidine-1-carboxylate (54).* Following GP7, compound 54 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (57 mg, 0.18 mmol, 1 equiv), *tert*-butyl 4-formylpiperidine-1-carboxylate (38 mg, 0.18 mmol, 1 equiv), glacial acetic acid (10 μL), $NaBH_3CN$ (34 mg, 0.54 mmol, 3 equiv) in 3 mL of MeOH and THF mixture (1:1). Purification: flash chromatography (4–10% MeOH in DCM). Yield: 27 mg (29%), dark yellow oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83–7.89 (m, 2H), 7.59 (d, $J$ = 8.2 Hz, 1H), 7.36–7.56 (m, 4H), 7.21 (t, $J$ = 8.2 Hz, 1H), 6.80 (d, $J$ = 3.5 Hz, 1H), 6.64 (d, $J$ = 7.6 Hz, 1H), 4.15 (t, $J$ = 5.3 Hz, 2H), 3.73–3.97 (m, 2H), 3.02 (t, $J$ = 5.3 Hz, 2H), 2.78–2.95 (m, 1H), 2.48–2.71 (m, 3H), 1.56–1.91 (m, 5H), 1.43 (s, 9H), 1.07–1.30 (m, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.1, 152.4, 138.3, 136.3, 133.9, 129.3, 126.8, 125.8, 124.9, 121.3, 106.7, 104.6, 79.4, 67.6, 52.7, 48.8, 47.5, 45.2, 44.3, 36.0, 29.0, 28.5, 24.5. Formula: $C_{27}H_{35}N_3O_5S$. HRMS: *m/z* found [$M + H^+$] 514.2440, $C_{27}H_{35}N_3O_5S$ requires 514.2375.

*4.1.17.25. Tert-butyl 4-(((2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy) ethyl)amino)methyl)piperidine-1-carboxylate (55).* Following GP7, compound 55 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (57 mg, 0.18 mmol, 1 equiv), *tert*-butyl 4-formylpiperidine-1-carboxylate (38 mg, 0.18 mmol, 1 equiv), glacial acetic acid (10 μL), $NaBH_3CN$ (34 mg, 0.54 mmol, 3 equiv) in 3 mL of MeOH and THF mixture (1:1). Purification: flash chromatography (4% MeOH in DCM). Yield: 50 mg (54%), yellow oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.90 (m, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.37–7.55 (m, 4H), 7.20 (t, $J$ = 8.2 Hz, 1H), 6.73–6.78 (m, 1H), 6.64 (d, $J$ = 8.2 Hz, 1H), 4.01–4.19 (m, 4H), 3.03 (t, $J$ = 5.3 Hz, 2H), 2.67 (t, $J$ = 12.6 Hz, 2H), 2.57 (d, $J$ = 6.5 Hz, 2H), 1.97 (br. s., 1H), 1.54–1.76 (m, 3H), 1.43–1.47 (m, 9H), 1.00–1.19 (m, 2H). Formula: $C_{27}H_{35}N_3O_5S$; MS: *m/z* 514 ($M + H^+$).

*4.1.17.26. 2-((1-(Phenylsulfonyl)-1H-indol-4-yl)oxy)-N-(pyridin-2-ylmethyl)ethan-1-amine (57).* Following GP7, compound 57 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (57 mg, 0.18 mmol, 1 equiv), 2-pyridinecarboxyaldehyde (17 μL, 0.18 mmol, 1 equiv), $NaBH_4$ (14 mg, 0.36 mmol, 2 equiv) in 3 mL of anhydrous MeOH. Purification: flash chromatography (6% MeOH in DCM). Yield: 37 mg (50%), orange oil. Purity 95% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.52–8.55 (m, 1H), 7.83–7.88 (m, 2H), 7.63 (dt, $J$ = 2.0, 7.6 Hz, 1H), 7.59 (d, $J$ = 8.6 Hz, 1H), 7.49–7.54 (m, 1H), 7.47 (d, $J$ = 3.4 Hz, 1H), 7.40–7.44 (m, 2H), 7.32 (d, $J$ = 7.5 Hz, 1H), 7.20 (t, $J$ = 8.0 Hz, 1H), 7.14–7.17 (m, 1H), 6.80–6.83 (m, 1H), 6.63 (d, $J$ = 8.0 Hz, 1H), 4.22 (t, $J$ = 5.4 Hz, 2H), 4.03 (s, 2H), 3.14 (t, $J$ = 5.2 Hz, 2H), 2.93 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 158.8, 152.3, 149.4, 138.3, 136.7, 136.3, 133.9, 129.3, 126.8, 125.8, 124.9, 122.4, 122.3, 121.4, 106.8, 106.5, 104.6, 67.5, 54.8, 48.3. Formula: $C_{22}H_{21}N_3O_3S$; MS: *m/z* 408 ($M + H^+$).

*4.1.17.27. 2-((1-(Phenylsulfonyl)-1H-indol-4-yl)oxy)-N-(pyridin-4-ylmethyl)ethan-1-amine (58).* Following GP7, compound 58 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (14) (57 mg, 0.18 mmol, 1 equiv), isonicotinaldehyde

(17 μL, 0.18 mmol, 1 equiv), glacial acetic acid (15 μL), $NaBH_3CN$ (16 mg, 0.25 mmol, 1.4 equiv) in 3 mL of MeOH and THF mixture (1:1). Purification: flash chromatography (5–10% MeOH in DCM). Yield: 23 mg (31%), yellow oil. Purity 96% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.50–8.55 (m, 2H), 7.83–7.90 (m, 2H), 7.61 (d, $J$ = 8.2 Hz, 1H), 7.37–7.56 (m, 4H), 7.25–7.30 (m, 2H), 7.21 (t, $J$ = 8.2 Hz, 1H), 6.74–6.78 (m, 1H), 6.65 (d, $J$ = 7.6 Hz, 1H), 4.18 (t, $J$ = 5.3 Hz, 2H), 3.89 (s, 2H), 3.05 (t, $J$ = 5.0 Hz, 2H), 1.91 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.2, 149.8, 149.2, 138.2, 136.2, 133.8, 129.2, 126.7, 125.6, 124.8, 122.8, 121.2, 106.7, 106.1, 104.5, 67.6, 52.3, 48.1. Formula: $C_{22}H_{21}N_3O_3S$; MS: *m/z* 408 ($M + H^+$).

*4.1.17.28. N-((5-Methylfuran-2-yl)methyl)-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (59).* Following GP7, compound 59 was prepared using 2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy) ethan-1-amine (14) (57 mg, 0.18 mmol, 1.3 equiv), 5-methyl-2-furalaldehyde (14 μL, 0.14 mmol, 1 equiv), glacial acetic acid (3.6 μL), $NaBH_3CN$ (26 mg, 0.42 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (3% MeOH in DCM). Yield: 25 mg (44%), yellow oil. Purity 100% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.85 (d, $J$ = 8.0 Hz, 2H), 7.59 (d, $J$ = 8.0 Hz, 1H), 7.48–7.53 (m, 1H), 7.46 (d, $J$ = 4.0 Hz, 1H), 7.37–7.43 (m, 2H), 7.19 (t, $J$ = 8.0 Hz, 1H), 6.78 (d, $J$ = 4.0 Hz, 1H), 6.62 (d, $J$ = 8.0 Hz, 1H), 6.06 (d, $J$ = 2.9 Hz, 1H), 5.86 (d, $J$ = 1.7 Hz, 1H), 4.15 (t, $J$ = 5.2 Hz, 2H), 3.80 (s, 2H), 3.05 (t, $J$ = 5.2 Hz, 2H), 2.23 (s, 3H), 2.09 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.4, 151.8, 151.6, 138.3, 136.3, 133.9, 129.3, 126.8, 125.8, 124.9, 121.3, 108.1, 106.8, 106.5, 106.1, 104.7, 67.7, 47.8, 46.3, 13.7. Formula: $C_{22}H_{22}N_2O_4S$; MS: *m/z* 411 ($M + H^+$).

*4.1.17.29. N-Benzyl-2-((1-(phenylsulfonyl)indolin-4-yl)oxy)ethan-1-amine (60).* Following GP7, compound 60 was prepared using 2-((1-(phenylsulfonyl)indolin-4-yl)oxy)ethan-1-amine (21) (57 mg, 0.18 mmol, 1 equiv), benzaldehyde (18 μL, 0.18 mmol, 1 equiv), glacial acetic acid (3.6 μL), $NaBH_3CN$ (34 mg, 0.54 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (4% MeOH in DCM). Yield: 22 mg (30%), colourless oil. Purity 98% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.77–7.81 (m, 2H), 7.50–7.55 (m, 1H), 7.40–7.45 (m, 2H), 7.27–7.31 (m, 5H), 7.22–7.26 (m, 1H), 7.11–7.16 (m, 1H), 6.50 (d, $J$ = 8.0 Hz, 1H), 4.05 (t, $J$ = 5.2 Hz, 2H), 3.91 (t, $J$ = 8.6 Hz, 2H), 3.83 (s, 2H), 2.96 (t, $J$ = 5.2 Hz, 2H), 2.82 (t, $J$ = 8.6 Hz, 2H), 1.90 (br. s, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.4, 143.4, 140.0, 137.0, 133.2, 129.3, 129.1, 128.6, 128.2, 127.4, 127.2, 119.2, 108.0, 107.0, 67.6, 53.8, 50.3, 48.0, 25.0. Formula: $C_{23}H_{24}N_2O_3S$; MS: *m/z* 409 ($M + H^+$).

