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Research paper

Design, synthesis and biological evaluation of new phthalimide and saccharin derivatives with alicyclic amines targeting cholinesterases, beta-secretase and amyloid beta aggregation

Dawid Panek [a, 1], Anna Więckowska [a, 1], Tomasz Wichur [a], Marek Bajda [a], Justyna Godyń [a], Jakub Jończyk [a], Kamil Mika [a], Jana Janockova [b], Ondrej Soukup [b], Damijan Knez [c], Jan Korabecny [b], Stanislav Gobec [c], Barbara Malawska [a, *]

[a] Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland
[b] Biomedical Research Centre, University Hospital Hradec Kralove, Czech Republic
[c] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

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ABSTRACT

The complexity of Alzheimer's disease (AD) calls for search of multifunctional compounds as potential candidates for effective therapy. A series of phthalimide and saccharin derivatives linked by different alicyclic fragments (piperazine, hexahydropyrimidine, 3-aminopyrrolidine or 3-aminopiperidine) with phenylalkyl moieties attached have been designed, synthesized, and evaluated as multifunctional anti-AD agents with cholinesterase, β-secretase and β-amyloid inhibitory activities. *In vitro* studies showed that the majority of saccharin derivatives with piperazine moiety and one phthalimide derivative with 3-aminopiperidine fragment exhibited inhibitory potency toward acetylcholinesterase (AChE) with *Ee*AChE IC_{50} values ranging from 0.83 μM to 19.18 μM. The target compounds displayed inhibition of human β-secretase-1 (*h*BACE1) ranging from 26.71% to 61.42% at 50 μM concentration. Among these compounds, two multifunctional agents (**26**, [2-(2-(4-benzylpiperazin-1-yl)ethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide] and **52**, 2-(2-(3-(3,5-difluorobenzylamino)piperidin-1-yl)ethyl)isoindoline-1,3-dione) have been identified. Compound **26** exhibited the highest inhibitory potency against *Ee*AChE (IC_{50} = 0.83 μM) and inhibitory activity against *h*BACE1 (33.61% at 50 μM). Compound **52** is a selective AChE inhibitor ($IC_{50\ AChE}$ = 6.47 μM) with BACE1 inhibitory activity (26.3% at 50 μM) and it displays the most significant Aβ anti-aggregating properties among all the obtained compounds (39% at 10 μM). Kinetic and molecular modeling studies indicate that **26** may act as non-competitive AChE inhibitor able to interact with both catalytic and peripheral active site of the enzyme.

1. Introduction

Alzheimer's disease (AD) is a chronic, progressive form of dementia that constitutes 50–75% of all the cases. Patients with AD are primarily the elderly, 84% of them are over 74 years old. According to the latest data, the number of people living with dementia today is estimated at about 40 million worldwide, and this number is expected to triple by 2050 [1]. These numbers are correlated with the observed aging of population, especially in the developed countries of Europe and North America [2]. The analysis of epidemiological data does not allow drawing optimistic conclusions for the future, especially in the light of the lack of the effective treatment of the disease.

AD is a complex neurodegenerative disease with multifaceted pathomechanism, which despite significant progress in the field, remains unclear [3]. Researchers came up with many hypotheses that try to explain the disease process. Currently, there are few well recognized and acceptable theories, one of which is *β-amyloid hypothesis* [4]. As the major cause of the disease it indicates the accumulation of aberrant, misfolded β-amyloid (Aβ) peptide in the central nervous system (CNS) [5,6]. Aβ is a toxic polypeptide built from 37 to 43 amino acids that is formed by a proteolysis of the amyloid precursor protein (APP). APP can be metabolized in two

* Corresponding author.
E-mail address: mfmalaws@cyf-kr.edu.pl (B. Malawska).
[1] These authors contributed equally to this work.

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pathways: non-amyloidogenic and amyloidogenic. In the latter, APP is firstly cleaved by β-secretase (β-site APP-cleaving enzyme 1, BACE1) into a soluble amyloid precursor protein beta (sAPPβ) and polypeptide C-99. Subsequently γ-secretase cleaves polypeptide C-99, yielding fibrillogenic β-amyloid peptides, that consist of 37–43 amino acids [7–9]. The most toxic of them, 42-unit fragments, are able to aggregate and accumulate extracellular, forming Aβ plaques and leading to multidirectional neurotoxicity and neuron death. Exposition to Aβ causes oxidative stress and inflammatory damage [10,11]. Moreover, some evidence indicate that intracellular tangles of tau proteins accompanying AD could be created as a response to the formation of Aβ [12]. However, researchers are not sure which comes first, "the chicken or the egg" since there are as many reports suggesting primary role of tau protein in the pathogenesis of AD [13–15]. Nonetheless, there is no doubt about the critical role of Aβ in the ethiopathogenesis and development of AD. Therefore, preventing the formation of Aβ seems to be the reasonable objective for treating of AD. If we analyze a process of Aβ formation we can assume that BACE1, which is involved in the first and rate-limiting step of this process, is particularly attractive biological target [16,17].

Cholinergic hypothesis assumes that memory impairments in AD are associated with the damage of cholinergic neurons in the CNS [18,19]. This results in a reduction of cholinergic neurotransmission leading to decline in memory, problems with communication, time and space disorientation and others. In a healthy brain, the action of acetylcholine (ACh) is terminated mainly by acetylcholinesterase and additionally by butyrylcholinesterase (AChE, E.C. 3.1.1.7, and BuChE, E.C. 3.1.1.8, respectively). However, with the progression of AD, the level of AChE significantly decreases. The activity of BuChE, on the other hand, is increased, especially in hippocampus and temporal cortex [7,20]. Although AChE is recognized as a symptomatic drug target, numerous studies have shown that this enzyme plays also non-enzymatic roles in neurite growth, differentiation, adhesion and also synaptic maintenance. Furthermore, according to some *in vitro* studies, AChE can initiate the formation of Aβ fibrils and Aβ plaques [21,22]. This process is mediated by the interaction between Aβ and the peripheral anionic site (PAS) of AChE. These findings led to the development of dual-binding site AChE inhibitors, able to block both catalytic active site (CAS) and peripheral binding site (PAS), as potential anti-AD agents [23–25].

Currently there are only four drugs used in the pharmacotherapy of AD: donepezil, rivastigmine, galantamine and memantine [26]. Three of them are AChE inhibitors that improve cholinergic neurotransmission in the brain by inhibition of acetylcholine degradation [27,28]. Memantine is used complementarily and acts by blocking *N*-methyl-D-aspartate (NMDA) receptors. These drugs do not cure the disease but only delay the progression of its symptoms [29]. This makes AD treatment one of the biggest unmet medical needs and one of the biggest challenges for pharmaceutical research. Regarding current status of anti-AD drugs in clinical trials (phase II and III) most of them focus on β-amyloid as biological target. These include vaccines, antibodies and inhibitors or modulators of γ- and β-secretases. It is interesting to note that among small molecules in ongoing phase III clinical trials, two compounds belong to the class of non-peptidomimetic inhibitors of BACE1 [30].

Diseases with multifactorial pathophysiology, such as AD, may require treatment that modulates more than one biological target simultaneously (polypharmacology). An alternative approach for traditional cocktail of drugs and multicomponent drugs is the strategy that utilizes multi-target-directed-ligands (MTDLs). MTDLs are compounds that can act on two or more independent biological targets. Over the last years, a number of multifunctional ligands for the potential treatment of AD have been developed [31–33]. Most of them are AChE and/or BuChE inhibitors endowed with some additional biological properties such as: Aβ-aggregation inhibition, antioxidant or metal-chelating activity. Although many of these molecules showed promising activity *in vitro*, only some were active *in vivo* in preclinical (ladostigil [34], bis-(7)-tacrine [35] and memoquin [36]) or even clinical studies (ladostigil) [34,37–39].

Among MTDL, compounds with inhibitory activity towards symptomatic and disease modifying targets - cholinesterases and BACE1 - seem to be of a special interest. In Fig. 1 we present few examples of such compounds (**I**–**IV**) with balanced potencies against both biological targets [40–43].

In this paper we describe the design, synthesis and biological evaluation of phthalimide or saccharin derivatives linked by different alicyclic fragments with phenylalkylmoieties, as novel multi-target anti-AD agents. *In vitro* evaluation of the compounds included the assessment of AChE, BuChE, BACE1 and Aβ aggregation inhibitory activity. Furthermore, molecular modeling studies were carried out to investigate binding mode of the compounds with AChE and BACE1 and structure-activity relationship of these new compounds.

2. Results and discussion

2.1. Design

Our research interests focus on multi-functional compounds against Alzheimer's disease [44–47]. Based on previous experience we aimed at developing new MTDLs with cholinesterases and BACE1 inhibitory activity as well as Aβ anti-aggregation activity. Recently, we have reported two lead structures derived from donepezil - compounds **V** and **VI** (Fig. 2). Compound **V** [45] was found to be a potent and selective human AChE inhibitor (IC_{50} = 0.268 μM) with anti-Aβ aggregation activity (65.96% at 10 μM) and neuroprotective effect against Aβ toxicity at 1 μM and 3 μM. Compound **VI** [47] was the most potent and selective human AChE inhibitor in the series (IC_{50} = 33 nM) with the ability to inhibit Aβ aggregation (22.19% at 10 μM). Molecular modeling showed that compounds **V** and **VI** bind concomitantly to both catalytic and peripheral active site of AChE. The phthalimide or saccharin fragments were engaged in the interactions with PAS while the benzylamine fragment created interactions with amino acids of CAS of AChE. Starting with these findings, we modified structures of compounds **V** and **VI** with an idea of expanding their biological activity on BACE1 (Fig. 2). Instead of a simple alkyl chain we introduced hexahydropyrimidine, piperazine or 3-aminopiperidine as diaminoalkyl motives. We assumed that these alicyclic amines containing two basic nitrogen atoms would provide interactions with the catalytic dyad of BACE1 that is composed of two aspartic acid residues: Asp32 and Asp228. Piperazine and pyrimidine-diamine fragments were reported previously as responsible for the interactions with BACE1 [43,48]. Benzylamine fragment was modified by the introduction of one or two fluorine atoms that are abundant in BACE1 inhibitors [49].

2.2. Chemistry

The general procedure for the synthesis of the target compounds **23**–**48** is presented in Scheme 1. Compounds **1**–**6** were prepared by alkylation of saccharin and phthalimide salts with the appropriate α,ω-dibromoalkanes. *N*-phenyl-alkyl-piperazine derivatives (**7**–**12**) were obtained in a reaction of N-alkylation of piperazine with the appropriate fluoro-substituted phenyl-alkyl halides. Hexahydropyrimidine derivatives (**15** and **16**) were synthesized in a two-step pathway. In the first step, propane-1,3-diamine reacted with 1-(chloromethyl)-2-fluorobenzene or 1-

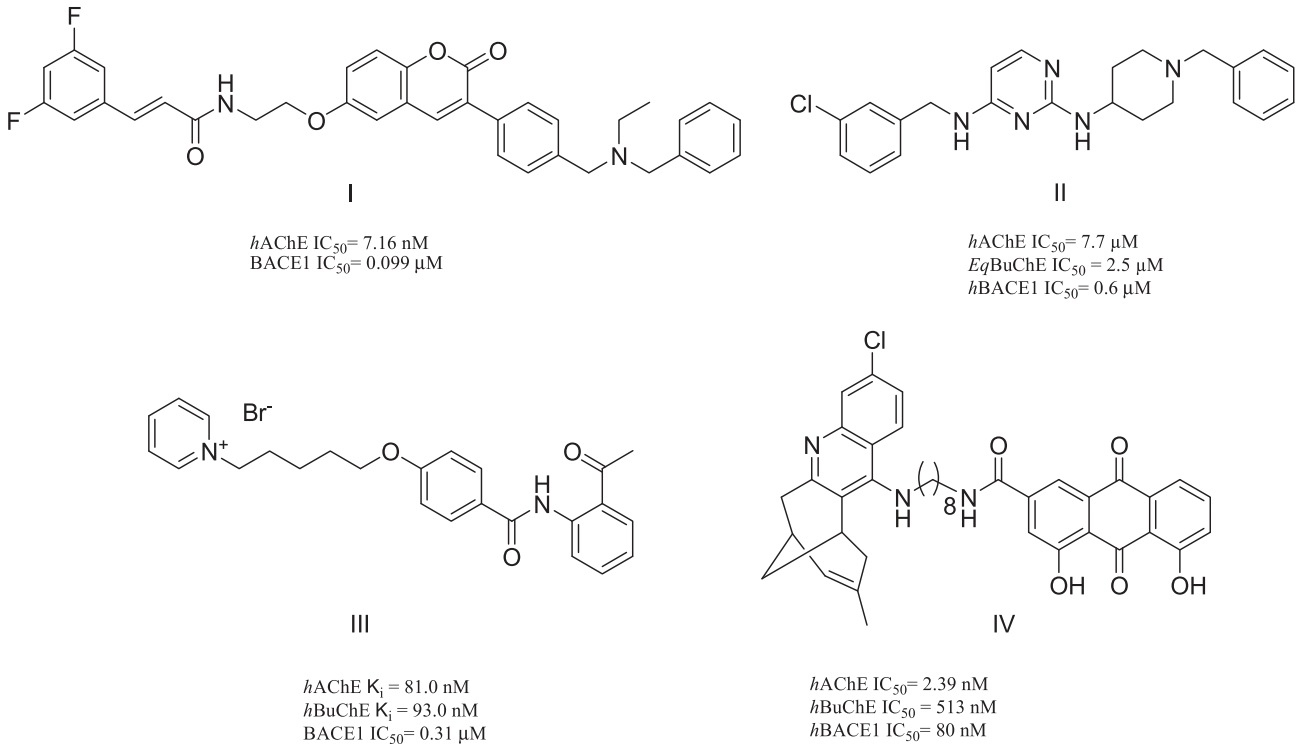


Fig. 1. Previously described, selected MTDLs, inhibitors of AChE and BACE1.

(bromomethyl)-3,5-difluorobenzene to give compounds **13** and **14**. In the next step, the reaction of **13** and **14** with formaldehyde led to the formation of the hexahydropyrimidine ring and gave compounds **15** and **16**. To obtain piperidine-3-amine derivatives **20**–**22** at first *tert*-butyl piperidin-3-ylcarbamate was alkylated with fluorine-substituted or non-substituted benzyl halides to give intermediates **17**–**19**. Boc-deprotection of **17**–**19** resulted in primary amines **20**–**22**. Finally, compounds **1**–**6** were used as alkylating agents in the reaction of nucleophilic substitution with *N*-phenyl-alkyl-heterocyclic derivatives (**7**–**12**, **15**, **16**, **20**–**22**) and provided products **23**–**48**.

To obtain *N*-benzylpiperidine-3-amine derivatives **51** and **52** we utilized method depicted in Scheme 2. At first, *tert*-butyl piperidin-3-ylcarbamate was alkylated with 2-(2-bromoethyl)isoindoline-1,3-dione. The resulting compound **49** was then Boc-deprotected and the obtained primary amine **50** was alkylated by 1-(chloromethyl)-2-fluorobenzene or 1-(bromomethyl)-3,5-difluorobenzene to give final products **51** and **52**.

The method described above failed in case of saccharin derivatives **64** and **65**. One of the semi-products contained a primary amine group and a saccharine moiety, what caused self-aminolysis and, as a consequence, an opening of the saccharin ring. Therefore, we developed a synthetic pathway where we introduced a saccharin moiety in the last possible reaction step (Scheme 2). First, *tert*-butyl piperidin-3-ylcarbamate was alkylated with 2-bromoethyl acetate to give compound **53**. Deprotection and subsequent alkylation of the resulting primary amine with either 1-(chloromethyl)-2-fluorobenzene or 1-(bromomethyl)-3,5-difluorobenzene gave compounds **54** and **55**. In the next step secondary amine groups of compounds **54** and **55** were Boc-protected in the reaction with di-*tert*-butyl dicarbonate. The resulting esters **56** and **57** were then hydrolyzed to alcohols **58** and **59** which were then reacted with methanesulfonyl chloride in anhydrous dichloromethane in the presence of triethylamine. The obtained mesylates **60** and **61** were used to alkylate sodium saccharin to get compounds **62** and **63**. The final products **64** and **65** in form of hydrochloride salts were obtained by Boc-deprotection with HCl in ethyl acetate. Purity and structures of the obtained compounds were confirmed by chromatographic (TLC, LC-MS) and spectroscopic (NMR) methods.

2.3. Biological activity

2.3.1. Inhibition of cholinesterases and BACE1

We determined the cholinesterase inhibitory profiles of the synthesized derivatives using the method established by Ellman et al. [50]. Compounds were evaluated against AChE from *electric eel* (*Ee*AChE) and BuChE from *equine serum* (*Eq*BuChE). In the first step, we performed assays using a 10 μM screening concentration of the tested compounds. We determined IC_{50} value for compounds which exhibited inhibitory potency higher than 50% at 10 μM. Tacrine and donepezil were used as the reference compounds. Results of the biological evaluation are presented in Table 1.

All the compounds were also evaluated against human recombinant BACE1 (*h*BACE1) (see Table 1). A biochemical spectrofluorometric assay (fluorescence resonance energy transfer, FRET-based), which utilizes the cleavage of a peptide substrate mimicking the human APP sequence with the Swedish mutation, was used [17]. As a reference we used well-known inhibitor of BACE1 – **Inhibitor IV** – for which we established the IC_{50} value of 0.046 μM that is in accordance with the published data [51]. The compounds were tested at 50 μM concentration and their activities were reported as percentage of *h*BACE1 inhibition (Table 1).

