ACS Chemical Neuroscience

Subscriber access provided by READING UNIV

Article

Design, synthesis and biological evaluation of 1-benzylamino-2-hydroxyalkyl derivatives as new potential disease-modifying multifunctional anti-Alzheimer's agents

Dawid Panek, Anna Wi#ckowska, Jakub Jo#czyk, Justyna Gody#, Marek W. Bajda, Tomasz Wichur, Anna Pasieka, Damijan Knez, Anja Pislar, Jan Korabecny, Ondrej Soukup, Vendula Sepsova, Raimon Sabaté, Janko Kos, Stanislav Gobec, and Barbara Malawska

ACS Chem. Neurosci., **Just Accepted Manuscript** • DOI: 10.1021/acschemneuro.7b00461 • Publication Date (Web): 18 Jan 2018

Downloaded from http://pubs.acs.org on January 19, 2018

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

ACS Publications

ACS Chemical Neuroscience is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Design, synthesis and biological evaluation of 1-benzylamino-2-hydroxyalkyl derivatives as new potential disease-modifying multifunctional anti-Alzheimer's agents

Dawid Panek[a1], Anna Więckowska[a1], Jakub Jończyk[a], Justyna Godyń[a], Marek Bajda[a], Tomasz Wichur[a], Anna Pasieka[a], Damijan Knez[b], Anja Pišlar[b], Jan Korabecny[c,d], Ondrej Soukup[c,d], Vendula Sepsova[c,d], Raimon Sabaté[e,f], Janko Kos[b], Stanislav Gobec[b], Barbara Malawska[a]*

[a]Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland

[b]Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

[c]Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

[d]Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defense, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

[e]Department of Pharmacy and Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy and Food Science, University of Barcelona, Av Joan XXIII 27-31, 08028 Barcelona, Spain

[f]Institute of Nanoscience and Nanotechnology (IN2UB), University of Barcelona, Av Joan XXIII, S/N, 08028 Barcelona, Spain

*Correspondence: mfmalaws@cyf-kr.edu.pl

[1] These authors contributed equally to this work

Abstract

The multitarget approach is a promising paradigm in drug discovery, potentially leading to new treatment options for complex disorders, such as Alzheimer's disease. Herein, we present the discovery of a unique series of 1-benzylamino-2-hydroxyalkyl derivatives combining inhibitory activity against butyrylcholinesterase, β-secretase, β-amyloid and tau protein aggregation, all related to mechanisms which underpin Alzheimer's disease. Notably, diphenylpropylamine derivative **10** showed balanced activity against both disease-modifying targets – inhibition of β-secretase ($IC_{50\ hBACE\text{-}1}$ = 41.60 μM), inhibition of amyloid β aggregation ($IC_{50\ A\beta}$ = 3.09 μM), inhibition of tau aggregation (55% at 10 μM), as well as against symptomatic targets: butyrylcholinesterase inhibition ($IC_{50\ hBuChE}$ = 7.22 μM). It might represent an encouraging starting point for development of multifunctional disease-modifying anti-Alzheimer's agents.

Keywords: Alzheimer's disease; multifunctional agents; butyrylcholinesterase inhibitors; BACE-1 inhibitors; Aβ aggregation; tau aggregation; molecular docking

1. Introduction

Nearly 36 million are affected people today, and three times more projected to be affected in 2050; these alarming numbers show that Alzheimer's disease (AD) remains a serious economic and social problem.[1,2] Search for novel drugs and effective therapies against AD is one of the largest challenges for modern medicine and pharmacotherapy.[3] Although the cause of the disease is not fully understood, a variety of mechanisms that might be involved in its pathogenesis have been described. Among them, deposition of amyloid β (Aβ) peptide, hyperphosphorylation and aggregation of the tau protein, oxidative stress, and cholinergic

neurotransmission deficiency are the main players in the etiopathogenetic spectacle.[4–7] The cholinergic system is linked with cognitive processes in the brain; therefore, impaired cholinergic neurotransmission leads to memory-associated symptoms of AD.[8–10] Current research has revealed that the deficiency of acetylcholine (ACh) is not the primary cause of AD but a consequence contributing to symptoms related to neurodegeneration,. Physiologically, the activity of ACh is terminated in the synapses by acetylcholinesterase (AChE, E.C. 3.1.1.7) and butyrylcholinesterase (BuChE, E.C. 3.1.1.8). While AChE hydrolyses the majority of acetylcholine in a healthy brain, with the progression of the disease, BuChE takes over the role of AChE, reaching almost 80% of the overall cholinesterase activity.[11,12] Three of the four currently available drugs registered between 1997 and 2003 (donepezil, rivastigmine, galantamine) act as AChE and/or BuChE inhibitors.[13–15] The effectiveness of these drugs is limited since they are able to ameliorate the symptoms only in the early stages of the disease. However, cholinesterases still represent valuable targets in the search for new anti-AD agents.

A widely accepted amyloid hypothesis have postulated the formation and aggregation of the β-amyloid (Aβ) peptide as the main cause of AD.[16,17] Aβ is a neurotoxic peptide composed of 37-42 amino acids that is formed as a consequence of an enzymatic cleavage of the amyloid precursor protein (APP) by β-secretase (β-site APP-cleaving enzyme 1, BACE-1) and γ-secretase. Aβ peptide, particularly the 42-amino-acid form, has high propensity for aggregation leading to neuroinflammation, oxidative stress, loss of neurons and finally to cognitive and psychological impairment. Accordingly, reduction of Aβ levels in the central nervous system (CNS) is considered a rational therapeutic approach in the search for new anti-Alzheimer's agents.[18–21]

The formation of neurofibrillary tangles (NFTs) is another pathological process occurring in the course of AD. NFTs are composed of hyperphosphorylated microtubule-associated tau

proteins. In the healthy brain, tau stabilizes microtubules, but when hyperphosphorylated, it forms paired helical filaments that aggregate into NFTs. NFTs promote the instability of microtubules and impair axonal transport, leading to neurotoxicity and cell death. Ever since the correlation between tau aggregation, neuron death and clinical dementia was proven, it has become one of the key targets in AD.[6,22,23] Despite the very important role of tau protein in the development of AD, there is a limited number of compounds with tau-inhibitory activity.[24–26] Several studies have provided evidence that the formation of tau tangles can be induced by Aβ.[27–29] Therefore, compounds with inhibitory activities against Aβ and tau-aggregation might have disease-modifying effects, which are of special interest for modern drug discovery.

Taken together, AD is a complex disease of a multi-factorial nature, and there are multiple therapeutic targets that could be the key to the effective treatment of the disease. Therefore, AD is considered a disease that requires combination therapy or, as an alternative, development of multi-target directed ligands (MTDLs) for its effective therapy. MTDLs are molecules that can interact simultaneously with more than one biological target. Design and discovery of new multipotent ligands as potential anti-AD drugs is an attractive and widely explored approach in drug discovery, as reflected in a number of current research papers.[30–32]

Researchers are still exploring new and emerging routes to develop effective therapies for AD. The appropriate selection of biological targets, including those designated as being disease-modifying, is crucial for the design of potent MTDLs. It is, however, challenging to obtain compounds with balanced activities against the chosen targets that concomitantly retain the drug-like properties. Herein, we present the results of our research that meet all the above criteria. Indeed, 1-benzylamino-2-hydroxyalkyl derivatives represent a novel class of MTDLs purposely designed as anti-AD agents, inhibitors of AChE, BuChE, BACE-1, amyloid β and tau aggregation. Due to their balanced activity profile against the selected targets they could

display potential symptomatic as well as disease-modifying effects. Their druglike properties were calculated prior to their synthesis as a part of preliminary data (see Supplementary data). Molecular modelling studies were performed to explain enzyme-ligand interactions and to support the structure-activity-relationship discussion.

2. **Results and discussion**

2.1 Design

The presented research is a continuation of our previous investigation dedicated to the search for MTDLs with cholinesterase-, BACE-1- and amyloid-β- inhibitory activities.[33] Recently, we have reported a series of donepezil-related compounds with alicyclic amines containing two basic nitrogen atoms in their structures (**compound I, Fig. 1.**). We assumed that such a basic core would interact with the catalytic dyad of BACE-1 consisting of two aspartic acid residues: Asp32 and Asp228.[34] In the compounds presented herein, we replaced the alicyclic amines with 2-hydroxyethyleneamine, a transition state analogue, in order to improve their inhibitory properties against BACE-1. The designed structures were based on a benzylamine scaffold taken from donepezil that could interact with the catalytic active site (CAS) of AChE, as reported previously.[35] Moreover, substituted benzylamines are commonly used in potent BACE-1 inhibitors **NVP-BXD-552**[34,36] and **GRL-8234**[37,38] (**Fig. 1.**), among others.

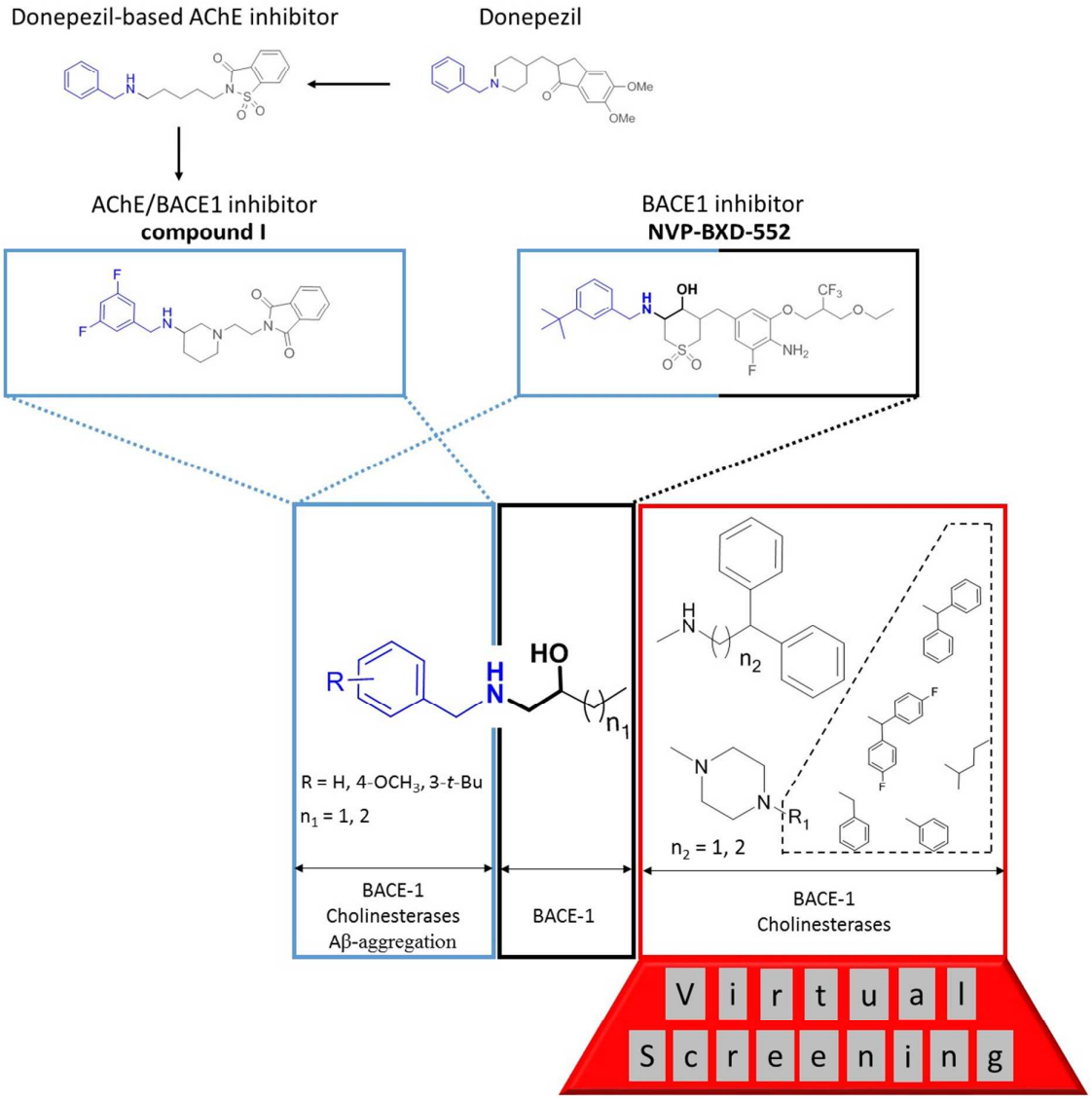


Figure 1. Design strategy of new MTDLs targeting cholinesterases and BACE-1.

The benzyl fragment (**Fig. 2, fragment A**) was connected through a 2-hydroxyethyleneamine linker (**Fig. 2, fragment B**) with the fragments selected from the virtual-screening results of the building blocks available from Sigma Aldrich against BACE-1, AChE and BuChE (**Fig. 2, fragment C**). The fragment data based included primary and secondary amines with aliphatic or aromatic substituents. Building blocks were directed to occupy the hydrophobic S1, S3 and polar S2, S4 sub-pockets of BACE-1 as well as the peripheral active sites of AChE and BuChE.[39] From the top-scored docking poses, we selected 4-(diphenylmethyl)piperazine and

4-(bis(4-fluorophenyl)methyl)piperazine, as well as their elongated analogues 2,2-diphenylethanamine or 3,3-diphenylpropan-1-amine, and truncated analogues 4-phenylpiperazine, 4-benzylpiperazine or 4-(pentan-2-yl)piperazine.

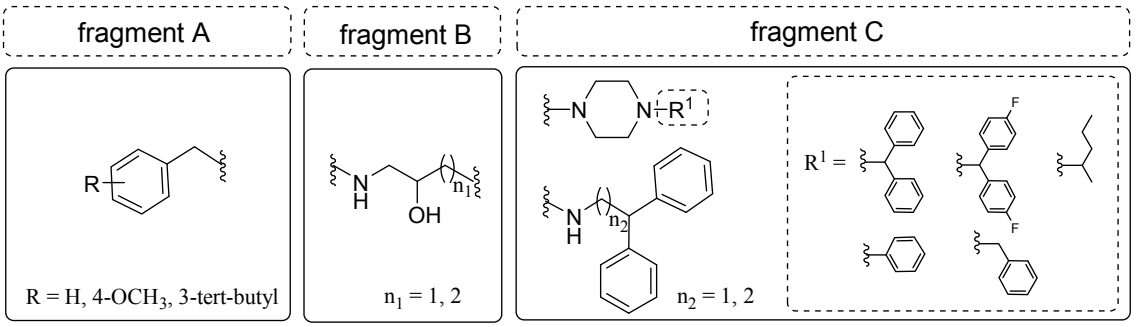


Figure 2. Combinations of different fragments yielding novel multipotent hybrids to confront AD

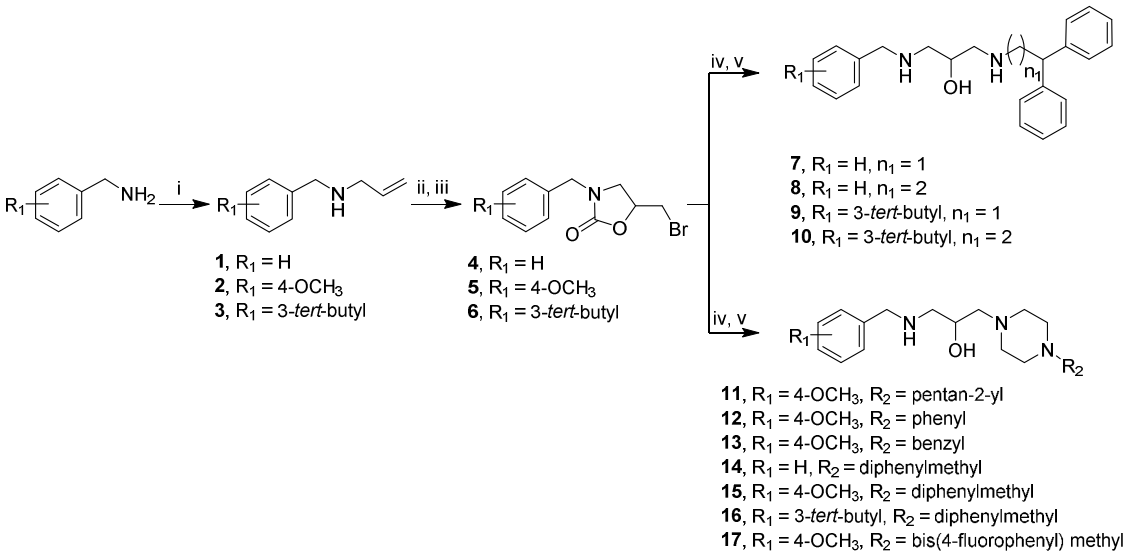
We expected that the designed molecules could also have anti-aggregation properties against neurotoxic β-amyloid because our recent studies showed that several series of substituted benzylamine derivatives possess Aβ anti-aggregation potency.[40–43] Analysis of a set of over 100 compounds revealed that more than 40% of them have Aβ anti-aggregation activity at a 10 μM screening concentration. Molecular docking revealed that the compounds containing a benzylamine moiety are able to anchor to the amyloid fibre along its axis between two beta-sheet fragments.[44] The benzyl fragment formed hydrophobic interactions with alanine (A21), leucine (L34) and valine (V36), while the protonated amine group formed ionic interactions with the carboxyl group of aspartic acid (D23). Therefore, we decided to evaluate Aβ anti-aggregation properties of the described novel benzylamine derivatives. It was also shown for several moieties and compounds that inhibition of Aβ aggregation correlates with inhibition of tau protein aggregation.[45,46] Accordingly, we explored the propensity of our novel hybrids to inhibit tau aggregation.

Considering the importance of physicochemical properties of compounds in relation to their drug-likeness and success in the drug development process we calculated the most important parameters of the newly designed MTDLs[47]. A majority of the compounds meet the criteria set by Lipinski's rule of five[48] and the requirements of bioavailability.