*4.1.17.30. N-(3-Methylbenzyl)-2-((1-(phenylsulfonyl)indolin-4-yl) oxy)ethan-1-amine (61).* Following GP7, compound 61 was prepared using 2-((1-(phenylsulfonyl)indolin-4-yl)oxy)ethan-1-amine (21) (57 mg, 0.18 mmol, 1 equiv), *m*-tolualdehyde (21 μL, 0.18 mmol, 1 equiv), glacial acetic acid (3.6 μL), $NaBH_3CN$ (34 mg, 0.54 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (4% MeOH in DCM). Yield: 43 mg (56%), pale yellow oil. Purity 95% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.78–7.82 (m, 2H), 7.52–7.56 (m, 1H), 7.41–7.46 (m, 2H), 7.30 (d, $J$ = 8.0 Hz, 1H), 7.18–7.22 (m, 1H), 7.05–7.17 (m, 4H), 6.52 (d, $J$ = 8.0 Hz, 1H), 4.06 (t, $J$ = 5.2 Hz, 2H), 3.93 (t, $J$ = 8.3 Hz, 2H), 3.80 (s, 2H), 2.97 (t, $J$ = 5.4 Hz, 2H), 2.83 (t, $J$ = 8.6 Hz, 2H), 2.33 (s, 3H), 1.91 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.3, 143.2, 139.8, 138.1, 136.9, 133.1, 129.1, 129.0, 128.8, 128.3, 127.8, 127.2, 125.0, 119.1, 107.8, 106.9, 67.5, 53.6, 50.2, 48.0, 24.9, 21.3. Formula: $C_{24}H_{26}N_2O_3S$; MS: *m/z* 423 ($M + H^+$).

*4.1.17.31. Tert-butyl 3-(((2-((1-(phenylsulfonyl)indolin-4-yl)oxy) ethyl)amino)methyl)piperidine-1-carboxylate (62).* Following GP7, compound 62 was prepared using 2-((1-(phenylsulfonyl)indolin-4-yl)oxy)ethan-1-amine (21) (57 mg, 0.18 mmol, 1 equiv), *tert*-butyl 3-formylpiperidine-1-carboxylate (38 mg, 0.18 mmol, 1 equiv), glacial acetic acid (3.6 μL), $NaBH_3CN$ (34 mg, 0.54 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (5% MeOH in DCM). Yield: 42 mg (45%), yellowish oil. Purity 97% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.76–7.81 (m, 2H), 7.50–7.55 (m, 1H), 7.39–7.45 (m, 2H), 7.23–7.29 (m, 1H), 7.12 (t, $J$ = 8.3 Hz, 1H), 6.49 (d, $J$ = 8.0 Hz, 1H), 3.99–4.06 (m, 2H), 3.91 (t, $J$ = 8.6 Hz, 2H), 3.85 (d, $J$ = 12.0 Hz, 1H), 3.68–3.79 (m, 1H), 2.90–2.97 (m, 2H), 2.83 (t, $J$ = 8.6 Hz, 2H), 2.59–2.67 (m, 1H), 2.47–2.58 (m, 2H), 1.77–1.83 (m, 1H), 1.68 (br. s., 1H), 1.59 (dd, $J$ = 4.3, 9.5 Hz, 1H), 1.34–1.48 (m, 11H), 1.08–1.19 (m, 1H), 0.67–0.92 (m, 1H). Formula: $C_{27}H_{37}N_3O_5S$; MS: *m/z* 516 ($M + H^+$).

*4.1.17.32. 4-(((2-((1-(phenylsulfonyl)indolin-4-yl)oxy)ethyl)amino) methyl)phenyl acetate (63).* Following GP7, compound 63 was prepared using 2-((1-(phenylsulfonyl)indolin-4-yl)oxy)ethan-1-amine (21) (57 mg, 0.18 mmol, 1 equiv), 4-acetoxybenzaldehyde (25 μL, 0.18 mmol, 1 equiv), glacial acetic acid (3.6 μL), $NaBH_3CN$ (34 mg, 0.54 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (5% MeOH in DCM), preparative HPLC. Yield: 10 mg (12%), white solid. Purity 100% (UPLC/MS). $^1$H NMR – formate salt (500 MHz, $CDCl_3$) $\delta$ 8.20 (br. s., 1H), 7.78 (d, $J$ = 7.5 Hz, 2H), 7.48–7.55 (m, 1H), 7.39–7.45 (m, 2H), 7.35 (d, $J$ = 8.0 Hz, 2H), 7.28 (d, $J$ = 8.0 Hz, 1H), 7.10 (t, $J$ = 8.0 Hz, 1H), 7.03 (d, $J$ = 8.0 Hz, 2H), 6.86 (br. s., 2H), 6.43 (d, $J$ = 8.6 Hz, 1H), 4.08 (t, $J$ = 4.6 Hz, 2H), 3.93 (s, 2H), 3.89 (t, $J$ = 8.3 Hz, 2H), 3.05 (t, $J$ = 4.6 Hz, 2H), 2.80 (t, $J$ = 8.6 Hz, 2H), 2.27 (s, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 169.3, 154.5, 150.7, 143.4, 136.8, 133.2, 132.0, 130.3, 129.3, 129.0, 127.2, 122.0, 119.1, 108.3, 106.7, 64.8, 51.2, 50.2, 46.0, 24.8, 21.1. Formula: $C_{25}H_{26}N_2O_5S$; MS: *m/z* 467 ($M + H^+$).

*4.1.17.33. N-Benzyl-2-((1-(naphthalen-1-ylsulfonyl)-1H-indol-4-yl) oxy)ethan-1-amine (64).* Following GP7, compound 64 was prepared using 2-((1-(naphthalen-1-ylsulfonyl)-1*H*-indol-4-yl)oxy) ethan-1-amine (15) (55 mg, 0.15 mmol, 1 equiv), benzaldehyde (15 μL, 0.15 mmol, 1 equiv), glacial acetic acid (3 μL), $NaBH_3CN$ (28 mg, 0.45 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (4% MeOH in DCM). Yield: 35 mg (51%), yellow oil. Purity 97% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.68–8.71 (m, 1H), 8.07 (dd, $J$ = 1.2, 7.5 Hz, 1H), 8.01 (d, $J$ = 8.6 Hz, 1H), 7.85 (d, $J$ = 8.0 Hz, 1H), 7.69 (d, $J$ = 3.4 Hz, 1H), 7.60–7.64 (m, 1H), 7.53 (dt, $J$ = 1.2, 7.5 Hz, 1H), 7.47 (t, $J$ = 8.3 Hz, 1H), 7.41 (d, $J$ = 8.6 Hz, 1H), 7.29–7.36 (m, 4H), 7.22–7.27 (m, 1H), 7.13 (t, $J$ = 8.3 Hz, 1H), 6.79 (d, $J$ = 3.4 Hz, 1H), 6.59 (d, $J$ = 8.0 Hz, 1H), 4.16 (t, $J$ = 5.2 Hz, 2H), 3.87 (s, 2H), 3.05 (t, $J$ = 5.4 Hz, 2H), 2.08 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.3, 139.9, 136.0, 135.4, 134.2, 133.9, 129.1, 129.0, 128.7, 128.4, 128.1, 128.1, 127.1, 127.0, 125.5, 125.2, 124.0, 123.9, 120.8, 106.4, 105.3, 104.3, 67.6, 53.6, 47.9. Formula: $C_{27}H_{24}N_2O_3S$; MS: *m/z* 457 ($M + H^+$).

*4.1.17.34. N-(3-Methylbenzyl)-2-((1-(naphthalen-1-ylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (65).* Following GP7, compound 65 was prepared using 2-((1-(naphthalen-1-ylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (15) (42 mg, 0.11 mmol, 1 equiv), *m*-tolualdehyde (13 μL, 0.11 mmol, 1 equiv), glacial acetic acid (2.2 μL), $NaBH_3CN$ (21 mg, 0.33 mmol, 3 equiv) in 1.5 mL MeOH. Purification: flash chromatography (4% MeOH in DCM). Yield: 24 mg (46%), yellow oil. Purity 96% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.69 (d, $J$ = 9.2 Hz, 1H), 8.07 (dd, $J$ = 1.2, 7.5 Hz, 1H), 8.01 (d, $J$ = 8.6 Hz, 1H), 7.85 (d, $J$ = 8.6 Hz, 1H), 7.69 (d, $J$ = 3.4 Hz, 1H), 7.60–7.64 (m, 1H), 7.51–7.56 (m, 1H), 7.47 (t, $J$ = 7.7 Hz, 1H), 7.40 (d, $J$ = 8.6 Hz, 1H), 7.18–7.23 (m, 1H), 7.10–7.16 (m, 3H), 7.06 (d, $J$ = 7.5 Hz, 1H), 6.79 (d, $J$ = 3.4 Hz, 1H), 6.59 (d, $J$ = 8.0 Hz, 1H), 4.16 (t, $J$ = 5.2 Hz, 2H), 3.84 (s, 2H), 3.05 (t, $J$ = 5.2 Hz, 2H), 2.32 (s, 3H), 2.16 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.3, 139.7, 138.1, 136.0, 135.4, 134.2, 133.9,

129.1, 129.0, 128.9, 128.7, 128.3, 128.1, 127.8, 127.1, 125.5, 125.2, 125.1, 124.0, 123.9, 120.8, 106.4, 105.3, 104.3, 67.5, 53.6, 47.9, 21.3. Formula: $C_{28}H_{26}N_2O_3S$; MS: *m/z* 471 ($M + H^+$).