The assay was purchased from Life TechnologiesWe found that nine of piperazine and one of 3-aminopiperidine derivatives showed activity against *Ee*AChE with IC_{50} values ranging from 0.830 μM to 19.18 μM. Their potency was comparable with the literature value of IC_{50} for galantamine (IC_{50} = 0.623–0.665 μM) [52,53] but lower in comparison with compounds **V** and **VI** as well

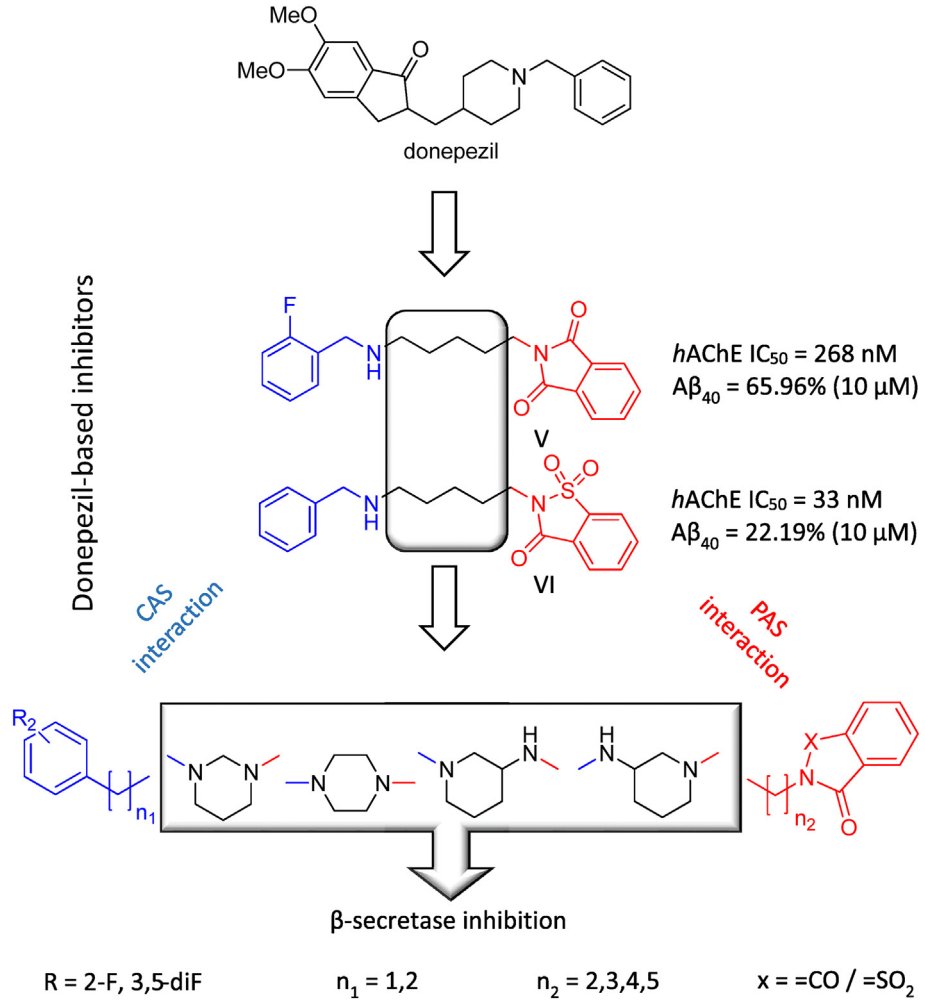


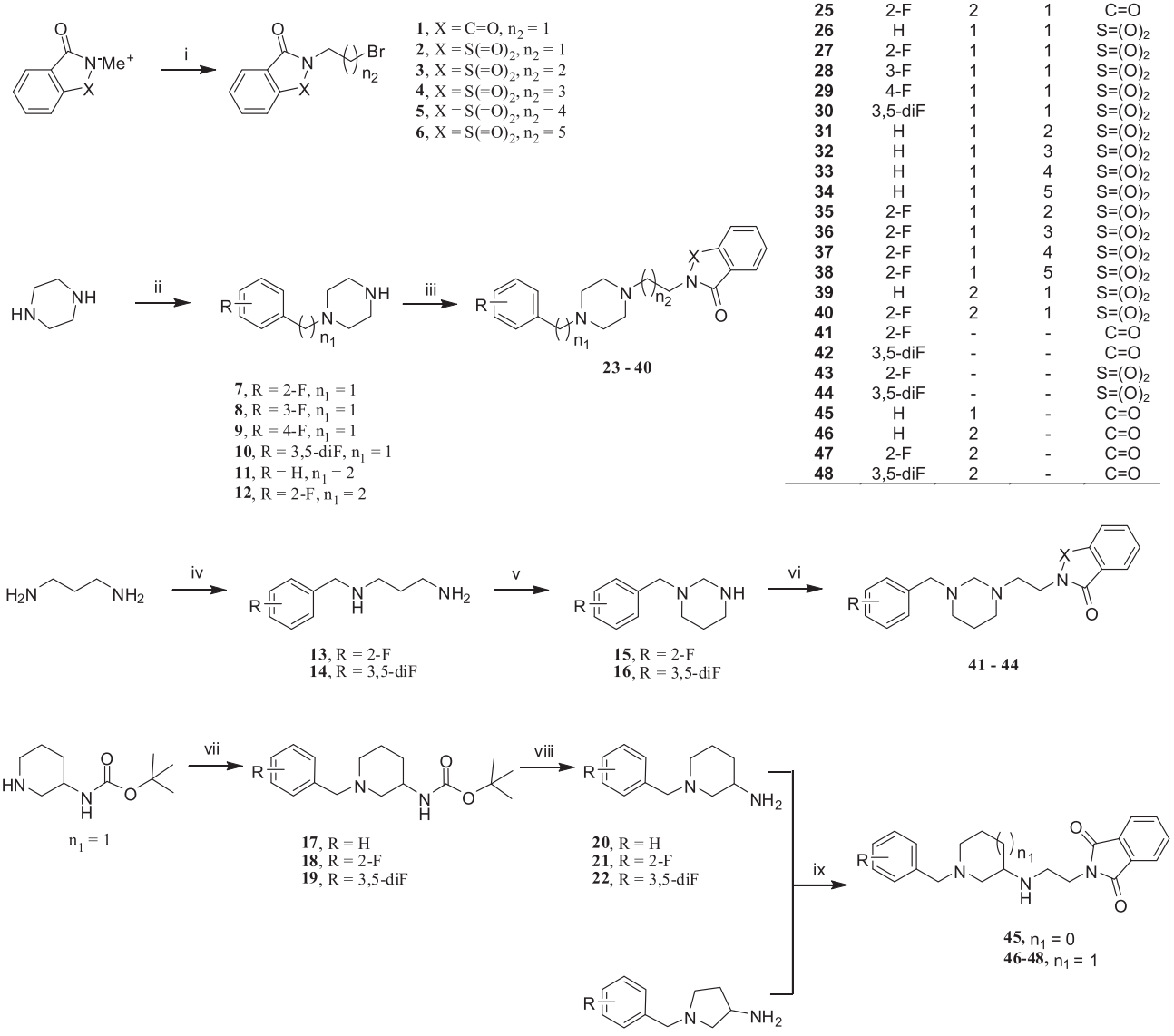
Fig. 2. Design strategy of new phthalimide and saccharin derivatives with alicyclic amines targeting cholinesterases, BACE1 and Aβ aggregation.

as references tacrine and donepezil. Other compounds were rather weak inhibitors (18.40%–43.06% inhibition at 10 μM) or were deprived of activity. From the presented data several conclusions can be drawn regarding: (1) the presence of saccharin or phthalimide, (2) length of the carbon linker between saccharin/phthalimide moiety and alicyclic amine ring, (3) various effects of substitution at the phenyl ring. As the majority of the active compounds belong to piperazine derivatives, it can be concluded that among the tested alicyclic fragments, piperazine is the optimal one. Furthermore, all the potent piperazine derivatives contain saccharin which is superior to phthalimide. The effect of the length of carbon linker between saccharin and piperazine fragment depends on the substitution pattern at the phenyl ring. Among the unsubstituted compounds **26**, **31**–**34**, the most active were **26** with ethylene linker and **32** with buthylene linker. Within 2-fluorine substituted compounds **27**, **35**–**38** the most potent were **36, 37** and **38** with buthylene, pentylene and hexylene linkers, respectively. The most significant decrease in activity was observed for compounds **31** and **35** with propylene linkers. The most potent compound was **26** with unsubstituted phenyl ring. None of the modifications within the phenyl ring (2-F, 3-F, 4-F, 3,5-di-F) brought beneficial effect to the inhibitory potency. The slightest decrease of potency was observed for 3-F and 4-F substituted compounds **28** and **29**. A major decrease of activity was noticed when the linker connecting piperazine and phenyl ring was elongated from methylene to ethylene (**26** vs. **39** and **27** vs. **40**). It is worth noting that the rearrangement of 3-aminopiperidine fragment by setting the secondary amine in the direction of benzylamine brought activity to 3,5-difluoro substituted compound **52** (Fig. 3). All of the *Ee*AChE inhibitors were selective for this enzyme since none of the tested compounds reached 50% of inhibition at the screening concentration (10 μM) against *Eq*BuChE.

Inhibitory potencies of the tested compounds towards *h*BACE1 were ranging from 21.74% to 61.42% at 50 μM concentration with the most potent derivative of saccharine **65** bearing 3-aminopiperidine core and 3,5-difluoro substituted *N*-benzylamine moiety. Considering rather similar results for all the compounds it is hard to discuss their structure-activity relationship.

2.3.2. Kinetic study of AChE inhibition

As compound **26** showed the highest potency toward *Ee*AChE, it was selected as a representative for kinetic studies to gain insight into the mode of inhibition. Analysis of the Lineweaver-Burk reciprocal plot (1/*V* versus 1/S) (Fig. 4A) showed that compound **26** displays linear non-competitive inhibition, as increased slopes and preserved intercepts at increasing concentrations of the inhibitor were observed. Non-competitive type of inhibition was further confirmed by Cornish – Bowden plot (S/*V* versus [I]) (Fig. 4B). Non-competitive inhibition indicates preferential interactions with the PAS rather than the CAS of the enzyme and is also characteristic for donepezil [54].



Cmp.	R	n_1	n_2	X
23	2-F	1	1	C=O
24	3,5-diF	1	1	C=O
25	2-F	2	1	C=O
26	H	1	1	$S=(O)_2$
27	2-F	1	1	$S=(O)_2$
28	3-F	1	1	$S=(O)_2$
29	4-F	1	1	$S=(O)_2$
30	3,5-diF	1	1	$S=(O)_2$
31	H	1	2	$S=(O)_2$
32	H	1	3	$S=(O)_2$
33	H	1	4	$S=(O)_2$
34	H	1	5	$S=(O)_2$
35	2-F	1	2	$S=(O)_2$
36	2-F	1	3	$S=(O)_2$
37	2-F	1	4	$S=(O)_2$
38	2-F	1	5	$S=(O)_2$
39	H	2	1	$S=(O)_2$
40	2-F	2	1	$S=(O)_2$
41	2-F	-	-	C=O
42	3,5-diF	-	-	C=O
43	2-F	-	-	$S=(O)_2$
44	3,5-diF	-	-	$S=(O)_2$
45	H	1	-	C=O
46	H	2	-	C=O
47	2-F	2	-	C=O
48	3,5-diF	2	-	C=O

Scheme 1. Synthesis of compounds **23**–**48**. Reagents and conditions: i) α,ω-dibromoalkane, acetonitrile, K_2CO_3, 80 °C, 24 h; ii) phenyl-alkyl-halide derivative, acetonitrile, R.T., 24 h; iii) compounds **1**–**6**, acetonitrile, K_2CO_3, 80 °C, 24 h; iv) 1-(chloromethyl)-2-fluorobenzene/1-(bromomethyl)-3,5-difluorobenzene, acetonitrile, 0 °C/R.T., 20 h; v) formaldehyde, acetonitrile/water, R.T., 1 h; vi) compounds **1** or **2**, DMF, K_2CO_3, 110 °C, 24 h; vii) benzyl halide derivative, acetonitrile, K_2CO_3, R.T., 44 h; viii) HCl, methanol, R.T., 24 h; ix) **1**, acetonitrile, K_2CO_3, 80 °C, 24 h.

2.3.3. *Inhibition of Aβ$_{1-42}$ aggregation*

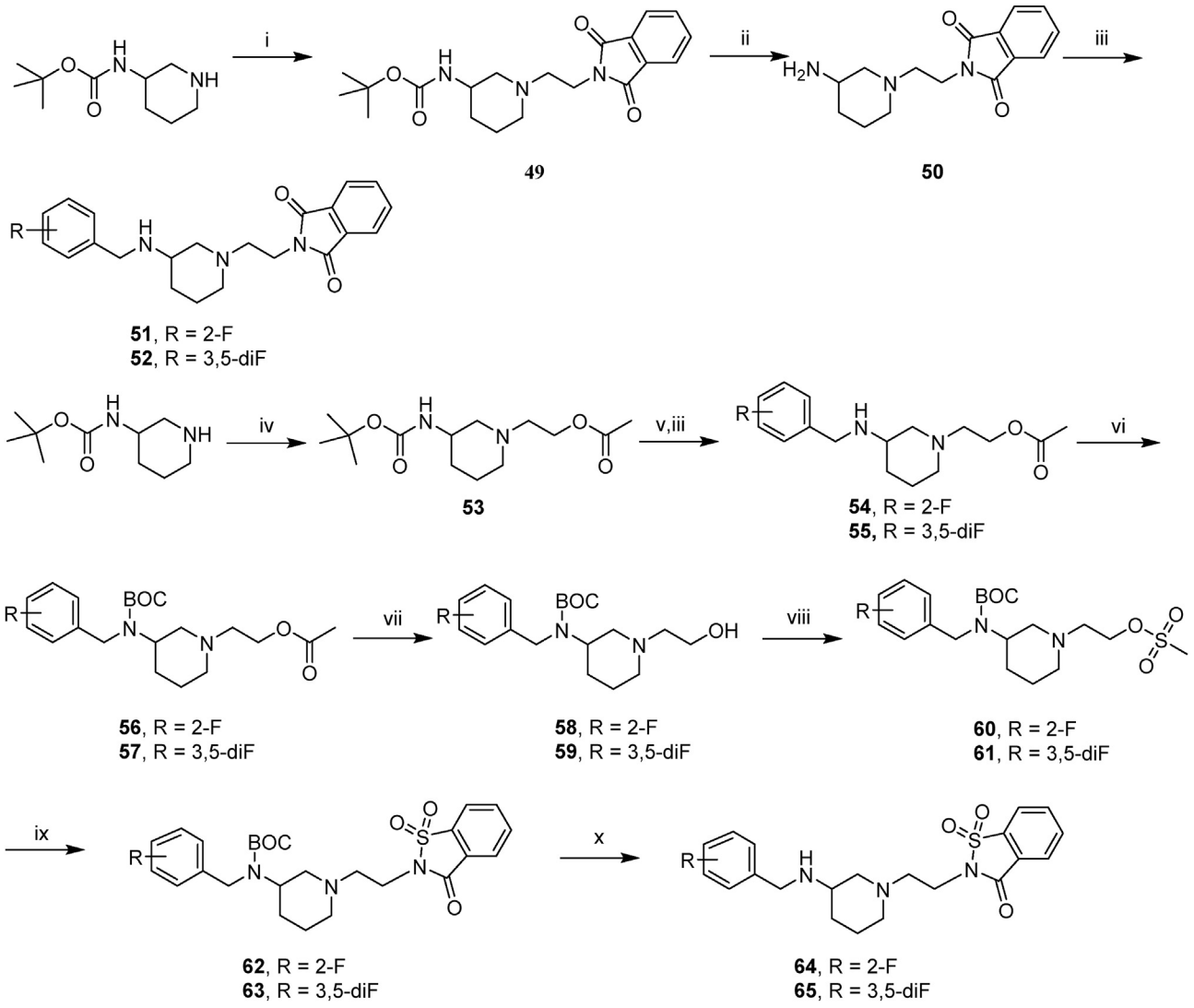
The ability of the target compounds to inhibit Aβ aggregation was tested in Thioflavin-T based assay [55] (Table 2). As a screening concentration we used 10 μM, the same as in Ellman's assay. Although donepezil has been described as a weak inhibitor in this assay [45] we tested it for the comparison with our compounds which were structurally derived from this drug. Most of the tested compounds showed inhibitory activity less than 10%, but for five of them (**23**, **27**, **40**, **44** and **52**) we found activities ranging from 13.3% to 39.0%. These activities are comparable or higher with that of donepezil but not so high as our previous inhibitor (**V**) [45].

2.3.4. *Blood-brain barrier (BBB) permeation*

The CNS is the expected site of action of the compounds presented here therefore it is essential to know if they permeate the BBB. The ability of the selected representative compounds to cross BBB was studied using a parallel artificial membrane permeability assay (PAMPA) [56,57]. Nine standard drugs with known BBB accessibility were used as the references. The following ranges of permeability (P_e 10^{-6} cm s^{-1}) were established: $P_e > 4.0$ for compounds with predicted high BBB permeability, $P_e \leq 2.0$ for compounds with predicted low permeability and $2.0 > P_e \geq 4.0$ for compounds with uncertain BBB permeability (Table 3). Compounds **32**, **36** and **37** with the values of P_e over 4.0 showed high probability to cross the BBB via passive diffusion. Compounds **26** and **29** fall into the interval of P_e values between 2.0 and 4.0 therefore their ability to permeate through BBB is uncertain.

2.4. *Docking studies on AChE and BACE1*

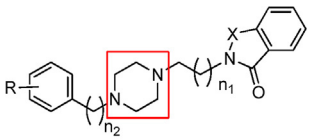
Novel compounds were docked into the *Torpedo californica* AChE crystal structure as well as to human BACE1 to find the possible



Scheme 2. Synthesis of compounds **51**, **52**, **64** and **65**. Reagents and conditions: i) 2-(2-bromoethyl)isoindoline-1,3-dione, acetonitrile, K_2CO_3, NaI, 80 °C, 24 h, ii) TFA, dichloromethane, R.T., 4 h, iii) 1-(chloromethyl)-2-fluorobenzene/1-(bromomethyl)-3,5-difluorobenzene, acetonitrile, K_2CO_3, 80 °C, 24 h, iv) 2-bromoethyl acetate, acetonitrile, K_2CO_3, 80 °C, 24 h; v) TFA, dichloromethane, R.T., 2 h; vi) di-*tert*-butyl dicarbonate, TEA, dichloromethane, R.T., 2 h; vii) methanol-water mixture, K_2CO_3, 65 °C, 2 h, viii) methanesulfonyl chloride, dichloromethane, TEA, R.T., 2 h; ix) sodium saccharin, acetonitrile, K_2CO_3, 80 °C, 24 h; x) 1 M HCl in ethyl acetate, R.T., 24 h.