2.2 Chemistry

We obtained 24 final compounds that can be classified into two groups: 1,3-diaminopropan-2-ol derivatives (**7**–**17**) and 1,4-diaminobutan-2-ol derivatives (**28**–**38**, **45**, **46**).

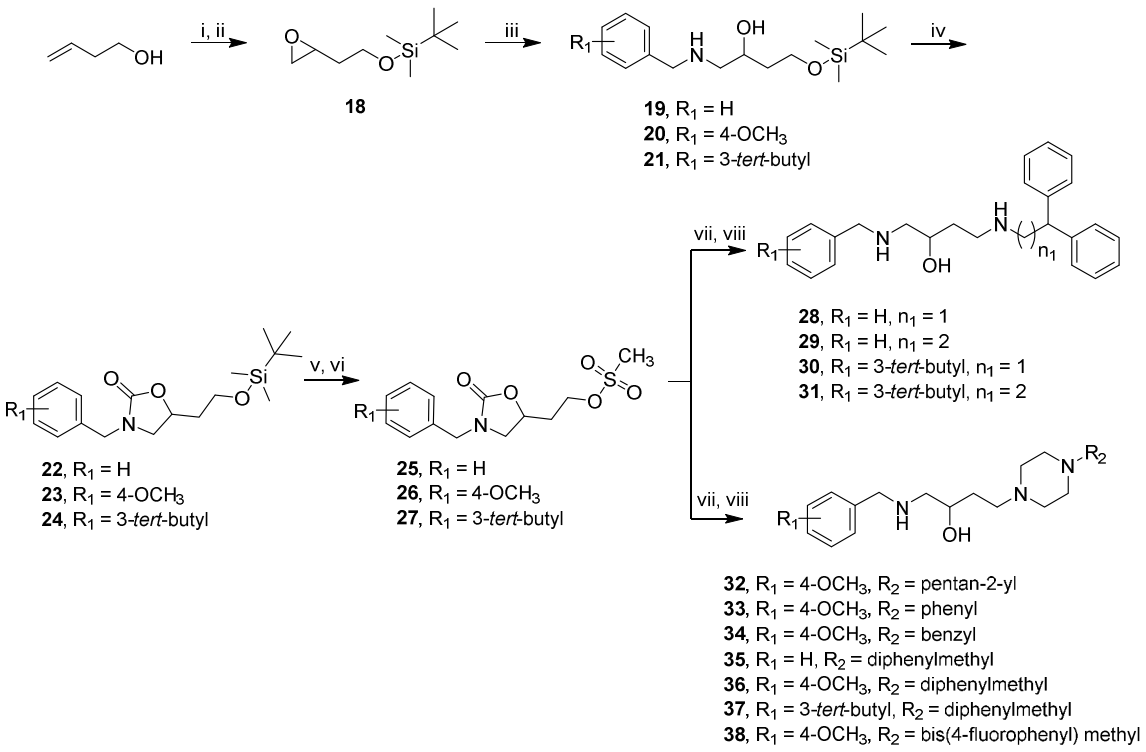
The first class, bearing 1,3-diaminopropan-2-ol, was synthesized according to the **Scheme 1**. In the first step, *N*-allylbenzylamine derivatives (**1**–**3**) were obtained by alkylation of non-substituted or appropriately substituted (4-OCH_3, 3-*tert*-butyl) benzylamines with allyl chloride. The secondary amine groups in the resulting products were then *N*-BOC protected. Next, the reaction with *N*-bromosuccinimide led to the appropriate bromohydrins that, in the conditions used, spontaneously formed oxazolidin-2-one rings that served as protecting groups for the amino and hydroxyl groups. This yielded the compounds (**4**–**6**) used in the next step as alkylating agents for different *N*-substituted amines. The final compounds (**7**–**17**) were prepared by alkaline hydrolysis of oxazolidin-2-ones, that led to the 1,3-diaminopropan-2-ol moiety in the core.



Scheme 1. Reagents and conditions: i) allyl chloride, MeCN, K_2CO_3, 80 °C, 16 h; ii) di-*tert*-butyl dicarbonate, TEA, DCM, R.T., 4 h; iii) *N*-bromosuccinimide, water, MeCN, R.T., 16 h; iv) 4-(pentan-2-yl)piperazine/4-phenylpiperazine/4-benzylpiperazine/*N*-(diphenylmethyl)piperazine/4-(bis(4-fluorophenyl)methyl) piperazine/2,2-diphenylethanamine/3,3-diphenylpropan-1-amine, MeCN/ toluene/DMF (microwave-assisted), K_2CO_3, 60–80 °C, 1–16 h; v) ethanol, NaOH, 78 °C, 6 h.

To obtain 1,4-diaminobutan-2-ol derivatives, we used the synthetic route depicted in **Scheme 2**. First, the hydroxyl group of but-3-en-1-ol was protected with a *tert*-butyl dimethyl silyl group. Then, the double bond was oxidized with *meta*-chloroperoxybenzoic acid. The resulting epoxide **18** was used as an alkylating agent in the reaction with the appropriate benzylamines to provide 1,2-aminoalcohols **19**–**21**. In the next step the amino and hydroxyl groups were protected by transformation to oxazolidin-2-one derivatives using carbonyldiimidazole. Then, the *tert*-butyl dimethyl silyl group was removed from **22**–**24** by tetra-*n*-butylammonium fluoride and the resulting alcohol was reacted with methanesulfonyl chloride. Mesylates **25**–**27** were used to alkylate the appropriate primary or secondary amines. Some of these alkylations were carried out using microwave radiation, which reduced the

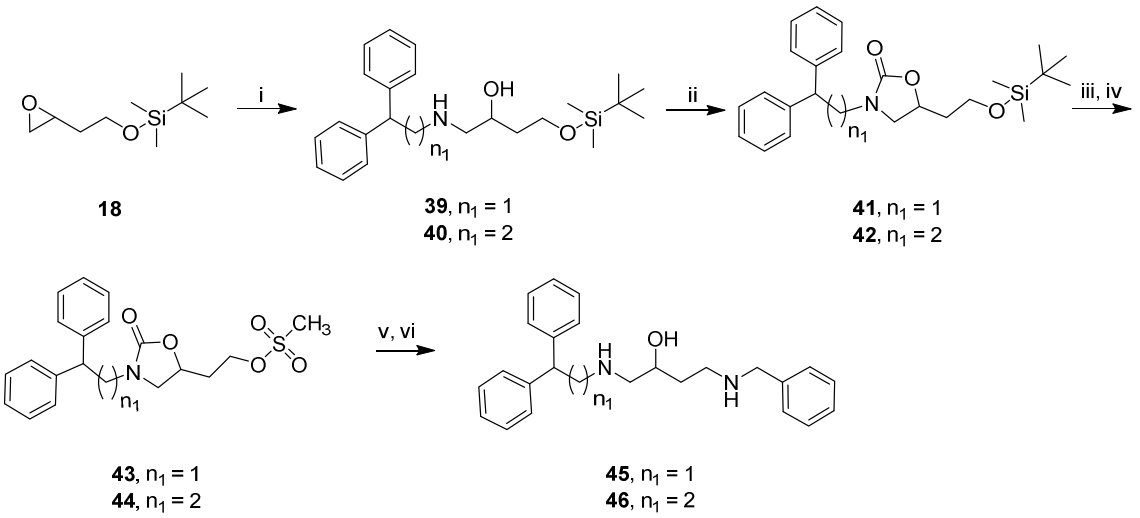
time of reaction from 16 hours to 1 hour. The final compounds **28**–**38** were obtained after alkaline hydrolysis of oxazolidin-2-one.



Scheme 2. Reagents and conditions: i) *tert*-butylchlorodimethylsilane, DCM, imidazole, R.T., 5 h, ii) *meta*-chloroperoxybenzoic acid, DCM, R.T. 16 h, iii) phenylmethanamine/(4-methoxyphenyl)methanamine/(3-(*tert*-butyl)phenyl)methanamine, *n*-propanol, pyridine, 97 °C, iv) carbonyldiimidazole, DCM, TEA, R.T., 5 h, v) tetra-*n*-butylammonium fluoride, THF, R.T., 16 h; vi) methanesulfonyl chloride, DCM, TEA, R.T., 2 h; vii) 4-(pentan-2-yl)piperazine/4-phenylpiperazine/*N*-benzylpiperazine/*N*-(diphenylmethyl)piperazine/4-(bis(4-fluorophenyl)methyl)piperazine/2,2-diphenylethanamine/3,3-diphenylpropan-1-amine, MeCN/toluene/DMF (microwave assisted), K_2CO_3, 80 °C, 1–16 h; viii) ethanol, NaOH, 78 °C, 6 h.

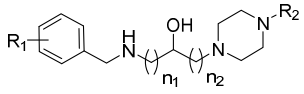
Compounds **45** and **46,** with the four-carbon fragment B, (**Fig. 2**) were prepared by a slightly modified synthetic pathway presented in **Scheme 3**. The principal difference was the

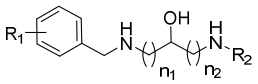
order of amine substitution; herein, ω, ω'-diphenylalkylamines were alkylated prior to attachment of the benzylamine moiety in the last step.



Scheme 3. Reagents and conditions: i) 2,2-diphenylethanamine/3,3-diphenylpropan-1-amine, *n*-propanol, pyridine, 97 °C, ii) carbonyldiimidazole, DCM, TEA, R.T., 5 h, iii) tetra-*n*-butylammonium fluoride, THF, R.T., 16 h; iv) methanesulfonyl chloride, DCM, TEA, R.T., 2 h; v) phenylmethanamine, MeCN, K_2CO_3, 80 °C, 16 h; vi) ethanol, NaOH, 78 °C, 6 h.

Table 1. Inhibition of *ee*AChE, *eq*BuChE, *h*BuChE, *h*BACE-1 and Aβ aggregation by **7**–**17**, **28**–**38**, **45**, **46**.[a]

Cmp.	R_1	R_2	n_1	n_2	*ee*AChE IC_{50} [μM]/ % inh.[b]	*eq*BuChE IC_{50} [μM]/ % inh.[c]	*h*BuChE IC_{50} [μM]/ % inh.[d]	*h*BACE1 IC_{50} [μM]/ % inh.[e]	Inh. of Aβ aggr. IC_{50} [μM]/ % inh.[f]
									
11	4-OMe	pentan-2-yl	1	1	<10%	20.03% ± 0.57	n.d.[h]	31.08% ± 4.14	15.9% ± 1.1
12	4-OMe	phenyl	1	1	<10%	39.57% ± 2.27	n.d.[h]	23.67% ± 4.43	<10%
13	4-OMe	benzyl	1	1	<10%	24.91% ± 1.39	n.d.[h]	24.65% ± 1.66	<10%
14	H	diphenylmethyl	1	1	<10%	30.72% ± 2.55	n.d.[h]	43.55% ± 2.53	21.08% ± 0.5
15	4-OMe	diphenylmethyl	1	1	<10%	24.50% ± 0.70	n.d.[h]	47.45% ± 2.98	16.3% ± 5.2
16	3-*tert*-butyl	diphenylmethyl	1	1	14.13% ± 4.03	16.61 ± 0.35	<50%	41.59% ± 4.83	77.2% ± 2.1
17	4-OMe	bis(4-fluorophenyl)methyl	1	1	14.04% ± 2.32	7.12 ± 0.15	<50%	79.68 ± 2.72	21.8% ± 10.5
32	4-OMe	pentan-2-yl	1	2	3.62 ± 0.07	9.36 ± 0.08	<50%	35.03% ± 5.52	23.7% ± 8.9
33	4-OMe	phenyl	1	2	<10%	<10%	n.d.[h]	22.43% ± 2.37	<10%
34	4-OMe	benzyl	1	2	36.62% ± 1.85	35.55% ± 0.64	n.d.[h]	34.88% ± 7.73	32.3% ± 14.0
35	H	diphenylmethyl	1	2	17.08% ± 7.92	21.01% ± 5.13	n.d.[h]	42.95% ± 3.60	24.2% ± 9.9

36	4-OMe	diphenylmethyl	1	2	13.63% ± 0.98	31.52% ± 3.05	n.d.[h]	33.89% ± 3.28	15.2% ± 8.1
37	3-*tert*-butyl	phenylmethyl	1	2	<10%	9.16 ± 0.24	n.d.[h].	41.43% ± 3.68	81.4% ± 7.9
38	4-OMe	bis(4-fluorophenyl)methyl	1	2	25.08% ± 1.10	45.57% ± 1.76	n.d.[h]	101.90 ± 2.76	<10%
									
7	H	2,2-diphenylethylamine	1	1	17.94% ± 3.13	1.49 ± 0.03	<50%	76.47 ± 3.60	33.6% ± 1.2
8	H	3,3-diphenylpropylamine	1	1	<10%	1.59 ± 0.05	6.76 ± 0.18	55.42% ± 7.36	53.3% ± 2.7
9	3-*tert*-butyl	2,2-diphenylethylamine	1	1	<10%	1.55 ± 0.05	7.22 ± 0.32	64.48 ± 2.55	88.7% ± 5.5
10	3-*tert*-butyl	3,3-diphenylpropylamine	1	1	21.78% ± 5.46	2.92 ± 0.1	5.74 ± 0.26	41.60 ± 1.47	88.2% ± 5.9 3.09 ± 1.64
28	H	2,2-diphenylethylamine	1	2	13.22% ± 0.42	2.90 ± 0.04	5.18 ± 0.16	63.76 ± 3.27	29.7% ± 6.3
29	H	3,3-diphenylpropylamine	1	2	<10%	6.74 ± 0.12	<50%	39.68 ± 2.11	<10%
30	3-*tert*-butyl	2,2-diphenylethylamine	1	2	<10%	2.09 ± 0.08	2.69 ± 0.12	49.24% ± 16.11	87.6% ± 3.1
31	3-*tert*-butyl	3,3-diphenylpropylamine	1	2	<10%	5.61 ± 016	<50%	32.17 ± 1.46	84.9% ± 0.8 1.22 ± 0.89
45	-	2,2-diphenylethylamine	2	1	<10%	6.64 ± 0.086	<50%	36.24% ± 2.96	44.8% ± 1.3
46	-	3,3-diphenylpropylamine	2	1	<10%	2.34 ± 0.044	4.99 ± 0.18	22.26% ± 13.86	45.4% ± 5.0
					References				
		Tacrine			0.023±0.0004	0.015±0.0001		n.d.[h]	
		Donepezil			0.011±0.0002	1.83±0.04		n.d.[h]	13.80% ± 6.8
		Inhibitor IV[g]			n.d.[h]	n.d.[h]		0.046 ± 0.001	
		Resveratrol							78.50 ± 3.1

- the most interesting values

[a] Values are expressed as the 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*BACE-1 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 BuChE or percent inhibition with inhibitor at 10 μM.

[e] IC_{50} inhibitory concentration of human recombinant BACE-1 and substrate (Rh-EVNLDAEFK-quencher) or percent inhibition with inhibitor at 50 mM.

[f] IC_{50} inhibitory concentration of self-induced $A\beta_{1-42}$ aggregation or percent inhibition with inhibitor at 10 μM.

[g] Calbiochem, Merck; Nottingham, UK.

[h] Not determined.

2.3 Inhibition of *ee*AChE, *eq*BuChE and *h*BuChE

We evaluated the abilities of the synthesized compounds to inhibit AChE from electric eel (*ee*AChE) and BuChE from equine serum (*eq*BuChE) using Ellman's method.[49] Initially, we performed a screening assay using a single concentration of inhibitors (10 μM) and determined their activity as a percentage of enzyme inhibition. Based on the preliminary data, the compounds with inhibitory potencies higher than 50% were established, and their inhibitory potencies were expressed as IC_{50} values. Tacrine and donepezil were used as positive reference compounds. The results of the assay are presented in **Table 1**.

Among the tested compounds, only compound **32** displayed inhibitory activity against both *ee*AChE and *eq*BuChE, with IC_{50} values of 3.62 μM and 9.36 μM, respectively. At the same time, we found some potent inhibitors of *eq*BuChE with IC_{50} values ranging from 1.49 to 16.61 μM: **7**–**10**, **16**, **17**, **28**–**31**, **37**, **45**, and **46,** with **7** being the most promising. Since these compounds were almost inactive against *ee*AChE they are considered as being selective *eq*BuChE inhibitors. A structure-activity relationship analysis revealed that the most active compounds contain a 2,2-diphenylethylamine or 3,3-diphenylpropylamine moiety in their structures (**Fig. 2, fragment C**). Replacement of these fragments with 4-piperazine resulted in a noticeable reduction of their inhibitory activity (**14** *vs.* **7** and **8**, **35** *vs.* **28** and **29**), although this trend was not observed in the compounds with a 3-*tert*-butyl-benzylamine fragment (**Fig. 2, fragment A**) (**16** *vs.* **9** and **10**, **37** *vs.* **30** and **31**). The *tert*-butyl substituent improved the activity of *N*-(diphenylmethyl)piperazine derivatives as compared to unsubstituted ones (**14**

vs. **16**, **35** *vs.* **37**) but it did not have the same effect on ω, ω'-diphenylalkylamine derivatives (**7** *vs.* **9, 8** *vs.* **10**, **28** *vs.* **30**, **29** *vs.* **31**). Regarding the length of an alkylene core (**Fig. 2, fragment B**), we observed slightly higher activities for 1,3-diaminopropan-2-ol derivatives than for 1,4-diaminobutan-2-ol derivatives (**28** *vs.* **7**, **30** *vs.* **9**, **29** *vs.* **8**, **31** *vs.* **10**). Based on the results we can say that a secondary ω, ω'-diphenylalkylamine group (**Fig. 2, fragment C**) is more beneficial here than the two tertiary amine groups of the piperazine fragment (**Fig. 2, fragment C**).