*4.1.17.35. Tert-butyl 3-(((2-((1-(naphthalen-1-ylsulfonyl)-1H-indol-4-yl)oxy)ethyl)amino)methyl) piperidine-1-carboxylate (66).* Following GP7, compound 66 was prepared using 2-((1-(naphthalen-1-ylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (15) (55 mg, 0.15 mmol, 1 equiv), *tert*-butyl 3-formylpiperidine-1-carboxylate (32 mg, 0.15 mmol, 1 equiv), glacial acetic acid (3 μL), $NaBH_3CN$ (28 mg, 0.45 mmol, 3 equiv) in 2 mL MeOH. Purification: flash chromatography (5% MeOH in DCM). Yield: 34 mg (40%), yellow oil. Purity 96% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.69 (d, $J$ = 8.6 Hz, 1H), 8.07 (br. s., 1H), 8.01 (d, $J$ = 8.0 Hz, 1H), 7.85 (d, $J$ = 8.0 Hz, 1H), 7.69 (d, $J$ = 4.0 Hz, 1H), 7.62 (ddd, $J$ = 1.4, 7.0, 8.7 Hz, 1H), 7.51–7.56 (m, 1H), 7.48 (t, $J$ = 7.7 Hz, 1H), 7.41 (d, $J$ = 8.6 Hz, 1H), 7.13 (t, $J$ = 8.0 Hz, 1H), 6.87 (br. s., 1H), 6.59 (d, $J$ = 8.0 Hz, 1H), 4.09–4.20 (m, 2H), 3.83 (d, $J$ = 6.3 Hz, 1H), 3.63 (d, $J$ = 9.2 Hz, 1H), 3.05 (br. s., 3H), 2.78 (d, $J$ = 7.5 Hz, 1H), 2.61 (br. s., 2H), 1.74–1.90 (m, 2H), 1.59 (td, $J$ = 1.2, 2.3 Hz, 1H), 1.36–1.49 (m, 11H), 1.11–1.27 (m, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.2, 136.0, 135.4, 134.2, 133.9, 129.1, 129.1, 128.7, 128.1, 127.2, 125.5, 125.2, 124.0, 124.0, 123.9, 120.9, 106.5, 104.3, 53.4, 48.6, 28.4, 28.4, 28.4, 28.3, 28.3, 24.1, 24.1. Formula: $C_{31}H_{37}N_3O_5S$. MS: *m/z* 564 ($M + H^+$).

*4.1.17.36. 4-(((2-((1-(Naphthalen-1-ylsulfonyl)-1H-indol-4-yl)oxy) ethyl)amino)methyl)phenyl acetate (67).* Following GP7, compound 67 was prepared using 2-((1-(naphthalen-1-ylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (15) (40 mg, 0.11 mmol, 1 equiv), 4-acetoxybenzaldehyde (15 μL, 0.11 mmol, 1 equiv), glacial acetic acid (2.2 μL), $NaBH_3CN$ (21 mg, 0.33 mmol, 3 equiv) in 1.5 mL MeOH. Purification: flash chromatography (3–4% MeOH in DCM). Yield: 18 mg (32%), orange oil. Purity 95% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.65–8.71 (m, 1H), 7.97–8.09 (m, 2H), 7.81–7.88 (m, 1H), 7.65–7.72 (m, 1H), 7.58–7.64 (m, 1H), 7.50–7.56 (m, 1H), 7.44–7.50 (m, 1H), 7.38–7.42 (m, 1H), 7.35 (d, $J$ = 8.6 Hz, 2H), 7.08–7.15 (m, 1H), 7.02 (d, $J$ = 8.6 Hz, 2H), 6.75–6.82 (m, 1H), 6.57 (d, $J$ = 8.0 Hz, 1H), 4.14 (t, $J$ = 4.9 Hz, 2H), 3.87 (s, 2H), 3.03 (t, $J$ = 5.2 Hz, 2H), 2.41 (br. s., 1H), 2.28 (s, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 170.3, 169.7, 152.3, 152.1, 149.9, 136.1, 135.5, 129.3, 129.3, 129.2, 128.8, 128.2, 127.3, 125.6, 125.4, 124.1, 124.0, 121.7, 121.0, 106.6, 105.4, 104.4, 67.2, 52.9, 23.4, 21.2. Formula: $C_{29}H_{26}N_2O_5S$; MS: *m/z* 515 ($M + H^+$).

*4.1.17.37. N-Benzyl-2-((1-benzyl-1H-indol-4-yl)oxy)ethan-1-amine (68).* Following GP7, compound 68 was prepared using 2-((1-benzyl-1*H*-indol-4-yl)oxy)ethan-1-amine (16) (213 mg, 0.80 mmol, 1.1 equiv), benzaldehyde (74 μL, 0.73 mmol, 1 equiv), glacial acetic acid (15 μL), $NaBH_3CN$ (138 mg, 2.19 mmol, 3 equiv) in 7 mL of MeOH and THF mixture (1:1). Purification: flash chromatography (0–5% MeOH in DCM). Yield: 86 mg (33%), yellowish oil. Purity 100% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.36–7.39 (m, 2H), 7.32–7.36 (m, 2H), 7.22–7.30 (m, 4H), 7.05–7.11 (m, 3H), 7.03 (d, $J$ = 2.9 Hz, 1H), 6.91 (d, $J$ = 8.0 Hz, 1H), 6.64–6.66 (m, 1H), 6.53 (d, $J$ = 8.0 Hz, 1H), 5.30 (s, 2H), 4.27 (t, $J$ = 5.4 Hz, 2H), 3.92 (s, 2H), 3.12 (t, $J$ = 5.3 Hz, 2H), 2.07 (br. s, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.6, 140.1, 137.9, 137.5, 128.7, 128.4, 128.1, 127.5, 127.0, 126.8, 126.7, 122.5, 119.4, 103.3, 100.5, 99.0, 67.3, 53.6, 50.2, 48.2. Formula: $C_{24}H_{24}N_2O$; MS: *m/z* 357 ($M + H^+$).

*4.1.17.38. 2-((1-Benzyl-1H-indol-4-yl)oxy)-N-(3-methylbenzyl) ethan-1-amine (69).* Following GP7, compound 69 was prepared using 2-((1-benzyl-1*H*-indol-4-yl)oxy)ethan-1-amine (16) (100 mg, 0.37 mmol, 1.1 equiv), *m*-tolualdehyde (40 μL, 0.34 mmol, 1 equiv), glacial acetic acid (7.5 μL), $NaBH_3CN$ (64 mg, 1.02 mmol, 3 equiv) in 3.5 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 46 mg (37%), brownish oil. Purity 100% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.15–7.31 (m, 6H), 7.05–7.10 (m, 4H), 7.03 (d, $J$ = 2.9 Hz, 1H), 6.91 (d, $J$ = 8.0 Hz, 1H), 6.64–6.67 (m, 1H), 6.53 (d, $J$ = 8.0 Hz, 1H), 5.30 (s, 2H), 4.28 (t, $J$ = 5.2 Hz, 2H), 3.89 (s, 2H), 3.12 (t, $J$ = 5.2 Hz, 2H), 2.35 (s, 3H), 2.10 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.6, 139.9, 138.0, 137.8, 137.5, 128.9, 128.7, 128.3, 127.7, 127.5, 126.8, 126.7, 125.2, 122.5, 119.4, 103.3, 100.5, 99.0, 67.3, 53.6, 50.2, 48.2, 21.4. Formula: $C_{25}H_{26}N_2O$; MS: *m/z* 371 ($M + H^+$).

*4.1.17.39. Tert-butyl 3-(((2-((1-benzyl-1H-indol-4-yl)oxy)ethyl) amino)methyl)piperidine-1-carboxylate (70).* Following GP7, compound 70 was prepared using 2-((1-benzyl-1*H*-indol-4-yl)oxy) ethan-1-amine (16) (100 mg, 0.37 mmol, 1.1 equiv), *tert*-butyl 3-formylpiperidine-1-carboxylate (72 mg, 0.33 mmol, 1 equiv), glacial acetic acid (7.5 μL), $NaBH_3CN$ (64 mg, 1.02 mmol, 3 equiv) in 3.5 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 50 mg (33%), colourless oil. Purity 95% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.20–7.31 (m, 3H), 7.01–7.11 (m, 4H), 6.86–6.94 (m, 1H), 6.60–6.71 (m, 1H), 6.45–6.55 (m, 1H), 5.32–5.32 (m, 1H), 5.30–5.34 (m, 2H), 4.23 (t, $J$ = 4.6 Hz, 2H), 3.89–3.98 (m, 1H), 3.04–3.15 (m, 2H), 2.85–2.96 (m, 1H), 2.73–2.82 (m, 1H), 2.52–2.71 (m, 3H), 1.76–1.96 (m, 2H), 1.57–1.75 (m, 2H), 1.36–1.50 (m, 9H), 1.09–1.24 (m, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.1, 152.5, 137.8, 128.9, 127.7, 127.1, 126.7, 122.7, 119.2, 103.5, 100.3, 99.1, 79.6, 67.0, 52.9, 50.3, 49.0, 48.6, 44.1, 36.2, 28.6, 24.5. Formula: $C_{28}H_{37}N_3O_3$. HRMS: *m/z* found [$M + H^+$] 464.2955, $C_{28}H_{37}N_3O_3$ requires 464.2913.

*4.1.17.40. N-Benzyl-2-((1-benzylindolin-4-yl)oxy)ethan-1-amine (71).* Following GP7, compound 71 was prepared using 2-((1-benzylindolin-4-yl)oxy)ethan-1-amine (17) (83 mg, 0.31 mmol, 1.1 equiv), benzaldehyde (28.5 μL, 0.28 mmol, 1 equiv), glacial acetic acid (6 μL), $NaBH_3CN$ (53 mg, 0.84 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 32 mg (32%), brownish oil. Purity 95% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.31–7.39 (m, 8H), 7.24–7.29 (m, 2H), 7.02 (t, $J$ = 8.0 Hz, 1H), 6.28 (d, $J$ = 8.0 Hz, 1H), 6.22 (d, $J$ = 8.0 Hz, 1H), 4.25 (s, 2H), 4.14 (t, $J$ = 5.2 Hz, 2H), 3.90 (s, 2H), 3.33 (t, $J$ = 8.3 Hz, 2H), 3.03 (t, $J$ = 5.2 Hz, 2H), 2.93 (t, $J$ = 8.3 Hz, 2H), 2.74 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.5, 154.4, 139.5, 138.6, 128.8, 128.6, 128.6, 128.4, 128.0, 127.3, 127.2, 116.3, 102.4, 101.2, 67.1, 53.7, 53.7, 53.5, 48.1, 25.7. Formula: $C_{24}H_{26}N_2O$; MS: *m/z* 359 ($M + H^+$).