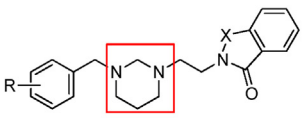
binding mode and to explain the reasons for varied potencies. We used the previously developed methods for docking to both targets and assessment of binding modes [58,59]. In the case of AChE all derivatives were arranged along the active gorge and bound to the catalytic active site as well as peripheral anionic site. The binding mode of the most active inhibitor **26** is shown in Fig. 5. The benzyl moiety was responsible for π-π stacking with Trp84 in the CAS. The protonated piperazine nitrogen atom forms cation-π interactions with Phe330 and a hydrogen bond network with Tyr121 via a water molecule. The other part of piperazine ring and short two carbon linker interacted with aromatic side chains of Phe290, Phe331, and Tyr334 in the middle of the active gorge. The saccharin fragment was engaged in π-π stacking with Trp279 and CH-π interactions with Tyr70 in the PAS. The hydrogen bonds were formed by the carbonyl group with Tyr121 and one oxygen atom from sulfone with main chain of Phe288 via water molecules. Such binding mode provided the highest activity against AChE for this compound. It was observed that other compounds from piperazine series gave worse fit to the active gorge, that might result in lower potency. Introduction of one or two fluorine atoms on the benzyl or phenethyl moiety did not provide any extra interactions. Moreover, 3,5-substitution led to unbeneficial excessive occupation of limited space close to the catalytic triad (Ser200, His440, Glu327). Phthalimide established weaker hydrogen bonds compared to saccharin and thus led to diminished activity. With regard to the length of linker it seems that optimal tether between phenyl ring and piperazine moiety is equal one methylene group. The chain between piperazine and saccharin/phthalimide might be of different length due to its fitting to enzyme by conformational changes. However, it seems that two carbon linker (compound **26** and **28**) and four carbon (**32** and **36**) gave a little bit better fit than the others. It is also worth to note that linearity of molecules provided by di-substituted piperazine is very important for binding to AChE. The compounds from series of hexahydropyrimidine and 3-aminopiperidine derivatives were not linear. Such geometry of their molecules resulted in non-optimal fit into the active site and brought significantly reduced activity. Moreover, in case of series A of 3-aminopiperidine derivatives (**47**, **48**) the protonation of secondary amine group led to the lack of cation-π interaction with Phe330. The strength of binding with AChE was assessed by

Table 1
Inhibition of *Ee*AChE, *Eq*BuChE, *h*BACE1 by compounds **23**–**48**, **51**, **52**, **64** and **65**.[a]



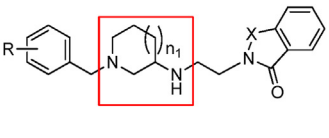
piperazine derivatives

23 - 40



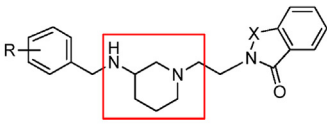
hexahydropyrimidine derivatives

41 - 44



3-aminopiperidine and 3-aminopyrrolidine derivatives

45 - 48



3-aminopiperidine derivatives

51, 52, 64, 65

Cmp.	R	X	n_1	n_2	*Ee*AChE	*Eq*BuChE	*h*BACE1
					IC_{50} [μM]/ % inh.[b]	IC_{50} [μM]/ % inh.[c]	% inh.[d]
piperazine derivatives							
23	2-F	=CO	1	1	41.82% ± 2.83	<10%	38.06% ± 7.98
24	3,5-diF	=CO	1	1	<10%	14.22% ± 4.21	45.00% ± 6.73
25	2-F	=CO	1	2	<10%	20.28% ± 4.09	48.36% ± 7.44
26	H	$=S(O)_2$	1	1	0.830 ± 0.02	22.15% ± 2.11	33.61% ± 3.27
27	2-F	$=S(O)_2$	1	1	17.80 ± 0.12	16.12% ± 6.60	36.01% ± 2.01
28	3-F	$=S(O)_2$	1	1	2.14 ± 0.08	<10%	28.61% ± 4.01
29	4-F	$=S(O)_2$	1	1	6.28 ± 0.30	47.49% ± 0.77	36.00% ± 10.09
30	3,5-diF	$=S(O)_2$	1	1	19.18 ± 0.11	11.00% ± 1.63	42.03% ± 4.78
31	H	$=S(O)_2$	2	1	34.16% ± 2.49	11.74% ± 2.20	50.70% ± 3.45
32	H	$=S(O)_2$	3	1	3.01 ± 0.09	41.45% ± 0.99	37.31% ± 5.21
33	H	$=S(O)_2$	4	1	43.06% ± 2.43	30.39% ± 2.06	21.74% ± 0.23
34	H	$=S(O)_2$	5	1	41.74% ± 1.03	41.8% ± 2.68	45.44% ± 5.79
35	2-F	$=S(O)_2$	2	1	<10%	12.01% ± 1.79	32.74% ± 4.96
36	2-F	$=S(O)_2$	3	1	4.25 ± 0.17	29.56% ± 4.46	28.84% ± 9.11
37	2-F	$=S(O)_2$	4	1	7.36 ± 0.18	21.94% ± 3.73	39.66% ± 3.33
38	2-F	$=S(O)_2$	5	1	8.43 ± 0.28	31.57% ± 3.79	36.17% ± 9.82
39	H	$=S(O)_2$	1	2	<10%	18.43% ± 1.45	36.55% ± 4.08
40	2-F	$=S(O)_2$	1	2	<10%	11.32% ± 3.61	36.71% ± 3.28
hexahydropyrimidine derivatives							
41	2-F	=CO	–	–	<10%	27.76% ± 3.91	31.72% ± 1.20
42	3,5-diF	=CO	–	–	<10%	<10%	28.04% ± 4.37
43	2-F	$=S(O)_2$	–	–	18.40% ± 2.84	21.60% ± 4.12	32.96% ± 6.37
44	3,5-diF	$=S(O)_2$	–	–	25.42% ± 3.68	16.41% ± 3.61	26.71% ± 4.76
3-aminopyrrolidine derivative							
45	H	=CO	0	–	25.7% ± 1.36	21.38% ± 2.66	36.01% ± 2.61
3-aminopiperidine derivatives (Series A)							
46	H	=CO	1	–	<10%	16.04% ± 2.03	37.04% ± 1.38
47	2-F	=CO	1	–	18.95% ± 4.61	28.34% ± 5.37	36.32% ± 3.82
48	3,5-diF	=CO	1	–	<10%	14.19% ± 1.22	36.34% ± 1.57
3-aminopiperidine derivatives (Series B)							
51	2-F	=CO	–	–	15.28% ± 4.61	<10%	45.01% ± 1.38
52	3,5-diF	=CO	–	–	6.47 ± 0.25	20.01% ± 1.43	26.30% ± 1.61
64	2-F	$=S(O)_2$	–	–	<10%	<10%	30.00% ± 6.40
65	3,5-diF	$=S(O)_2$	–	–	<10%	<10%	61.42% ± 2.64
reference compounds							
Tacrine	–	–	–	–	0.023 ± 0.0004	0.002 ± 0.0001	n.d.[f]
Donepezil	–	–	–	–	0.011 ± 0.0002	1.83 ± 0.04	n.d.[f]
Inhibitor IV[e]	–	–	–	–	n.d.[f]	n.d.[f]	0.046 ± 0.001[g]

[a] Values are expressed as means ± the standard error of the mean (SEM) of at least three experiments (n = 3), each performed in triplicate (*Ee*AChE, *Eq*BuChE and *h*BACE1 inhibition).
[b] IC_{50} inhibitory concentration of AChE from *electric eel* or percent inhibition with inhibitor at 10 μM.
[c] IC_{50} inhibitory concentration of BuChE from horse serum or percent inhibition with inhibitor at 10 μM.
[d] IC_{50} inhibitory concentration of human recombinant *h*BACE1 and substrate (Rh-EVNLDAEFK-quencher) or percent inhibition with inhibitor at 50 μM.
[e] Calbiochem, Merck; Nottingham, UK.
[f] Not determined.
[g] IC_{50} value.

ChemScore function. The most active derivative **26** obtained value equal to 44.84 which was still lower than the score for donepezil (49.48). This stays in accordance with the results of the biological assay.

The investigated compounds revealed diverse binding modes within the active site of BACE1. The basic nitrogen atoms of alicyclic amines such as hexahydropyrimidine, piperazine or 3-aminopiperidine interacted with the active-site aspartate residues Asp32 and Asp228 (catalytic dyad). The distance between these basic centers and catalytic aspartates was different for more and less active compounds. In case of the more potent derivatives (**65**, **24**, **25**, **31**, **34** and **51**) it was shorter while the less active inhibitors (most of the remaining compounds) were more distant from dyad. The remaining parts of molecules were located in the neighboring pockets (S2′, S2 and S3). The binding mode of the most active inhibitor **65** is shown in Fig. 6. The protonated secondary amine group created salt bridge with Asp228 as well as hydrogen bond with Gly34. The substituted benzyl moiety occupied S2′ pocket and

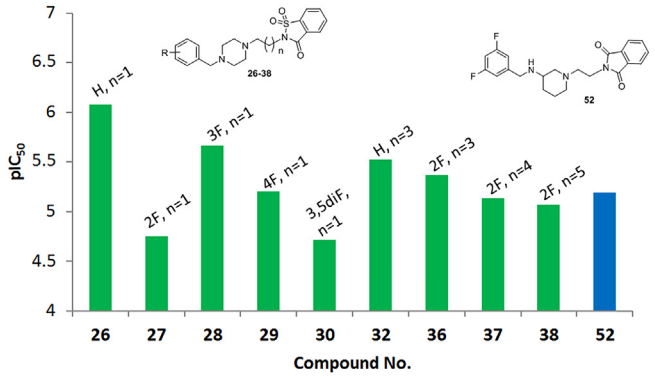


Fig. 3. Comparison of *Ee*AChE pIC_{50} values of the most active compounds.

Table 2
Inhibition of Aβ-aggregation by compounds **23**, **27**, **40**, **44** and **52**.

Compound	Aβ aggregation % inh[a,b]
23	13.3 ± 6.5
27	18.6 ± 7.4
40	15.0 ± 2.7
44	13.5 ± 3.6
52	39.0 ± 3.4
Donepezil	13.80 ± 6.8

[a] % Inhibition of $A\beta_{1-42}$ aggregation (10 μM compound concentration, 1.5 μM $A\beta_{1-42}$). Experiment was performed in quadruplicates.
[b] ($p < 0.05$) statistically different compared to control experiments ($A\beta_{1-42}$ alone); one-way analysis of variance (ANOVA), followed by *post hoc* Bonferroni *t*-test (SigmaPlot v 12.0).

interacted with Tyr71, Val69 and Tyr198. The saccharin fragment was surrounded by the following residues: Asn233 and Arg235 from S2 pocket as well as Thr231 at the edge of S3 pocket. The low occupancy of S3 site may be the reason of relatively low activity due to the importance of binding within this pocket for high inhibition of BACE1 [60]. The other compounds with at least 45% inhibition of enzyme, such as inhibitor **24**, **25** or **51**, presented similar binding mode as compound **65** but one main change appeared. In these cases, saccharin was replaced by phthalimide, and this moiety was slightly shifted towards the center of S3 pocket. However, this did not improve the activity because the phthalimide was not engaged in any highly specific interactions. In case of compounds with lower activity (*e.g.* **28, 40**, **42**, **44**) the orientation of the molecules in the active site was reversed. The benzyl moiety occupied the S3 pocket instead of S2'. This can be non-specific nature of binding interactions and may be responsible for reduced potency. The strength of binding with BACE1 was assessed by GoldScore function. The most active compound **65** obtained value 69.16 in comparison with 102.69 for the reference ligand - NVP-BXD552 [60].

Table 3
In vitro blood-brain barrier permeability prediction for compounds: **26**, **29**, **32**, **36** and **37**.

Compound	BBB penetration estimation	
	P_e ± SEM ($*10^{-6}$ cm s^{-1})	CNS (+/−)[a]
26	3.75 ± 0.7	CNS (+/−)
29	3.65 ± 0.1	CNS (+/−)
32	10.6 ± 1.1	CNS (+)
36	11.0 ± 1.5	CNS (+)
37	9.6 ± 1.4	CNS (+)
Donepezil	7.3 ± 0.9	CNS (+)
Rivastigmine	6.6 ± 0.5	CNS (+)
Tacrine	5.3 ± 0.19	CNS (+)
Testosterone	11.3 ± 1.6	CNS (+)
Chlorpromazine	5.6 ± 0.6	CNS (+)
Hydrocortisone	2.85 ± 0.1	CNS (+/−)
Piroxicam	2.2 ± 0.15	CNS (+/−)
Theophylline	1.07 ± 0.18	CNS (−)
Atenolol	1.02 ± 0.37	CNS (−)

CNS (−) (low BBB permeation predicted); Pe (10-6 cm s^{-1}) ≤ 2.0.
CNS (+/−) (BBB permeation uncertain); P_e (10^{-6} cm s^{-1}) ≥ 4.0 > 2.0.
[a] CNS (+) (high BBB permeation predicted); P_e (10^{-6} cm s^{-1}) > 4.0.

3. Conclusions

Multi-target-directed ligand approach is widely used in the search for new treatment of Alzheimer's disease that remains unresolved therapeutic problem. Our previous studies resulted in obtaining saccharin and phthalimide derivatives as MTDLs with cholinesterase inhibitory activity and anti-Aβ aggregation properties. In the project presented herein we have tried to endow the selected lead compounds **- V** and **VI -** with BACE1 inhibitory activity. Therefore we introduced in their structure fragments containing diaminoalkyl motives that could provide interactions with the catalytic site of BACE1. The activity of all the synthesized compounds was evaluated against *ee*AChe, *eq*BuChE and *h*BACE1, we have also determined their Aβ anti-aggregating properties and ability to permeate BBB for the selected compounds. The novel compounds display decreased anti-cholinesterase activity compering to the lead structures but they all gained BACE1 inhibitory activity. Among the synthesized and tested thirty compounds

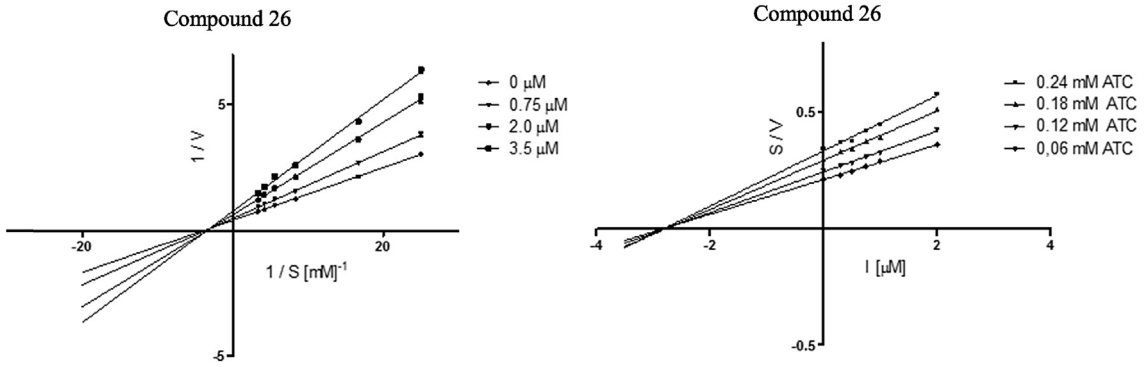


Fig. 4. Lineweaver-Burk (A) and Cornish-Bowden (B) plots illustrating non-competitive *Ee*AChE inhibition by compound **26**. S = acetylthiocholine; *V* = initial velocity rate; I = inhibitor concentration.

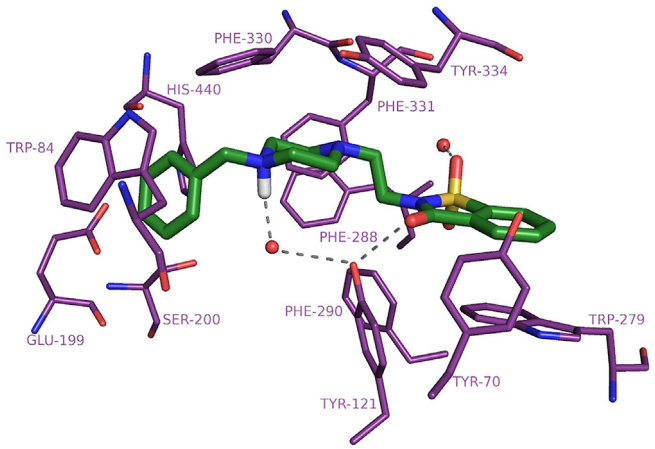


Fig. 5. The binding mode of compound **26** within the active site of AChE. The red spheres represent conserved water molecules engaged in the hydrogen bond network. Hydrogen bonds marked by grey dotted line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

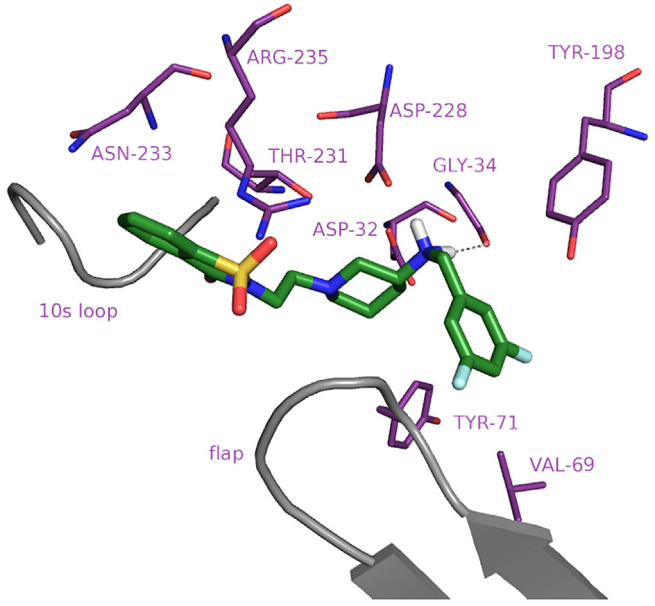


Fig. 6. The binding mode of compound **65** within the active site of BACE1. Asp32 and Asp228 constitute catalytic dyad. The 10s loop and flap are the most flexible parts of BACE1. The S2′ pocket contains Tyr71, Val69 and Tyr198. Asn233 and Arg235 belong to the S2 site, and Thr231 to the S3 pocket. Hydrogen bonds marked by grey dotted line.

we identified piperazine derivative **26** and 3-aminopiperidine derivative **52** as interesting hits for further development. Both compounds are selective AChE inhibitors (**26**: $IC_{50\ AChE} = 0.83$ μM; **52**: $IC_{50\ AChE} = 6.47$ μM) with BACE1 inhibitory activity (**26**: 33.6%; **52**: 26.3% at 50 μM). Additionally compound **52** displays the most significant Aβ anti-aggregating properties (39% at 10 μM). The SAR analysis of these series of compounds gives us directions for further development of novel multifunctional anti-AD agents.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

^{1}H NMR spectra were recorded on Varian Mercury 300 at 300 MHz. The chemical shifts for ^{1}H NMR and ^{13}C NMR are referenced to TMS via residual solvent signals ($CDCl_3$ at 7.26 ppm and 77.16 ppm DMSO-d_6 at 2.50 ppm and 39.52). Mass spectra (MS) were obtained on an UPLC-MS/MS system consisting of a Waters ACQUITY® UPLC® (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). Analytical thin layer chromatography (TLC) was done using aluminum sheets precoated with silica gel 60 F_{254}. Column chromatography was performed on Merck silica gel 60 (63–200 μm). Flash chromatography was performed on Isolera™ Spectra (Biotage). The purity of the final compounds was determined using an analytical RPLC-MS on Waters Acquity TQD using an Aquity UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm) at 214 nm and 254 nm CH_3CN/H_2O gradient with 0.1% HCOOH was used as the mobile phase at a flow rate of 0.3 mL/min. All the compounds showed purity of >95%, as determined by RPLC. All of the reagents were purchased from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) and dichloromethane (DCM) were distilled under nitrogen immediately before use. The drying agent used for THF was sodium/benzophenone ketyl, and for DCM, calcium hydride.

The following compounds: 2-(2-bromoethyl)isoindoline-1,3-dione **1** [61], 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide **2** [62], 2-(3-bromopropyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide **3** [62], 2-(4-bromobutyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide **4** [62], 2-(5-bromopentyl)benzo[*d*]isothiazol-3(2H)-one 1,1-dioxide **5** [62], 2-(6-bromohexyl)benzo[*d*]isothiazol-3(2H)-one 1,1-dioxide **6** [62], have been previously reported.

4.1.2. General procedure for the preparation of hydrochloride salts

All the final compounds were evaluated *in vitro* as hydrochloride salts. The hydrochloride salts were prepared by dissolving the compounds in a minimum quantity of ethyl acetate. Then the solution was treated with 5 M solution of HCl in propan-2-ol, evaporated under reduced pressure and dried.