The selectivity of the presented compounds against BuChE over AChE may be a consequence of the size of **fragment C** (**Fig. 2**). Larger fragments, such as those containing benzhydryl moieties, are too bulky to fit into the narrow gorge of the AChE catalytic site. On the other hand, the BuChE active site is more spacious and accepts larger molecules; therefore, the presented compounds are selective BuChE inhibitors. The only exception is compound **32**, with a smaller and more flexible 4-(pent-2-yl)piperazine fragment, which is an inhibitor of both AChE and BuChE. Similar observations have already been described in previously published papers.[41,50,51]

For the most potent inhibitors of *eq*BuChE we also evaluated their inhibitory potencies against human BuChE (*h*BuChE). We found that six of the tested compounds (**8**–**10**, **28**, **30**, **46**) inhibit this enzyme more than 50%, and for those six compounds, we established IC_{50} values ranging from 2.69 to 7.22 μM. Only the compounds with 2,2-diphenylethylamine or 3,3-diphenylpropylamine moieties displayed activities against *h*BuChE. Additionally, the activities were lower than they were for *eq*BuChE. The effect of the *tert*-butyl substituent in the benzyl ring keeps us from drawing an unambiguous SAR. The *tert*-butyl substituent significantly improved the activity of **9** compared with unsubstituted **7** but improved the activities of **10** and **30** only to a small extent compared to **8** and **28,** respectively. The role of the linker length was crucial for the inhibition of *h*BuChE. Derivatives of 1,3-diaminopropan-

2-ol with 3,3-diphenylpropylamine (**8**, **10**) exhibited higher inhibition potency compared to their 2,2-diphenylethylamine counterparts (**7** and **9**), while derivatives of 1,4-diaminobutan-2-ol (**28**, **30**) were more active in combination with 2,2-diphenylethylamine than with the 3,3-diphenylpropylamine fragment (**29**, **31**).

In the search for new anti-Alzheimer's agents, both AChE and BuChE are important biological targets. With the progression of the disease the role of BuChE in the regulation of cholinergic neurotransmission systematically grows at the expense of AChE. Additionally, there is evidence for non-enzymatic functions of BuChE that are relevant for AD treatment, e.g., reduction of Aβ levels.[52–56] Due to the above facts, MTDLs with inhibitory potency toward BuChE presented in this study may be relevant for the treatment of both the symptoms and the causes of the disease[57].

2.4 Kinetic studies of *eq*BuChE inhibition

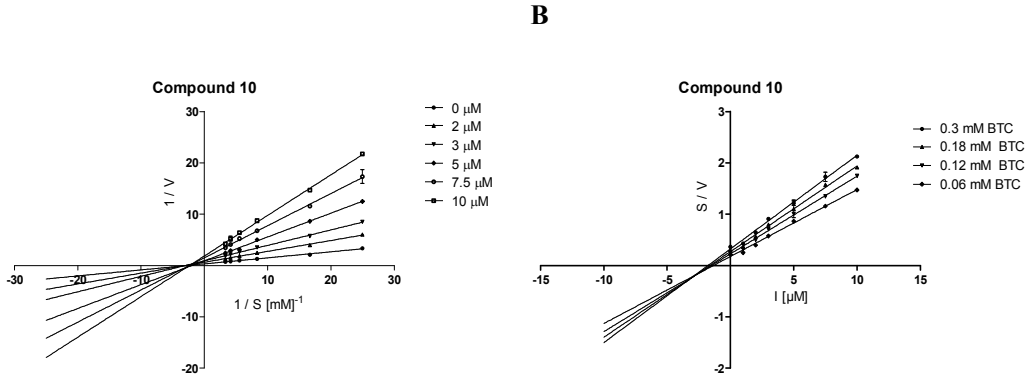


Figure 3. Lineweaver-Burk (A) and Cornish-Bowden (B) plots illustrating mixed types of *eq*BuChE inhibition by compound **10**. S = butyrylthiocholine; *V* = initial velocity rate; I = inhibitor concentration.

The kinetic studies of *eq*BuChE inhibition were performed with compound **10** as one of the most potent *eq*BuChE inhibitors presented herein. Although the Lineweaver-Burk reciprocal

plots are ambiguous (**Fig. 3A**), the calculated K_m values and Cornish–Bowden plots are characteristic for the mixed types of inhibition (**Fig. 3B**). Mixed types of enzyme inhibition with increasing K_m at increasing concentrations of **10** indicates a higher affinity of the inhibitor to free enzyme than to the enzyme-substrate complex and suggests preferential interaction of the inhibitor with the catalytic active site than with the peripheral anionic site of *eq*BuChE; however, both interactions are possible at the same time.

To date, a number of selective pseudo-irreversible BuChE inhibitors have been described, including carbamate analogues of cymserine[58] or isosorbide.[59] However, the pseudo-irreversible mechanism of inhibition may cause non-specific or off-target reactivity-based toxicities.[60,61] Therefore, selective reversible BuChE inhibitors are now of great interest, due to their potential for use in AD treatment. Such inhibitors have already been reported[52,62–65], however, compound **10** as the selective reversible BuChE inhibitor with mixed types of enzyme inhibition and a broad multifunctional profile of biological activity, seems to be unique.

2.5 Inhibition of BACE-1

We determined the inhibitory activities against human recombinant BACE-1 (*h*BACE-1) of all the compounds in the study using a spectrofluorometric assay (fluorescence resonance energy transfer, FRET based). This assay is based on the cleavage of a modified peptide substrate (analogue of the APP sequence with the Swedish mutation), leading to an increase in fluorescence.[66] For the reference compound, inhibitor IV, we established an IC_{50} value of 0.046 μM, which corresponds to the literature data.[67] First, we tested the ability of the new compounds to inhibit *h*BACE-1 at a single concentration of inhibitors (50 μM). For the selected compounds we evaluated IC_{50} values ranging from 32.17 to 101.90 μM (**Table 1**). The most potent compounds contain 2,2-diphenylethylamine (**7**, **9**, **28**), 3,3-

diphenylpropylamine (**8**, **10**, **29**, **31**) or 4-(bis(4-fluorophenyl) methyl)piperazine (**17** and **38**) fragments.

The starting point for the design of the MTDLs presented herein was the selective BACE-1 inhibitor NVP-BXD-552. To expand the activity profile of the compound towards cholinesterases, we discarded the sulfone moiety embedded in the cyclic hydroxyethylamine scaffold. Although it creates significant interactions with the flap of BACE-1 (H-bond with Thr72 and Gly73), it does not provide optimal interactions with cholinesterases. The obtained compounds displayed significantly reduced activity against BACE-1 when compared to selective BACE-1 inhibitors, but they displayed well-balanced activity towards all biological targets what is an important feature of MTDLs.

2.6 Inhibition of Aβ aggregation

We determined the Aβ anti-aggregation profiles of the obtained compounds by a Thioflavin-T assay.[68] First, we carried out a screening assay at a 10 μM concentration of each compound. Seven of the tested compounds inhibited Aβ aggregation by more than 50% (**8**–**10**, **16**, **30**, **31**, **37**) with comparable or higher activities to the reference compound resveratrol. The IC_{50} values were established for the two selected compounds: **10** (IC_{50} = 3.09 μM) and **31** (IC_{50} = 1.22 μM). To exclude the interference of the compounds with the readout of Thioflavin T fluorescence and to confirm the inhibitory activity, we also performed a dot blot assay for these two derivatives. Both reduced the formation of Aβ fibrils, which was detected using a fibril-specific LOC antibody (46.0% ± 3.8% and 40.6% ± 4.6% inhibition of Aβ fibril formation for **10** and **31**, respectively). Analysis of the results (**Table 1.**) showed that all the active compounds contained a diphenylmethyl moiety in their structures. The *tert*-butyl substituent in the benzylamine fragment has a major impact on the activity of the compounds, as exemplified by the comparisons of **7** *vs.* **9**, **8** *vs.* **10**, **14** *vs.* **16**, **28** *vs.* **30** and **35** *vs.* **37**. The

results obtained confirm our expectation that the target compounds may inhibit beta-amyloid aggregation. This is a valuable property of these molecules, which can further inhibit the formation of amyloid plaques.

2.7 Inhibition of tau aggregation

The role of tau proteins in the aetiopathogenesis of AD is unambiguous, and the inhibition of its aggregation may modify the course of the disease. Based on their inhibitory activities against Aβ aggregation we selected four compounds (**9**, **10**, **31** and **37)** and tested their abilities to inhibit tau aggregation. We tested the compounds using a Thioflavin-T-based assay using truncated and full-length forms of tau protein.[69] All the tested compounds displayed inhibitory potencies ranging from 45% to 70% at 10 μM concentration (**Table 2**). The most potent compound **37** inhibited both truncated and full-length tau aggregation by 68%.

Table 2. Inhibition of full-length and truncated tau aggregation by compounds **9**, **10**, **30** and **37**.

Compound	Inhibition of full-lenght tau aggregation [% ± SEM][a]	Inhibition of truncated tau aggregation [% ± SEM][a]
9	55.1 ± 3.6	54.5 ± 2.5
10	44.4 ± 4.5	53.8 ± 5.0
30	62.7 ± 1.2	59.7 ± 2.3
37	68.0 ± 3.4	68.7 ± 3.7

[a] 10 μM compound concentration, 40 μM tau concentration, experiments performed in triplicate.

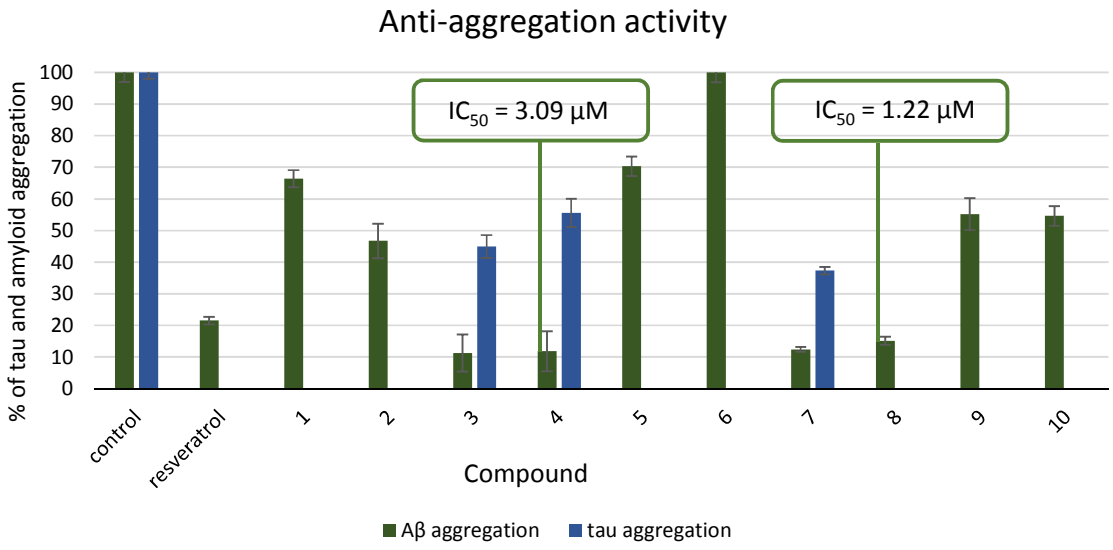


Figure 4. The effect of the synthesized compounds on Aβ and tau aggregation.

Among multitarget agents with inhibitory activities towards symptomatic and disease-modifying targets in AD, only a few groups of compounds possess activities towards the inhibition of aggregation of both Aβ and tau. Compared with the data presented herein (**Fig. 4**), the potencies of these inhibitors are similar or lower against tau aggregation and lower against Aβ aggregation. Furthermore, some of these compounds with similar multifunctional profiles are relatively large, with a high molecular weight, large numbers of condensed aromatic rings and very high lipophilicities[70,71,25]. On the other hand, there is a number of aggregation inhibitors with high hydrophilicity and therefore low bioavailability e.g. polyphenols, anthraquinones, phenylthiazolylhydrazides and benzothiazoles[24,72]. Not rare aggregation inhibitors can be classified as PAINS (Pan Assay Interference Compounds)[73] so they can non-specifically react with a number of undesirable proteins. Herein, we have proposed non-PAINS with anti-aggregation properties, relatively small molecular weights and optimized druglike properties which constitutes an interesting approach for further development.

2.8 Blood brain barrier (BBB) permeation

Since one of the basic features of anti-AD drugs is their ability to reach therapeutic targets in CNS, it is important to evaluate this parameter at the earliest stage of the development of novel anti-AD agents. To evaluate whether our compounds are centrally active, we used a parallel artificial membrane permeability assay (PAMPA).[74] The data obtained for the new compounds were compared to those of standard drugs with known CNS permeability, which were also confirmed using PAMPA. Prediction of BBB penetration is summarized in **Table 3**. All the tested compounds (**7**, **8**, **9**, **17**, **31**, **32**, **37**, **45**) displayed P_e values higher than 4.0, thus showing high probability for penetration of the BBB *via* passive diffusion. One of the major challenges in the design of MTDLs is optimization of their druglike properties. These preliminary results are optimistic forecasts for advanced pharmacokinetic studies and the possibility of further development of these compounds as anti-AD agents.

Table 3. *In vitro* blood-brain barrier permeation assay for compounds **7**, **8**, **9**, **17**, **31**, **32**, **37** and **45**.

Compound	BBB penetration estimation	
	$P_e \pm$ SEM ($*10^{-6}$ cm s^{-1})	CNS (+/–)[a]
7	8.9 ± 3.3	CNS (+)
8	5.2 ± 0.2	CNS (+)
9	7.4 ± 1.4	CNS (+)
17	7.1 ± 1.2	CNS (+)
31	5.7 ± 1.4	CNS (+)
32	6.5 ± 0.9	CNS (+)
37	9.1 ± 0.2	CNS (+)
45	4.9 ± 0.7	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 (–)

[a] CNS (+) (high BBB permeation predicted), P_e (10^{-6} cm s^{-1}) > 4.0; CNS () (low BBB permeation predicted), P_e (10^{-6} cm s^{-1}) ≤ 2.0; CNS (+/) (BBB permeation uncertain), 4.0 ≥ P_e (10^{-6} cm s^{-1}) > 2.0. [75]

2.9 Docking studies on BACE-1 and BuChE

Molecular modelling studies helped us better understand the binding modes of the active compounds within the active sites of *h*BACE-1 and *h*BuChE. All BACE-1 inhibitors presented one dominant conformation when docked to the enzyme. We chose the most active ligand, **31**, to present the observed interactions (**Figure 5**). The hydroxyethylamine group, used as a transition state analogue, was the main grip point of molecule **31**. It formed characteristic ionic and hydrogen bonds with the catalytic dyad (Asp32 and Asp228) and additional hydrogen bonds with Gly34, Thr220 and the ligand. The benzylamine fragment provided interactions within the S1' and S2' sub-pockets of the enzyme. A substituted benzylamine fragment together with the hydroxyethylamine moiety allows for effective pharmacophore binding to the BACE-1 active site. The positions that these fragments take in the enzyme are the same as those observed in the X-ray structures of potent BACE-1 inhibitors, including the previously mentioned NVP-BXD-552 and GRL-8234.[36,76,37,38] The diphenylmethyl moiety was located within the confines of the aromatic S1 and S3 sub-pockets and interacted with the s10 loop. These interactions are crucial for the effective inhibition of the enzyme both by strong, single target inhibitors of BACE-1 and by the presented MTDLs. Ligands with smaller substituents like benzyl or phenyl fragments, occupying only one sub-pocket, were less active. Additionally, the position of the diphenylmethyl fragment enables

further extension of the compounds in the S2 and S3 sub-pockets and makes the enhancement of their inhibitory activity possible.

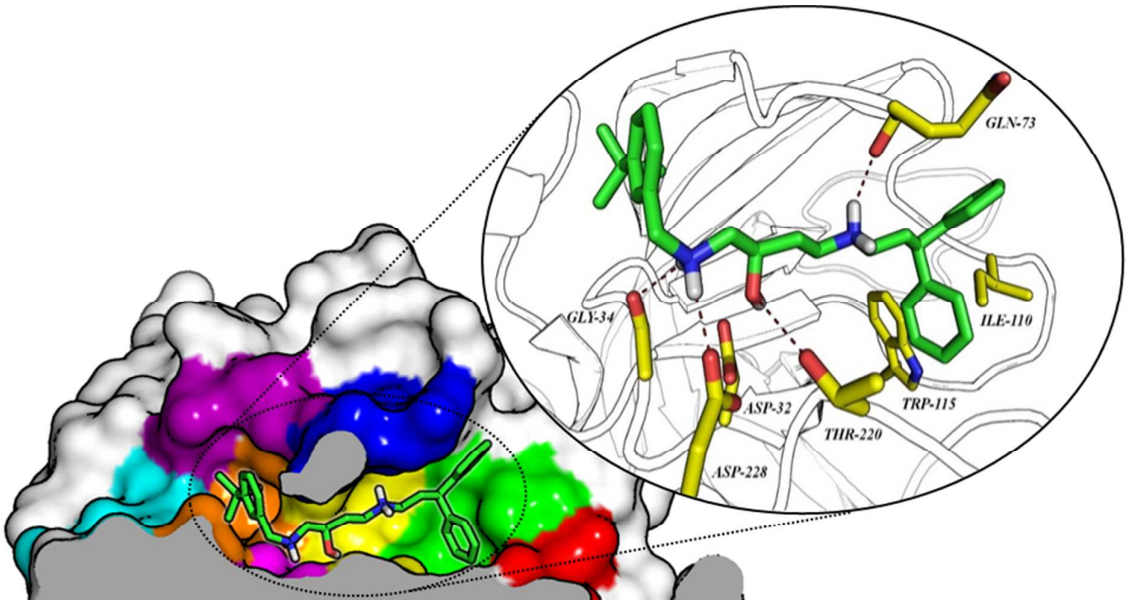


Figure 5. Predicted binding mode of compound **31** (green) with BACE-1. The general view presents the position of the ligand in the active site of the enzyme with coloured subsites S4'–S4.[77] In the detailed representation, the most important amino acids are marked in yellow; red dots represent hydrogen bonds.