*4.1.17.41. 2-((1-Benzylindolin-4-yl)oxy)-N-(3-methylbenzyl)ethan-1-amine (72).* Following GP7, compound 72 was prepared using 2-((1-benzylindolin-4-yl)oxy)ethan-1-amine (17) (83 mg, 0.31 mmol, 1.1 equiv), *m*-tolulaldehyde (33 μL, 0.28 mmol, 1 equiv), glacial acetic acid (6 μL), $NaBH_3CN$ (53 mg, 53 mmol, 3 equiv) in 3 mL MeOH. Purification: flash chromatography (4% MeOH in DCM). Yield: 44 mg (42%), brownish oil. Purity 95% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.33–7.41 (m, 4H), 7.15–7.32 (m, 4H), 7.01–7.12 (m, 2H), 6.31 (d, $J$ = 8.0 Hz, 1H), 6.24 (d, $J$ = 8.0 Hz, 1H), 4.27 (s, 2H), 4.15 (t, $J$ = 5.4 Hz, 2H), 3.87 (s, 2H), 3.35 (t, $J$ = 8.6 Hz, 2H), 3.04 (t, $J$ = 5.2 Hz, 2H), 2.96 (t, $J$ = 8.6 Hz, 2H), 2.37 (s, 3H), 2.20 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.6, 154.4, 140.0, 138.7, 138.2, 129.0, 128.8, 128.6, 128.5, 128.0, 127.9, 127.2, 125.3, 116.4, 102.5, 101.2, 67.4, 53.8, 53.8, 53.7, 48.4, 25.7, 21.6. Formula: $C_{25}H_{28}N_2O$; MS: *m/z* 373 ($M + H^+$).

*4.1.17.42. Tert-butyl 3-(((2-((1-benzylindolin-4-yl)oxy)ethyl)amino) methyl)piperidine-1-carboxylate (73).* Following GP7, compound 73 was prepared using 2-((1-benzylindolin-4-yl)oxy)ethan-1-amine (17) (134 mg, 0.50 mmol, 1.1 equiv), *tert*-butyl 3-

formylpiperidine-1-carboxylate (96 mg, 0.45 mmol, 1 equiv), glacial acetic acid (9 μL), $NaBH_3CN$ (85 mg, 1.35 mmol, 3 equiv) in 4.5 mL MeOH. Purification: flash chromatography (5% MeOH in DCM). Yield: 87 mg (41%), colourless oil. Purity 96% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.29–7.37 (m, 3H), 7.24–7.29 (m, 1H), 7.02 (br. s., 1H), 6.20–6.30 (m, 2H), 4.23 (s, 2H), 4.10 (t, $J$ = 4.3 Hz, 2H), 3.91 (br. s., 1H), 3.75 (d, $J$ = 12.6 Hz, 1H), 3.30 (t, $J$ = 8.6 Hz, 2H), 2.95–3.04 (m, 2H), 2.91 (d, $J$ = 4.6 Hz, 3H), 2.77 (t, $J$ = 11.5 Hz, 1H), 2.48–2.68 (m, 4H), 1.76–1.89 (m, 1H), 1.59–1.70 (m, 1H), 1.39–1.44 (m, 11H), 1.15 (t, $J$ = 11.5 Hz, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.4, 155.1, 154.4, 138.6, 128.8, 128.5, 128.0, 127.2, 116.3, 102.4, 101.2, 79.5, 66.9, 53.7, 53.5, 52.5, 49.0, 28.9, 28.5, 28.5, 25.7, 24.4. Formula: $C_{28}H_{39}N_3O_3$; MS: $m/z$ 466 ($M + H^+$).

*4.1.17.43. 1-Benzyl-4-(2-(benzylamino)ethoxy)indolin-2-one (74).* Following GP7, compound 74 was prepared using 4-(2-aminoethoxy)-1-benzylindolin-2-one (19) (40 mg, 0.14 mmol, 1.3 equiv), benzaldehyde (11 μL, 0.11 mmol, 1 equiv), glacial acetic acid (2.2 μL), $NaBH_3CN$ (21 mg, 0.33 mmol, 3 equiv) in 1.5 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 15 mg (37%), brown oil. Purity 98% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.33–7.35 (m, 3H), 7.22–7.30 (m, 7H), 7.09 (t, $J$ = 8.0 Hz, 1H), 6.55 (d, $J$ = 8.6 Hz, 1H), 6.38 (d, $J$ = 8.0 Hz, 1H), 4.88 (s, 2H), 4.14 (t, $J$ = 5.2 Hz, 2H), 3.89 (s, 2H), 3.53 (s, 2H), 3.04 (t, $J$ = 5.2 Hz, 2H); NH signal not detected. $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 175.3, 154.6, 145.5, 139.5, 136.0, 129.0, 128.7, 128.5, 128.1, 127.5, 127.3, 127.2, 111.2, 106.4, 102.7, 67.5, 53.6, 47.8, 43.9, 33.4. Formula: $C_{24}H_{24}N_2O_2$; MS: $m/z$ 373 ($M + H^+$).

*4.1.17.44. 1-Benzyl-4-(2-((3-methylbenzyl)amino)ethoxy)indolin-2-one (75).* Following GP7, compound 75 was prepared using 4-(2-aminoethoxy)-1-benzylindolin-2-one (19) (56 mg, 0.20 mmol, 1.3 equiv), *m*-tolualdehyde (17.7 μL, 0.15 mmol, 1 equiv), glacial acetic acid (2.5 μL), $NaBH_3CN$ (28 mg, 0.45 mmol, 3 equiv) in 1.5 mL MeOH. Purification: flash chromatography (0–5% MeOH in DCM). Yield: 16 mg (28%), brownish oil. Purity 95% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.20–7.32 (m, 6H), 7.06–7.17 (m, 4H), 6.55 (d, $J$ = 8.0 Hz, 1H), 6.38 (d, $J$ = 7.5 Hz, 1H), 4.88 (s, 2H), 4.14 (t, $J$ = 5.2 Hz, 2H), 3.85 (s, 2H), 3.53 (s, 2H), 3.04 (t, $J$ = 5.2 Hz, 2H), 2.34 (s, 3H), NH signal not detected. $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 175.3, 154.5, 145.5, 139.1, 138.2, 136.0, 129.0, 129.0, 128.7, 128.4, 128.0, 127.5, 127.3, 125.2, 111.2, 106.4, 102.7, 67.4, 53.5, 47.7, 43.9, 33.5, 21.4. Formula: $C_{25}H_{26}N_2O_2$; MS: $m/z$ 387 ($M + H^+$).

*4.1.17.45. Tert-butyl 3-(((2-((1-benzyl-2-oxoindolin-4-yl)oxy)ethyl)amino)methyl)piperidine-1-carboxylate (76).* Following GP7, compound 76 was prepared using 4-(2-aminoethoxy)-1-benzylindolin-2-one (19) (60 mg, 0.21 mmol, 1.1 equiv), *tert*-butyl 3-formylpiperidine-1-carboxylate (40 mg, 0.19 mmol, 1 equiv), glacial acetic acid (4 μL), $NaBH_3CN$ (36 mg, 0.57 mmol, 3 equiv) in 2 mL of MeOH and THF mixture (1:1). Purification: flash chromatography (4% MeOH in DCM). Yield: 15 mg (16%), brown oil. Purity 96% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.22–7.32 (m, 5H), 7.01–7.14 (m, 1H), 6.47–6.60 (m, 1H), 6.38 (d, $J$ = 6.9 Hz, 1H), 4.88 (s, 2H), 4.03–4.31 (m, 2H), 3.73–3.98 (m, 2H), 3.45–3.71 (m, 3H), 3.02 (d, $J$ = 17.8 Hz, 2H), 2.70–2.92 (m, 2H), 2.44–2.68 (m, 2H), 1.85 (d, $J$ = 12.0 Hz, 2H), 1.50–1.74 (m, 2H), 1.42 (s, 9H), 0.76–0.88 (m, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 175.5, 168.3, 145.6, 136.1, 134.2, 129.2, 128.8, 127.6, 127.4, 126.8, 123.4, 102.9, 65.2, 48.6, 44.0, 37.4, 33.6, 29.8, 28.6, 28.5, 22.8, 14.2. Formula: $C_{28}H_{37}N_3O_4$; MS: $m/z$ 480 ($M + H^+$).

*4.1.18. General procedure for the synthesis of final compounds 77–79 (GP10)*

To a solution of 2-((1-benzyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-4-yl)oxy)ethan-1-aminium chloride (26) (1.3 equiv) in 1:1 mixture of MeOH and THF 1,4-diazabicyclo[2.2.2]octane (DABCO) (7.0 equiv) and aldehyde (1.0 equiv) were added. After 1 h of stirring at ambient temperature, the reaction mixture was cooled on an ice bath and $NaBH_3CN$ (3.0 equiv) was added. The reaction was stirred 18 h at rt then the solvents were removed in vacuo, the residue was treated with water and extracted with EtOAC. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (5% MeOH in DCM).