4.1.3. Procedure for the synthesis of compounds 7–12 (procedure A)

To a solution of piperazine (4 equiv.) in acetonitrile a solution of the appropriate phenyl-alkyl-halide derivative was added dropwise at 0 °C. After the addition the reaction mixture was stirred at room temperature for 24 h. Concentration under reduced pressure resulted in solid residue. The crude product was purified by silica gel column chromatography in DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v).

4.1.3.1. 1-(2-Fluorobenzyl)piperazine (7). Following the procedure **A**, reaction of piperazine (237 mg, 2.76 mmol) with 1-(chloromethyl)-2-fluorobenzene (100 mg, 0.69 mmol) in acetonitrile (10 + 5 mL) was performed. Purification by column chromatography gave the product **7** (120 mg, yield 89%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v) $R_f = 0.34$. MW 194.25. Formula: $C_{11}H_{15}FN_2$. MS *m/z* 195.22 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.38 (td, $J = 7.50$, 1.67 Hz, 1H), 7.19–7.26 (m, 1H), 7.07–7.14 (m, 1H), 7.03 (ddd, $J = 9.87$, 8.34, 1.28 Hz, 1H), 3.58 (d, $J = 1.54$ Hz, 2H), 2.86–2.95 (m, 4H), 2.41–2.53 (m, 4H), 1.89 (br. s., 1H).

4.1.3.2. 1-(3-Fluorobenzyl)piperazine (8). Following the procedure **A**, reaction of piperazine (911.4 mg, 10.58 mmol) with 1-(bromomethyl)-3-fluorobenzene (500 mg, 2.65 mmol) in acetonitrile (30 + 15 mL) was performed. Purification by column chromatography gave the product **8** (417 mg, yield 81%) as a colorless oil. TLC in DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v) $R_f = 0.40$. MW 194.25. Formula: $C_{11}H_{15}FN_2$. MS *m/z* 195.22 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.21–7.30 (m, 1H), 7.02–7.12 (m, 2H), 6.87–6.98 (m,

1H), 3.47 (s, 2H), 2.85 (t, J = 4.90 Hz, 4H), 2.41 (t, J = 4.40 Hz, 4H), 1.71 (s, 1H).

4.1.3.3. 1-(4-Fluorobenzyl)piperazine (9). Following the procedure **A**, reaction of piperazine (1170 mg, 13.60 mmol) with 1-(chloromethyl)-4-fluorobenzene (414 μL, 3.4 mmol) in acetonitrile (40 + 15 mL) was performed. Purification by column chromatography gave the product **9** (579 mg, yield 88%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v) R_f = 0.28. MW 194.25. Formula: $C_{11}H_{15}FN_2$. MS *m/z* 195.22 (M +H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.26 (tt, J = 8.80, 2.90 Hz, 2H), 6.98 (tt, J = 8.80, 1.80 Hz, 2H), 3.44 (s, 2H), 2.89 (t, J = 4.70 Hz, 4H), 2.41 (t, J = 4.70 Hz, 4H), 2.13 (s, 1H).

4.1.3.4. 1-(3,5-Difluorobenzyl)piperazine (10). Following the procedure **A**, reaction of piperazine (166.4 mg, 1.932 mmol) with 1-(bromomethyl)-3,5-difluorobenzene (100 mg, 0.483 mmol) in acetonitrile (10 + 5 mL) was performed. Purification by column chromatography gave the product **10** (79 mg, yield 77%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v) R_f = 0.31. MW 212.24. Formula: $C_{11}H_{14}F_2N_2$. MS *m/z* 213.16 (M+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.85–6.91 (m, 2H), 6.68 (tt, J = 8.98, 2.31 Hz, 1H), 3.46 (s, 2H), 2.89–2.96 (m, 4H), 2.24–2.55 (m, 5H).

4.1.3.5. 1-Phenethylpiperazine (11). Following the procedure **A**, reaction of piperazine (1858 mg, 21.570 mmol) with (2-bromoethyl) benzene (731 μL, 5.393 mmol) in acetonitrile (40 + 15 mL) was performed. Purification by column chromatography gave the product **11** (791 mg, yield 77.1%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v) R_f = 0.41. MW 190.28. Formula: $C_{12}H_{18}N_2$. MS *m/z* 191.14 (M+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.08–7.25 (m, 5H), 2.84 (t, J = 5.30 Hz, 4H), 2.47–2.78 (br. s, 4H), 2.43 (br. s, 4H), 2.17 (s, 1H).

4.1.3.6. 1-(2-Fluorophenethyl)piperazine (12). Following the procedure **A**, reaction of piperazine (170 mg, 1.970 mmol) with 1-(bromoethyl)-2-fluorobenzene (100 mg, 0.492 mmol) in acetonitrile (10 + 5 mL) was performed. Purification by column chromatography gave the product **12** (88 mg, yield 86%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v) R_f = 0.40. MW 208.28. Formula: $C_{12}H_{17}FN_2$. MS *m/z* 209.24 (M+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.13–7.25 (m, 2H), 6.96–7.10 (m, 2H), 2.97 (t, J = 4.90 Hz, 2H), 2.85 (t, J = 11.30 Hz, 2H), 2.46–2.64 (m, 9H).

4.1.4. Procedure for the synthesis of compounds 13 and 14 (procedure B)

To a solution of propane-1,3-diamine (4 equiv.) in acetonitrile a solution of the appropriate phenyl-alkyl-halide derivative was added dropwise at 0 °C. After the addition the reaction mixture was stirred at room temperature for 24 h, Subsequently the mixture was concentrated under reduced pressure. Resulting residue was dissolved in 20 mL of 1 M NaOH solution and extracted with DCM (3 × 20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v), yielding a colorless oil.

4.1.4.1. N-(2-fluorobenzyl)propane-1,3-diamine (13). Following the procedure **B**, reaction of propane-1,3-diamine (1.13 mL, 13.49 mmol) with 1-(chloromethyl)-2-fluorobenzene (0.40 mL, 3.37 mmol) in acetonitrile was performed. Purification by column chromatography gave the product **13** (505 mg, yield 82%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v) R_f = 0.19. MW 182.24. Formula: $C_{10}H_{15}FN_2$. MS *m/z* 183.18 (M+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.32 (td, J = 7.57, 1.80 Hz, 1H), 7.17–7.25 (m, 1H), 7.06–7.13 (m, 1H), 7.02 (ddd, J = 10.00, 8.34, 1.15 Hz, 1H), 3.83 (s, 2H), 2.76 (t, J = 6.80 Hz, 2H), 2.68 (t, J = 6.82 Hz, 2H), 1.65 (quin, J = 6.86 Hz, 2H), 1.57 (s, 1H), 1.24 (s, 1H).

4.1.4.2. N-(3,5-difluorobenzyl)propane-1,3-diamine (14). Following the procedure **B**, reaction of propane-1,3-diamine (1.13 mL, 13.49 mmol) with 1-(bromomethyl)-3,5-difluorobenzene (0.44 mL, 3.37 mmol) in acetonitrile was performed. Purification by column chromatography gave the product **14** (467 mg, yield 69%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.1, v/v/v) R_f = 0.21. MW 200.23. Formula: $C_{10}H_{14}F_2N_2$. MS *m/z* 201.14 (M+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.80–6.91 (m, 1H), 6.62–6.72 (m, 2H), 3.77 (s, 2H), 2.79 (t, J = 6.80 Hz, 2H), 2.67 (t, J = 6.92 Hz, 2H), 1.66 (quin, J = 6.67 Hz, 2H), 1.61 (s, 1H), 1.24 (s, 1H).

4.1.5. Procedure for the synthesis of compounds 15 and 16 (procedure C)

To a solution of the appropriate propane-1,3-diamine derivative (1 equiv.) in ethanol a 37% solution of formaldehyde (1.7 equiv.) and 20% NaOH aq. solution (0.2 equiv.) were added at 0 °C. After the addition, the reaction mixture was stirred at room temperature for 1 h, and concentrated under reduced pressure. To the resulting residue 5 mL of saturated solution of $NaHCO_3$ was added, and extracted with DCM (3 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography DCM/MeOH (9/1, v/v).

4.1.5.1. 1-(2-Fluorobenzyl)-hexahydropyrimidine (15). Following the procedure **C**, reaction of *N*-(2-fluorobenzyl)propane-1,3-diamine (**13**) (200 mg, 1.1 mmol) with 37% formaldehyde solution (0.14 mL, 1.87 mmol) and 20% $NaOH_{(aq)}$ solution (0.04 mL, 0.22 mmol) in ethanol (5 mL) was performed. Purification by extraction and column chromatography gave product **15** (198 mg, yield 93%) as a colorless oil. TLC DCM/MeOH (9/1, v/v) R_f = 0.51. MW 194.25. Formula: $C_{11}H_{15}FN_2$. MS: *m/z* 181.16 (M-(CH_2)+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.36 (td, J = 7.37, 1.92 Hz, 1H), 7.17–7.26 (m, 1H), 7.10 (td, J = 7.44, 1.28 Hz, 1H), 6.98–7.06 (m, 1H), 3.49 (d, J = 1.54 Hz, 2H), 3.45 (s, 2H), 2.81 (t, J = 5.40 Hz, 2H), 2.62 (t, J = 5.40 Hz, 2H), 1.73 (s, 1H), 1.61 (quin, J = 5.51 Hz, 2H).

4.1.5.2. 1-(3,5-Difluorobenzyl)-hexahydropyrimidine (16). Following the procedure **C**, reaction of *N*-(3,5-difluorobenzyl)propane-1,3-diamine (**14**) (200 mg, 1 mmol) with 37% formaldehyde solution (0.13 mL, 1.70 mmol) and 20% $NaOH_{(aq)}$ solution (0.04 mL, 0.2 mmol) in ethanol (5 mL) was performed. Purification by extraction and column chromatography gave the product **16** (185 mg, yield 87%) as a colorless oil. TLC DCM/MeOH (9/1, v/v) R_f = 0.54. MW 212.24. Formula: $C_{11}H_{14}F_2N_2$. MS: *m/z* 199.21 (M-(CH_2)+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.81–6.90 (m, 2H), 6.66 (tt, J = 8.94, 2.34 Hz, 1H), 3.47 (s, 2H), 3.27 (s, 2H), 2.66 (t, J = 5.10 Hz, 2H), 2.55 (t, J = 4.90 Hz, 2H), 1.78 (br. s., 1 H), 1.63 (q, J = 5.60 Hz, 2H).

4.1.6. Procedure for the synthesis of compounds 17–19 (procedure D)

To a solution of *tert*-butyl piperidin-3-ylcarbamate (1 equiv.) in acetonitrile the appropriate benzyl halide derivative (1.1 equiv.) was added at room temperature. After the addition the reaction mixture was stirred at room temperature for 44 h. When the reaction was finished, the solvent was evaporated under reduced pressure, and the resulting residue was dissolved in 20 mL of saturated solution of $NaHCO_3$ and extracted with DCM (2 × 20 mL).

The organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography in petroleum ether/ethyl acetate (8/2, v/v).

4.1.6.1. Tert-butyl 1-benzylpiperidin-3-ylcarbamate (17). Following the general procedure **D** reaction of *tert*-butyl piperidin-3-ylcarbamate (298 mg, 1.49 mmol) with (chloromethyl)benzene (208 mg, 1.64 mmol) in acetonitrile (30 mL) was performed. Purification gave product **17** (248 mg, yield 57.4%) as a colorless oil. TLC petroleum ether/ethyl acetate (8/2, v/v) R_f = 0.22. MW 290.40. Formula: $C_{17}H_{26}N_2O_2$. MS *m/z* 291.22 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.18–7.39 (m, 5H), 4.89–5.11 (m, 1H), 3.67–3.83 (m, 1H), 3.47 (s, 2H), 2.49 (m., 4H), 1.50–1.76 (m, 4H), 1.43 (s, 9H).

4.1.6.2. Tert-butyl 1-(2-fluorobenzyl)piperidin-3-ylcarbamate (18). Following the general procedure **D** reaction of *tert*-butyl piperidin-3-ylcarbamate (401 mg, 2.00 mmol) with 1-(chloromethyl)-2-fluorobenzene (318 mg, 2.20 mmol) in acetonitrile (50 mL) was performed. Purification gave product **18** (345 mg, yield 56%) as a colorless oil. TLC petroleum ether/ethyl acetate (8/2, v/v) R_f = 0.16. MW 308.39. Formula: $C_{17}H_{25}FN_2O_2$. MS *m/z* 309.28 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.35 (dt, J = 1.80, 7.44 Hz, 1H), 7.18–7.25 (m, 1H), 7.07–7.13 (m, 1H), 7.01 (ddd, J = 1.28, 8.34, 9.87 Hz, 1H), 4.98 (br. s, 1H), 3.75 (br. s, 1H), 3.55 (s, 2H), 2.51–2.64 (m, 1H), 2.18–2.50 (m, 3H), 1.47–1.78 (m, 4H), 1.43 (s, 9H).

4.1.6.3. Tert-butyl 1-(3,5-difluorobenzyl)piperidin-3-ylcarbamate (19). Following the general procedure **D** reaction of *tert*-butyl piperidin-3-ylcarbamate (401 mg, 2.00 mmol) with 1-(bromomethyl)-3,5-difluorobenzene (455 mg, 2.20 mmol) in acetonitrile (50 mL) was performed. Purification gave product **19** (515 mg, yield 79%) as a colorless oil. TLC petroleum ether/ethyl acetate (8:2, v/v) R_f = 0.32. MW 326.38. Formula: $C_{17}H_{24}N_2F_2O_2$. MS *m/z* 327.30 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.80–6.89 (m, 2H), 6.67 (tt, J = 2.34, 8.94 Hz, 1H), 4.95 (br. s, 1H), 3.70–3.83 (m, 1H), 3.43 (s, 2H), 2.47–2.61 (m, 1H), 2.18–2.46 (m, 3H), 1.50–1.76 (m, 4H), 1.44 (s, 9H).

4.1.7. Procedure for the synthesis of compounds 20–22 (procedure E)

To the appropriate *tert*-butyl piperidin-3-ylcarbamate derivative 10% solution of HCl in MeOH was added. The reaction mixture was stirred at room temperature for 24 h. When the reaction was finished, the solvent and hydrogen chloride were evaporated under reduced pressure, producing a residue that was then suspended in ethyl acetate with a small amount of diethyl ether. The obtained hydrochloride salt was then dissolved in saturated solution of $NaHCO_3$ and extracted with DCM (6 × 20 mL). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum.

4.1.7.1. 1-Benzylpiperidin-3-amine (20). Following the procedure **E,** reaction of *tert*-butyl 1-benzylpiperidin-3-ylcarbamate (**17**) (248 mg, 0.855 mmol) with hydrochloric acid (15 mL) was performed to give product **20** (148 mg, yield 91.0%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.05, v/v/v) R_f = 0.15. MW 190.28. Formula: $C_{12}H_{18}N_2$. MS *m/z* 191.14 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.18–7.35 (m, 5H), 3.47–3.52 (m, 2H), 2.81–2.92 (m, 1H), 2.74 (d, J = 10.55 Hz, 1H), 2.53–2.66 (m, 1H), 2.00–2.14 (m, 1H), 1.75–1.92 (m, 2H), 1.64–1.75 (m, 1H), 1.39–1.63 (m, 4H).

4.1.7.2. 1-(2-Fluorobenzyl)piperidin-3-amine (21). Following the procedure **E** reaction of *tert*-butyl 1-(2-fluorobenzyl)piperidin-3-ylcarbamate (**18**) (935 mg, 3.03 mmol) with hydrochloric acid (30.3 mL) was performed to give product **21** (600 mg, yield 95%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.05, v/v/v) R_f = 0.27. MW 208.28. Formula: $C_{12}H_{17}FN_2$. MS *m/z* 209.18 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.36 (dt, J = 2.05, 7.44 Hz, 1H), 7.17–7.24 (m, 1H), 7.05–7.12 (m, 1H), 7.01 (ddd, J = 1.28, 8.34, 9.87 Hz, 1H), 3.57 (s, 2H), 2.81–2.91 (m, 1H), 2.73–2.81 (m, 1H), 2.58–2.69 (m, 1H), 2.04–2.14 (m, 1H), 1.89 (t, J = 9.49 Hz, 1H), 1.47–1.83 (m, 3H), 1.25–1.31 (m, 2H), 0.99–1.16 (m, 1H).

4.1.7.3. 1-(3,5-Difluorobenzyl)piperidin-3-amine (22). Following the procedure **E,** reaction of *tert*-butyl 1-(3,5-difluorobenzyl)piperidin-3-ylcarbamate (**19**) (500 mg, 1.532 mmol) with hydrochloric acid (15 mL) was performed to give product **22** (316 mg, yield 89%) as a colorless oil. TLC DCM/MeOH/25% $NH_{3(aq)}$ (9/1/0.05, v/v/v) R_f = 0.25. MW 226.27. Formula: $C_{12}H_{16}F_2N_2$. MS *m/z* 227.19 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 6.77–6.87 (m, 2H), 6.63 (tt, J = 2.34, 8.94 Hz, 1H), 3.33–3.47 (m, 2H), 2.78–2.92 (m, 1H), 2.62–2.73 (m, 1H), 2.46–2.58 (m, 1H), 1.97–2.10 (m, 1H), 1.72–1.91 (m, 2H), 1.43–1.72 (m, 4H), 0.99–1.16 (m, 1H).

4.1.8. Procedure for the synthesis of compounds 23–25 (procedure F)

2-(2-Bromoethyl)isoindoline-1,3-dione (**1**) (1 equiv.) with the appropriate piperazine derivative (**7**, **10** or **12**) (1–1.1 equiv.) in the presence of K_2CO_3 (1.2–2 equiv.) in acetonitrile was refluxed for 24 h. When the reaction was finished the solvent was evaporated under reduced pressure and the resulting residue was dissolved in 5 mL of ethyl acetate and washed with saturated solution of $NaHCO_3$ (3 × 5 mL) and saturated solution of NaCl (5 mL). The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product (**23**, **24** or **25**) was purified by column chromatography (petroleum ether/ethyl acetate 4.5/5.5, v/v for **23**, 5/5, v/v for **24**, 3/7, v/v for **25**).