Considering the results of the docking studies performed with *h*BuChE, we divided the ligands into two groups: more potent derivatives with bent conformations (2,2-diphenylethylamine and 3,3-diphenylpropylamine derivatives) and less potent derivatives with extended conformations (piperazine derivatives). A bent conformation allows the formation of additional CH−π interactions between the benzyl ring and Trp231, as presented in **Figure 6**. In the extended conformation, the benzyl ring is situated at the entrance of the active site, where it creates only weak interactions with the amino acids of PAS. Additionally, the nitrogen atoms in 2,2-diphenylethylamine and 3,3-diphenylpropylamine derivatives are

preferentially protonated compared to the piperazine nitrogen atom and provide cation−π interactions with Trp82 and Tyr332. The diphenylmethyl moiety in all the compounds creates hydrophobic interactions with Trp82 and Trp430. In addition, the hydroxyethylamine fragment provides strong interactions with Pro285 by forming two hydrogen bonds.

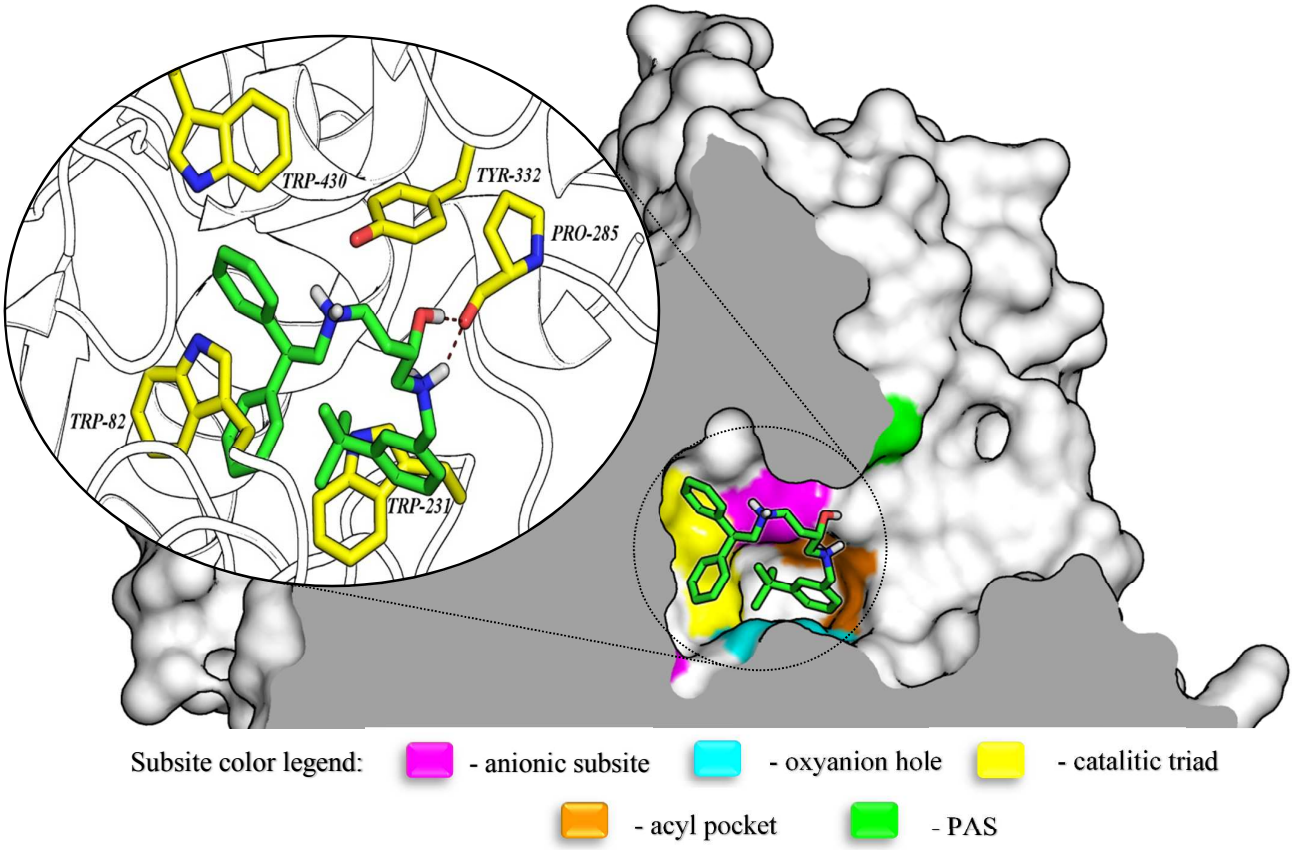


Figure 6. Predicted binding mode of compound **30** (green) with BuChE. The general view presents the position of the ligand in the active site of the enzyme with coloured subsites. In the detailed representation, the most important amino acids are marked in yellow; red dots represent hydrogen bonds.[78]

3 Conclusions

With a rapidly growing number of patients with AD, a lack of effective treatments has rendered AD one of the largest issues in current pharmaceutical research. Due to the multifactorial nature of the disease, designing multifunctional ligands is considered a rational strategy in the search for new anti-Alzheimer's treatments. In the selection of biological targets for the novel MTDLs presented herein, we turned our attention mainly to disease-modifying mechanisms. As a starting point, we used compounds on the basis of their cholinesterase inhibition and modified them to treat the symptoms of the disease. Based on the structure of BACE-1 inhibitor (NVP-BXD-552) and on our previous studies, we designed, synthesized and evaluated 24 novel multitarget-directed ligands targeting AChE, BuChE, BACE-1 and the aggregation of Aβ and tau proteins. Generally, we found that the compounds exhibited potency towards all chosen biological targets in the micromolar range, and some of them even showed broad and well-balanced profiles of activities directed at all of the targets. For the representative compounds, we confirmed the ability to permeate the BBB by the BBB-PAMPA assay.

We highlight compounds **9** and **10** as being the most interesting due to their potencies as well as their broad and well-balanced spectra of biological activities. Both compounds selectively inhibit eqBuChE and *h*BuChE over *ee*AChE. However, more importantly, **9** and **10** are also able to inhibit disease-modifying targets BACE-1 and the aggregation of both Aβ and tau proteins. Compared to previously reported compounds[79] we have broadened the profile in these new multifunctional agents, conferring upon them a broader spectrum of activities and improved potencies against BACE-1 and Aβ aggregation. We have tried to compile the results with the current knowledge about AD. The pathogenesis based on amyloidopathy and tauopathy starts can even start 25 years before the first symptoms. The appearance of amyloid in the CSF and brain slightly precede the occurrence of tau pathology, nevertheless these processes are progressing simultaneously therefore they are just as important. There is

continued progress in the diagnostics of AD, and perhaps, soon, it will be possible to diagnose AD long before the first symptoms. Therefore, the dual Aβ and tau anti-aggregation profile in combination with the inhibition of BACE-1 is of much interest for disease-modifying anti-Alzheimer's agents. However, if the first memory disorders are already present, the patients should be treated with cholinesterase inhibitors, for instance; even at that stage, it is worth inhibiting pathological processes leading to the formation of both senile plaques and neurofibrillary tangles to try to stop the progression of AD. To the best of our knowledge, compounds combining inhibitory activities against BuChE, BACE-1, tau and A*β* aggregation have not been published to date. Therefore, this is the first report of multi-targeted compounds combining these functionalities and taking into account potential disease-modifying and symptomatic activities. The presented results will be a good starting point for further development of analogues derived from compound **10** and for further studies of these valuable candidates.

4 Methods

4.1 Chemistry

4.1.1 General methods

^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Mercury 300 at 300 MHz. The chemical shifts for ^{1}H NMR are referenced to TMS *via* residual solvent signals (^{1}H, $CDCl_3$ at 7.26 ppm, DMSO-d_6 at 2.50 ppm). Mass spectra (MS) were recorded on 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). For the TLC and column chromatography following solvents were used:

dichloromethane (DCM), methanol (MeOH), petroleum ether, chloroform, ethyl acetate, 25% ammonia water solution. 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 x 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 above 95%. All of the reagents were purchased from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) and 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: *N*-benzylprop-2-en-1-amine (**1**)[80], *N*-(4-methoxybenzyl)prop-2-en-1-amine (**2**)[80], 1-(4-benzhydrylpiperazin-1-yl)-3-(benzylamino)propan-2-ol (**14**)[81], *tert*-butyl-dimethyl(2-(oxiran-2-yl)ethoxy)silane (**18**)[82] have been reported previously.

4.1.2 Procedure for the synthesis of compounds: 1–3 (procedure A)

To a mixture of benzylamine derivative (1.5 equiv.) and K_2CO_3 (3.0 equiv.) refluxed in acetonitrile (MeCN) a solution of allyl chloride (1.0 equiv.) in MeCN was added dropwise and the reaction mixture was refluxed for 16 h. After the mixture was cooled to the room temperature, the solvent was removed and the residue was treated with saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was then purified by flash column chromatography.

4.1.2.1 *N*-Benzylprop-2-en-1-amine (1)

Following the procedure A, reaction of benzylamine (0.428 mL, 3.92 mmol) dissolved in 10 mL MeCN with allyl chloride (0.214 mL, 2.61 mmol) dissolved in 5 mL MeCN in the presence of K_2CO_3 (1.080 g, 7.84 mmol) was performed. Extraction: 10 mL sat. $NaHCO_{3(aq)}$,

3 × 10 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.8/0.2/0.05, v/v/v). Yield: 180 mg (47%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.8/0.2/0.05, v/v/v) R_f = 0.13. MW 147.22. Formula $C_{10}H_{13}N$. MS m/z 148.13 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 - 7.37 (m, 5H), 5.94 (ddt, J = 17.18, 10.19, 5.93, 5.93 Hz, 1H), 5.20 (dq, J = 17.18, 1.71 Hz, 1H), 5.12 (dq, J = 10.26, 1.45 Hz, 1H), 3.80 (s, 2H), 3.28 (dt, J = 5.96, 1.51 Hz, 2H), 1.51 (br. s, 1H).

4.1.2.2 *N*-(4-Methoxybenzyl)prop-2-en-1-amine (2)

Following the procedure A, reaction of 4-methoxybenzylamine (0.512 mL, 3.92 mmol) dissolved in 10 mL MeCN with allyl chloride (0.214 mL, 2.61 mmol) dissolved in 5 mL MeCN in the presence of K_2CO_3 (1.080 g, 7.84 mmol) was performed. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.8/0.2/0.05, v/v/v). Yield: 221 mg (48%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.8/0.2/0.05, v/v/v) R_f = 0.16. MW 177.24. Formula $C_{11}H_{15}NO$. MS m/z 178.04 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.21 - 7.27 (m, 2H), 6.83 - 6.89 (m, 2H), 5.85 - 6.01 (m, 1H), 5.19 (dq, J = 17.28, 1.68 Hz, 1H), 5.10 (dq, J = 10.26, 1.45 Hz, 1H), 3.79 (s, 3H), 3.73 (s, 2H), 3.26 (dt, J = 5.96, 1.51 Hz, 2H), 1.42 (br. s, 1H).

4.1.2.3 *N*-(3-(*Tert*-butyl)benzyl)prop-2-en-1-amine (3)

Following the procedure A, reaction of (3-(*tert*-butyl)phenyl)methanamine (1.000 g, 6.13 mmol) dissolved in 20 mL MeCN with allyl chloride (0.312 g, 4.08 mmol) dissolved in 10 mL MeCN in the presence of K_2CO_3 (1.693 g, 12.25 mmol) was performed. Extraction: 20 mL sat. $NaHCO_{3(aq)}$, 3 × 20 mL DCM. Purification: flash chromatography in DCM/MeOH (9.5/0.5, v/v). Yield: 507 mg (61%), TLC (DCM/MeOH, 9.8/0.2 v/v) R_f = 0.17. MW 203.32. Formula $C_{14}H_{21}N$. MS m/z 204.09 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.12 - 7.38 (m,

4H), 5.87 - 6.04 (m, 1H), 5.22 (dd, *J* = 17.29, 1.47 Hz, 1H), 5.13 (dd, *J* = 10.26, 1.47 Hz, 1H), 3.80 (s, 2H), 3.31 (dt, *J* = 6.15, 1.32 Hz, 2H), 1.58 (br. s, 1H), 1.33 (s, 9H).

4.1.3 General procedure for the synthesis of compounds: 4–6 (procedure B)

To an ice-cooled solution of the amine **4–6** (1.1 equiv.) and anhydrous TEA (3.0 equiv.) in anhydrous THF a solution of di-*tert*-butyl dicarbonate (1.2 equiv.) in THF was added dropwise and the reaction mixture was stirred at room temperature for 4 h. Then the solvent was removed and the resulting residue was treated with water and extracted with DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. To the resulting residue dissolved in MeCN and cooled on an ice-bath, a solution of *N*-bromosuccinimide (1.2 equiv.) in water was added dropwise, and the reaction mixture was stirred at room temperature for 16 h. Then the MeCN was evaporated and water phase was extracted with ethyl acetate. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under the reduced pressure. The resulting residue was purified by flash column chromatography in 20–60% ethyl acetate in petroleum ether.

4.1.3.1 3-Benzyl-5-(bromomethyl)oxazolidin-2-one (4)

Following the procedure B, solution of di-*tert*-butyl dicarbonate (1.636 g, 7.50 mmol) in 5 mL anhydrous THF was added dropwise to a solution of *N*-benzylprop-2-en-1-amine (**1**) (0.920 g, 6.25 mmol) in 15 mL anhydrous THF in the presence of anhydrous TEA (2.620 mL, 18.75 mmol). Extraction: 3 × 20 mL DCM. To the resulting *tert*-butyl allyl(benzyl)carbamate (1.761 g, 7.12 mmol) dissolved in 13 mL MeCN, a solution of *N*-bromosuccinimide (1.521 g, 8.54 mmol) in 20 mL water was added. Extraction 3 × 20 mL ethyl acetate. Yield: 760 mg (38%), TLC (petroleum ether/ethyl acetate, 5/5, v/v) R_f = 0.49. MW 270.12. Formula $C_{11}H_{12}BrNO_2$. MS m/z 270.16 (M+), 272.15 (M+2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 - 7.41 (m, 5H), 4.63 - 4.74 (m, 1H), 4.35 - 4.51 (m, 2H), 3.38 - 3.59 (m, 3H), 3.27 (dd, *J* = 9.38, 5.86 Hz, 1H).

4.1.3.2 5-(Bromomethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (5)

Following the procedure B, solution of di-*tert*-butyl dicarbonate (0.993 g, 4.55 mmol) in 3 mL anhydrous THF was added dropwise to a solution of *N*-(4-methoxybenzyl)prop-2-en-1-amine (**2**) (0.672 g, 3.79 mmol) in 10 mL anhydrous THF in the presence of anhydrous TEA (1.588 mL, 11.37 mmol). Extraction: 3 × 15 mL DCM. To the resulting *tert*-butyl allyl(benzyl)carbamate (1.215 g, 4.38 mmol) dissolved in 10 mL MeCN, a solution of *N*-bromosuccinimide (0.936 g, 5.26 mmol) in 15 mL water was added. Extraction 3 × 15 mL ethyl acetate. Yield: 669 mg (59%), TLC (petroleum ether/ethyl acetate, 5/5, v/v) R_f = 0.46. MW 300.15. Formula $C_{12}H_{14}BrNO_3$. MS m/z 299.98 (M), 301.98 (M+2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 - 7.25 (m, 2H), 6.83 - 6.92 (m, 2H), 4.59 - 4.72 (m, 1H), 4.28 - 4.45 (m, 2H), 3.77 - 3.84 (m, 3H), 3.48 - 3.56 (m, 2H), 3.40 (dd, *J* = 10.52, 7.69 Hz, 1H), 3.24 (dd, *J* = 8.98, 5.90 Hz, 1H).

4.1.3.3 5-(Bromomethyl)-3-(3-(*tert*-butyl)benzyl)oxazolidin-2-one (6)

Following the procedure B, solution of di-*tert*-butyl dicarbonate (0.330 g, 1.51 mmol) in 1.5 mL anhydrous THF was added dropwise to a solution of *N*-(3-(*tert*-butyl)benzyl)prop-2-en-1-amine (**3**) (0.256 g, 1.26 mmol) in 5 mL anhydrous THF in the presence of anhydrous TEA (0.526 mL, 3.78 mmol). Extraction: 3 × 8 mL DCM. To the resulting *tert*-butyl allyl(3-(*tert*-butyl)benzyl)carbamate (0.378 g, 1.25 mmol) dissolved in 5 mL MeCN, a solution of *N*-bromosuccinimide (0.266 mg ,1.50 mmol) in 10 mL water was added. Extraction 3 × 10 mL ethyl acetate. Yield: 211 mg (51%), TLC (petroleum ether/ethyl acetate, 5/5, v/v) R_f = 0.62. MW 326.23. Formula $C_{15}H_{20}BrNO_2$. MS m/z 325.96 (M), 327.97 (M+2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 - 7.38 (m, 3H), 7.06 - 7.11 (m, 1H), 4.63 - 4.75 (m, 1H), 4.43 (s, 2H), 3.49 - 3.58 (m, 2H), 3.45 (dd, *J*= 10.55, 7.03 Hz, 1H), 3.28 (dd, *J* = 8.79, 5.86 Hz, 1H), 1.32 (s, 9H).

4.1.4 General procedure for the synthesis of compounds: 7–17, 28–38 (procedure C)

An adequate 3-benzyl-5-(bromomethyl)oxazolidin-2-one derivative (**4**–**6**) or 2-(3-benzyl-2-oxooxazolidin-5-yl)ethyl methanesulfonate derivative (**25**–**27**) (1.0– 1.1 equiv.), K_2CO_3 (1.2– 3.0 equiv.) and a corresponding amine (0.8– 3.0 equiv.) in MeCN or toluene were refluxed for 16 h or heated in DMF in microwave reactor at 80 ^oC for 1 h. Then the solvent was removed and the resulting residue was treated with saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. The resulting product was purified by column chromatography. To the obtained amine dissolved in ethanol, NaOH (10.0 equiv.) was added and the reaction mixture was refluxed for 6 h. After the reaction mixture was cooled to room temperature, the solution was neutralized with glacial acetic acid, ethanol was removed and the resulting residue was treated with water and extracted with DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting product was purified by column chromatography.