*4.1.18.1. 1-Benzyl-4-(2-(benzylamino)ethoxy)-1,3-dihydro-2H-benzo[d]imidazole-2-one (77).* Following GP10, compound 77 was prepared using 2-((1-benzyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-4-yl)oxy)ethan-1-aminium chloride (26) (64 mg, 0.20 mmol), DABCO (121 mg, 1.08 mmol), benzaldehyde (15 μL, 0.15 mmol), $NaBH_3CN$ (28 mg, 0.45 mmol) in 2 mL of MeOH and THF mixture (1:1). Yield: 41 mg (73%), brownish oil. Purity 100% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.32–7.36 (m, 2H), 7.19–7.30 (m, 8H), 6.86 (t, $J$ = 8.0 Hz, 1H), 6.59 (d, $J$ = 8.6 Hz, 1H), 6.47 (d, $J$ = 8.0 Hz, 1H), 4.95 (s, 2H), 4.19 (t, $J$ = 5.2 Hz, 2H), 3.89 (s, 2H), 3.05 (t, $J$ = 4.9 Hz, 2H); both NH signals not detected. $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.3, 143.2, 139.1, 136.3, 131.2, 128.6, 128.4, 128.3, 127.5, 127.3, 127.1, 121.5, 117.9, 106.2, 102.4, 68.1, 53.3, 47.7, 44.6. Formula: $C_{23}H_{23}N_3O_2$; MS: $m/z$ 374 ($M + H^+$).

*4.1.18.2. 1-Benzyl-4-(2-((3-methylbenzyl)amino)ethoxy)-1,3-dihydro-2H-benzo[d]imidazole-2-one (78).* Following GP10, compound 78 was prepared using 2-((1-benzyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-4-yl)oxy)ethan-1-aminium chloride (26) (64 mg, 0.20 mmol), DABCO (121 mg, 1.08 mmol), *m*-tolualdehyde (18 μL, 0.15 mmol), $NaBH_3CN$ (28 mg, 0.45 mmol) in 2 mL of MeOH and THF mixture (1:1). Yield: 28 mg (48%), brownish oil. Purity 98% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.20–7.29 (m, 5H), 7.12–7.19 (m, 3H), 7.03 (d, $J$ = 6.9 Hz, 1H), 6.83–6.88 (m, 1H), 6.58 (d, $J$ = 8.0 Hz, 1H), 6.47 (d, $J$ = 8.0 Hz, 1H), 4.95 (s, 2H), 4.19 (t, $J$ = 5.2 Hz, 2H), 3.87 (s, 2H), 3.07 (t, $J$ = 5.2 Hz, 2H), 2.28 (s, 3H), both NH signals not detected. $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.4, 143.3, 138.9, 138.3, 136.5, 131.4, 129.3, 128.8, 128.5, 128.1, 127.6, 127.4, 125.6, 121.6, 118.0, 106.3, 102.5, 68.0, 53.3, 47.8, 44.7, 21.4. Formula: $C_{24}H_{25}N_3O_2$; MS: $m/z$ 388 ($M + H^+$).

*4.1.18.3. Tert-butyl 3-(((2-((1-benzyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazole-4-yl)oxy)ethyl)amino)methyl)piperidine-1-carboxylate (79).* Following GP10, compound 79 was prepared using 2-((1-benzyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-4-yl)oxy)ethan-1-aminium chloride (26) (64 mg, 0.20 mmol), DABCO (121 mg, 1.08 mmol), *tert*-butyl 3-formylpiperidine-1-carboxylate (32 mg, 0.15 mmol), $NaBH_3CN$ (28 mg, 0.45 mmol) in 2 mL of MeOH and THF mixture (1:1). Yield: 24 mg (33%), brownish oil. Purity 96% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.18–7.31 (m, 5H), 6.85 (t, $J$ = 8.3 Hz, 1H), 6.53–6.59 (m, 1H), 6.47 (d, $J$ = 8.0 Hz, 1H), 4.95–5.07 (m, 2H), 4.14–4.25 (m, 2H), 3.53 (br. s., 1H), 3.27–3.43 (m, 2H), 3.05–3.14 (m, 1H), 2.94–3.05 (m, 4H), 2.57–2.74 (m, 2H), 1.97 (d, $J$ = 11.5 Hz, 1H), 1.79 (br. s., 1H), 1.59–1.67 (m, 1H), 1.40–1.53 (m, 9H), both NH signals not detected. $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 155.2, 155.0, 143.2, 136.5, 131.4, 128.7, 127.6, 127.4, 121.5, 117.8, 105.2, 102.3, 80.1, 67.2, 50.8, 50.5, 48.4, 45.8, 45.4, 44.7, 33.8, 28.5, 23.1. Formula: $C_{27}H_{36}N_4O_4$; MS: $m/z$ 481 ($M + H^+$).

*4.1.19. Synthesis of N-benzyl-N-methyl-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (28)*

To *N*-benzyl-2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (27) (164 mg, 0.40 mmol, 1 equiv) dissolved in MeOH and 37% $HCHO_{(aq)}$ (149 μL, 2 mmol, 5 equiv) glacial acetic acid (40 μL)

was added. After 1 h of stirring at ambient temperature, the reaction mixture was cooled on an ice bath and $NaBH_3CN$ (126 mg, 2 mmol, 5 equiv) was added. The reaction was stirred 18 h at rt then, the solvents were removed in vacuo, the residue was treated with 0.1 M $NaOH_{(aq)}$ and extracted with DCM. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (20–50% EtOAc in petroleum ether). Yield: 57 mg (34%), colourless oil. Purity 96% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83–7.91 (m, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.16–7.55 (m, 10H), 6.76–6.80 (m, 1H), 6.62 (d, $J$ = 7.6 Hz, 1H), 4.18 (t, $J$ = 5.9 Hz, 2H), 3.64 (s, 2H), 2.88 (t, $J$ = 5.9 Hz, 2H), 2.37 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.4, 138.9, 138.2, 136.2, 133.8, 129.2, 129.0, 128.3, 127.1, 126.7, 125.7, 124.7, 121.3, 106.5, 104.3, 66.7, 62.7, 55.6, 43.0. Formula: $C_{24}H_{24}N_2O_3S$; MS: $m/z$ 421 ($M + H^+$).

#### 4.1.20. Synthesis of N,N-dimethyl-3-(((2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethyl)amino)methyl)aniline (37)

To a suspension of 3-(aminomethyl)-*N*,*N*-dimethylaniline (81 mg, 0.54 mmol, 3 equiv) and $K_2CO_3$ (75 mg, 0.54 mmol, 3 equiv) in 3 mL of acetonitrile, 4-(2-bromoethoxy)-1-(phenylsulfonyl)-1*H*-indole (8) dissolved in 1 mL of acetonitrile was added dropwise. The reaction was refluxed for 18 h; then, the solvent was removed in vacuo, the residue was treated with water and extracted with DCM. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (2–10% methanol in DCM). Yield: 63 mg (78%), yellow oil. Purity 95% (UPLC/MS). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83–7.90 (m, 2H), 7.60 (d, $J$ = 8.2 Hz, 1H), 7.38–7.56 (m, 4H), 7.15–7.28 (m, 2H), 6.78 (d, $J$ = 3.5 Hz, 1H), 6.61–6.75 (m, 4H), 4.19 (t, $J$ = 5.3 Hz, 2H), 3.85 (s, 2H), 3.08 (t, $J$ = 5.3 Hz, 2H), 2.93 (s, 6H), 1.94 (br. s., 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.4, 150.9, 140.8, 138.2, 136.2, 133.8, 129.2, 129.1, 126.7, 125.7, 124.8, 121.3, 116.4, 112.3, 111.4, 106.6, 106.4, 104.6, 67.7, 54.2, 48.0, 40.6. Formula: $C_{25}H_{27}N_3O_3S$; MS: $m/z$ 450 ($M + H^+$).

#### 4.1.21. Synthesis of 4-(4-benzylpiperazin-1-yl)-1-(phenylsulfonyl)-1H-indole (49)

To a mixture of 1-(phenylsulfonyl)-4-(piperazin-1-yl)-1*H*-indole (54 mg, 0.16 mmol, 1 equiv) and $K_2CO_3$ (49 mg, 0.35 mmol, 2.2 equiv) in 3 mL of acetonitrile, benzyl bromide (22.5 μL, 0.19, 1.2 equiv) was added. The reaction was stirred at rt for 18 h. After that time the solvent was removed in vacuo, the residue was treated with water and extracted with DCM. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (30% EtOAc in petroleum ether). Yield: 55 mg (80%), beige oil. Purity 98% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.85–7.89 (m, 2H), 7.65 (d, $J$ = 8.6 Hz, 1H), 7.49–7.55 (m, 2H), 7.40–7.45 (m, 2H), 7.31–7.39 (m, 4H), 7.27–7.30 (m, 1H), 7.21 (t, $J$ = 8.3 Hz, 1H), 6.72 (d, $J$ = 7.5 Hz, 1H), 6.68 (d, $J$ = 2.9 Hz, 1H), 3.62 (s, 2H), 3.17 (br. s., 4H), 2.68 (br. s., 4H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 146.2, 138.3, 136.0, 133.8, 129.4, 129.3, 128.4, 127.4, 126.9, 125.6, 124.8, 124.3, 110.9, 107.9, 107.5, 63.1, 53.4, 51.5. Formula: $C_{25}H_{25}N_3O_2S$; MS: $m/z$ 432 ($M + H^+$).