4.1.8.1. 2-(2-(4-(2-Fluorobenzyl)piperazin-1-yl)ethyl)isoindoline-1,3-dione (23). Following the procedure **F**, reaction of 1-(2-fluorobenzyl)piperazine (**7**) (60 mg, 0.310 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (90 mg, 0.310 mmol) and K_2CO_3 (86 mg, 0.62 mmol) in acetonitrile (5 mL) was performed. Purification by extraction and column chromatography gave product **23** (35 mg, yield 28%). TLC petroleum ether/ethyl acetate (4.5/5.5, v/v) R_f = 0.22. MW 367.42. Formula: $C_{21}H_{22}N_3O_2$. MS *m/z* 368.31 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.79–7.87 (m, 2H), 7.67–7.74 (m, 2H), 7.35 (dt, J = 1.80,7.44 Hz, 1H), 7.17–7.24 (m, 1H), 7.05–7.11 (m, 1H), 7.00 (ddd, J = 1.28, 8.34, 9.87 Hz, 1H), 3.80 (t, J = 6.67 Hz, 2H), 3.55 (d, J = 1.28 Hz, 2H), 2.63 (t, J = 6.67 Hz, 2H), 2.38–2.59 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.31, 161.35 (d, $J_{C\text{-}F}$ = 246.2 Hz), 133.80, 132.17, 131.57 (d, $J_{C\text{-}F}$ = 4.6 Hz), 128.65 (d, $J_{C\text{-}F}$ = 8.2 Hz), 124.61 (d, $J_{C\text{-}F}$ = 14.7 Hz), 123.75 (d, $J_{C\text{-}F}$ = 3.5 Hz), 123.15, 115.17 (d, $J_{C\text{-}F}$ = 22.3 Hz), 55.66, 55.13 (d, $J_{C\text{-}F}$ = 1.9 Hz), 52.99, 52.77, 35.28.

4.1.8.2. 2-(2-(4-(3,5-Difluorobenzyl)piperazin-1-yl)ethyl)isoindoline-1,3-dione (24). Following the procedure **F**, reaction of 1-(3,5-difluorobenzyl)piperazine (**10**) (78 mg, 0.370 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (93 mg, 0.370 mmol) and K_2CO_3 (61 mg, 0.440 mmol) in acetonitrile (2 mL) was performed. Purification by extraction and column chromatography gave the product **24** (75 mg, yield 53%). TLC petroleum ether/ethyl acetate (5/5, v/v) R_f = 0.41. MW 385.41. Formula: $C_{21}H_{21}F_2N_3O_2$. MS *m/z* 386.33 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.80–7.88 (m, 2H), 7.67–7.75 (m, 2H), 6.79–6.89 (m, 2H), 6.66 (tt, J = 2.31, 8.98 Hz, 1H), 3.81 (t, J = 6.67 Hz, 2H), 3.42 (s, 2H), 2.64 (t, J = 6.67 Hz, 2H), 2.31–2.60 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.31, 162.93

(dd, J_{C-F} = 247.69, 12.72 Hz), 142.71 (t, J_{C-F} = 8.7 Hz), 133.81, 132.17, 123.15, 111.14–111.57 (m), 102.29 (t, J_{C-F} = 25.4 Hz), 62.01 (t, J_{C-F} = 2.21 Hz), 55.64, 53.01, 52.95, 35.25.

4.1.8.3. 2-(2-(4-(2-Fluorophenethyl)piperazin-1-yl)ethyl)isoindoline-1,3-dione (25). Following the procedure **F**, reaction of 2-(2-fluorophenethyl)piperazine (**12**) (77 mg, 0.370 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (94 mg, 0.370 mmol) and K_2CO_3 (61 mg, 0.440 mmol) in acetonitrile (2 mL) was performed. Purification by extraction and column chromatography gave the product **25** (64 mg, yield 45.6%). TLC petroleum ether/ethyl acetate (3/7, v/v) R_f = 0.24. MW 381.44. Formula: $C_{22}H_{24}N_3O_2$. MS *m/z* 382.34 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.80–7.87 (m, 2H), 7.67–7.74 (m, 2H), 7.12–7.22 (m, 2H), 6.94–7.07 (m, 2H), 3.82 (t, J = 6.54 Hz, 2H), 2.78–2.86 (m, 2H), 2.46–2.68 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.31, 161.07 (d, J_{C-F} = 244.9 Hz), 133.81, 132.15, 130.92 (d, J_{C-F} = 4.9 Hz), 127.82 (d, J_{C-F} = 8.0 Hz), 126.77 (d, J_{C-F} = 16.0 Hz), 123.97 (d, J_{C-F} = 3.6 Hz), 123.15, 115.18 (d, J_{C-F} = 22.1 Hz), 58.45, 55.60, 52.82, 52.65, 35.18, 26.44 (d, J_{C-F} = 2.0 Hz).

4.1.9. Procedure for the synthesis of compounds 26–40, 45–49, 51, 52 (procedure G)

The appropriate saccharine derivative (**2**–**6**) or isoindoline-1,3-dione derivative (**1**, **50**) (1 equiv.) with the appropriate amine derivative (1 equiv.) in the presence of K_2CO_3 (1–3 equiv.) in acetonitrile was heated at reflux for 24 h. When the reaction was finished the solvent was evaporated under reduced pressure, producing a residue that was then dissolved in 5 mL of ethyl acetate and extracted with saturated solution of $NaHCO_3$ (2 × 5 mL) and saturated solution of NaCl (5 mL). The organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography in petroleum ether/ethyl acetate (5/5 to 1/9, v/v) or DCM/MeOH (10/0.5, v/v).

4.1.9.1. 2-(2-(4-benzylpiperazin-1-yl)ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (26). Following the procedure **G**, reaction of *N*-benzylpiperazine (91 mg, 0.517 mmol) with 2-(2-bromoethyl) benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (150 mg, 0.517 mmol) and K_2CO_3 (107 mg, 0.776 mmol) in acetonitrile (5 mL) was performed. Purification by extraction and column chromatography gave the product **26** (24 mg, yield 14%). TLC petroleum ether/ethyl acetate (1/9, v/v) R_f = 0.36. MW 385.48. Formula: $C_{20}H_{23}N_3O_3S$. MS *m/z* 386.46 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.02–8.07 (m, 1H), 7.77–7.94 (m, 3H), 7.19–7.35 (m, 5H), 3.89 (t, J = 6.90 Hz, 2H), 3.51 (s, 2H), 2.78 (t, J = 6.70 Hz, 2H), 2.40–2.69 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.90, 137.66, 134.69, 134.29, 129.46, 128.33, 128.28, 127.38, 127.30, 125.14, 120.91, 62.70, 55.46, 52.79, 52.62, 36.64.

4.1.9.2. 2-(2-(4-(2-Fluorobenzyl)piperazin-1-yl)ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (27). Following the procedure **G**, reaction of 1-(2-fluorobenzyl)piperazine (**7**) (60 mg, 0.310 mmol) with 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (90 mg, 0.310 mmol) and K_2CO_3 (86 mg, 0.620 mmol) in acetonitrile (5 mL) was performed. Purification by extraction and column chromatography gave the product **27** (35 mg, yield 28%). TLC ethyl acetate/petroleum ether (5/5, v/v) R_f = 0.22. MW 403.47. Formula: $C_{20}H_{22}FN_3O_3S$. MS *m/z* 404.34 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.02–8.06 (m, 1H), 7.78–7.93 (m, 3H), 7.36 (dt, J = 1.92, 7.50 Hz, 1H), 7.18–7.25 (m, 1H), 6.97–7.13 (m, 2H), 3.88 (t, J = 7.18 Hz, 2H), 3.59 (s, 2H), 2.77 (t, J = 7.18 Hz, 2H), 2.46–2.67 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.35 (d, J_{C-F} = 246.2 Hz), 158.82, 137.63, 134.63, 134.23, 131.59 (d, J_{C-F} = 4.5 Hz), 128.73 (d, J_{C-F} = 8.2 Hz), 127.37, 125.09, 124.39 (d, J_{C-F} = 14.5 Hz), 123.78 (d, J_{C-F} = 3.6 Hz), 120.85, 115.17 (d, J_{C-F} = 22.3 Hz), 55.49, 55.04 (d, J_{C-F} = 1.8 Hz), 52.90, 52.65, 36.60.

4.1.9.3. 2-(2-(4-(3-Fluorobenzyl)piperazin-1-yl)ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (28). Following the procedure **G**, reaction of 1-(3-fluorobenzyl)piperazine (**8**) (80 mg, 0.414 mmol) with 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (120 mg, 0.414 mmol) and K_2CO_3 (86 mg, 0.621 mmol) in acetonitrile (5 mL) was performed. Purification by extraction and column chromatography gave the product **28** (24 mg, yield 14%). TLC ethyl acetate/petroleum ether (7/3, v/v) R_f = 0.41. MW 403.47. Formula: $C_{20}H_{22}FN_3O_3S$. MS *m/z* 404.34 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.01–8.09 (m, 1H), 7.77–7.95 (m, 3H), 7.20–7.31 (m, 1H), 7.01–7.11 (m, 2H), 6.87–6.98 (m, 1H), 3.90 (t, J = 7.18 Hz, 2H), 3.50 (s, 2H), 2.79 (t, J = 7.05 Hz, 2H), 2.39–2.71 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 163.39 (d, J_{C-F} = 244.92 Hz), 158.89, 140.73 (d, J_{C-F} = 7.74 Hz), 137.68, 134.67, 134.28, 129.59 (d, J_{C-F} = 8.29 Hz), 127.40, 125.13, 124.59 (d, J_{C-F} = 2.76 Hz), 120.91, 115.79 (d, J_{C-F} = 21.56 Hz), 113.93 (d, J_{C-F} = 21.56 Hz), 62.29 (d, J_{C-F} = 2.21 Hz), 55.53, 52.97, 52.90, 36.66.

4.1.9.4. 2-(2-(4-(4-Fluorobenzyl)piperazin-1-yl)ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (29). Following the procedure **G**, reaction of 1-(4-fluorobenzyl)piperazine (**9**) (100 mg, 0.517 mmol) with 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (150 mg, 0.517 mmol) and K_2CO_3 (108 mg, 0.78 mmol) in acetonitrile (3 mL) was performed. Purification by extraction and column chromatography gave the product **29** (99 mg, yield 47.5%). TLC ethyl acetate/petroleum ether (7/3, v/v) R_f = 0.27. MW 403.47. Formula: $C_{20}H_{22}FN_3O_3S$. MS *m/z* 404.13 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.00–8.09 (m, 1H), 7.76–7.96 (m, 3H), 7.20–7.35 (m, 2H), 6.99 (tt, J = 8.80, 2.90 Hz, 2H), 3.89 (t, J = 7.03 Hz, 2H), 3.51 (s, 2H), 2.74–2.84 (m, 2H), 2.43–2.72 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 162.14 (d, J_{C-F} = 245.31 Hz), 158.92, 137.69, 134.70, 134.29, 132.80 (d, J_{C-F} = 3.46 Hz), 130.91 (d, J_{C-F} = 8.06 Hz), 127.36, 125.12, 120.89, 115.09 (d, J_{C-F} = 21.88 Hz), 61.91, 55.44, 52.75, 52.60, 36.65.

4.1.9.5. 2-(2-(4-(3,5-Difluorobenzyl)piperazin-1-yl)ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (30). Following the procedure **G**, reaction of 1-(3,5-difluorobenzyl)piperazine (**10**) (62 mg, 0.290 mmol) with 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (85 mg, 0.290 mmol) and K_2CO_3 (52 mg, 0.380 mmol) in acetonitrile (5 mL) was performed. Purification by extraction and column chromatography gave the product **30** (25 mg, yield 20%). TLC ethyl acetate/petroleum ether (4/6, v/v) R_f = 0.24. MW 421.46. Formula: $C_{20}H_{21}F_2N_3O_3S$. MS *m/z* 422.29 [$M+H^+$]. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.01–8.09 (m, 1H), 7.79–7.95 (m, 3H), 6.81–6.91 (m, 2H), 6.67 (tt, J = 2.34, 8.94 Hz, 1H), 3.90 (t, J = 7.05 Hz, 2H), 3.46 (s, 2H), 2.79 (t, J = 7.05 Hz, 2H), 2.61 (br. s., 4H), 2.46 (br. s., 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 162.96 (dd, J_{C-F} = 247.69, 12.72 Hz), 158.89, 142.65 (t, J_{C-F} = 8.80 Hz), 137.70, 134.67, 134.28, 127.40, 125.13, 120.90, 111.16–111.59 (m), 102.34 (t, J_{C-F} = 25.40 Hz), 61.99 (t, J_{C-F} = 1.94 Hz), 55.52, 52.98, 52.94, 36.66.

4.1.9.6. 2-(3-(4-(Benzyl)piperazin-1-yl)propyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (31). Following the procedure **G**, reaction of 1-benzylpiperazine (96 mg, 0.545 mmol) with 2-(3-bromopropyl) benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**3**) (150 mg, 0.493 mmol) and K_2CO_3 (136 mg, 0.984 mmol) in acetonitrile (3 mL) was performed. Purification by extraction and column chromatography in DCM/MeOH (10/0.5, v/v) gave the product **31** (68 mg, yield 34.5%). TLC DCM/MeOH (10/0.5, v/v) R_f = 0.25. MW 399.51. Formula: $C_{21}H_{25}N_3O_3S$. MS *m/z* 400.15 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.01–8.07 (m, 1H), 7.78–7.93 (m, 3H), 7.21–7.34 (m, 5H), 3.84

(t, $J = 7.00$ Hz, 2H), 3.50 (s, 2H), 2.48 (t, $J = 6.74$ Hz, 10H), 2.02 (quin, $J = 7.60$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.97, 137.73, 134.63, 134.25, 130.05, 129.29, 128.20, 127.49, 127.09, 125.07, 120.88, 62.93, 55.27, 52.90, 37.67, 25.37, 23.84.

4.1.9.7. 2-(4-(4-(Benzyl)piperazin-1-yl)butyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (32). Following the procedure **G**, reaction of 1-benzylpiperazine (90 mg, 0.511 mmol) with 2-(4-bromobutyl) benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**4**) (150 mg, 0.471 mmol) and K_2CO_3 (128 mg, 0.926 mmol) in acetonitrile (3 mL) was performed. Purification by extraction (residues resulting after solvent evaporation was dissolved in 10 mL DCM, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 10 mL DCM) and column chromatography in DCM/MeOH (10/0.5, v/v) gave the product **32** (121 mg, yield 62.1%). TLC DCM/MeOH (10/0.5, v/v) $R_f = 0.14$. MW 413.53. Formula: $C_{22}H_{27}N_3O_3S$. MS *m/z* 414.17 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.01–8.08 (m, 1H), 7.75–7.95 (m, 3H), 7.18–7.38 (m, 5H), 3.79 (t, $J = 7.33$ Hz, 2H), 3.50 (s, 2H), 2.34–2.60 (m, 10H), 1.87 (quin, $J = 7.47$ Hz, 2H), 1.61 (quin, $J = 7.60$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.92, 138.02, 134.66, 134.26, 129.22, 128.17, 127.41, 127.01, 125.10, 120.86, 63.02, 57.76, 53.09, 52.96, 39.23, 26.47, 23.98.

4.1.9.8. 2-(5-(4-(Benzyl)piperazin-1-yl)pentyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (33). Following the procedure **G**, reaction of 1-benzylpiperazine (88 mg, 0.499 mmol) with 2-(5-bromopentyl) benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**5**) (150 mg, 0.452 mmol) and K_2CO_3 (125 mg, 0.904 mmol) in acetonitrile (3 mL) was performed. Purification by extraction (residues resulting after solvent evaporation was dissolved in 10 mL DCM, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 5 mL DCM) and column chromatography in DCM/MeOH (10/0.5, v/v) gave the product **33** (95 mg, yield 49.2%). TLC DCM/MeOH (10/0.5, v/v) $R_f = 0.17$. MW 427.56. Formula: $C_{23}H_{20}N_3O_3S$. MS *m/z* 428.13 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.00–8.11 (m, 1 H), 7.77–7.95 (m, 3H), 7.20–7.34 (m, 5H), 3.77 (t, $J = 7.60$ Hz, 2H), 3.51 (s, 2H), 2.31–2.63 (m, 10H), 1.87 (quin, $J = 7.62$ Hz, 2H), 1.51–1.66 (m, 2H), 1.35–1.50 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 159.24, 138.69, 134.64, 134.26, 132.19, 131.53, 129.22, 128.19, 127.03, 125.10, 120.88, 62.99, 58.29, 53.12, 52.84, 39.30, 28.27, 26.10, 24.75.

4.1.9.9. 2-(6-(4-(Benzyl)piperazin-1-yl)hexyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (34). Following the procedure **G**, reaction of 1-benzylpiperazine (104 mg, 0.590 mmol) with 2-(6-bromohexyl) benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**6**) (186 mg, 0.537 mmol) and K_2CO_3 (148 mg, 1.071 mmol) in acetonitrile (3 mL) was performed. Purification by extraction (dissolved in 10 mL DCM, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 10 mL DCM) and column chromatography in DCM/MeOH (10/0.5, v/v) gave the product **34** (173 mg, yield 73.0%). TLC DCM/MeOH (10/0.5, v/v) $R_f = 0.20$. MW 441.59. Formula: $C_{24}H_{31}N_3O_3S$. MS *m/z* 442.15 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.02–8.09 (m, 1H), 7.78–7.95 (m, 3H), 7.19–7.36 (m, 5H), 3.76 (t, $J = 7.60$ Hz, 2H), 3.50 (s, 2H), 2.23–2.63 (m, 10H), 1.85 (quin, $J = 7.00$ Hz, 2H), 1.25–1.57 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.92, 138.14, 134.61, 134.23, 132.77, 129.21, 128.16, 127.45, 126.97, 125.10, 120.86, 63.07, 58.55, 53.21, 53.04, 39.37, 38.33, 28.33, 27.02, 26.70.

4.1.9.10. 2-(3-(4-(2-Fluorobenzyl)piperazin-1-yl)propyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (35). Following the procedure **G**, reaction of 1-(2-fluorobenzyl)piperazine (**7**) (64 mg, 0.330 mmol) with 2-(3-bromopropyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**3**) (100 mg, 0.330 mmol) and K_2CO_3 (55 mg, 0.400 mmol) in acetonitrile (2 mL) was performed. Purification by extraction (dissolved in 10 mL ethyl acetate, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 5 mL ethyl acetate) and column chromatography in DCM/MeOH (10/0.5, v/v) gave the product **35** (109 mg, yield 79%). The final product was obtained in the form of hydrochloride salt. TLC DCM/MeOH (10/0.5, v/v) $R_f = 0.25$. MW 417.50. Formula: $C_{21}H_{25}N_3O_3S$. MS *m/z* 418.24 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.99–8.08 (m, 1H), 7.77–7.93 (m, 3H), 7.34 (dt, $J = 1.80$, 7.44 Hz, 1H), 7.17–7.24 (m, 1H), 7.05–7.13 (m, 1H), 7.01 (ddd, $J = 1.15$, 8.27, 9.81 Hz, 1H), 3.83 (t, $J = 7.44$ Hz, 2H), 3.56 (d, $J = 1.03$ Hz, 2H), 2.34–2.61 (m, 10H), 1.94–2.08 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.34 (d, $J_{C\text{-}F} = 246.1$ Hz), 158.92, 137.64, 134.59, 134.21, 131.58 (d, $J_{C\text{-}F} = 4.6$ Hz), 128.67 (d, $J_{C\text{-}F} = 8.2$ Hz), 127.43, 125.03, 124.48 (d, $J_{C\text{-}F} = 14.7$ Hz), 123.74 (d, $J_{C\text{-}F} = 3.6$ Hz), 120.83, 115.16 (d, $J_{C\text{-}F} = 22.3$ Hz), 55.31, 55.13 (d, $J_{C\text{-}F} = 1.9$ Hz), 52.94, 52.71, 37.68, 25.36.