4.1.4.1 1-(Benzylamino)-3-((2,2-diphenylethyl)amino)propan-2-ol (7)

The reaction of 3-benzyl-5-(bromomethyl)oxazolidin-2-one (**4**) (100 mg, 0.370 mmol) with 2,2-diphenylethanamine (219 mg, 1.110 mmol) in the presence of K_2CO_3 (102 mg, 0.740 mmol) in 3 mL MeCN was performed according to the procedure C. Extraction: 10 mL water, 3 × 10 mL ethyl acetate. Purification: flash chromatography (1– 3% MeOH in DCM). The resulting 3-benzyl-5-(((2,2-diphenylethyl)amino)methyl)oxazolidin-2-one (71 mg, 0.184 mmol) was dissolved in 2 mL ethanol and a reaction with sodium hydroxide (74 mg, 1.840 mmol) was performed. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.1, v/v/v). Yield: 53 mg (40%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.1, v/v/v) R_f = 0.36. MW 360.49. Formula

$C_{24}H_{28}N_2O$. MS m/z 361.20 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.14 - 7.38 (m, 15H), 4.18 (t, J = 7.91 Hz, 1H), 3.69 - 3.85 (m, 3H), 3.16 - 3.35 (m, 2H), 2.98 (br. s, 3H), 2.70 - 2.84 (m, 2H), 2.59 (ddd, J = 14.65, 12.31, 7.03 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.41, 142.38, 137.97, 128.72, 128.69, 128.58, 128.45, 127.99, 127.94, 127.53, 126.72, 126.69, 67.28, 54.23, 53.41, 53.18, 52.52, 50.84.

4.1.4.2 1-(Benzylamino)-3-((3,3-diphenylpropyl)amino)propan-2-ol (8)

The reaction of 3-benzyl-5-(bromomethyl)oxazolidin-2-one (**4**) (82 mg, 0.310 mmol) with 3,3-diphenylpropan-1-amine (197 mg, 0.930 mmol) in the presence of K_2CO_3 (86 mg, 0.620 mmol) in 2 mL MeCN was performed according to the procedure C. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL ethyl acetate. Purification: column chromatography (DCM/diethyl ether/MeOH/$NH_3 \cdot H_2O$, 9.0/1.0/0.25/0.025, v/v/v/v). The resulting 3-benzyl-5-(((3,3-diphenylpropyl)amino)methyl)oxazolidin-2-one (106 mg, 0.265 mmol) was dissolved in 3 mL ethanol and a reaction with sodium hydroxide (106 mg, 2.650 mmol) was performed. Extraction: 10 mL water, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 90 mg (78%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.42. MW 374.52. Formula $C_{25}H_{30}N_2O$. MS m/z 375.29 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.12 - 7.38 (m, 15H), 4.00 (t, J = 7.62 Hz, 1H), 3.69 - 3.85 (m, 3H), 2.45 - 2.75 (m, 9H), 2.25 (q, J = 7.03 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.59, 139.92, 128.49, 128.45, 128.11, 127.76, 127.07, 126.23, 68.18, 53.80, 53.39, 52.89, 49.00, 48.19, 35.66.

4.1.4.3 1-((3-(*Tert*-butyl)benzyl)amino)-3-((2,2-diphenylethyl)amino)propan-2-ol (9)

The reaction of 5-(bromomethyl)-3-(3-(*tert*-butyl)benzyl)oxazolidin-2-one (**6**) (120 mg, 0.368 mmol) with 3,3-diphenylpropan-1-amine (233 mg, 1.104 mmol) in the presence of K_2CO_3

(102 mg, 0.736 mmol) in 3 mL MeCN was performed according to the procedure C. Extraction: 10 mL water, 3 × 10 mL ethyl acetate. Purification: column chromatography (DCM/diethyl ether/MeOH/$NH_3 \cdot H_2O$, 9.0/1.0/0.25/0.025, v/v/v/v). The resulting 3-(3-(*tert*-butyl)benzyl)-5-(((3,3-diphenylpropyl)amino)methyl)oxazolidin-2-one (101 mg, 0.221 mmol) was dissolved in 2.5 mL ethanol and a reaction with sodium hydroxide (88 mg, 2.210 mmol) was performed. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.7/0.3/0.025, v/v/v). Yield: 60 mg (38%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.41. MW 416.60. Formula $C_{28}H_{36}N_2O$. MS m/z 417.29 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.15 - 7.36 (m, 13H), 7.09 (d, J = 7.03 Hz, 1H), 4.18 (s, 1H), 3.77 (d, J = 4.10 Hz, 3H), 3.18 - 3.35 (m, 2H), 3.12 (br. s, 3H), 2.71 - 2.82 (m, 2H), 2.63 (td, J = 11.70, 7.03 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.48, 142.62, 142.56, 138.12, 128.69, 128.66, 128.25, 128.02, 127.99, 126.65, 126.62, 125.46, 125.38, 124.37, 67.61, 54.35, 53.67, 53.42, 52.69, 51.06, 34.67, 31.38.

4.1.4.4 1-((3-(*Tert*-butyl)benzyl)amino)-3-((3,3-diphenylpropyl)amino)propan-2-ol (10)

The reaction of 5-(bromomethyl)-3-(3-(tert-butyl)benzyl)oxazolidin-2-one (**6**) (120 mg, 0.368 mmol) with 2,2-diphenylethanamine (218 mg, 1.103 mmol) in the presence of K_2CO_3 (102 mg, 0.736 mmol) in 3 mL MeCN was performed according to the procedure C. Extraction: 10 mL water, 3 × 10 mL ethyl acetate. Purification: column chromatography (DCM/diethyl ether/MeOH/$NH_3 \cdot H_2O$, 9.0/1.0/0.25/0.025, v/v/v/v). The resulting 3-(3-(*tert*-butyl)benzyl)-5-(((2,2-diphenylethyl)amino)methyl)oxazolidin-2-one (106 mg, 0.239 mmol) was dissolved in 2.5 mL ethanol and reaction with sodium hydroxide (96 mg, 2.390 mmol) was performed. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 56 mg (37%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.47. MW 430.62. Formula $C_{29}H_{38}N_2O$.

MS m/z 431.25 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 - 7.39 (m, 14H), 4.00 (t, J = 8.20 Hz, 1H), 3.70 - 3.88 (m, 3H), 3.25 (br. s, 3H), 2.71 (s, 2H), 2.50 - 2.66 (m, 4H), 2.29 (q, J = 7.60 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.40, 144.38, 138.98, 128.54, 128.20, 127.74, 126.29, 125.27, 125.20, 124.20, 67.66, 53.99, 53.38, 52.90, 48.97, 48.06, 35.08, 34.65, 31.38.

4.1.4.5 1-((4-Methoxybenzyl)amino)-3-(4-(pentan-2-yl)piperazin-1-yl)propan-2-ol (11)

The reaction of 5-(bromomethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**5**) (115 mg, 0.384 mmol) with 1-(pentan-2-yl)piperazine (50 mg, 0.320 mmol) in the presence of K_2CO_3 (66 mg, 0.480 mmol) in 2 mL MeCN was performed according to the procedure C. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 8 mL DCM. Purification: flash chromatography (1– 10% MeOH in DCM). The resulting 3-(4-methoxybenzyl)-5-((4-(pentan-2-yl)piperazin-1-yl)methyl)oxazolidin-2-one (60 mg, 0.160 mmol) was dissolved in 1.5 mL ethanol and a reaction with sodium hydroxide (64 mg, 1.600 mmol) was performed. Extraction: 8 mL 5% $NH_3 \cdot H_2O$, 3 × 10 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.5/0.5/0.05, v/v/v). Yield: 35 mg (26%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.14. MW 349.51. Formula $C_{20}H_{35}N_3O_2$. MS m/z 350.30 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 - 7.25 (m, 2H), 6.81 - 6.88 (m, 2H), 3.84 (td, J = 6.80, 3.59 Hz, 1H), 3.78 (s, 3H), 3.73 (d, J = 2.05 Hz, 2H), 2.25 - 2.70 (m, 14H), 1.42 - 1.54 (m, 1H), 1.15 - 1.42 (m, 4H), 0.96 (d, J = 6.41 Hz, 3H), 0.89 (t, J = 7.05 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.28, 128.61, 114.63, 114.16, 66.91, 65.84, 59.48, 59.21, 58.29, 56.29, 55.24, 54.22, 53.38, 53.00, 20.07, 14.25.

4.1.4.6 1-((4-Methoxybenzyl)amino)-3-(4-phenylpiperazin-1-yl)propan-2-ol (12)

The reaction of 5-(bromomethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**5**) (100 mg, 0.333 mmol) with 1-phenylpiperazine (49 mg, 0.303 mmol) in the presence of K_2CO_3 (63 mg, 0.454 mmol) in 2 mL MeCN was performed according to the procedure C. Extraction: 5 mL sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: flash chromatography (50– 80% ethyl acetate in petroleum ether). The resulting 3-(4-methoxybenzyl)-5-((4-phenylpiperazin-1-yl)methyl)oxazolidin-2-one (56 mg, 0.147 mmol) was dissolved in 2 mL ethanol and a reaction with sodium hydroxide (59 mg, 1.470 mmol) was performed. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.5/0.5/0.025, v/v/v). Yield: 47 mg (40%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.025, v/v/v) R_f = 0.07. MW 355.47. Formula $C_{21}H_{29}N_3O_2$. MS m/z 356.15 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 - 7.31 (m, 4H), 6.82 - 6.96 (m, 5H), 3.85 - 3.95 (m, 1H), 3.80 (s, 3H), 3.77 (d, J = 2.82 Hz, 2H), 3.20 (ddd, J = 5.96, 3.91, 1.67 Hz, 4H), 2.68 - 2.84 (m, 4H), 2.45 - 2.65 (m, 5H), 2.34 - 2.42 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.67, 151.20, 132.11, 129.33, 129.10, 119.79, 116.07, 113.80, 65.99, 62.04, 55.28, 55.25, 53.37, 52.87, 49.23.

4.1.4.7 1-(4-Benzylpiperazin-1-yl)-3-((4-methoxybenzyl)amino)propan-2-ol (13)

The reaction of 5-(bromomethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**5**) (100 mg, 0.333 mmol) with 1-benzylpiperazine (53 mg, 0.303 mmol) in the presence of K_2CO_3 (63 mg, 0.454 mmol) in 2 mL MeCN was performed according to the procedure C. Extraction: 5 mL sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: flash chromatography (50– 80% ethyl acetate in petroleum ether). The resulting 5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(4-methoxybenzyl)oxazolidin-2-one (75 mg, 0.190 mmol) was dissolved in 2 mL ethanol and a reaction with sodium hydroxide (76 mg, 1.900 mmol) was performed. Extraction: 8 mL 5% $NH_3 \cdot H_2O$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 61 mg (50%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v)

R_f = 0.14. MW 369.50. Formula $C_{22}H_{31}N_3O_2$. MS m/z 370.17 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 - 7.36 (m, 7H), 6.81 - 6.90 (m, 2H), 3.78 - 3.89 (m, 4H), 3.74 (d, J = 2.82 Hz, 2H), 3.50 (s, 2H), 2.27 - 2.84 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.64, 138.02, 132.17, 129.31, 129.18, 128.20, 127.05, 113.77, 65.86, 63.00, 61.91, 55.27, 55.24, 53.32, 53.08, 52.91.

4.1.4.8 1-(4-Benzhydrylpiperazin-1-yl)-3-(benzylamino)propan-2-ol (14)

The reaction of 3-benzyl-5-(bromomethyl)oxazolidin-2-one (**4**) (150 mg, 0.555 mmol) with 1-benzhydrylpiperazine (133 mg, 0.528 mmol) in the presence of K_2CO_3 (154 mg, 1.111 mmol) in 3 mL MeCN was performed according to the procedure C. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 8 mL DCM. Purification: flash chromatography (30– 70% ethyl acetate in petroleum ether). The resulting 5-((4-benzhydrylpiperazin-1-yl)methyl)-3-benzyloxazolidin-2-one (103 mg, 0.233 mmol) was dissolved in 3 mL ethanol and a reaction with sodium hydroxide (93 mg, 2.330 mmol) was performed. Extraction: 10 mL 5% $NH_3 \cdot H_2O$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 82 mg (36%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.21. MW 415.57. Formula $C_{27}H_{33}N_3O$. MS m/z 416.23 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14 - 7.48 (m, 15H), 4.23 (s, 1H), 3.76 - 3.92 (m, 3H), 2.98 (br. s, 2H), 2.26 - 2.77 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.69, 140.03, 128.48, 128.42, 128.16, 127.91, 127.00, 126.94, 76.22, 65.86, 61.92, 53.96, 53.59, 53.06, 51.94.

4.1.4.9 1-(4-Benzhydrylpiperazin-1-yl)-3-((4-methoxybenzyl)amino)propan-2-ol (15)

The reaction of 5-(bromomethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**5**) (100 mg, 0.333 mmol) with 1-benzhydrylpiperazine (77 mg, 0.303 mmol) in the presence of K_2CO_3 (63 mg, 0.454 mmol) in 2 mL MeCN was performed according to the procedure C. Extraction: 5 mL

sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: flash chromatography (gradient 5/5 to 2/8 of petroleum ether/ethyl acetate). The resulting 5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(4-methoxybenzyl)oxazolidin-2-one (83 mg, 0.176 mmol) was dissolved in 2 mL ethanol and reaction with sodium hydroxide (70 mg, 1.760 mmol) was performed. Extraction: 8 mL 5% $NH_3 \cdot H_2O$, 3 × 10 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.5/0.5/0.05, v/v/v). Yield: 48 mg (32%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.17. MW 445.60. Formula $C_{28}H_{35}N_3O_2$. MS m/z 446.21 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 - 7.44 (m, 4H), 7.22 - 7.32 (m, 6H), 7.12 - 7.21 (m, 2H), 6.81 - 6.92 (m, 2H), 4.19 (s, 1H), 3.85 - 3.95 (m, 1H), 3.75 - 3.84 (m, 5H), 3.54 (br. s., 2H), 2.27 - 2.80 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.95, 142.62, 130.46, 129.74, 128.47, 127.86, 126.94, 113.91, 76.19, 65.34, 61.94, 55.26, 53.64, 52.89, 52.68, 51.84.

4.1.4.10 1-(4-Benzhydrylpiperazin-1-yl)-3-((3-(*tert*-butyl)benzyl)amino)propan-2-ol (16)

The reaction of 5-(bromomethyl)-3-(3-(*tert*-butyl)benzyl)oxazolidin-2-one (**6**) (108 mg, 0.331 mmol) with 1-benzhydrylpiperazine (70 mg, 0.276 mmol) in the presence of K_2CO_3 (82 mg, 0.596 mmol) in 3 mL MeCN was performed according to the procedure C. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 8 mL DCM. Purification: flash chromatography (10– 50% ethyl acetate in petroleum ether). The resulting 5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(3-(*tert*-butyl)benzyl)oxazolidin-2-one (60 mg, 0.121 mmol) was dissolved in 3 mL ethanol and a reaction with sodium hydroxide (48 mg, 1.210 mmol) was performed. Extraction: 10 mL 5% $NH_3 \cdot H_2O$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 46 mg (30%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.18. MW 471.68. Formula $C_{31}H_{41}N_3O$. MS m/z 472.39 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 - 7.48 (m, 4H), 7.10 - 7.36 (m, 10H), 4.23 (s, 1H), 3.78 - 3.91 (m, 3H), 2.28 - 2.78 (m, 14H), 1.31 - 1.38 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.83, 152.16, 141.59,

130.89, 128.74, 128.68, 127.64, 127.38, 126.91, 126.87, 126.20, 75.40, 62.82, 59.75, 53.06, 51.73, 50.20, 49.01, 31.15, 21.92.

4.1.4.11 1-(4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)-3-((4-methoxybenzyl)amino)propan-2-ol (17)

The reaction of 5-(bromomethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**5**) (100 mg, 0.333 mmol) with 1-(bis(4-fluorophenyl)methyl)piperazine (62 mg, 0.303 mmol) in the presence of K_2CO_3 (63 mg, 0.454 mmol) in 2 mL MeCN was performed according to the procedure C. Extraction: 5 mL sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: flash chromatography (50–80% ethyl acetate in petroleum ether). The resulting 5-((4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)methyl)-3-(4-methoxybenzyl)oxazolidin-2-one (80 mg, 0.158 mmol) was dissolved in 2 mL ethanol and a reaction with sodium hydroxide (63 mg, 1.580 mmol) was performed. Extraction: 8 mL sat.$NaHCO_{3aq}$, 3 × 10 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.5/0.5/0.025, v/v/v). Yield: 44 mg (27%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.025, v/v/v) R_f = 0.11. MW 481.58. Formula $C_{28}H_{33}F_2N_3O_2$. MS m/z 482.23 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 - 7.37 (m, 4H), 7.20 - 7.25 (m, 2H), 6.92 - 6.99 (m, 4H), 6.83 - 6.87 (m, 2H), 4.19 (s, 1H), 3.77 - 3.87 (m, 5H), 3.74 (d, *J*=1.80 Hz, 2H), 2.24 - 2.74 (m, 13H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.78 (d, *J* = 245.48 Hz), 158.67, 138.16 (d, *J* = 3.32 Hz), 131.96, 129.23, 129.25 (d, *J* = 16.04 Hz), 115.39 (d, *J* = 21.01 Hz), 113.78, 74.47, 65.80, 61.86, 55.25 (d, *J* = 2.21 Hz), 53.48, 53.31, 52.89, 51.79.