#### 4.1.22. Synthesis of 2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)-N-(piperidin-3-ylmethyl)ethan-1-amine (53)

To a solution of *tert*-butyl 3-(((2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethyl)amino) methyl)piperidine-1-carboxylate (54) (51 mg, 0.10 mmol) dissolved in 2 mL DCM, TFA (0.5 mL) was added. The reaction was stirred at rt for 1 hthen, the mixture was diluted with DCM and a saturated solution of $NaHCO_{3(aq)}$. The mixture's pH was adjusted to ca. 10 with 0.1 M $NaOH_{(aq)}$ and it was extracted with DCM. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by column chromatography (DCM/MeOH/petroleum ether/$NH_{(aq)}$ 600/225/90/45). Yield: 33 mg (80%), yellowish oil. Purity 95% (UPLC/MS). $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.90 (dd, $J$ = 1.2, 8.6 Hz, 2H), 7.63 (d, $J$ = 8.6 Hz, 1H), 7.54–7.62 (m, 2H), 7.46–7.52 (m, 2H), 7.26 (t, $J$ = 8.0 Hz, 1H), 7.05 (d, $J$ = 4.6 Hz, 1H), 6.80 (d, $J$ = 7.5 Hz, 1H), 4.40–4.45 (m, 2H), 3.52–3.57 (m, 3H), 3.33–3.39 (m, 1H), 3.06–3.19 (m, 2H), 2.89–3.01 (m, 1H), 2.78–2.88 (m, 1H), 2.34–2.45 (m, 1H), 1.99–2.06 (m, 1H), 1.91–1.99 (m, 1H), 1.75–1.88 (m, 1H), 1.35–1.45 (m, 1H), both NH signals not detected. $^{13}$C NMR (126 MHz, $CD_3OD$) $\delta$ 152.9, 139.3, 137.7, 135.4, 130.6, 128.0, 126.9, 126.6, 108.6, 107.9, 107.4, 106.2, 74.1, 64.8, 51.8, 47.5, 45.1, 32.8, 27.5, 22.8. Formula: $C_{25}H_{27}N_3O_3S$; MS: $m/z$ 414 ($M + H^+$).

#### 4.1.23. Synthesis of N-methyl-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine (56)

To a solution of *N*-benzyl-*N*-methyl-2-((1-(phenylsulfonyl)-1*H*-indol-4-yl)oxy)ethan-1-amine (28) (54 mg, 0.13 mmol) dissolved in 3 mL EtOH, HCOOH (50 μL) and 10% Pd/C (10 mg, 19% w/w) was added. The reaction was hydrogenated at rt for 5 h. After that time, the solution was filtered through Celite® and concentrated in vacuo. The residue was treated with water and extracted with DCM. Combined organic fractions were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by preparative HPLC (2–10% methanol in DCM). Yield: 12 mg (28%), brownish oil. Purity 100% (UPLC/MS). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.85 (dd, $J$ = 1.15, 8.6 Hz, 2H), 7.59 (d, $J$ = 8.6 Hz, 1H), 7.49–7.54 (m, 1H), 7.46 (d, $J$ = 3.4 Hz, 1H), 7.38–7.44 (m, 2H), 7.20 (t, $J$ = 8.0 Hz, 1H), 6.79 (d, $J$ = 3.4 Hz, 1H), 6.63 (d, $J$ = 8.0 Hz, 1H), 4.16 (t, $J$ = 5.2 Hz, 2H), 3.01 (t, $J$ = 5.2 Hz, 2H), 2.50 (s, 3H), 2.41 (br. s., 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.1, 138.3, 136.3, 133.9, 129.3, 126.8, 125.8, 124.9, 121.3, 106.9, 106.2, 104.6, 69.7, 50.2, 35.8. Formula: $C_{17}H_{18}N_2O_3S$; MS: $m/z$ 331 ($M + H^+$).

### 4.2. X-ray crystallography of hBuChE in complex with 27, 54 and 70

Crystallization. Recombinant human BChE (*h*BuChE) was produced in CHO–K1 cells (Sigma Aldrich) as previously described [75]. The protein was purified using *h*BuChE specific affinity chromatography (Hupresin®; CHEMFORASE, Rouen, France) and size exclusion chromatography (Superdex 200, GE Healthcare), as previously described [76]. Crystallization was carried out at 293K with the hanging drop vapor diffusion method in 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES) pH 6.5, 2.15 M $(NH_4)_2SO_4$ buffer. 27 ligand was prepared at 0.1 M in 100% DMSO and protein-ligand complex was obtained by soaking crystals at 1 mM ligand final concentration in mother liquor (0.1 M MES pH 6.5, 2.15 M $(NH_4)_2SO_4$, 1% DMSO). Crystals were cryo-protected in a solution of 0.1 M MES pH 6.5, 2.15 M $(NH_4)_2SO_4$, 20% Glycerol, 1 mM 27, 1% DMSO before flash cooling into liquid nitrogen. Crystals in complex with 54 and 70 were obtained similarly, except that initial ligand solutions were prepared in 100% MeOH instead of DMSO.

Structure Determination. X-ray diffraction data were collected at 100 K on the ID30B beamline of the European Synchrotron Research Facility (ESRF, Grenoble, France) for 27 or the PROMIXA-1 beamline of the SOLEIL Synchrotron (Saint Aubin, France) for 54 and 70. Images recorded on a PILATUS3 S 6 M detector (Dectris) for ID30B or an EIGER-X-16 M (Dectris) for PROXIMA-1 were respectively processed automatically with autoPROC [77] or manually treated with XDS [78]. Data analysis was realized using the Phenix software suite [79]. Initial models were obtained by molecular replacement using Phaser-MR and the *h*BuChE structure (PDB entry 1p0i) devoid of any ligand, glycans or water molecules. Extra electron density was observed close to the active site gorge that allowed fitting of the respective ligands. Ligand geometry restraints were processed using Phenix eLBOW [80] and the semi-empirical

quantum mechanical method (AM1). The models were refined by iterative cycles of Phenix.refine and model building using *Coot* [81]. For final refinement cycles of 27 and 54 bound structure, a partial occupancy was assigned to a first group composed of residues 285 to 287 and 27 or 54 with alternate position A and a partial occupancy for a second group composed of residues 285 to 287 with alternate position B, to consider the displacement of the acyl-loop upon ligand binding. Human BuChE structures in complex with 27, 54 and 70 were respectively deposited into the Protein Data Bank under accession numbers 7awg, 7awh, 7awi. Structure representations were realized using the PyMOL software (Schrodinger LLC).

Molecular dynamic simulations. The structure of hBuChE was prepared from the crystal structure of the monomeric apoprotein (PDB entry 1p0i). Missing loops and mutated regions were built and minimized as previously described [82]. The eqBuChE model was generated from the primary sequence (UniProt entry Q9N1N9) using the Phyre2 (Protein Homology/analogy Recognition Engine v2.0) server [83]. Molecular dynamics simulations were carried out using GROMACS versions 2019.4 [84] using the Amber14sb forcefield [85], 300 K temperature coupling schemes using a Berendsen thermostat, and the particle-mesh Ewald method for determining long-range electrostatics (10 Å cutoff). The protein was immersed in a periodic cubic water box with a minimal distance of 10 Å to any edge. The box was solvated using the TIP4P solvation model and chloride and sodium counter ions at a concentration of 100 mM were added to neutralize the simulation system. A steepest descent method was used for energy minimization, then the system was subjected to equilibration at 300 K and 1 bar for 100 ps applying position restraints for heavy atoms. Finally, full MD simulation was performed for 10 ns at 300 K, using 2 fs timesteps. Each simulation, human and equine BuChE, was independently realized 3 times on a dual decacore Xeon® workstation with 128 GB RAM and two Nvidia Titan X (Pascal) GPU. Final trajectories were treated in order to center the studied protein into the cubic water box to avoid protein slippage. For analysis, the average backbone b factor was calculated from the trajectory using gmx_rmsf, included in the GROMACS package. Representations were realized using PyMOL software (Schrodinger LLC).

### 4.3. In silico BuChE binding mode analysis

Crystal structure of *h*BuChE in complex with compound 27 was prepared using a software package from Schrödinger (Small molecule discovery suite, Release 2017-2, Maestro version 11.2.0.13, MMshare version 3.8.013, Schrödinger, LLC, New York, NY, 2017). Co-crystallised ligand 27 or *N*-benzyl-2-((1-(phenylsulfonyl)-1H-indol-4-yl)oxy)ethan-1-amine was identified at the active site of chain A. Receptor grids were computed for further docking experiments using Glide Receptor Grid Generation from Small molecule discovery suite (Release 2017-2, Glide version 75013, Schrödinger LLC) [86,87]. Docking volume was defined as a centroid of a co-crystallised ligand with midpoint box size of 10 × 10 × 10 Å. No rotatable groups on the receptor were defined and no explicit exclusion volumes were used. Generated Glide grids were used in subsequent docking experiments with Glide software (Glide version 75013, Schrödinger LLC) [88]. Docking parameters were defined as following: VdW scaling factor was set to 0.80 and partial charge cutoff parameter set to 0.15. Scoring function was Glide SP with flexible ligand sampling together with sampling of nitrogen inversions and ring conformations and final post-docking minimization. Ligands were prepared using Schrödinger Ligprep tool with Epik setting (pH of 7.4) and OPLS3 force field [89]. Re-docking of a crystal structure ligand 27 in the *h*BuChE active site reproduced the crystal structure binding mode with an-atom RMSD of 1.18 Å. Designed compounds were all prepared and docked in an analogous manner and their predicted binding modes examined. No constraints were used during docking/scoring runs.

### 4.4. Molecular modeling with 5-$HT_6$R

Computer-aided design was all about prediction of protein-ligand interactions through molecular docking to the previously developed homology models of the 5-$HT_6$ receptors. Procedure for obtaining ligand-optimized models of high predictive value, utilizing induced fit docking (IFD), was characterized in detail previously [57,90,91]. The 5-$HT_6$ receptor model was based on the 5-$HT_{1B}$ serotonin receptor crystal structure (PDB ID: 4IAR) [92]. Ligand structures were optimized using LigPrep tool and docked by Glide SP flexible docking procedure using OPLS3 force field. H-bond constraint, as well as centroid of a grid box were located on Asp3.32106 residue. Selection of the binding mode was based on scoring function and manual interaction analysis. The docking procedure was verified using various chemical classes of high affinity 5-$HT_6$ receptor ligands. Small-Molecule Drug Discovery Suite (Schrödinger, Inc.) was licensed for Jagiellonian University Medical College.