4.1.9.11. 2-(4-(4-(2-Fluorobenzyl)piperazin-1-yl)butyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (36). Following the procedure **G**, reaction of 1-(2-fluorobenzyl)piperazine (**7**) (78 mg, 0.400 mmol) with 2-(4-bromobutyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**4**) (127 mg, 0.400 mmol) and K_2CO_3 (62 mg, 0.480 mmol) in acetonitrile (2.5 mL) was performed. Purification by extraction (dissolved in 10 mL ethyl acetate, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 5 mL ethyl acetate) and column chromatography in DCM/MeOH (10/0.5, v/v) gave the product **36** (133 mg, yield 77%). TLC DCM/MeOH (10/0.5, v/v) $R_f = 0.25$. MW 431.52. Formula: $C_{22}H_{26}FN_3O_3S$. MS *m/z* 432.33 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.02–8.06 (m, 1H), 7.78–7.93 (m, 3H), 7.35 (dt, $J = 1.80$, 7.44 Hz, 1H), 7.18–7.24 (m, 1H), 7.05–7.12 (m, 1H), 7.01 (ddd, $J = 1.28$, 8.34, 9.87 Hz, 1H), 3.79 (t, $J = 7.44$ Hz, 2H), 3.59 (d, $J = 1.28$ Hz, 2H), 2.51 (br. s., 8H), 2.39 (t, $J = 7.70$ Hz, 2H), 1.86 (quin, $J = 7.50$ Hz, 2H), 1.53–1.66 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.39 (d, $J_{C\text{-}F} = 246.0$ Hz), 158.92, 137.66, 134.65, 134.26, 131.61 (d, $J_{C\text{-}F} = 4.6$ Hz), 128.70 (d, $J_{C\text{-}F} = 8.2$ Hz), 127.39, 125.11, 124.51 (d, $J_{C\text{-}F} = 14.8$ Hz), 123.76 (d, $J_{C\text{-}F} = 3.5$ Hz), 120.86, 115.21 (d, $J_{C\text{-}F} = 22.3$ Hz), 57.75, 55.18 (d, $J_{C\text{-}F} = 1.9$ Hz), 53.08, 52.69, 39.23, 26.47, 24.03.

4.1.9.12. 2-(5-(4-(2-Fluorobenzyl)piperazin-1-yl)pentyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (37). Following the procedure **G**, reaction of 1-(2-fluorobenzyl)piperazine (**7**) (66 mg, 0.340 mmol) with 2-(5-bromopentyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**5**) (112 mg, 0.340 mmol) and K_2CO_3 (57 mg, 0.410 mmol) in acetonitrile (2.5 mL) was performed. Purification by extraction (dissolved in 10 mL ethyl acetate, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 5 mL ethyl acetate) and column chromatography in DCM/MeOH (10/0.5, v/v) gave the product **37** (80 mg, yield 53%). TLC (DCM/MeOH 10/0.5) $R_f = 0.21$. MW 445.55. Formula: $C_{23}H_{28}FN_3O_3S$. MS *m/z* 446.29 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.02–8.07 (m, 1H), 7.78–7.94 (m, 3H), 7.35 (dt, $J = 1.80$, 7.44 Hz, 1H), 7.17–7.24 (m, 1H), 7.05–7.12 (m, 1H), 7.01 (ddd, $J = 1.03$, 8.34, 9.87 Hz, 1H), 3.76 (t, $J = 7.44$ Hz, 2H), 3.59 (d, $J = 1.28$ Hz, 2H), 2.51 (br. s., 8H), 2.30–2.38 (m, 2H), 1.86 (quin, $J = 15.10$ Hz, 2H), 1.48–1.62 (m, 2H), 1.35–1.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.37 (d, $J_{C\text{-}F} = 246.2$ Hz), 158.89, 137.62, 134.63, 134.25, 131.60 (d, $J_{C\text{-}F} = 4.6$ Hz), 128.69 (d, $J_{C\text{-}F} = 8.2$ Hz), 127.37, 125.07, 124.45 (d, $J_{C\text{-}F} = 14.8$ Hz), 123.74 (d, $J_{C\text{-}F} = 3.6$ Hz), 120.85, 115.19 (d, $J_{C\text{-}F} = 22.3$ Hz), 58.30, 55.16 (d, $J_{C\text{-}F} = 1.8$ Hz), 53.13, 52.62, 39.28, 28.27, 26.21, 24.73.

4.1.9.13. 2-(2-(4-(2-Fluorobenzyl)piperazin-1-yl)hexyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (38). Following the procedure **G**, reaction of 1-(2-fluorobenzyl)piperazine (**7**) (93 mg, 0.480 mmol) with 2-(6-bromohexyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**6**) (167 mg, 0.480 mmol) and K_2CO_3 (80 mg, 0.580 mmol) in acetonitrile (2.5 mL) was performed. Purification by extraction

(dissolved in 10 mL ethyl acetate, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 5 mL ethyl acetate) and column chromatography in DCM/MeOH (10/0.5, v/v) gave product **38** (130 mg, yield 58%). TLC DCM/MeOH (10/0.5, v/v) R_f = 0.22. MW 459.58. Formula: $C_{24}H_{30}FN_3O_3S$. MS *m/z* 460.32 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 8.01–8.07 (m, 1H), 7.77–7.94 (m, 3H), 7.35 (dt, J = 1.80, 7.44 Hz, 1H), 7.17–7.24 (m, 1H), 7.05–7.13 (m, 1H), 7.01 (ddd, J = 1.15, 8.27, 9.81 Hz, 1H), 3.75 (t, J = 7.69 Hz, 2H), 3.59 (d, J = 1.28 Hz, 2H), 2.52 (br. s., 8H), 2.29–2.36 (m, 2H), 1.84 (quin, J = 7.37 Hz, 2H), 1.28–1.56 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.37 (d, $J_{C\text{-}F}$ = 246.2 Hz), 158.90, 137.62, 134.62, 134.24, 131.61 (d, $J_{C\text{-}F}$ = 4.6 Hz), 128.69 (d, $J_{C\text{-}F}$ = 8.2 Hz), 127.38, 125.07, 124.43 (d, $J_{C\text{-}F}$ = 14.8 Hz), 123.74 (d, $J_{C\text{-}F}$ = 3.6 Hz), 120.85, 115.19 (d, $J_{C\text{-}F}$ = 22.3 Hz), 58.47, 55.17 (d, $J_{C\text{-}F}$ = 1.8 Hz), 53.12, 52.59, 39.31, 28.30, 26.97, 26.66, 26.60.

4.1.9.14. 2-(2-(4-phenethylpiperazin-1-yl)ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (39). Following the procedure **G**, reaction of 2-(phenethyl)piperazine (**11**) (200 mg, 1.051 mmol) with 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (335 mg, 1.155 mmol) and K_2CO_3 (291 mg, 2.102 mmol) in acetonitrile (5 mL) was performed. Purification by extraction and column chromatography in DCM/MeOH (10/0.5, v/v) gave product (**39**) (57 mg, yield 13.6%). TLC DCM/MeOH (10/0.5, v/v) R_f = 0.16. MW 399.51. Formula: $C_{21}H_{25}N_3O_3S$. MS *m/z* 400.15 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 8.03–8.12 (m, 1H), 7.78–7.95 (m, 3H), 7.16–7.33 (m, 5H), 3.91 (t, J = 7.03 Hz, 2H), 2.47–2.85 (m, 14H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 158.91, 140.24, 137.71, 134.67, 134.28, 128.68, 128.37, 126.04, 125.13, 120.89, 60.43, 55.56, 53.09, 52.96, 36.66, 33.52.

4.1.9.15. 2-(2-(4-(2-Fluorophenethyl)piperazin-1-yl)ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (40). Following the procedure **G**, reaction of 2-(2-fluorophenethyl)piperazine (**12**) (83 mg, 0.400 mmol) with 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (116 mg, 0.400 mmol) and K_2CO_3 (55 mg, 0.400 mmol) in acetonitrile (2 mL) was performed. Purification by extraction and column chromatography gave product **40** (40 mg, yield 24%). TLC DCM/MeOH (10/0.5, v/v) R_f = 0.27. MW 417.50. Formula: $C_{21}H_{24}FN_3O_3S$. MS *m/z* 418.24 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 8.02–8.07 (m, 1H), 7.77–7.94 (m, 3H), 7.11–7.23 (m, 2H), 6.94–7.08 (m, 2H), 3.90 (t, J = 7.05 Hz, 2H), 2.74–2.87 (m, 4H), 2.49–2.70 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.11 (d, $J_{C\text{-}F}$ = 244.9 Hz), 158.88, 137.67, 134.67, 134.27, 130.92 (d, $J_{C\text{-}F}$ = 5.0 Hz), 127.75 (d, $J_{C\text{-}F}$ = 8.1 Hz), 127.40, 127.06 (d, $J_{C\text{-}F}$ = 16.0 Hz), 125.12, 123.95 (d, $J_{C\text{-}F}$ = 3.5 Hz), 120.89, 115.18 (d, $J_{C\text{-}F}$ = 22.2 Hz), 58.68, 55.56, 53.00, 52.98, 36.64, 26.71 (d, $J_{C\text{-}F}$ = 2.0 Hz).

4.1.9.16. 2-(2-(1-Benzylpyrrolidin-3-ylamino)ethyl)isoindoline-1,3-dione (45). Following the procedure **G**, reaction of 1-benzylpyrrolidin-3-amine (273 mg, 1.549 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (394 mg, 1.549 mmol) and K_2CO_3 (325 mg, 2.352 mmol) in acetonitrile (5 mL) was performed. Purification by extraction and flash column chromatography gave product **45** (198 mg, yield 36.6%). TLC DCM/MeOH/25% $NH_{3(aq)}$ (9.5/0.5/0.05, v/v/v) R_f = 0.15. MW 349.43. Formula: $C_{21}H_{23}N_3O_2$. MS *m/z* 350.19 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.79–7.86 (m, 2H), 7.65–7.74 (m, 2H), 7.16–7.37 (m, 5H), 3.78 (t, J = 5.90 Hz, 2H), 3.56 (s, 2H), 3.24–3.38 (m, 1H), 2.84 (d, J = 5.86 Hz, 2H), 2.70 (dd, J = 9.38, 6.45 Hz, 1H), 2.54–2.64 (m, 1H), 2.47 (td, J = 8.65, 6.15 Hz, 1H), 2.31 (dd, J = 9.38, 5.27 Hz, 1H), 2.01–2.15 (m, 1H), 1.45–1.58 (m, 1H), NH not detected; ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.45, 138.93, 133.89, 132.11, 128.78, 128.17, 126.87, 123.21, 60.64, 60.44, 56.98, 52.93, 46.23, 38.08, 32.06.

4.1.9.17. 2-(2-((1-benzylpiperidin-3-yl)amino)ethyl)isoindoline-1,3-dione (46). Following the procedure **G,** reaction of 1-benzylpiperidin-3-amine (**20**) (128 mg, 0.673 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (171 mg, 0.673 mmol) and K_2CO_3 (134 mg, 1.010 mmol) in acetonitrile (3 mL) was performed. Purification by extraction (dissolved in 10 mL DCM, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 10 mL DCM) and column chromatography in DCM/MeOH/25% $NH_{3(aq)}$ (9.7/0.3/0.025, v/v/v) gave product **46** (72 mg, yield 29.4%). TLC DCM/MeOH/25% $NH_{3(aq)}$ (9.7/0.3/0.025, v/v/v) R_f = 0.21. MW 363.45. Formula: $C_{22}H_{25}N_3O_2$. MS *m/z* 364.19 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.79–7.88 (m, 2H), 7.65–7.74 (m, 2H), 7.21–7.31 (m, 5H), 3.77 (td, J = 6.59, 2.05 Hz, 2H), 3.39–3.53 (m, 2H), 2.88 (t, J = 6.74 Hz, 2H), 2.66–2.78 (m, 2H), 2.47–2.60 (m, 1H), 2.01–2.14 (m, 1H), 1.88–2.00 (m, 1H), 1.70–1.84 (m, 2H), 1.44–1.70 (m, 2H), NH not detected; ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.48, 138.38, 133.86, 132.14, 129.03, 128.13, 126.86, 123.21, 63.23, 59.37, 53.68, 53.64, 44.98, 38.40, 30.71, 23.42.

4.1.9.18. 2-(2-(1-(2-Fluorobenzyl)piperidin-3-ylamino)ethyl)isoindoline-1,3-dione (47). Following the procedure **G**, reaction of 1-(2-fluorobenzyl)piperidin-3-amine (**21**) (104 mg, 0.500 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (127 mg, 0.500 mmol) and K_2CO_3 (69 mg, 0.500 mmol) in acetonitrile (2 mL) was performed. Purification by extraction (dissolved in 20 mL DCM, 20 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 20 mL DCM) and column chromatography in DCM/MeOH (10/0.5, v/v) and then in $CHCl_3$/MeOH/25% $NH_{3(aq)}$ (8/1/0.1, v/v/v) gave product **47** (109 mg, yield 57.0%). TLC DCM/MeOH (10/0.5, v/v) R_f = 0.16. MW 381.44. Formula: $C_{22}H_{24}FN_3O_2$. MS *m/z* 382.34 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.80–7.87 (m, 2H), 7.68–7.75 (m, 2H), 7.35 (dt, J = 1.92, 7.50 Hz, 1H), 7.17–7.25 (m, 1H), 6.95–7.12 (m, 2H), 3.79 (t, J = 6.50 Hz, 2H), 3.56 (d, J = 1.03 Hz, 2H), 2.90 (t, J = 6.54 Hz, 2H), 2.68–2.83 (m, 2H), 2.54–2.65 (m, 1H), 2.07–2.19 (m, 1H), 1.99 (t, J = 8.85 Hz, 1H), 1.72–1.83 (m, 1H), 1.45–1.71 (m, 2H), 1.05–1.22 (m, 1H), NH not detected; ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.47, 161.30 (d, $J_{C\text{-}F}$ = 245.8 Hz), 133.86, 132.09, 131.41 (d, $J_{C\text{-}F}$ = 4.7 Hz), 128.52 (d, $J_{C\text{-}F}$ = 8.2 Hz), 124.75 (d, $J_{C\text{-}F}$ = 14.7 Hz), 123.74 (d, $J_{C\text{-}F}$ = 3.6 Hz), 123.20, 115.11 (d, $J_{C\text{-}F}$ = 22.4 Hz), 59.06, 55.34 (d, $J_{C\text{-}F}$ = 1.8 Hz), 53.63, 53.34, 44.96, 38.34, 30.55, 23.42.

4.1.9.19. 2-(2-(1-(3,5-Difluorobenzyl)piperidin-3-ylamino)ethyl)isoindoline-1,3-dione (48). Following the procedure **G**, reaction of 1-(3,5-difluorobenzyl)piperidin-3-amine (**22**) (113 mg, 0.500 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (127 mg, 0.500 mmol) and K_2CO_3 (69 mg, 0.500 mmol) in acetonitrile (2 mL) was performed. Purification by extraction (dissolved in 20 mL DCM, 20 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 20 mL DCM) and flash column chromatography in DCM/MeOH (10/0.5, v/v) gave product **48** (142 mg, yield 71.0%). TLC DCM/MeOH (10/0.5, v/v) R_f = 0.24. MW 399.43. Formula: $C_{22}H_{23}F_2N_3O_2$. MS *m/z* 400.29 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.80–7.87 (m, 2H), 7.67–7.74 (m, 2H), 6.79–6.88 (m, 2H), 6.65 (tt, J = 2.44, 8.98 Hz, 1H), 3.74–3.82 (m, 2H), 3.42 (s, 2H), 2.84–2.95 (m, 2H), 2.67–2.78 (m, 2H), 2.46–2.56 (m, 1H), 2.02–2.13 (m, 1H), 1.88–2.00 (m, 1H), 1.71–1.84 (m, 1H), 1.43–1.71 (m, 3H), 1.17 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.51, 162.93 (dd, $J_{C\text{-}F}$ = 247.69, 12.72 Hz), 143.08 (t, $J_{C\text{-}F}$ = 8.57 Hz), 133.91, 132.08, 123.24, 110.99–111.43 (m), 102.22 (t, $J_{C\text{-}F}$ = 25.40 Hz), 62.34 (t, $J_{C\text{-}F}$ = 2.21 Hz), 59.50, 53.69, 53.55, 44.98, 38.32, 30.45, 23.36.

4.1.9.20. Tert-butyl 1-(2-(1,3-dioxoisoindolin-2-yl)ethyl)piperidin-3-ylcarbamate (49). Following the procedure **G**, reaction of *tert*-butyl piperidin-3-ylcarbamate (600 mg, 2.996 mmol) with 2-(2-

bromoethyl)isoindoline-1,3-dione (**1**) (761 mg, 2.996 mmol) and K_2CO_3 (620 mg, 4.486 mmol) in acetonitrile (30 mL) was performed. Purification by extraction (dissolved in 20 mL DCM, 20 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 20 mL DCM) and flash column chromatography in petroleum ether/ethyl acetate (5/5, v/v) gave product **49** (843 mg, yield 75.3%). TLC petroleum ether/ethyl acetate (5/5, v/v) $R_f = 0.38$. MW 373.45. Formula: $C_{20}H_{27}N_3O_4$. MS *m/z* 374.30 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.82 (dd, $J = 5.51, 2.95$ Hz, 2H), 7.66–7.73 (m, 2H), 3.79 (t, $J = 6.54$ Hz, 2H), 3.46 (d, $J = 5.90$ Hz, 1H), 2.87–3.14 (m, 1H), 2.78 (d, $J = 3.59$ Hz, 1H), 2.59 (t, $J = 6.54$ Hz, 2H), 2.36–2.51 (m, 2H), 1.93–2.09 (m, 1H), 1.57–1.80 (m, 1H), 1.39–1.56 (m, 3H), 1.15–1.31 (s, 9H).

4.1.9.21. 2-(2-(3-(2-Fluorobenzylamino)piperidin-1-yl)ethyl)isoindoline-1,3-dione (51). *Tert*-butyl 1-(2-(1,3-dioxoisoindolin-2-yl)ethyl) piperidin-3-ylcarbamate (**49**) (843 mg, 2.257 mmol) was Boc-deprotected using 60% solution of trifluoroacetic acid in DCM (10 mL) to 2-(2-(3-aminopiperidin-1-yl)ethyl)isoindoline-1,3-dione (**50)** (562 mg, yield 91.2%). Then, following the procedure **G**, reaction of 2-(2-(3-aminopiperidin-1-yl)ethyl)isoindoline-1,3-dione (**50**) (200 mg, 0.732 mmol) with 1-(chloromethyl)-2-fluorobenzene (761 mg, 0.732 mmol) and K_2CO_3 (215 mg, 1.560 mmol) in acetonitrile (15 mL) was performed. Purification by extraction (dissolved in 10 mL DCM, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 10 mL DCM) and flash column chromatography in petroleum ether/ethyl acetate (5/5, v/v) gave product **51** (92 mg, yield 32.9%). TLC petroleum ether/ethyl acetate (5/5, v/v) $R_f = 0.46$. MW 381.44. Formula: $C_{22}H_{24}FN_3O_2$. MS *m/z* 382.20 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.76–7.88 (m, 2H), 7.65–7.75 (m, 2H), 7.41 (t, $J = 7.03$ Hz, 1H), 7.17–7.24 (m, 1H), 7.04–7.12 (m, 1H), 6.98 (ddd, $J = 9.82, 8.35, 1.17$ Hz, 1H), 3.87 (s, 2H), 3.81 (t, $J = 6.45$ Hz, 2H), 2.87 (d, $J = 10.55$ Hz, 1H), 2.67–2.75 (m, 1H), 2.62 (t, $J = 6.45$ Hz, 2H), 2.08–2.35 (m, 3H), 1.59–1.81 (m, 2H), 1.23–1.56 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.37, 161.45 (d, $J_{C\text{-}F} = 246.0$), 133.80, 132.16, 130.58 (d, $J_{C\text{-}F} = 4.61$ Hz), 128.77 (d, $J_{C\text{-}F} = 8.20$ Hz), 124.60 (d, $J_{C\text{-}F} = 14.30$ Hz), 124.15 (d, $J_{C\text{-}F} = 3.46$ Hz), 123.15, 115.18 (d, $J_{C\text{-}F} = 21.88$ Hz), 58.79 (d, $J_{C\text{-}F} = 6.91$ Hz), 56.03, 53.74, 53.39, 44.08, 35.50, 30.09, 23.23.