4.1.4.12 1-(Benzylamino)-4-((2,2-diphenylethyl)amino)butan-2-ol (28)

The reaction of 2-(3-benzyl-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**25**) (202 mg, 0.670 mmol) with 2,2-diphenylethanamine (397 mg, 2.010 mmol) in the presence of K_2CO_3 (280 mg, 2.020 mmol) in 2 mL DMF was performed according to the procedure C. Extraction: 8

mL sat. $NaHCO_{3(aq)}$, 3 × 8 mL DCM. Purification: column chromatography (20-50% ethylacetate in DCM, then 10% MeOH in DCM). The resulting 3-benzyl-5-(2-((2,2-diphenylethyl)amino)ethyl)oxazolidin-2-one (84 mg, 0.209 mmol) was dissolved in 2 mL ethanol and a reaction with sodium hydroxide (74 mg, 2.090 mmol) was performed. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 46 mg (18%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.21. MW 374.52. Formula $C_{25}H_{30}N_2O$. MS m/z 375.29 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14 - 7.39 (m, 15H), 4.16 (t, J = 7.62 Hz, 1H), 3.82 - 3.92 (m, 1H), 3.79 (d, J = 3.52 Hz, 2H), 3.17 - 3.33 (m, 2H), 2.74 - 3.02 (m, 5H), 2.51 - 2.67 (m, 2H), 1.48 - 1.64 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.44, 139.88, 128.70, 128.39, 128.17, 127.93, 127.00, 126.67, 71.20, 55.04, 54.13, 53.71, 50.91, 48.11, 33.07.

4.1.4.13 1-(Benzylamino)-4-((3,3-diphenylpropyl)amino)butan-2-ol (29)

The reaction of 2-(3-benzyl-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**25**) (143 mg, 0.477 mmol) with 3,3-diphenylpropan-1-amine (303 mg, 1.431 mmol) in the presence of K_2CO_3 (198 mg, 1.430 mmol) in 2 mL DMF was performed according to the procedure C Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 8 mL DCM. Purification: column chromatography (1– 6% MeOH in DCM). The resulting 3-benzyl-5-(2-((3,3-diphenylpropyl)amino)ethyl)oxazolidin-2-one (81 mg, 0.195 mmol) was dissolved in 2 mL ethanol and a reaction with sodium hydroxide (78 mg, 1.950 mmol) was performed. Extraction: 10 mL water, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 70 mg (38%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.21. MW 388.55. Formula $C_{26}H_{32}N_2O$. MS m/z 389.31 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 - 7.40 (m, 15H), 3.99 (t, J = 7.60 Hz, 1H), 3.86 - 3.95 (m, 1H), 3.76 - 3.85 (m, 2H), 2.89 (dt, J = 11.72, 4.69 Hz, 1H), 2.46 - 2.77 (m, 6H), 2.23 (q, J = 7.23 Hz, 2H), 1.48 - 1.63 (m, 2H), 1.20 - 1.37 (m,

2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.29, 139.90, 128.53, 128.41, 128.17, 127.74, 127.01, 126.31, 70.78, 55.06, 53.75, 48.94, 47.88, 47.72, 35.00, 32.98.

4.1.4.14 1-((3-(*Tert*-butyl)benzyl)amino)-4-((2,2-diphenylethyl)amino)butan-2-ol (30)

The reaction of 2-(3-(3-(*tert*-butyl)benzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**27**) (146 mg, 0.410 mmol) with 2,2-diphenylethanamine (203 mg, 1.027 mmol) in the presence of K_2CO_3 (113 mg, 0.820 mmol) in 2 mL toluene was performed according to the procedure C. Extraction: 8 mL water, 3 × 8 mL DCM. Purification: flash chromatography (50– 80% ethyl acetate in petroleum ether). The resulting 3-(3-(*tert*-butyl)benzyl)-5-(2-((2,2-diphenylethyl)amino)ethyl)oxazolidin-2-one (103 mg, 0.226 mmol) was dissolved in 2.5 mL ethanol and reaction with sodium hydroxide (90 mg, 2.260 mmol) was performed. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 72 mg (41%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.28. MW 430.62. Formula $C_{29}H_{38}N_2O$. MS m/z 431.48 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 - 7.36 (m, 14H), 4.17 (t, J = 7.60 Hz, 1H), 3.84 - 3.93 (m, 1H), 3.78 (d, J = 4.69 Hz, 2H), 3.16 - 3.33 (m, 2H), 2.76 - 3.02 (m, 5H), 2.53 - 2.70 (m, 2H), 1.48 - 1.63 (m, 2H), 1.25 - 1.39 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.42, 142.26, 138.35, 128.74, 128.22, 127.96, 126.75, 125.50, 125.42, 124.30, 70.39, 54.64, 53.97, 53.71, 50.61, 47.74, 34.67, 32.94, 31.38.

4.1.4.15 1-((3-(*Tert*-butyl)benzyl)amino)-4-((3,3-diphenylpropyl)amino)butan-2-ol (31)

The reaction of 2-(3-(3-(*tert*-butyl)benzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**27**) (122 mg, 0.340 mmol) with 3,3-diphenylpropan-1-amine (216 mg, 1.020 mmol) in the presence of K_2CO_3 (142 mg, 1.030 mmol) in 2 mL DMF was performed according to the

procedure C. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 8 mL DCM. Purification: column chromatography (18– 50% ethyl acetate in DCM, then 10% MeOH in DCM). The resulting 3-(3-(*tert*-butyl)benzyl)-5-(2-((3,3-diphenylpropyl)amino)ethyl)oxazolidin-2-one (70 mg, 0.149 mmol) was dissolved in 2 mL ethanol and a reaction with sodium hydroxide (60 mg, 1.490 mmol) was performed. Extraction: 10 mL water, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 38 mg (25%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.34. MW 444.65 Formula $C_{30}H_{40}N_2O$. MS m/z 445.41 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 - 7.39 (m, 14H), 3.98 (t, J = 8.20 Hz, 1H), 3.87 - 3.94 (m, 1H), 3.72 - 3.85 (m, 2H), 3.44 (br. s, 3H), 2.86 - 2.98 (m, 1H), 2.71 - 2.80 (m, 1H), 2.50 - 2.70 (m, 4H), 2.29 (q, J = 7.60 Hz, 2H), 1.49 - 1.73 (m, 2H), 1.29 - 1.34 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.51, 143.86, 138.02, 128.61, 128.28, 127.71, 126.43, 125.55, 125.52, 124.40, 68.96, 54.37, 53.64, 48.87, 47.73, 46.71, 34.67, 33.80, 32.41, 31.38.

4.1.4.16 1-((4-Methoxybenzyl)amino)-4-(4-(pentan-2-yl)piperazin-1-yl)butan-2-ol (32)

The reaction of 2-(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**26**) (127 mg, 0.384 mmol) with 1-(pentan-2-yl)piperazine (50 mg, 0.320 mmol) in the presence of K_2CO_3 (66 mg, 0.480 mmol) in 2 mL MeCN was performed according to the procedure C. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 4 × 8 mL DCM. Purification: flash chromatography (1– 10% MeOH in DCM). The resulting 3-(4-methoxybenzyl)-5-(2-(4-(pentan-2-yl)piperazin-1-yl)ethyl)oxazolidin-2-one (60 mg, 0.198 mmol) was dissolved in 1.5 mL ethanol and reaction with sodium hydroxide (79 mg, 1.980 mmol) was performed. Extraction: 8 mL 5% $NH_3 \cdot H_2O$, 6 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 62 mg (53%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.18. MW 363.54. Formula $C_{21}H_{37}N_3O_2$. MS m/z 364.32 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 - 7.25 (m, 2H), 6.80 - 6.86 (m, 2H), 3.90 (ddd, J = 8.85, 5.90, 2.69 Hz, 1H),

3.77 (s, 3H), 3.73 (s, 2H), 2.29 - 2.77 (m, 14H), 1.66 (dd, *J* = 9.62, 4.49 Hz, 1H), 1.30 - 1.52 (m, 6H), 0.92 (d, *J* = 6.67 Hz, 3H), 0.87 (t, *J* = 6.92 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.28, 128.61, 114.63, 114.16, 66.91, 65.84, 59.48, 59.21, 58.29, 56.29, 55.30, 55.24, 54.22, 53.38, 53.00, 20.07, 14.25.

4.1.4.17 1-((4-Methoxybenzyl)amino)-4-(4-phenylpiperazin-1-yl)butan-2-ol (33)

The reaction of 2-(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**26**) (328 mg, 0.995 mmol) with 1-phenylpiperazine (193 mg, 1.194 mmol) in the presence of K_2CO_3 (206 mg, 1.492 mmol) in 5 mL MeCN was performed according to the procedure C. Extraction: 5 mL sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: flash chromatography (1– 6% MeOH in DCM). The resulting 3-(4-methoxybenzyl)-5-(2-(4-phenylpiperazin-1-yl)ethyl)oxazolidin-2-one (195 mg, 0.493 mmol) was dissolved in 5 mL ethanol and reaction with sodium hydroxide (197 mg, 4.930 mmol) was performed. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL ethyl acetate. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.025, v/v/v). Yield: 117 mg (32%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.025, v/v/v) R_f = 0.08. MW 369.50. Formula $C_{22}H_{31}N_3O_2$. MS m/z 370.24 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 - 7.31 (m, 4H), 6.81 - 6.95 (m, 5H), 3.89 - 4.00 (m, 1H), 3.79 (s, 3H), 3.76 (d, *J* = 1.54 Hz, 2H), 3.18 (t, *J* = 5.00 Hz, 5H), 2.50 - 2.83 (m, 9H), 1.68 - 1.83 (m, 1H), 1.48 - 1.61 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.69, 160.68, 160.34, 134.31, 133.84, 131.97, 123.48, 123.09, 57.53, 56.57, 54.76, 44.00, 43.50, 35.82, 28.21, 21.51.

4.1.4.18 4-(4-Benzylpiperazin-1-yl)-1-((4-methoxybenzyl)amino)butan-2-ol (34)

The reaction of 2-(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**26**) (147 mg, 0.446 mmol) with 1-benzylpiperazine (94 mg, 0.535 mmol) in the presence of K_2CO_3 (92 mg, 0.669 mmol) in 3 mL MeCN was performed according to the procedure C.

Extraction: 5 mL sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: flash chromatography (1– 6% MeOH in DCM). The resulting 5-(2-(4-benzylpiperazin-1-yl)ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (76 mg, 0.186 mmol) was dissolved in 3 mL ethanol and reaction with sodium hydroxide (74 mg, 1.860 mmol) was performed. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 52 mg (30%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.11. MW 383.53. Formula $C_{23}H_{33}N_3O_2$. MS m/z 384.14 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 - 7.37 (m, 7H), 6.80 - 6.90 (m, 2H), 3.91 (td, *J* = 4.68, 2.44 Hz, 1H), 3.59 - 3.84 (m, 7H), 3.48 (s, 2H), 2.22 - 2.86 (m, 12H), 1.67 (dd, *J*=9.75, 4.36 Hz, 1H), 1.38 - 1.54 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.60, 137.90, 132.07, 129.39, 129.16, 128.22, 127.08, 113.73, 72.05, 62.91, 57.26, 55.25, 55.11, 53.18, 53.08, 53.00, 29.64.

4.1.4.19 4-(4-Benzhydrylpiperazin-1-yl)-1-(benzylamino)butan-2-ol (35)

The reaction of 2-(3-benzyl-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**25**) (213 mg, 0.710 mmol) with 1-benzhydrylpiperazine (270 mg, 1.070 mmol) in the presence of K_2CO_3 (300 mg, 2.130 mmol) in 3 mL DMF was performed according to the procedure C. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 8 mL DCM. Purification: column chromatography (5– 50% ethyl acetate in DCM, then 10% MeOH in DCM). The resulting 5-(2-(4-benzhydrylpiperazin-1-yl)ethyl)-3-(3-(*tert*-butyl)benzyl)oxazolidin-2-one (207 mg, 0.454 mmol) was dissolved in 4 mL ethanol and a reaction with sodium hydroxide (182 mg, 4.540 mmol) was performed. Extraction: 10 mL $NH_3 \cdot H_2O$, 3 × 10 mL DCM. Purification: column chromatography (2-14% MeOH in DCM. Yield: 161 mg (53%), TLC (DCM/MeOH, 9/1, v/v) R_f = 0.14. MW 429.60. Formula $C_{28}H_{35}N_3O$. MS m/z 430.39 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 - 7.43 (m, 15H), 4.16 - 4.23 (m, 1H), 3.89 - 4.00 (m, 1H), 3.82 - 3.89 (m, 2H), 3.59 - 3.81 (m, 2H), 2.26 - 2.77 (m, 12H), 1.68 (dtd, *J* = 14.36, 9.82, 9.82, 4.69 Hz, 1H), 1.45 - 1.57 (m, 1H). ^{13}C NMR

(75 MHz, $CDCl_3$) δ 142.48, 139.25, 128.47, 128.42, 128.32, 127.87, 127.13, 126.96, 76.03, 71.56, 57.00, 54.91, 53.59, 53.40, 51.72, 29.60.

4.1.4.20 4-(4-Benzhydrylpiperazin-1-yl)-1-((4-methoxybenzyl)amino)butan-2-ol (36)

The reaction of 2-(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**26**) (205 mg, 0.621 mmol) with 1-benzhydrylpiperazine (188 mg, 0.745 mmol) in the presence of K_2CO_3 (129 mg, 0.931 mmol) in 4 mL MeCN was performed according to the procedure C. Extraction: 10 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: flash chromatography (1–6% MeOH in DCM). The resulting 5-(2-(4-benzhydrylpiperazin-1-yl)ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (160 mg, 0.329 mmol) was dissolved in 5 mL ethanol and reaction with sodium hydroxide (132 mg, 3.290 mmol) was performed. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 94 mg (34%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.24. MW 459.62. Formula $C_{29}H_{37}N_3O_2$. MS m/z 460.22 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 - 7.44 (m, 4H), 7.12 - 7.31 (m, 8H), 6.80 - 6.88 (m, 2H), 4.19 (s, 1H), 3.89 (td, J = 4.62, 2.31 Hz, 1H), 3.79 (s, 3H), 3.73 (d, J = 1.54 Hz, 2H), 3.32 - 3.44 (m, 1H), 2.17 - 2.83 (m, 13H), 1.66 (dd, J = 9.87, 4.49 Hz, 1H), 1.41 - 1.53 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.53, 142.55, 132.38, 129.31, 128.47, 127.89, 127.88, 126.94, 113.70, 76.08, 72.19, 57.28, 55.22, 53.48, 53.36, 53.26, 51.88, 29.63.

4.1.4.21 4-(4-Benzhydrylpiperazin-1-yl)-1-((3-(*tert*-butyl)benzyl)amino)butan-2-ol (37)

The reaction of 2-(3-(3-(*tert*-butyl)benzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**27**) (128 mg, 0.360 mmol) with 1-benzhydrylpiperazine (136 mg, 0.540 mmol) in the presence of K_2CO_3 (149 mg, 1.080 mmol) in 3 mL dimethylformamide (DMF) was performed according

to the procedure C. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 8 mL DCM. Purification: column chromatography (5– 50% ethyl acetate in DCM, then 10% MeOH in DCM). The resulting 5-((4-benzhydrylpiperazin-1-yl)methyl)-3-(3-(*tert*-butyl)benzyl)oxazolidin-2-one (71 mg, 0.139 mmol) was dissolved in 3 mL ethanol and a reaction with sodium hydroxide (56 mg, 1.390 mmol) was performed. Extraction: 10 mL water, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v). Yield: 53 mg (30%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.34. MW 485.70. Formula $C_{32}H_{43}N_3O$. MS m/z 486.42 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 - 7.44 (m, 14H), 4.18 (s, 1H), 3.95 – 4.14 (m, 3H), 3.80 - 3.95 (m, 2H), 2.13 - 2.79 (m, 10H), 1.47 - 1.83 (m, 2H), 1.23 - 1.35 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.58, 142.41, 136.90, 128.51, 128.29, 127.84, 127.01, 125.79, 125.75, 124.63, 75.99, 70.42, 56.53, 54.26, 53.39, 51.51, 34.70, 31.37, 30.18, 29.58.

4.1.4.22 4-(4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)-1-((4-methoxybenzyl)amino)butan-2-ol (38)

The reaction of 2-(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**26**) (90 mg, 0.272 mmol) with 1-(bis(4-fluorophenyl)methyl)piperazine (94 mg, 0.326 mmol) in the presence of K_2CO_3 (68 mg, 0.489 mmol) in 2 mL MeCN was performed according to the procedure C. Extraction: 5 mL sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: flash chromatography (1– 5% MeOH in DCM). The resulting 5-(2-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (82 mg, 0.157 mmol) was dissolved in 2 mL ethanol and reaction with sodium hydroxide (63 mg, 1.570 mmol) was performed. Extraction: 8 mL sat. $NaHCO_{3(aq)}$, 3 × 10 mL DCM. Purification: column chromatography (DCM/MeOH/$NH_3 \cdot H_2O$, 9.7/0.3/0.025, v/v/v). Yield: 37 mg (23%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.7/0.3/0.025, v/v/v) R_f = 0.16. MW 495.60. Formula $C_{29}H_{35}F_2N_3O_2$. MS m/z 496.22 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (ddd, *J*

= 8.59, 5.64, 2.44 Hz, 4H), 7.18 - 7.25 (m, 2H), 6.92 - 6.99 (m, 4H), 6.80 - 6.88 (m, 2H), 4.19 (s, 1H), 3.83 - 3.93 (m, 1H), 3.78 (s, 3H), 3.73 (s, 2H), 2.19 - 2.83 (m, 14H), 1.58 - 1.75 (m, 1H), 1.42 – 1.51 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.79 (d, J = 245.47 Hz), 158.53, 138.02 (d, J = 3.87 Hz), 132.47, 129.27, 129.16 (d, J = 1.11 Hz), 115.39 (d, J = 21.56 Hz), 113.70, 74.36, 72.21, 57.23, 55.25, 55.23, 53.31, 51.73, 29.66.