### 4.5. 5-$HT_6$ receptor Binding assay [65]

Preparation of solutions of test and reference compounds. 10 mM stock solutions of tested compounds were prepared in DMSO. Serial dilutions of compounds were prepared in 96-well microplate in assay buffers using automated pipetting system epMotion 5070 (Eppendorf). Each compound was tested in 8 concentrations from 10 to 5 to 10−12 M (final concentration). Radioligand binding was performed using membranes from CHO−K1 cells stably transfected with the human 5-$HT_6$ receptor (PerkinElmer). All assays were carried out in duplicates. 50 μl working solution of the tested compounds, 50 μl [$^3$H]-LSD (final concentration 2.5 nM) and 150 μl diluted membranes (15 μg protein per well) prepared in assay buffer (50 mM Tris, pH 7.4, 10 mM $MgCl_2$, 0.1 mM EDTA) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). Methiothepin (10 μM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 60 min at 27 °C. The reaction was terminated by rapid filtration through GF/B filter mate presoaked with 0.5% polyethyleneimine for 30 min. Ten rapid washes with 200 μl 50 mM Tris buffer (4 °C, pH 7.4) were performed using automated harvester system Harvester-96 MACH III FM (Tomtec). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 5 min. Radioactivity was counted in MicroBeta2 scintillation counter (PerkinElmer). Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software) and $K_i$ values were estimated from the Cheng−Prusoff equation.

### 4.6. Functional assays for 5-$HT_6$ receptor

Materials and methods. Test and reference compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM. Serial dilutions were prepared in 96-well microplate in assay buffer and 8 to 10 concentrations were tested. For the 5-HT6,

adenylyl cyclase activity were monitored using cryopreserved 1321N1 cells with expression of the human serotonin 5-HT6 receptor (PerkinElmer, USA). Thawed cells were resuspended in stimulation buffer (HBSS, 5 mM HEPES, 0.5 IBMX, and 0.1% BSA at pH 7.4) at $3 \times 10^5$ cells/ml. The same volume (10 μl) of cell suspension was added to tested compounds. Samples were loaded onto a white opaque half area 96-well microplate. The antagonist response experiment was performed with 22 nM serotonin as the reference agonist for 5-HT6 receptor. The agonist and antagonist were added simultaneously. Cell stimulation was performed for 30 min at room temperature. After incubation, cAMP measurements were performed with homogeneous TR-FRET immunoassay using the LANCE Ultra cAMP kit (PerkinElmer, USA). 10 μl of EucAMP Tracer Working Solution and 10 μl of ULight-*anti*-cAMP Tracer Working Solution were added, mixed, and incubated for 1 h. The TR-FRET signal was read on an EnVision microplate reader (PerkinElmer, USA). $IC_{50}$ and $EC_{50}$ were determined by nonlinear regression analysis using GraphPad Prism 7.0 software.

### 4.7. Cholinesterases in vitro inhibitory activity (eeAChE, eqBuChE, hBuChE)

The reagents used to perform cholinesterases inhibitory activity assays were purchased from Sigma–Aldrich (Steinheim, Germany); only human butyrylcholinesterase (*h*BuChE) isolated from human plasma was from Vivonics (Bedford, MA, USA). The protocol based on spectrophotometric Ellman's method [64] was followed, with small modifications, using 96-well microplates. Aqueous stock solutions of the enzymes (*ee*AChE, *eq*BuChE, and *h*BuChE) in the concentrations of 5U/mL were diluted before use, giving the final concentrations of 0.384 U/mL. Stock solutions of the tested compounds were prepared in DMSO. Prior to starting the reaction, the mixture of 25 μL of the target compound (or water or mixture of DMSO/water in appropriate ratio; i.e. blank samples) was incubated in 0.1 M phosphate buffer (200 μL, pH = 8.0) with DTNB (20 μL; 0.0025 M) and the enzyme (20 μL; *ee*AChE, *eq*BuChe or *h*BuChE). The incubation and final reactions were performed at room temperature (25 °C) for animal enzymes (*ee*AChE or *eq*BuChe) and 36 °C for the human enzyme (*h*BuChE). After 5 min of pre-incubation, the final reactions were started by adding 20 μL of ATC (0.00375 M) or BTC (0.00375 M) solutions (depending on the enzyme used). Following the next 5 min, the changes in absorbance were measured at 412 nm, using a microplate reader (EnSpire Multimode; PerkinElmer, Waltham, MA, USA). All the target compounds were tested at the screening concentration of 10 μM. Based on equation 100-(S/B) × 100 (where S and B were the respective enzyme activities with and without the test sample, respectively) the percent of inhibition of each enzyme for each compound was calculated. Compounds with the enzyme inhibitory activity at 10 μM better than 50%, were further evaluated to obtain $IC_{50}$ values. To determine $IC_{50}$ value, seven different concentrations of each compound were tested giving enzyme inhibition between 5% and 95%. The experiments were performed in triplicate. The $IC_{50}$ values were calculated using nonlinear regression (GraphPad Prism 5; GraphPad Software, San Diego, CA, USA) by plotting the residual enzyme activities against the applied inhibitor concentration. Tacrine was used as the reference compound.

### 4.8. Kinetics of eqBuChE inhibition for selected compounds

The protocol described above was followed, based on Ellman's method [64]. Aqueous enzyme stock solution (5 U/mL) was diluted before use to the concentration of 0.384 U/mL. Aqueous substrate BTC stock solution (0.02125 M) was also diluted to give six final concentrations in the wells: 0.3, 0.24, 0.18, 0.12, 0.06, and 0.04 mM. Stock solutions of the target compounds were prepared in DMSO and diluted in demineralized water to obtain six different concentrations of each inhibitor, further resulting in the percent of enzyme inhibition between 30% and 80%. For each concentration of the target compound, all six BTC solutions were used to perform the Ellman's assays. Each experiment was performed in triplicate. $V_{max}$ and $K_m$ values of the Michaelis–Menten kinetics were calculated by nonlinear regression from substrate–velocity curves. Lineweaver-Burk and Cornish-Bowden plots were obtained using linear regression in GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

### 4.9. 100-Fold dilution assay

Following the modified protocol described by Kosak et al. [67], the recovery of *eq*BuChE activity was measured after 30 min, 60 min, and 120 min of the pre-incubation of the mixture containing 10-fold the $IC_{50}$ of the target compound and 100-fold the final enzyme concentration (at room temperature 25 °C). After the pre-incubation time, 2.85 μL of mixture was diluted into each well's solution containing 242.15 μL of phosphate buffer (0.1 M; pH = 8) and 20 μL of DTNB (0.0025 M). Next, 20 μL of BTC (0.00375 M) was added, to start the final reaction. After 5 min, the absorbance was measured at 412 nm by a microplate reader (EnSpire Multimode; PerkinElmer, Waltham, MA, USA). Tacrine and donepezil (reversible enzyme inhibitors), as well as rivastigmine (pseudo-irreversible enzyme inhibitor), were used as the controls. The blank samples were prepared analogically to the tested ones, using preincubated enzyme with water mixture (or enzyme with appropriate ratio of water/DMSO) instead of the mixture of enzyme and the target compound. Two independent experiments were performed for the tested compounds, each one in quadruplicate.

### 4.10. $A\beta_{42}$ aggregation inhibition - thioflavin-T (ThT) fluorometric assay [66]

The inhibition of $A\beta_{42}$ aggregation was measured fluorimetrically as described previously [93]. Briefly, HFIP-pretreated $A\beta_{42}$ (Merck Millipore, Darmstadt, Germany) at 1.5 μM, the test compound (10 μM final concentration) and Thioflavin-T (10 μM final concentration) were incubated at room temperature in 96-well microplate covered with aluminum foil with continuous shaking for 24–48 h. The DMSO was always at 3% (v/v). The fluorescence intensity ($\lambda_{ex} = 440$ nm; $\lambda_{em} = 490$ nm) was measured every 3 min (Synergy™ H4 plate reader, BioTek Instruments, Inc. VT, USA). The assay was run in quadruplicates.

### 4.11. Metabolic stability in mouse liver microsomes (MLMs) [58]

Materials and Methods. In general, the reaction was carried out at 37 °C using 1 mg/mL of mouse liver microsomes (MLMs) (Sigma-Aldrich, St. Louis, MO, USA) in 200 μL of reaction buffer containing 0.1 M Tris-HCl (pH 7.4), NADPH Regeneration System (Promega, Madison, WI, USA) and 50 μM concentration of the tested compound. The reaction was initiated by adding 50 μL of the Regeneration System after 5 min of compound preincubation at 37 °C. The reaction was terminated after 120 min by adding cold methanol. The microsomes were centrifuged and the supernatant was collected next for UPLC-MS analyses.

### 4.12. Hepatotoxicity [58]

Materials and Methods. Hepatotoxicity was estimated using hepatoma HepG2 (ATCC® HB-8065™) cell line. The cells were cultured in modified Eagle's medium with 2 mM glutamine, 1%

non-essential amino acids (NEAA), and 10% FBS (Gibco, Carlsbad, CA, USA) at 37 °C in an atmosphere containing 5% of $CO_2$. The CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay from Promega (Madison, WI, USA) was used for estimation of cells viability. The compounds were investigated in quadruplicate at four concentrations (0.1, 1, 10, and 100 μM) for 72 h. The antiproliferative drug doxorubicin (DX) in dose 1 μM and mitochondrial toxin carbonyl cyanide 3-chlorophenyl-hydrazone (CCCP, 10 μM) were used as positive controls.