4.1.9.22. 2-(2-(3-(3,5-Difluorobenzylamino)piperidin-1-yl)ethyl)isoindoline-1,3-dione (52). *Tert*-butyl 1-(2-(1,3-dioxoisoindolin-2-yl) ethyl)piperidin-3-ylcarbamate (**49**) was Boc-deprotected using 60% solution of trifluoroacetic acid in DCM (10 mL) to 2-(2-(3-aminopiperidin-1-yl)ethyl)isoindoline-1,3-dione (**50)**. Then following the procedure **G,** reaction of 2-(2-(3-aminopiperidin-1-yl)ethyl)isoindoline-1,3-dione (**50**) (200 mg, 0.732 mmol) with 1-(bromomethyl)-3,5-difluorobenzene (152 mg, 0,732 mmol) and K_2CO_3 (304 mg, 2.196 mmol) in acetonitrile (15 mL) was performed. Purification by extraction (dissolved in 10 mL DCM, 10 mL saturated solution of $NaHCO_3$, then aqueous phase was extracted by 2 × 10 mL DCM) and flash column chromatography in petroleum ether/ethyl acetate (5/5, v/v) gave the product **52** (92 mg, yield 31.4%). TLC petroleum ether/ethyl acetate (5/5, v/v) $R_f = 0.22$. MW 399.43. Formula: $C_{22}H_{23}F_2N_3O_2$. MS *m/z* 400.21 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.76–7.85 (m, 2H), 7.64–7.72 (m, 2H), 6.81–6.94 (m, 2H), 6.59–6.69 (m, 1H), 3.81 (td, $J = 6.45, 3.52$ Hz, 2H), 3.73–3.77 (m, 2H), 2.81 (d, $J = 9.96$ Hz, 1H), 2.62 (td, $J = 6.45, 2.34$ Hz, 3H), 2.46–2.58 (m, 2H), 2.29 (t, $J = 8.50$ Hz, 1H), 1.83–2.00 (m, 1H), 1.56–1.75 (m, 1H), 1.38–1.55 (m, 1H), 1.22–1.36 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.37, 162.62 (dd, $J_{C\text{-}F} = 247.61, 13.82$ Hz), 144.96 (t, $J_{C\text{-}F} = 9.20$ Hz), 133.82, 132.11, 123.09, 110.29–110.77 (m), 102.03 (t, $J_{C\text{-}F} = 25.30$ Hz), 58.79, 55.94, 53.93, 53.15, 50.02 (d, $J_{C\text{-}F} = 2.30$ Hz), 35.43, 30.88, 23.11.

4.1.10. Procedure for the synthesis of compounds 41–44 (procedure H)

The appropriate hexahydropyrimidine derivative (**15** or **16**) (1.1 equiv.) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) or 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (1 equiv.) in the presence of K_2CO_3 (2.5 equiv.) was heated in DMF at temperature 110 °C for 24 h. When the reaction was finished the solvent was evaporated under reduced pressure, producing a residue that was then dissolved in 15 mL of ethyl acetate and washed with water (3 × 10 mL). The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography in petroleum ether/ethyl acetate gradient from 8/2 to 5/5, v/v.

4.1.10.1. 2-(2-(3-(2-Fluorobenzyl)-tetrahydropyrimidin-1(2H)-yl) ethyl)isoindoline-1,3-dione (41). Following the procedure **H**, reaction of 1-(2-fluorobenzyl)-hexahydropyrimidine (**15**) (70 mg, 0.362 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (84 mg, 0,329 mmol) and K_2CO_3 (114 mg, 0.823 mmol) in DMF (3 mL) was performed. Purification by extraction and column chromatography gave the product **41** (56 mg, yield 46%) as a colorless oil. TLC DCM/MeOH (9.5/0.5, v/v) $R_f = 0.35$. MW 367.42. Formula: $C_{21}H_{22}FN_3O_2$. MS *m/z* 356.42 ($M-(CH_2)+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.87–7.89 (m, 2H), 7.78–7.86 (m, 2H), 7.34 (td, $J = 7.57, 1.80$ Hz, 1H), 7.19 (ddd, $J = 7.89, 5.58, 1.92$ Hz, 1H), 7.02–7.11 (m, 1H), 6.97 (ddd, $J = 9.87, 8.34, 1.28$ Hz, 1H), 3.78 (t, $J = 6.80$ Hz, 2H), 3.57 (d, $J = 1.03$ Hz, 2H), 3.28 (s, 2H), 2.60–2.74 (m, 4H), 2.55 (t, $J = 4.60$ Hz, 2H), 1.65 (quin, $J = 5.45$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.37, 161.29 (d, $J_{C\text{-}F} = 245.48$ Hz), 133.84, 132.16, 131.32 (d, $J_{C\text{-}F} = 4.42$ Hz), 128.62 (d, $J_{C\text{-}F} = 8.29$ Hz), 124.96 (d, $J_{C\text{-}F} = 14.37$ Hz), 123.85 (d, $J_{C\text{-}F} = 3.87$ Hz), 123.16, 115.13 (d, $J_{C\text{-}F} = 22.12$ Hz), 75.29, 52.18, 51.88, 51.84, 51.47 (d, $J_{C\text{-}F} = 2.76$ Hz), 35.60, 22.53.

4.1.10.2. 2-(2-(3-(3,5-Difluorobenzyl)-tetrahydropyrimidin-1(2H)-yl) ethyl)isoindoline-1,3-dione (42). Following the procedure **H**, reaction of 1-(3,5-difluorobenzyl)-hexahydropyrimidine (**16**) (94 mg, 0.440 mmol) with 2-(2-bromoethyl)isoindoline-1,3-dione (**1**) (102 mg, 0,400 mmol) and K_2CO_3 (139 mg, 1.000 mmol) in DMF (3 mL) was performed. Purification by extraction and column chromatography gave the product **42** (31 mg, yield 20%) as a colorless oil. TLC DCM/MeOH (9.5/0.5, v/v) $R_f = 0.38$. MW 385.42. Formula: $C_{21}H_{21}F_2N_3O_2$. MS *m/z* 374.36 ($M-(CH_2)+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 7.80–7.88 (m, 2H), 7.67–7.76 (m, 2H), 6.78 (dd, $J = 8.46, 2.31$ Hz, 2H), 6.65 (tt, $J = 8.91, 2.50$ Hz, 1H), 3.78 (t, $J = 6.80$ Hz, 2H), 3.43–3.49 (s, 2H), 3.26 (s, 2H), 2.63–2.75 (m, 4H), 2.53 (t, $J = 5.90$ Hz, 2 H), 1.64 (quin, $J = 5.39$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 168.34, 162.96 (dd, $J_{C\text{-}F} = 247.69, 12.72$ Hz), 143.05 (t, $J_{C\text{-}F} = 8.80$ Hz), 133.87, 132.17, 123.18, 110.91–111.34 (m), 102.29 (t, $J_{C\text{-}F} = 25.40$ Hz), 75.14, 58.06, 52.08, 51.97 (d, $J_{C\text{-}F} = 2.76$ Hz), 35.65, 29.68, 22.20.

4.1.10.3. 2-(2-(3-(2-Fluorobenzyl)-tetrahydropyrimidin-1(2H)-yl) ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (43). Following the procedure **H**, reaction of 1-(2-fluorobenzyl)-hexahydropyrimidine (**15**) (249 mg, 1.280 mmol) with 2-(2-bromoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (336 mg, 1.163 mmol) and K_2CO_3 (402 mg, 2.909 mmol) in DMF (5 mL) was performed. Purification by extraction and column chromatography gave the product **43** (112 mg, yield 24%) as a colorless oil. TLC DCM/MeOH (9.5/0.5, v/v) $R_f = 0.46$. MW 403.47. Formula: $C_{20}H_{22}FN_3O_3S$. MS *m/z* 392.42 ($M-(CH_2)+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ ppm 8.02–8.07 (m, 1H), 7.87–7.94 (m, 1H), 7.80–7.86 (m, 2H), 7.41 (td, $J = 7.44, 1.80$ Hz, 1H), 7.20 (ddd, $J = 7.82, 5.51, 1.80$ Hz, 1H), 7.05–7.13 (m, 1H), 7.00 (ddd, $J = 9.87, 8.34, 1.28$ Hz, 1H), 3.87 (t, $J = 7.40$ Hz, 2H), 3.62 (s, 2H), 3.36

(s, 2H), 2.86 (t, J = 7.20 Hz, 2H), 2.71 (t, J = 5.10 Hz, 2H), 2.59 (t, J = 5.60 Hz, 2H), 1.70 (quin, J = 5.45 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 161.20 (d, J_{C-F} = 246.03 Hz), 158.85, 137.69, 134.64, 134.25, 131.32 (d, J_{C-F} = 4.42 Hz), 128.65 (d, J_{C-F} = 8.29 Hz), 127.44, 125.13, 124.92 (d, J_{C-F} = 13.82 Hz), 123.92 (d, J_{C-F} = 3.32 Hz), 120.88, 115.15 (d, J_{C-F} = 22.12 Hz), 75.11, 52.00, 51.85, 51.50 (d, J_{C-F} = 2.76 Hz), 37.07, 29.68, 22.55.

4.1.10.4. 2-(2-(3-(3,5-Difluorobenzyl)-tetrahydropyrimidin-1(2H)-yl) ethyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide (44). Following the procedure **H**, reaction of 1-(3,5-difluorobenzyl)-hexahydropyrimidine (**16**) (209 mg, 0.985 mmol) with 2-(2-bromoethyl)benzo[*d*] isothiazol-3(2*H*)-one 1,1-dioxide (**2**) (250 mg, 0.895 mmol) and K_2CO_3 (247 mg, 1.790 mmol) in DMF (5 mL) was performed. Purification by extraction and column chromatography gave the product **44** (137 mg, yield 33%) as a colorless oil. TLC DCM/MeOH (9.5/0.5, v/v) R_f = 0.52. MW 421.46. Formula: $C_{20}H_{21}F_2N_3O_3S$. MS *m/z* 410.39 (M-(CH_2)+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.03–8.08 (m, 1H), 7.88–7.94 (m, 1H), 7.81–7.87 (m, 2 H), 6.85 (dd, J = 8.46, 2.31 Hz, 2H), 6.66 (tt, J = 8.91, 2.50 Hz, 1H), 3.86 (t, J = 6.90 Hz, 2H), 3.51 (s, 2H), 3.32 (s, 2H), 2.86 (t, J = 7.40 Hz, 2H), 2.73 (t, J = 5.40 Hz, 2H), 2.54 (t, J = 5.10 Hz, 2H), 1.68 (quin, J = 5.51 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 162.88 (dd, J_{C-F} = 248.24, 12.72 Hz), 158.86, 142.96 (t, J_{C-F} = 8.80 Hz), 137.69, 134.69, 134.28, 127.40, 125.13, 120.91, 110.97–111.45 (m), 102.34 (t, J_{C-F} = 25.40 Hz), 75.13, 58.23, 52.04 (d, J_{C-F} = 3.87 Hz), 51.93, 37.09, 29.68, 22.31.

4.1.11. 2-(3-(Tert-butoxycarbonylamino)piperidin-1-yl)ethyl acetate (53)

Tert-butyl piperidin-3-ylcarbamate (1.027 g, 5.117 mmol) with 2-bromoethyl acetate (0.712 g, 4.264 mmol) and K_2CO_3 (0.589 g, 4.264 mmol) in acetonitrile (42 mL) was heated at reflux for 24 h. When the reaction was finished the solvent was evaporated under reduced pressure, producing a residue that was then dissolved in 20 mL of ethyl acetate and washed with saturated solution of $NaHCO_3$ (3 × 20 mL) and saturated solution of NaCl (30 mL). The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography in gradient of DCM/MeOH (9.6/0.4 to 9.4/0.6, v/v) yielding product **53** as an oil (0.675 g, yield 55%). TLC DCM/MeOH (9.5/0.5, v/v) R_f = 0.24. MW 286.37. Formula: $C_{14}H_{26}N_2O_4$. MS *m/z* 287.28 (M+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 4.94–5.09 (m, 1H), 4.18–4.22 (m, 1H), 4.11–4.18 (m, 2H), 3.80–3.85 (m, 1H), 2.43–2.61 (m, 4H), 2.26–2.42 (m, 2H), 2.05 (s, 3H), 1.47–1.62 (m, 3H), 1.43 (s, 9H).

4.1.12. General procedure for the synthesis of compounds 54, 55 (procedure I)

To a solution of 2-(3-(*tert*-butoxycarbonylamino)piperidin-1-yl) ethyl acetate (**53**) (1 equiv.) in DCM, TFA (1 mL/1.4 mmol substrate) was added. Reaction mixture was stirred for 2 h and then the solvent and TFA were evaporated under reduced pressure. The resulting product was heated under the reflux for 24 h with 1-(chloromethyl)-2-fluorobenzene or 1-(bromomethyl)-3,5-difluorobenzene (0.9 equiv.) in the presence of K_2CO_3 (3 equiv.) in acetonitrile. When the reaction was finished the solvent was evaporated under reduced pressure, and the resulting product was then dissolved in 20 mL of ethyl acetate and washed with saturated solution of $NaHCO_3$ (3 × 20 mL) and saturated solution of NaCl (20 mL). The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography in gradient of DCM/MeOH from 9.8/0.2, v/v to 9.2/0.8, v/v and petroleum ether/ethyl acetate (2/8, v/v).

4.1.12.1. 2-(3-(2-Fluorobenzylamino)piperidin-1-yl)ethyl acetate (54). Following the procedure **I** reaction of 2-(3-(*tert*-butoxycarbonylamino)piperidin-1-yl)ethyl acetate (**53**) (825 mg, 2.880 mmol) with TFA (2 mL) in DCM (7 mL) was performed. Product was dissolved in acetonitrile (20 mL), 1-(chloromethyl)-2-fluorobenzene (307 μL, 2.59 mmol) and K_2CO_3 (1.194 mg, 8.641 mmol) were added. After purification by extraction and column chromatography the product **54** as an oil (368 mg, yield 49%) was obtained. TLC DCM/MeOH (9.5/0.5, v/v) R_f = 0.30. MW 294.36. Formula: $C_{16}H_{23}FN_2O_2$. MS *m/z* 295.32 (M+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.34 (td, J = 7.50, 1.92 Hz, 1H), 7.16–7.25 (m, 1H), 7.04–7.11 (m, 1H), 6.96–7.04 (m, 1H), 4.16 (t, J = 5.90 Hz, 2H), 3.85 (s, 2H), 2.86 (dd, J = 10.90, 2.95 Hz, 1H), 2.57–2.76 (m, 4H), 2.10–2.28 (m, 4H), 2.02 (s, 3H), 1.81 (m., 1H), 1.62–1.74 (m, 1H), 1.52 (dd, J = 9.87, 3.72 Hz, 1H).

4.1.12.2. 2-(3-(3,5-Difluorobenzylamino)piperidin-1-yl)ethyl acetate (55). Following the procedure **I** reaction of 2-(3-(*tert*-butoxycarbonylamino)piperidin-1-yl)ethyl acetate (471 mg, 1.645 mmol) (**53**) with TFA (1.5 mL) in DCM (5 mL) was performed. The product was dissolved in acetonitrile (20 mL), 1-(bromomethyl)-3,5-difluorobenzene (182 μL, 1.4 mmol) and K_2CO_3 (568 mg, 4.113 mmol) were added. After purification by extraction and column chromatography product **55** (172 mg, 33%) was obtained. TLC DCM/MeOH (9.5/0.5, v/v) R_f = 0.33. MW 312.35. Formula: $C_{16}H_{22}F_2N_2O_2$. MS *m/z* 313.27 (M+H^+) ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.81–6.94 (m, 2H), 6.66 (tt, J = 9.00, 2.30 Hz, 1H), 4.18 (t, J = 6.03 Hz, 2H), 3.79 (s, 2H), 2.76–2.91 (m, 1H), 2.58–2.74 (m, 4H), 2.13–2.27 (m, 1H), 2.07 (dd, J = 1.03, 0.51 Hz, 1H), 2.05 (s, 3H), 1.43–1.84 (m, 4H), NH not detected.

4.1.13. General procedure for the synthesis of compounds 58 and 59 (procedure J)

To a solution of compound **54** or **55** (1 equiv.) in anhydrous tetrahydrofuran, di-*tert*-butyl dicarbonate (1.1 equiv.) was added portionwise in the presence of anhydrous triethylamine (3 equiv.) at 0 °C. Reaction mixture was stirred for 2 h in room temperature and the solvent was evaporated under reduced pressure. The resulting residue was then dissolved in 10 mL of ethyl acetate and washed with 10% ammonia solution (10 mL), saturated solution of $NaHCO_3$ (2 × 10 mL) and saturated solution of NaCl (10 mL). The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The resulted product **56** or **57** was dissolved in mixture of methanol and water (2 mL) and heated at 65 °C in the presence of K_2CO_3 (2 equiv.) for 2 h. When the reaction was finished the solvent was evaporated under reduced pressure, producing a residue that was then dissolved in 15 mL of ethyl acetate and washed with water (3 × 15 mL) and saturated solution of NaCl (15 mL). The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography in a gradient of DCM/MeOH (from 9.9/0.1 to 9/1, v/v).