4.1.4.23 4-(Benzylamino)-1-((2,2-diphenylethyl)amino)butan-2-ol (45)

The reaction of 2-(3-(2,2-diphenylethyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**43**) (249 mg, 0.667 mmol) with benzylamine (214 mg, 2.000 mmol) in the presence of K_2CO_3 (184 mg, 1.334 mmol) in 4 mL toluene was performed according to the procedure C. Extraction: 5 mL sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.5/0.5/0.05, v/v/v). The resulting 5-(2-(benzylamino)ethyl)-3-(2,2-diphenylethyl)oxazolidin-2-one (113 mg, 0.282 mmol) was dissolved in 2 mL ethanol and reaction with sodium hydroxide (113 mg, 2.820 mmol) was performed. Extraction: 10 mL 5% $NH_3 \cdot H_2O$, 3 × 12 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.5/0.5/0.05, v/v/v). Yield: 85 mg (34%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.46. MW 374.53. Formula $C_{25}H_{30}N_2O$. MS m/z 375.29 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 - 7.36 (m, 15H), 4.19 (t, J = 7.62 Hz, 1H), 3.70 - 3.88 (m, 3H), 3.63 (br. s., 3H), 3.17 - 3.33 (m, 2H), 2.73 - 2.97 (m, 2H), 2.69 (dd, J = 11.72, 4.10 Hz, 1H), 2.59 (dd, J = 12.31, 8.21 Hz, 1H), 1.52 - 1.68 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.69, 142.56, 137.77, 128.68, 128.64, 128.61, 128.51, 128.02, 127.99, 127.58, 126.63, 126.58, 70.12, 55.33, 54.26, 53.18, 50.96, 46.71, 32.85.

4.1.4.24 4-(Benzylamino)-1-((3,3-diphenylpropyl)amino)butan-2-ol (46)

The reaction of 2-(3-(3,3-diphenylpropyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (**44**) (154 mg, 0.397 mmol) with benzylamine (128 mg, 1.192 mmol) in the presence of K_2CO_3

(109 mg, 0.794 mmol) in 4 mL toluene was performed according to the procedure C. Extraction: 5 mL sat. $NaHCO_{3(aq)}$, 3 × 5 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.5/0.5/0.05, v/v/v). The resulting 5-(2-(benzylamino)ethyl)-3-(3,3-diphenylpropyl)oxazolidin-2-one (87 mg, 0.210 mmol) was dissolved in 2 mL ethanol and reaction with sodium hydroxide (83 mg, 2.100 mmol) was performed. Extraction: 5 mL 5% $NH_3 \cdot H_2O$, 3 × 8 mL DCM. Purification: column chromatography in DCM/MeOH/$NH_3 \cdot H_2O$ (9.5/0.5/0.05, v/v/v). Yield: 54 mg (35%), TLC (DCM/MeOH/$NH_3 \cdot H_2O$, 9.5/0.5/0.05, v/v/v) R_f = 0.42. MW 388.56. Formula $C_{26}H_{32}N_2O$. MS m/z 389.25 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 - 7.40 (m, 15H), 3.87 - 4.05 (m, 5H), 3.71 - 3.85 (m, 2H), 2.73 - 2.98 (m, 2H), 2.46 - 2.67 (m, 4H), 2.29 (q, J = 7.60 Hz, 2H), 1.48 - 1.69 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.95, 142.62, 130.46, 129.74, 128.47, 127.86, 126.94, 113.91, 76.19, 65.34, 61.94, 55.26, 53.64, 52.89, 52.68, 51.84.

4.1.5 Synthesis of *tert*-butyl-dimethyl(2-(oxiran-2-yl)ethoxy)silane (18)

To a stirred solution of but-3-en-1-ol (3.00 g, 41.60 mmol) and imidazole (3.40 g, 49.92 mmol) in DCM (120 mL) *tert*-butylchlorodimethylsilane (7.52 g, 49.92 mmol) was added portionwise and the reaction mixture was stirred at room temperature for 16 h. Then the reaction mixture was washed with water (3 × 100 mL) and saturated aqueous solution of NaCl (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. To the resulting product dissolved in DCM (120 mL) cooled on an ice-bath, *meta*-chloroperoxybenzoic acid (14.36 g, 83.20 mmol) was added, and the reaction mixture was stirred at room temperature for 16 h. After that time, sodium thiosulfate was added to neutralize the excess of *meta*-chloroperoxybenzoic acid, and the solvent was evaporated. The produced residue was dissolved in *n*-hexane (100 mL) and washed with saturated aqueous solution of $NaHCO_3$ (3 × 100 mL) and saturated aqueous solution of NaCl (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give compound

18 (8.11 g, 96%) Yield: 9.455 g (93%), TLC (petroleum ether/ethyl acetate, 7/3, v/v) R_f = 0.52. MW 202.37. Formula $C_{10}H_{22}O_2Si$. MS m/z 203.14 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 3.74 - 3.81 (m, 2H), 2.98 - 3.11 (m, 1H), 2.78 (dd, J = 4.87, 4.10 Hz, 1H), 2.52 (dd, J = 5.13, 2.82 Hz, 1H), 1.62 - 1.85 (m, 2H), 0.87 - 0.93 (s, 9H), 0.02 - 0.12 (s, 6H).

4.1.6 General procedure for the synthesis of compounds: 19–21 (procedure D).

Tert-butyl-dimethyl(2-(oxiran-2-yl)ethoxy)silane (**18**) (1.0 equiv.), corresponding benzylamine (1.2 equiv.) and a catalytic amount of pyridine in *n*-propanol were refluxed for 16 h. Then, the solvent was evaporated and the resulting residue was purified by flash column chromatography using a mixture of MeOH and DCM (gradient from 0% to 10% of MeOH).

4.1.6.1 1-(Benzylamino)-4-((*tert*-butyldimethylsilyl)oxy)butan-2-ol (19)

Following the procedure D, reaction of phenylmethanamine (1.019 mL, 9.33 mmol) with *tert*-butyl-dimethyl(2-(oxiran-2-yl)ethoxy)silane (**18**) (1.580 g, 7.78 mmol) and catalytic amount of pyridine in 20 mL *n*-propanol was performed. Yield: 1.360 g (56%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.16. MW 309.52. Formula $C_{17}H_{31}NO_2Si$. MS m/z 310.16 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 - 7.39 (m, 5H), 3.87 - 4.00 (m, 1H), 3.61 - 3.87 (m, 4H), 3.14 (br. s, 1H), 2.50 - 2.78 (m, 2H), 1.57 - 1.74 (m, 2H), 1.29 (s, 1H), 0.89 (s, 9H), 0.05 (s, 6H).

4.1.6.2 4-((*Tert*-butyldimethylsilyl)oxy)-1-((4-methoxybenzyl)amino)butan-2-ol (20)

Following the procedure D, reaction of (4-methoxyphenyl)methanamine (7.324 mL, 46.72 mmol) with *tert*-butyl-dimethyl(2-(oxiran-2-yl)ethoxy)silane (**18**) (7.879 g, 38.933 mmol) and catalytic amount of pyridine in 200 mL *n*-propanol was performed. Yield: 5.420 g (57%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.18. MW 339.55. Formula $C_{18}H_{33}NO_3Si$. MS m/z 340.20 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 - 7.29 (m, 2H), 6.81 - 6.90 (m, 2H), 3.86

- 3.96 (m, 1H), 3.75 - 3.86 (m, 7H), 2.70 (dd, *J* = 12.05, 3.59 Hz, 1H), 2.61 (dd, *J* = 12.05, 8.46 Hz, 1H), 2.40 (br. s, 1H), 1.56 - 1.75 (m, 2H), 1.21 - 1.32 (s, 1H), 0.89 (s, 9H), 0.06 (s, 6H).

4.1.6.3 1-((3-(*Tert*-butyl)benzyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)butan-2-ol (21)

Following the procedure D, reaction of (3-(*tert*-butyl)phenyl)methanamine (0.925 g, 5.664 mmol) with *tert*-butyl-dimethyl(2-(oxiran-2-yl)ethoxy)silane (**18**) (0.770 g, 4.720 mmol) and catalytic amount of pyridine in 20 mL *n*-propanol was performed. Yield: 0.780 g (45%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.23. MW 365.63. Formula $C_{21}H_{39}NO_2Si$. MS m/z 366.18 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 - 7.38 (m, 1H), 7.22 - 7.32 (m, 2H), 7.10 - 7.20 (m, 1H), 3.87 - 3.98 (m, 1H), 3.75 - 3.86 (m, 4H), 3.12 (br. s, 1H), 2.58 - 2.80 (m, 2H), 1.58 - 1.74 (m, 2H), 1.32 (s, 10H), 0.89 (s, 9H), 0.06 (s, 6H).

4.1.6.4 4-((*Tert*-butyldimethylsilyl)oxy)-1-((2,2-diphenylethyl)amino)butan-2-ol (39)

Following the procedure D, reaction of 2,2-diphenylethan-1-amine (0.585 g, 2.965 mmol) with *tert*-butyl-dimethyl(2-(oxiran-2-yl)ethoxy)silane (**18**) (0.502 g, 2.471 mmol) and catalytic amount of pyridine in 7 mL *n*-propanol was performed. Yield: 0.414 g (42%), TLC (DCM/MeOH, 9/1, v/v) R_f = 0.56. MW 399.65. Formula $C_{24}H_{37}NO_2Si$. MS m/z 400.35 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.12 - 7.39 (m, 10H), 4.37 (t, *J* = 7.60 Hz, 1H), 3.83 - 3.98 (m, 1H), 3.66 - 3.82 (m, 3H), 3.28 - 3.47 (m, 5H), 2.82 (dd, *J* = 12.31, 3.52 Hz, 1H), 2.67 (dd, *J* = 11.72, 8.79 Hz, 1H), 0.83 - 0.93 (m, 9H), 0.05 (s, 6H).

4.1.6.5 4-((*Tert*-butyldimethylsilyl)oxy)-1-((3,3-diphenylpropyl)amino)butan-2-ol (40)

Following the procedure D, reaction of 3,3-diphenylpropan-1-amine (0.627 g, 2.965 mmol) with *tert*-butyl-dimethyl(2-(oxiran-2-yl)ethoxy)silane (**18**) (0.500 g, 2.471 mmol) and catalytic amount of pyridine in 7 mL *n*-propanol was performed. Yield: 0.546 g (53%), TLC (DCM/MeOH, 9/1, v/v) R_f = 0.61. MW 413.68. Formula $C_{25}H_{39}NO_2Si$. MS m/z 414.37 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.11 - 7.34 (m, 10 H), 4.02 (t, J = 7.60 Hz, 1H), 3.88 - 3.98 (m, 1H), 3.76 (t, J = 5.90 Hz, 2H), 3.54 - 3.71 (br.s., 2H), 2.60 - 2.81 (m, 4H), 2.39 (q, J = 7.60 Hz, 2H), 1.60 (d, J = 4.69 Hz, 2H), 0.80 - 0.93 (m, 9H), -0.01 - 0.12 (m, 6H).

4.1.7 General procedure for the synthesis of compounds: 22–24 (procedure E).

To a solution of 2-aminoalcohol **29**–**21** (1.0 equiv.) and TEA (1.6 equiv.) in DCM, 1,1'-carbonyldiimidazol (1.1 equiv.) was added portionwise and the reaction mixture was stirred for 5 h at room temperature. Then the solvent was evaporated and the resulting residue was purified by flash column chromatography using a mixture of petroleum ether and ethyl acetate (gradient from 10% to 30% of ethyl acetate).

4.1.7.1 3-Benzyl-5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)oxazolidin-2-one (22)

Following the procedure E, reaction of 1-(benzylamino)-4-((*tert*-butyldimethylsilyl)oxy)butan-2-ol (**19**) (1.360 g, 4.390 mmol) with 1,1'-carbonyldiimidazole (0.805 g, 4.940 mmol) and TEA (0.951 mL, 6.815 mmol) in 20 mL DCM was performed. Yield: 1.030 g (70%), TLC (petroleum ether/ethyl acetate, 7/3, v/v) R_f = 0.48. MW 335.51. Formula $C_{18}H_{29}NO_3Si$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.24 - 7.39 (m, 5H), 4.58 - 4.70 (m, 1H), 4.34 - 4.48 (m, 2H), 3.70 - 3.77 (m, 2H), 3.48 (t, J = 8.7 Hz, 1H), 3.10 (dd, J = 8.7, 7.1 Hz, 1H), 1.71 - 1.97 (m, 2H), 0.85 (s, 9H), 0.02 (s, 6H).

4.1.7.2 5-(2-((*Tert*-butyldimethylsilyl)oxy)ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (23)

Following the procedure E, reaction of 4-((*tert*-butyldimethylsilyl)oxy)-1-((4-methoxybenzyl)amino)butan-2-ol (**20**) (5.420 g, 15.962 mmol) with 1,1'-carbonyldiimidazole (2.912 g, 17.958 mmol) and TEA (3.565 mL, 25.539 mmol) in 300 mL DCM was performed. Yield: 4.668 g (80%), TLC (petroleum ether/ethyl acetate, 7/3, v/v) R_f = 0.42. MW 365.54. Formula $C_{19}H_{31}NO_4Si$. MS m/z 366.18 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, J = 8.46 Hz, 2H), 6.87 (d, J = 8.46 Hz, 2H), 4.55 - 4.68 (m, 1H), 4.34 (d, J = 3.33 Hz, 2H), 3.80 (s, 3H), 3.67 - 3.76 (m, 2H), 3.46 (t, J = 8.46 Hz, 1H), 3.07 (dd, J = 8.72, 6.92 Hz, 1H), 1.70 - 1.97 (m, 2H), 0.85 (s, 9H), 0.02 (s, 6H).

4.1.7.3 3-(3-(*Tert*-butyl)benzyl)-5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)oxazolidin-2-one (24)

Following the procedure E, reaction of 1-((3-(*tert*-butyl)benzyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)butan-2-ol (**21**) (0.630 g, 1.720 mmol) with 1,1'-carbonyldiimidazole (0.314 g, 1.940 mmoland TEA (0.373 mL, 2.670 mmol) in 20 mL DCM was performed. Yield: 0.510 g (76%), TLC (petroleum ether/ethyl acetate, 7/3, v/v) R_f = 0.53. MW 391.62. Formula $C_{22}H_{37}NO_3Si$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.37 - 7.31 (m, 1H), 7.31 - 7.24 (m, 2H), 7.11 - 7.05 (m, 1H), 4.70 - 4.58 (m, 1H), 4.40 (d, J = 3.62 Hz, 2H), 3.68 - 3.81 (m, 2H), 3.43 - 3.54 (m, 1H), 3.05 - 3.14 (m, 1H), 1.87 - 1.99 (m, 1H), 1.73 - 1.85 (m, 1H), 1.31 (s, 9H), 0.85 (s, 9H), 0.02 (s, 6H).

4.1.7.4 5-(2-((*Tert*-butyldimethylsilyl)oxy)ethyl)-3-(2,2-diphenylethyl)oxazolidin-2-one (41)

Following the procedure E, reaction of 4-((*tert*-butyldimethylsilyl)oxy)-1-((2,2-diphenylethyl)amino)butan-2-ol (**39**) (0.546 g, 1.366 mmol) with 1,1'-carbonyldiimidazole (0.249 g, 1.537 mmol) and TEA (0.295 mL, 2.117 mmol) in 6 mL DCM was performed. Yield: 0.460 g (79%), TLC (petroleum ether/ethyl acetate, 7/3, v/v) R_f = 0.56. MW 425.64.

Formula $C_{25}H_{35}NO_3Si$. MS m/z 426.27 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 - 7.36 (m, 10H), 4.37 - 4.51 (m, 1H), 4.30 (t, J = 8.50 Hz, 1H), 3.87 (d, J = 8.21 Hz, 2H), 3.54 - 3.71 (m, 2H), 3.32 (t, J = 8.50 Hz, 1H), 2.92 (dd, J = 8.50, 6.74 Hz, 1H), 1.46 - 1.74 (m, 2H), 0.84 - 0.88 (s, 9H), -0.03 - 0.04 (s, 6H).

4.1.7.5 5-(2-((*Tert*-butyldimethylsilyl)oxy)ethyl)-3-(3,3-diphenylpropyl)oxazolidin-2-one (42)

Following the procedure E, reaction of 4-((*tert*-butyldimethylsilyl)oxy)-1-((3,3-diphenylpropyl)amino)butan-2-ol (**40**) (0.330 g, 0.798 mmol) with 1,1'-carbonyldiimidazole (0.145 g, 0.897 mmol) and TEA (0.172 mL, 1.235 mmol) in 5 mL DCM was performed. Yield: 0.224 g (64%), TLC (petroleum ether/ethyl acetate, 7/3, v/v) R_f = 0.61. MW 439.67. Formula $C_{26}H_{37}NO_3Si$. MS m/z 440.66 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.12 - 7.36 (m, 10H), 4.46 - 4.60 (m, 1H), 3.95 (t, J = 7.62 Hz, 1H), 3.69 - 3.77 (m, 2H), 3.52 (t, J = 8.50 Hz, 1H), 3.10 - 3.35 (m, 3H), 2.31 (q, J = 7.00 Hz, 2H), 1.69 - 1.95 (m, 2H), 0.89 (s, 9H), 0.05 (s, 6H).

4.1.8 General procedure for the synthesis of compounds: 25–27 (procedure F)

To an ice-cooled solution of 3-benzyl-5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)oxazolidin-2-one derivative (**22**–**24**) (1.0 equiv.) in THF tetra-*n*-butylammonium fluoride (1 M sol. in THF) (1.1 equiv.) was added dropwise and the reaction mixture was stirred at room temperature for 16 h. Then the solvent was evaporated and the resulting residue was treated with water and extracted with DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. The resulting residue was purified by flash column chromatography using a mixture of DCM and MeOH (2– 8% MeOH in DCMv). To the obtained alcohol dissolved in an anhydrous DCM, anhydrous TEA (1.3 equiv.) and methanesulfonyl chloride (1.3 quiv.) were subsequently added. The reaction mixture was stirred at room temperature for

2 h. After this time the solvent was evaporated and the produced residue was dissolved in ethyl acetate and washed with water and saturated aqueous solution of NaCl. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure.