### 4.13. Analysis of hepatotoxicity, neurotoxicity and immunotoxicity activity using cellular models

Cells preparation. The human hepatocellular carcinoma cells (HepG2), the mouse microglial cells (BV-2) and the mouse neuroblastoma cells (N2a) were cultured using standard procedures. The cells were cultured in flasks with an area of 175 $cm^2$ (Sarstedt), and incubated at 37 °C, 5% $CO_2$. For the test of compounds with the cells line, hepatocytes, microglial cells and neuroblastoma cells were seeded on 96- well culture plate (Falcon) at a density of $2 \times 10^4$ cells per well in fresh medium. Cells were grown for 24 h in the incubator (37 °C, 5% $CO_2$) before performing experiments. After that dilution of test compounds were added and incubated for 24 h in aseptic conditions [94]. Preparation of solutions of test and reference compounds. Stock solutions were prepared in the concentration of $10^{-2}$ M for test and reference compounds. Minimum 1 mg of each tested compound was weighed and dissolved in appropriate volume of dimethyl sulfoxide. Serial dilutions were prepared in DMSO and then the diluted compounds were transferred to PBS, mixed and putted to medium. For evaluation of cytotoxicity on HepG2 cell line, astemizole was used as a reference substance. All experiments were performed in duplicates, in three independent experiments. Performing of cytotoxicity experiments. To assess cytotoxicity effect of compound test was performed: PrestoBlue (ThermoFisher) Experiments were carried out on a POLARstar Omega, plate reader (BMG Labtech). Cell viability was measured using the PrestoBlue reagent (ThermoFisher) [95]. PrestoBlue reagent is a resazurin-based solution that functions as a cell viability indicator are used. Metabolically active cells are capable of reducing the PrestoBlue reagent, with the colorimetric changes used as an indicator to quantify the viability of cells in culture. This change can be determined by measuring the fluorescence. After 24 h of incubation with the compounds it was added PrestoBlue reagent to wells of a microplate in an amount equals to one tenth of the remaining medium volume. After 15 min of incubation at 37 °C, the fluorescence intensity (EX 530; EM 580 nm) was measured in a plate reader. Viability values were calculated as a percentage of live cells with respect to the control sample (DMSO). The negative control was medium without cells. Data analysis and quality control. In Presto Blue assay (Table S3–S5) representing the viability of cells (as a percentage of control). The viability of cells were calculated into control (vehicle) and representing as a percentage of live cells. The fluorescence of background (medium without cells) were subtract from all wells before analyzing.

### 4.14. Pharmacokinetic study [96]

Materials and Reagents. HPLC grade methanol, acetonitrile and reagent grade formic acid, hydrochloric acid, potassium dihydrogen phosphate, orthophosphoric acid and sodium chloride were purchased from Merck (Darmstadt, Germany). Control blood and brain were obtained from adult male CD-1 mice (Animal House, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland.), 13–15 weeks of age and weighing 20–25 g. Mice were anaesthetized by intraperitoneal (*i.p.*) injection of 50 mg/kg ketamine plus 8 mg/kg xylazine, and blood samples were collected to heparinized tubes after animal decapitation. The plasma was separated by centrifugation (1000×*g*, 10 min). Plasma and brain were stored at −80 °C pending analysis.

Instrumentation and operating conditions. The LC/ESI-MS/MS experiments were performed on an ABSciex (Concord, Ontario, Canada) API 3200 triple quadrupole mass spectrometer equipped with an electrospray (ESI) ionization interface. This instrument was coupled to Shimadzu (Shimadzu, Japan) LC system. Data acquisition and processing were accomplished using ABSciex Analyst 1.5.2 data collection and integration software.

Chromatographic conditions. The chromatographic separation was performed on a Hypersil GOLD analytical column (100 mm × 3 mm i.d., 5 μm, Thermo Scientific, USA) with the column temperature set at 30 °C. The mobile phase consisted of a mixture of acetonitrile with the addition of 0.1% formic acid (solvent A) and water with the addition of 0.1% formic acid (solvent B) and was set at a flow rate of 0.4 mL/min. Starting amount of solvent A is 10%, isocratic elution from 0 to 5 min, and then gradient up to 90% of solvent A, and maintained to 10 min, and then gradient down to 10% of solvent A, and maintained to 15 min.

Mass spectrometric conditions. To find the optimal parameters of ion path and ion source for studied compound the quantitative optimization was done by direct infusion of the molecule at a concentration of 1 μg/mL, and at a flow rate of 10 μL/min using a Hamilton syringe pump (Hamilton, Reno, Nevada). The ion source parameters were as follows: ion spray voltage: 4500 V; nebulizer gas (gas 1): 20 psi; turbo gas (gas 2): 20 psi; temperature of the heated nebulizer: 400 °C; curtain gas: 40 psi; collision gas: 6 psi. Mass spectra were acquired by SRM with precursor/predominant product ion transitions for the analyte. The mass spectral Q1 → Q3 transitions monitored for compound 50 and IS were *m*/*z* 413.5 → 140.2 and *m*/*z* 305 → 248, respectively. The peak widths of precursor and product ions were set to 0.7 full width half-height. Quantification was done via peak area ratio.

Sample pretreatment. The plasma and brain sample pretreatment procedure involved acetonitrile precipitation. A 5 μL aliquot of the internal standard (IS, PH003437, Merck, Darmstadt, Germany) working solution (5 μg/mL) was added to 100 μL of the collected mice plasma and brain sample, which was then vortex-mixed for 10 s. Thereafter, 200 μL of acetonitrile was added, vortexed during 20 min, and then centrifuged (10000 rpm, 10 min). The supernatant (200 μL) was then transferred to insert placed in an autosampler vial, and a 20 μL volume of this was injected onto the LC column. Brain samples were thawed before use, whole brain was weighted and placed in a glass mortar and pestle tissue grinder, and homogenized with an appropriate amount of phosphate buffer (pH 7.4) in 1:2.5 ratio. Afterward, 100 μL of tissue homogenates were transferred to new Eppendorf tubes and spiked with 5 μL of the internal standard working solution. All samples were stored on ice during the preparation process and followed by procedures similar to those described above.

Animals. A group of 96 adult male mice (CD-1, 20–25 g) were used in the experiment. The animals were purchased from the Animal House at the Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland. During the habituation period the groups of 6 mice were kept in a plastic cage at a controlled room temperature (22 ± 2 °C), humidity (55 ± 10%), full spectrum cold white light (350–400 lx), on 12 h light/12 dark cycles (the lights on at 7:00 a.m., and off at 19:00 p.m.), and had free access to standard laboratory pellet and tap water. For pharmacokinetic study compound 50 dissolved in saline were administered intravenously and by intragastric gavage at a dose of 3 mg/kg and 30 mg/kg, respectively. Blood samples were collected at 0 min (predose), 5 min, 15 min, 30 min, 60 min, 120 min, 240 min and 480 min after

compound administration. The blood and brain samples were collected under general anesthesia induced by *i.p.* injection of 50 mg/kg ketamine plus 8 mg/kg xylazine. The blood samples were taken into heparinized tubes, immediately centrifuged at 1000×*g* for 10 min, and plasma was collected. The plasma and brain samples were immediately frozen at −80 °C. All experimental procedures were carried out in accordance with EU Directive 2010/63/EU and approved by the I Local Ethics Committee for Experiments on Animals of the Jagiellonian University in Krakow, Poland.

Pharmacokinetic study. Pharmacokinetic parameters were calculated by a non-compartmental approach from the average concentration values, using Phoenix WinNonlin software (Certara, Princeton, NJ 08540 USA). The first-order elimination rate constant (λz) was calculated by linear regression of log concentration versus time. The area under the mean plasma and brain concentration versus time curve ($AUC_{0\rightarrow t}$) was calculated from zero to the last concentration point using the linear trapezoidal rule as:

$$AUC_{0\rightarrow t} = \sum_{i=1}^{n} \frac{C_i + C_{i+1}}{2} \cdot (t_{i+1} - t_i) \quad (1)$$

where $C_i$ is the concentration of the compound.

The area under the first-moment curve ($AUMC_{0\ \rightarrow t}$) was estimated by calculation of the total area under the first-moment curve:

$$AUMC_{0\rightarrow t} = \sum_{i=1}^{n} ((t_i \cdot C_i + t_{i+1} \cdot C_{i+1}) / 2) \cdot (t_{i+1} - t_i) \quad (2)$$

where $t_i$ is the time of the last sampling.

Mean residence time (MRT) was calculated as:

$$MRT = \frac{AUMC_{0\rightarrow t}}{AUC_{0\rightarrow t}} \quad (3)$$

Systemic clearance (Cl) was calculated as

$$Cl = \frac{D_{iv}}{AUC_{0\rightarrow t}} \quad (4)$$

The volume of distribution at steady state ($V_{ss}$) was calculated as:

$$V_{ss} = \frac{D_{iv} \cdot AUMC_{0\rightarrow t}}{(AUC_{0\rightarrow t})^2} \quad (5)$$

The absolute bioavailability (F) of compound 50 after *p.o.* administration was calculated as:

$$F(\%) = \frac{AUC_{p.o.}}{AUC_{i.v.}} \cdot \frac{D_{i.v.}}{D_{p.o.}} \cdot 100 \quad (6)$$

where $D_{i.v.}$ and $D_{p.o.}$ are *i.v.* and *p.o.* doses of 50, respectively.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113792.

## PDB codes

Human BuChE structures in complex with **27**, **54** and **70** were respectively deposited into the Protein Data Bank under accession numbers 7awg, 7awh and 7awi.

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