4.1.13.1. Tert-butyl 2-fluorobenzyl(1-(2-hydroxyethyl)piperidin-3-yl) carbamate (58). Following the procedure **J**, reaction of 2-(3-(2-fluorobenzylamino)piperidin-1-yl)ethyl acetate **54** (358 mg, 1.216 mmol) with di-*tert*-butyl dicarbonate (292 mg, 1.338 mmol) in the presence of anhydrous triethyloamine (510 μL, 3.648 mmol) in anhydrous tetrahydrofuran (20 mL) was performed. After extraction the semi-product **56** was dissolved in MeOH-water mixture and heated with K_2CO_3 (336 mg, 2.433 mmol). Purification by extraction and column chromatography gave the product **58** (350 mg, yield 82%) as a colorless oil. TLC DCM/MeOH (9.5/0.5, v/v) R_f = 0.44 MW 352.44. Formula: $C_{19}H_{29}FN_2O_3$. MS *m/z* 353.42 (M+H^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.13–7.30 (m, 2H), 7.08

(td, $J = 9.00$, 1.54 Hz, 1H), 6.98 (dd, $J = 9.20$, 7.95 Hz, 1H), 4.44 (br. s., 2H), 3.54 (t, 5.90 Hz, 2H), 2.66–2.91 (t, 2H), 2.48–2.65 (m, 2H), 1.95–2.14 (m, 1H), 1.88 (td, $J = 11.41$, 2.82 Hz, 1H), 1.55–1.75 (m, 5H), 1.51 (s, 9H), OH not detected.

4.1.13.2. Tert-butyl 3,5-difluorobenzyl(1-(2-hydroxyethyl)piperidin-3-yl)carbamate (59). Following the procedure **J**, reaction of 2-(3-(3,5-difluorobenzylamino)piperidin-1-yl)ethyl acetate (172 mg, 0.551 mmol) (**55**) with di-*tert*-butyl dicarbonate (144 mg, 0.662 mmol) in the presence of anhydrous triethyloamine (230 μL, 1.653 mmol) in anhydrous tetrahydrofuran (10 mL) was performed. After extraction the produced semi-product **57** was dissolved in MeOH-water mixture and heated with K_2CO_3 (152 mg, 1.102 mmol). Purification by extraction and column chromatography gave product **59** (195 mg, yield 96%) as a colorless oil. TLC DCM/MeOH (9.5/0.5, v/v) $R_f = 0.52$. MW 370.43. Formula: $C_{19}H_{28}F_2N_2O_3$. MS *m/z* 371.31 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.59–6.81 (m, 3H), 4.36 (br. s., 2H), 3.56 (t, $J = 5.60$ Hz, 2H), 2.83 (t, $J = 10.52$ Hz, 2H), 2.40–2.58 (m, 2H), 1.97–2.11 (m, 1H), 1.89 (td, $J = 11.61$, 2.69 Hz, 1H), 1.63–1.80 (m, 2H), 1.27–1.62 (m, 12H), OH not detected.

4.1.14. General procedure for the synthesis of compounds 62 and 63 (procedure K)

To a solution of compound **58** or **59** (1 equiv.) in anhydrous DCM in the presence of distilled triethylamine (1.5 equiv.) methanesulfonyl chloride (1.5 equiv.) was added. The reaction mixture was stirred for 2 h, than solvent was evaporated under reduced pressure, producing a residue that was then dissolved in 10 mL of ethyl acetate and washed with water (3 × 10 mL). The organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The product **60** or **61** was dissolved in acetonitrile (7 mL), saccharin sodium was added (1.05 equiv.) and heated in reflux in the presence of K_2CO_3 (1.3 equiv.) for 24 h. When the reaction was finished the solvent was evaporated under reduced pressure, producing a residue that was then dissolved in 15 mL of ethyl acetate and washed with saturated solution of $NaHCO_3$ (15 mL), water (2 × 15 mL) and saturated solution of NaCl (10 mL). The organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography in a gradient of petroleum ether/ethyl acetate (from 7/3 to 5/5, v/v).

4.1.14.1. Tert-butyl -N-[(2-fluorophenyl)methyl]-N-{1-[2-(1,1,3-trioxo-1,2- benzothiazol-2-yl) ethyl]piperidin-3-yl}carbamate (62). Following the procedure **K** reaction of *tert*-butyl 2-fluorobenzyl(1-(2-hydroxyethyl)piperidin-3-yl)carbamate (**58**) (204 mg, 0.58 mmol) with methanesulfonyl chloride (67 μL, 0.87 mmol) in the presence of triethylamine (121 μL, 0.87 mmol) in anhydrous DCM (5 mL) was performed. After extraction the produced semi-product **60** was dissolved in acetonitrile, saccharin sodium was added (123 mg, 0.602) and heated in the presence of K_2CO_3 (104 mg, 0.753 mmol). Purification by extraction and column chromatography gave the product **62** (208 mg, yield 70%) as a colorless oil. TLC petroleum ether/ethyl acetate (5/5, v/v) $R_f = 0.68$. MW 517.61. Formula: $C_{26}H_{32}FN_3O_5S$ MS *m/z* 518.42 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.99–8.11 (m, 1H), 7.76–7.94 (m, 3H), 7.13–7.29 (m, 2H), 7.03–7.12 (m, 1H), 6.93–7.02 (m, 1H), 4.45 (br. s., 2H), 3.94–4.17 (m, 1H), 3.77–3.91 (m, 2H), 2.82–3.06 (m, 2H), 2.62–2.80 (m, 2H), 2.25–2.55 (m, 1H), 2.06–2.24 (m, 1H), 1.93 (t, $J = 10.90$ Hz, 1H), 1.57–1.80 (m, 3H), 1.41 (s, 9H).

4.1.14.2. Tert-butyl-N-[(3,5-difluorophenyl)methyl]-N-{1-[2-(1,1,3-trioxo-1,2- benzothiazol-2-yl)ethyl] piperidin-3-yl}carbamate (63). Following the procedure **K** reaction of *tert*-butyl 3,5-difluorobenzyl(1-(2-hydroxyethyl)piperidin-3-yl)carbamate (**59**) (195 mg, 0.526 mmol) with methanesulfonyl chloride (58 μL, 0.737 mmol) in the presence of triethylamine (102 μL, 0.737 mmol) in anhydrous DCM (7 mL) was performed. After extraction the produced semi-product **61** was dissolved in acetonitrile, saccharin sodium was added (119 mg, 0.583) and heated in the presence of K_2CO_3 (101 mg, 0.729 mmol). Purification by extraction and column chromatography gave the product **63** (147 mg, yield 52%) as a colorless oil. TLC petroleum ether/ethyl acetate (5/5. v/v) $R_f = 0.58$. MW 535.60. Formula: $C_{26}H_{31}F_2N_3O_5S$. MS *m/z* 536.35 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.02–8.07 (m, 1H), 7.78–7.94 (m, 3H), 6.73 (d, = 6.16 Hz, 2H), 6.59–6.68 (m, 1H), 4.37 (br. s., 2H), 3.85 (t, $J = 7.05$ Hz, 2H), 2.89 (t, $J = 10.64$ Hz, 2H), 2.74 (t, $J = 7.05$ Hz, 2H), 2.16 (d, $J = 6.41$ Hz, 1H), 1.96 (t, $J = 11.28$ Hz, 1H), 1.52–1.77 (m, 5H), 1.40 (s, 9H).

4.1.15. Procedure for the synthesis of compounds 64 and 65 (procedure L)

Compound **62** or **63** (1 equiv.) was stirred in 1 M solution of HCl in ethyl acetate (11.5 mL/1 mmol substrate) for 24 h. Then solvent was evaporated and the final product was dried under reduced pressure.

4.1.15.1. 1-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)ethyl)-3-((2-fluorobenzyl) ammonio)piperidin-1-ium chloride (64). Following the procedure **L** reaction of *tert*-butyl-*N*-[(2-fluorophenyl)methyl]-*N*-{1-[2-(1,1,3-trioxo-1,2-benzothiazol-2-yl) ethyl]piperidin-3-yl}carbamate (**62**) (225 mg, 0.434 mmol) with HCl in ethyl acetate (5 mL) was performed. Product **64** was obtained as a white solid (175 mg, yield 96.5%). TLC DCM/MeOH (9/1, v/v) $R_f = 0.61$. MW 417.50. Formula: $C_{21}H_{26}Cl_2FN_3O_3S$. MS *m/z* 418,27 ($M+H^+$). ^{1}H NMR (300 MHz, METHANOL-d_4) δ ppm 8.10–8.21 (m, 2H) 7.95–8.08 (m, 2H) 7.69 (t, $J = 7.18$ Hz, 1H) 7.47–7.59 (m, 1H) 7.19–7.36 (m, 2H) 4.39–4.56 (m, 2H) 4.33 (t, $J = 5.40$ Hz, 2H) 4.10 (m, 1H) 3.83 (m, 2H) 3.71 (t, $J = 3.60$ Hz, 2H) 2.32–2.46 (m, 1H) 2.15–2.28 (m, 1H) 1.75–2.09 (m, 3H) 1.20–1.41 (m, 2H).

4.1.15.2. 3-((3,5-difluorobenzyl)ammonio)-1-(2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)ethyl)piperidin-1-ium chloride (65). Following the procedure **L** reaction of *tert*-butyl-*N*-[(3,5-difluorophenyl)methyl]-*N*-{1-[2-(1,1,3-trioxo-1,2-benzothiazol-2-yl) ethyl]piperidin-3-yl}carbamate (**63**) (64 mg, 0.147 mmol) with HCl in ethyl acetate (2 mL) was performed. Product **65** was obtained as a white solid (44 mg, yield 89.2%). TLC DCM/MeOH (9/1, v/v) $R_f = 0.58$. MW 435.49. Formula: $C_{21}H_{25}Cl_2F_2N_3O_3S$. MS *m/z* 436.39 ($M+H^+$). ^{1}H NMR (300 MHz, METHANOL-d_4) δ ppm 8.10–8.20 (m, 2H) 8.03 (quin, $J = 7.05$ Hz, 2H) 7.34 (d, $J = 5.64$ Hz, 2H) 7.10 (t, $J = 9.10$ Hz, 1H) 4.28–4.64 (m, 4H) 4.06–4.26 (m, 1H) 3.80–4.01 (m, 2H) 3.67–3.78 (m, 2H) 2.32–2.50 (m, 1H) 2.13–2.31 (m, 1H) 1.80–2.12 (m, 3H) 1.14–1.47 (m, 2H).

4.2. Biological activity

4.2.1. In vitro inhibition of AChE and BuChE

Inhibitory activity of the synthesized compounds against the cholinesterases was measured using the spectrophotometric method described by Ellman et al. [49], modified for 24-well microplates. AChE from electric eel (*Ee*AChE) and BuChE from equine serum (*Eq*BuChE) were used. 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATC), butyrylthiocholine

iodide (BTC), and both cholinesterases were purchased from Sigma–Aldrich. The enzymes were prepared by dissolving 500 U of each in demineralized water to give stock solutions of 5 U/mL. AChE and BuChE were further diluted before use to a final concentration of 1.918 U/mL. In the first step of Ellman's method 25 μL of the tested compound (or water; *i.e.* blank samples) was incubated in 0.1 M phosphate buffer (765 μl, pH = 8.0) with the enzyme (20 μL; *Ee*AChE or *Eq*BuChE) at 25 °C. After an incubation period (5 min), 20 μL of DTNB and 20 μL of acetylthiocholine iodide (ATC) or butyrylthiocholine iodide (BTC) solutions (depending on the enzyme used) were added. After another 5 min of incubation, changes in absorbance were measured at 412 nm, using a microplate reader (EnSpire Multimode; PerkinElmer). All the compounds were tested at the screening concentration of 10 μM. Percent of inhibition was calculated from the equation: 100-(S/B) × 100, where S and B were the enzyme activities with and without the tested compound, respectively. For compounds with inhibitory potency higher than 50%, IC_{50} values were determined. For the IC_{50} measurements, six different concentrations of each compound, were used to obtain enzyme activities between 5% and 95%. The IC_{50} values were calculated using nonlinear regression (GraphPad Prism 5. GraphPad Software, San Diego California USA) by plotting the residual enzyme activities against the applied inhibitor concentration. All reactions were performed in triplicate.

4.2.2. Kinetic characterization of AChE inhibition

The kinetic studies were performed with compound **26**, which is the most potent *Ee*AChE inhibitor among synthesized compounds. We used Ellman's method [49], modified for 96-well microplates. The aqueous stock solution of *Ee*AChE (5 U/mL) was diluted before use to a final concentration of 0.384 U/mL (0.027 U/ml in the well). The stock solution (0.02125 M) of substrate acetylthiocholine iodide (ATC) was prepared in demineralized water and diluted before use. For each concentration of the test compound, ATC was used at concentrations of 0.3, 0.24, 0.18, 0.12, 0.06, and 0.04 mM in the wells. According to the modified Ellman's protocol, 25 μL of compound **26** (or water; *i.e.* blank samples) was incubated in 0.1 M phosphate buffer (200 μl, pH = 8.0) with 20 μL of *Ee*AChE and 20 μL of DTNB (0.0025 M; 0.18 mM in the well) in temperature 25 °C for 5 min. Six different concentrations of compound **26** were used to obtain enzyme activities between 30% and 80%. After an incubation period, 20 μL of ATC solutions in six concentrations were added. After a further 5 min, the change in absorbance was measured at 412 nm. 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 calculated using linear regression in GraphPad Prism 5.

4.2.3. In vitro BACE1 inhibitory activity

All were tested as *h*BACE1 inhibitors, using a PanVera's BACE1 fluorescence resonance energy transfer (FRET) Assay Kit, which includes purified baclovirus-expressed BACE1 and specific peptide substrate (Rh-EVNLDAEFK-quencher), based on the "Swedish" mutant of APP [63,64]. The assay was purchased from Life Technologies Polska Sp. z o. o. The analysis were carried out according to the supplied protocol with small modifications, using 384-well black microplates and a microplate reader (EnSpire Multimode; PerkinElmer). The wavelength was equal 553 nm for excitation and 576 nm for emission. Stock solutions of all tested compounds were prepared in DMSO and further diluted with assay buffer (50 mM sodium acetate; pH 4.5). In the first step 10 μL of BACE1 substrate was mixed with 10 μL of tested compound (or assay buffer; *i.e.* blank sample), then 10 μL of enzyme (1 U/mL) was added to start the reaction. After 60 min of incubation at 25 °C 10 μL of stop solution (2.5 M sodium acetate) was applied to stop the reaction. The fluorescence signal was read at 576 nm. Percent of inhibition was calculated from [1 − (S60 − S0)/(C60 − C0)] × 100, where S0 and S60 are fluorescence intensities of the tested sample (enzyme, substrate, test compound) at the beginning of the reaction and after 60 min respectively, while C0 and C60 are analogical fluorescence intensities of the blank sample (enzyme, substrate, buffer). All compounds were tested at screening concentration of 50 μM. Each compound was analyzed in triplicate. BACE1 Inhibitor IV (Calbiochem, Merck; Nottingham, UK) was used as the reference compound. To determine IC_{50} value of Inhibitor IV, eight different concentrations of this compound were used to obtain enzyme activities between 10% and 95%. The IC_{50} values were calculated using nonlinear regression (GraphPad Prism 5; GraphPad Software, San Diego California USA) by plotting the residual enzyme activities against the applied inhibitor concentration.

4.2.4. Inhibition of Aβ-aggregation

In order to investigate the inhibition of β-amyloid peptide aggregation by compounds, a Thioflavin T-based fluorometric assay was performed [56]. Recombinant human HFIP-pretreated $A\beta_{1-42}$ peptide (Lot 2665028, Merck Millipore, Darmstadt, Germany) was dissolved in DMSO to give 75 μM stock solution. The stock solution was further diluted in HEPES buffered solution (150 mM HEPES, pH 7.4, 150 mM NaCl), to 7.5 μM. $A\beta_{1-42}$ solution was then added to the test compounds in black-walled 96-well plate, and diluted with ThT solution (final concentration of 10 μM). Final mixture contained 1.5 μM $A\beta_{1-42}$, 10 μM of tested compound and 3% of DMSO. ThT fluorescence was measured every 300 s (excitation wavelength of 440 nm, emission wavelength of 490 nm), with the medium continuously shaking between measurements using a 96-well microplate reader (Synergy™ H4, BioTek Instruments, Inc., USA). The ThT emission of the $A\beta_{1-42}$ began to rise after 4–8 h, reached a plateau after 20 h, and remained almost unchanged for an additional 28 h of incubation. The fluorescence intensities at the plateau in the absence and presence of the tested compound were averaged, and the average fluorescence of the corresponding wells at t = 0 h was subtracted. The $A\beta_{1-42}$ aggregation inhibitory potency is expressed as the percentage inhibition (% inh= $(1 - F_i/F_0) \times 100\%$), where F_i is the increase in fluorescence of $A\beta_{1-42}$ treated with the tested compound, and F_0 is the increase in fluorescence of $A\beta_{1-42}$ alone.

4.2.5. PAMPA-BBB assay

In order to predict passive blood-brain penetration of compounds **26**, **29**, **32**, **36**, **37** modification of the parallel artificial membrane permeability assay (PAMPA) has been used based on reported protocol [57,66]. The filter membrane of the donor plate was coated with PBL (Polar Brain Lipid, Avanti, USA) in dodecane (4 μl of 20 mg/ml PBL in dodecane) and the acceptor well was filled with 300 μl of PBS pH 7.4 buffer (VD). Tested compounds were dissolved first in DMSO and that diluted with PBS pH 7.4 to reach the final concentration 100 μM in the donor well. Concentration of DMSO did not exceed 0.5% (V/V) in the donor solution. 300 μl of the donor solution was added to the donor wells (VA) and the donor filter plate was carefully put on the acceptor plate so that coated membrane was "in touch" with both donor solution and acceptor buffer. Test compound diffused from the donor well through the lipid membrane (Area = 0.28 cm^2) to the acceptor well. The concentration of the drug in both donor and the acceptor wells was assessed after 3, 4, 5 and 6 h of incubation in quadruplicate using the UV plate reader Synergy HT (Biotek, USA) at the maximum absorption wavelength of each compound. Concentration of the compounds was calculated from the standard curve and expressed as the permeability (Pe) according equation (1) [65,66]:

$$\log P_e = \log\{C \times -ln(1 - \frac{[drug]_{acceptor}}{[drug]_{equilibrium}})\}, \text{ where } C = (\frac{V_D \times V_A}{(V_D + V_A) \times Area \times time}) \quad (1)$$

4.3. Molecular modeling

The three-dimensional ligand structures were built with the Corina online tool (Molecular Networks GmbH, Erlangen, Germany). Subsequently, atom types and protonation states were checked and Gasteiger-Marsili charges were assigned using Sybyl-X 1.1 (Tripos, St. Louis, MO, USA). Finally, ligand structures were saved in the mol2 format.

Docking to *Torpedo californica* AChE (PDB code: 1EVE) and human BACE-1 (PDB code: 4D8C) was performed using Gold 5.1 program (The Cambridge Crystallographic Data Center, Cambridge, UK). Both targets were prepared as follows: all histidine residues were protonated at Nε, hydrogen atoms added, ligand molecules removed, and binding site defined as all amino acid residues within 10 Å from ligands: donepezil and NVP-BXD552, respectively. In case of BACE1 1 chain C was selected from oligomer, and numbers of residues were renumbered to the default. The presence of conserved water molecules was taken into account in case of acetylcholinesterase. A standard set of genetic algorithm with population size of 100 and number of operations of 100 000 was applied. As a result, 10 ligand conformations were obtained and sorted according to values of scoring function - ChemScore for AChE and GoldScore for BACE-1. Results were visualized by PyMOL 0.99rc2 (DeLano Scientific LLC, Palo Alto, CA, USA).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.09.078.

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