4.1.8.1 2-(3-Benzyl-2-oxooxazolidin-5-yl)ethyl methanesulfonate (25)

Following the procedure F reaction of 3-benzyl-5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)oxazolidin-2-one (**22**) (1.030 g, 3.070 mmol) with tetra-*n*-butylammonium fluoride (0.880 g, 3.380 mmol) in 10 mL THF was performed. Extraction: 15 mL water, 3 × 20 mL DCM. Purification: flash chromatography (2– 7% MeOH in DCM) to obtain 3-benzyl-5-(2-hydroxyethyl)oxazolidin-2-one. Yield: 0.620 g (91%), TLC (DCM/MeOH, 9/1, v/v) R_f = 0.72. MW 221.25. Formula $C_{12}H_{15}NO_3$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 - 7.40 (m, 5H) 4.62 - 4.75 (m, 1H) 4.40 (d, J = 5.28 Hz, 2H) 3.70 - 3.87 (m, 2H) 3.51 (t, J = 7.60 Hz, 1H) 3.10 (dd, J = 8.50, 7.33 Hz, 1H) 2.26 (br. s, 1H) 1.76 - 2.03 (m, 2H). The obtained 3-benzyl-5-(2-hydroxyethyl)oxazolidin-2-one (0.620 g, 2.800 mmol) was dissolved in 10 mL DCM, TEA (510 µL, 3.640 mmol) and methanesulfonyl chloride (296 µL, 3.640 mmol) were added. Extraction: 30 mL DCM, 3 × 30 mL water, 30 mL sat. $NaCl_{(aq)}$. Yield: 0.830 g (99%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.82. MW 299.34. Formula $C_{13}H_{17}NO_5S$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 - 7.40 (m, 5H), 4.60 - 4.73 (m, 1H), 4.42 (d, J = 2.34 Hz, 2H), 4.36 (t, J = 5.90 Hz, 2H), 3.55 (t, J = 8.50 Hz, 1H), 3.08 (dd, J = 8.79, 7.03 Hz, 1H), 3.00 (s, 3H), 2.08 (q, J = 6.25 Hz, 2H).

4.1.8.2 2-(3-(4-Methoxybenzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (26)

Following the procedure F reaction of 5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**23**) (0.350 g, 0.958 mmol) with tetra-*n*-butylammonium fluoride (0.275 g, 1.053 mmol) in 10 mL THF. Extraction: 20 mL water, 3 × 10 mL DCM. Purification flash chromatography (2– 7% MeOH in DCM) to obtain 5-(2-hydroxyethyl)-3-

(4-methoxybenzyl)oxazolidin-2-one. Yield: 0.240 g (100%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.46. MW 251.28. Formula $C_{13}H_{17}NO_4$. MS m/z 252.14 ($M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 - 7.23 (m, 2H) 6.83 - 6.91 (m, 2H) 4.67 (dd, J = 6.92, 4.87 Hz, 1H) 4.35 (d, J = 2.05 Hz, 2H) 3.75 - 3.87 (m, 5H) 3.50 (t, J = 8.59 Hz, 1H) 3.07 (dd, J = 8.72, 6.92 Hz, 1H) 1.77 - 2.04 (m, 2H) 1.67 (br. s, 1H). The obtained 5-(2-hydroxyethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (0.240 g, 0.955 mmol) was dissolved in 10 mL DCM, TEA (173 µL, 1.242 mmol) and methanesulfonyl chloride (97 µL, 1.242 mmol) were added. Extraction: 10 mL ethyl acetate, 2 × 10 mL water, 10 mL sat. $NaCl_{(aq)}$. Yield: 291 mg (93%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.87. MW 329.37. Formula $C_{14}H_{19}NO_6S$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14 - 7.21 (m, 2H), 6.82 - 6.89 (m, 2H), 4.54 - 4.72 (m, 1H), 4.30 - 4.37 (m, 4H), 3.78 (s, 3H), 3.52 (t, J = 8.40 Hz, 1H), 3.04 (dd, J = 8.79, 6.59 Hz, 1H), 2.99 (s, 3H), 2.04 (q, J = 5.70 Hz, 2H).

4.1.8.3 2-(3-(3-(*Tert*-butyl)benzyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (27)

Following the procedure F reaction of 3-(3-(*tert*-butyl)benzyl)-5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)oxazolidin-2-one (**24**) (0.510 g, 1.300 mmol) with tetra-*n*-butylammonium fluoride (0.370 g, 1.432 mmol) in 5 mL THF. Extraction: 10 mL water, 3 × 10 mL DCM. Purification flash chromatography (2– 7% MeOH in DCM) to obtain 3-(3-(*tert*-butyl)benzyl)-5-(2-hydroxyethyl)oxazolidin-2-one. Yield: 0.360 g (100%), TLC (DCM/MeOH, 9/1, v/v) R_f = 0.59. MW 277.36. Formula $C_{16}H_{23}NO_3$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 - 7.37 (m, 3H) 7.08 (s, 1H) 4.63 - 4.76 (m, 1H) 4.41 (d, J = 4.10 Hz, 2H) 3.70 - 3.88 (m, 2H) 3.52 (t, J = 8.50 Hz, 1H) 3.10 (dd, J = 8.79, 7.03 Hz, 1H) 1.78 - 2.03 (m, 3H) 1.26 - 1.38 (m, 9H). The obtained 3-(3-(*tert*-butyl)benzyl)-5-(2-hydroxyethyl)oxazolidin-2-one (**27**) (0.360 g, 1.298 mmol) was dissolved in 10 mL DCM, TEA (241 µL, 1.732 mmol) and methanesulfonyl chloride (142 µL, 1.732 mmol) were added. Extraction: 10 mL ethyl

acetate, 2 × 10 mL water, 10 mL sat. $NaCl_{(aq)}$. Yield: 440 mg (93%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.92. MW 355.45. Formula $C_{17}H_{25}NO_5S$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.24 - 7.39 (m, 3H), 7.04 - 7.11 (m, 1H), 4.60 - 4.73 (m, 1H), 4.33 - 4.46 (m, 4H), 3.56 (t, J = 8.7 Hz, 1H), 3.08 (dd, J = 8.7, 6.6 Hz, 1H), 3.01 (s, 3H), 2.03 - 2.13 (m, 2H), 1.32 (s, 9H).

4.1.8.4 2-(3-(2,2-Diphenylethyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (43)

Following the procedure F reaction of 5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-(2,2-diphenylethyl)oxazolidin-2-one (**41**) (0.480 g, 1.010 mmol) with tetra-*n*-butylammonium fluoride (0.294 g, 1.136 mmol) in 7 mL THF was performed. Extraction: 10 mL water, 3 × 10 mL DCM. Purification: flash chromatography (2– 7% MeOH in DCM) to obtain 3-(2,2-diphenylethyl)-5-(2-hydroxyethyl)oxazolidin-2-one. Yield: 0.306 g (97%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.52. MW 311.38. Formula $C_{19}H_{21}NO_3$. MS m/z 312.21 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.17 - 7.36 (m, 10H), 4.43 - 4.56 (m, 1H), 4.30 (t, J = 8.20 Hz, 1H), 3.87 (dd, J = 8.79, 5.28 Hz, 2H), 3.63 - 3.70 (m, 2H), 3.36 (t, J = 8.50 Hz, 1H), 2.94 (dd, J = 8.50, 6.74 Hz, 1H), 1.83 (br. s., 1H), 1.58 - 1.73 (m, 2H). The obtained 3-(2,2-diphenylethyl)-5-(2-hydroxyethyl)oxazolidin-2-one (0.306 g, 0.983 mmol) was dissolved in 5 mL DCM, TEA (164 µL, 1.180 mmol) and methanesulfonyl chloride (91 µL, 1.180 mmol) were added. Extraction: 10 mL DCM, 3 × 10 mL water, 10 mL sat. $NaCl_{(aq)}$. Yield: 0.341 g (89%), TLC (DCM/MeOH, 9.2/0.8, v/v) R_f = 0.76. MW 389.47. Formula $C_{20}H_{23}NO_5S$.

4.1.8.5 2-(3-(3,3-Diphenylpropyl)-2-oxooxazolidin-5-yl)ethyl methanesulfonate (44)

Following the procedure F reaction of 5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-(3,3-diphenylpropyl)oxazolidin-2-one (**42**) (0.224 g, 0.569 mmol) with tetra-*n*-butylammonium fluoride (0.150 g, 0.572 mmol) in 5 mL THF was performed. Extraction: 10 mL water, 3 × 10

mL DCM. Purification: flash chromatography (2– 7% MeOH in DCM) to obtain 3-(3,3-diphenylpropyl)-5-(2-hydroxyethyl)oxazolidin-2-one. Yield: 0.170 g (92%), TLC (DCM/MeOH, 9.5/0.5, v/v) R_f = 0.57. MW 325.41. Formula $C_{20}H_{23}NO_3$. MS m/z 326.23 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.14 - 7.34 (m, 10H), 4.49 - 4.64 (m, 1H), 3.95 (t, J = 7.62 Hz, 1H), 3.70 - 3.89 (m, 2H), 3.55 (t, J = 8.20 Hz, 1H), 3.08 - 3.36 (m, 3H), 2.32 (q, J = 7.60 Hz, 2H), 1.79 - 2.00 (m, 3H). The obtained 3-(3,3-diphenylpropyl)-5-(2-hydroxyethyl)oxazolidin-2-one (0.170 g, 0.522 mmol) was dissolved in 5 mL DCM, TEA (87 µL, 0.626 mmol) and methanesulfonyl chloride (48 µL, 0.626 mmol) were added. Extraction: 8 mL DCM, 3 × 8 mL water, 8 mL sat. $NaCl_{(aq)}$. Yield: 0.154 g (73%), TLC (DCM/MeOH, 9.2/0.8, v/v) R_f = 0.79. MW 403.49. Formula $C_{21}H_{25}NO_5S$.

4.2 Biological activity

4.2.1 *In vitro* inhibition of AChE and BuChE

Inhibitory activities of the synthesized compounds against the cholinesterases were measured using the spectrophotometric method described by Ellman et al.[49], as modified for 96-well microplates. AChE from *Electrophorus electricus* (*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 water to give stock solutions of 5 U/mL. *ee*AChE and *eq*BuChE were further diluted before use to a final concentration of 0.384 U/mL. In the first step of Ellman's method 25 µL of the test compound (or water; i.e. blank samples) was incubated in 0.1 M phosphate buffer (200 µL, pH = 8.0) with DTNB (20 µL; 0.0025 M) and the enzyme (20 µL; *ee*AChE or *eq*BuChE) in temperature 25°C. Then after an incubation period of 5 min., 20 µL of ATC (0.00375M) or BTC (0.00375M) solutions (depending on the enzyme used) were added. After a further 5 min, the

changes in absorbance were measured at 412 nm, using a microplate reader (EnSpire Multimode; PerkinElmer). All compounds were tested at screening concentration of 10 μM. Percent of inhibition was calculated from 100 – (S/B) x 100, where S and B were the respective enzyme activities with and without the test sample, respectively. For compounds with better than 50% inhibitory activity at 10 μM, IC_{50} values were determined. To determine IC_{50} value, six different concentrations of each compound were used to obtain enzyme activities between 5% and 95%. All reactions were performed in triplicate. The IC_{50} values were calculated using nonlinear regression (GraphPad Prism 5 [GraphPad Software, San Diego California USA 5.00]) by plotting the residual enzyme activities against the applied inhibitor concentration. The results of this assays are shown in **Table 1**. Donepezil and tacrine were used as the reference compounds.

4.2.2 *In vitro* inhibition of *h*BuChE

The BuChE inhibitory activity of the tested drugs was determined using Ellman´s method and is expressed as IC_{50}, the i.e. concentration that reduces the cholinesterase activity by 50%. Human plasmatic BuChE (BuChE; EC 3.1.1.8), 5,5´-dithiobis(2-nitrobenzoic acid) (Ellman´s reagent, DTNB), phosphate buffer (PB, pH 7.4) and butyrylthiocholine, were purchased from Sigma-Aldrich, Prague, Czech Republic. For measuring purposes – polystyrene Nunc 96-well microplates with flat bottom shape (ThermoFisher Scientific, USA) were utilized.

All the assays were carried out in 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 7.4. Enzyme solutions were prepared at 2.0 units/mL in 2 mL aliquots. The assay medium (100 μL) consisted of 40 μL of 0.1 M phosphate buffer (pH 7.4), 20 μL of 0.01 M DTNB, 10 μL of the enzyme, and 20 μL of 0.01 M substrate (ATC iodide solution).

All compounds were tested at 10 μM concentration, which was the highest concentration that followed with respect to their solubility in the assay. Only if the tested compound reduced

BuChE activity at least 50 %, solutions with inhibitor in concentration range 10^{-3} – 10^{-9} M were prepared and IC_{50} were calculated otherwise, IC_{50} could not be determined (n.d.). Tested compounds were preincubated for 5 min. The reaction was started by immediate addition of 20 μL of the substrate. The activity was determined by measuring the increase in absorbance at 412 nm at 37 °C at 2 min intervals - using a Multi-mode microplate reader Synergy 2 (Vermont, USA). Each concentration was assayed in triplicate. Software GraphPad Prizm 5 (San Diego, USA) was used for the statistical data evaluation.

4.2.3 Kinetic characterization of BuChE inhibition

The kinetic studies were performed with compound **31**. We used Ellman's method[49], modified for 96-well microplates. The stock solution of *eq*BuChE (5U/mL) was diluted before use to a final concentration of 0.384 U/mL. The stock solution (0.02125 M) of substrate BTC was prepared in demineralized water and diluted before use. Six different concentrations of inhibitor were used to obtain enzyme activities between 30% and 80%. For each concentration of the test compound, BTC was used at concentrations of 0.3, 0.24, 0.18, 0.12, 0.06, and 0.04 mM in the wells. The kinetic study was performed according to the protocol described in the section above. 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 Cornich-Bowden plots were calculated using linear regression in GraphPad Prism 5.

4.2.4 *In vitro* BACE-1 inhibitory activity

All the compounds were tested as *h*BACE-1 inhibitors, using a PanVera's BACE-1 fluorescence resonance energy transfer (FRET)[83,84] Assay Kit, which includes purified baclovirus-expressed BACE-1 and specific peptide substrate (Rh-EVNLDAEFK-quencher), based on the „Swedish" mutant of APP. 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 optimized for the 553 nm excitation and 576 nm emission. Stock solutions of all test 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 BACE-1 substrate was mixed with 10 μL of test 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)] x 100, where S0 and S60 are fluorescence intensities of the test 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 the compounds were tested at screening concentration of 50 μM. Each compound was analyzed in triplicate. BACE-1 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 5.00]) by plotting the residual enzyme activities against the applied inhibitor concentration.

4.2.5. Inhibition of Aβ-aggregation

4.2.5.1 Thioflavin T assay

In order to investigate the inhibition of β-amyloid peptide aggregation by compounds, a thioflavin T-based fluorometric assay was performed.[85] Recombinant human HFIP-pretreated $Aβ_{1–42}$ peptide (Lot 2691412 and 2718332, 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 test compound, and 3% 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 approximately 4 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 test compounds 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 – Fi / F0) ×100%), where Fi is the increase in fluorescence of $A\beta_{1-42}$ treated with the test compound, and F0 is the increase in fluorescence of $A\beta_{1-42}$ alone.

For the IC_{50} determination, the dilutions of compounds **10** and **31** were prepared covering concentrations from 30 nM to 30 μM (final concentrations). The percentage of inhibition was calculated as described above. The IC_{50} values were calculated using nonlinear regression (GraphPad Prism 6 [GraphPad Software, San Diego California USA 5.00]) by plotting the percentage of inhibition against the applied inhibitor concentration.

4.2.5.2 Dot Blot assay

Recombinant human 1,1,1,3,3,3-hexafluoro-2-propanol-pretreated $A\beta_{1-42}$ peptide (Lot 2718332 Merck Millipore, Darmstadt, Germany) was dissolved in DMSO to give 100 μM stock solution. Prior to the incubations, the $A\beta_{1-42}$ peptide stock solution was diluted in HEPES buffered solution (150 mM HEPES, pH 7.4, 150 mM NaCl) to give 10 μM $A\beta_{1-42}$.

Then 10 μL of this $A\beta_{1-42}$ solution was mixed with 10 μL of the compound (100 μM stock in HEPES buffered solution; 10 μM final concentration), and added to the black-walled 96-well plates in quadruplicate, and diluted with HEPES buffered solution to a final volume of 100 μL. 2 % DMSO in HEPES buffered solution was used to determine the blank value. The sealed 96-well plate was incubated at 37 ºC with continuous shaking for 24 h. After the incubation period, 10 μL of each sample was transferred to a nitrocellulose membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and blocked with 5% non-fat milk in TBST (50 mM Tris, pH 7.4, 150 mM NaCl, 0.01% Tween 20) for 30 min at room temperature. The membrane was then washed with TBST, and incubated for 1 h at room temperature with the amyloid-specific rabbit antibody LOC (Merck Millipore, Billerica, MA, USA; 1:10 000) in 2% non-fat milk in TBST. After washed with TBST, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit antibody (Merck Millipore; 1:10 000) for 1 h at room temperature. The immunoreactive dots were developed with chemiluminescence detection kit SuperSignal™ West Dura Extended Duration Substrate (Thermo Fischer Scientific, Waltham, MA, USA). The dots intensities were quantified using Sygene's GeneTools Software (Sygene, Cambridge, UK). The equation $(1 - (R_i - b) / (R_0 - b)) \times 100\%$ was used to quantify the inhibition of the $A\beta_{1-42}$ aggregation, where R_i represents the response of $A\beta_{1-42}$ treated with the test compounds, R_0 the response of $A\beta_{1-42}$ alone, and b the blank value.

4.2.6. Inhibition of tau aggregation[69]

4.2.6.1 Tau full-length in vitro assays

Preparation of *tau*-441: *tau*-441 was obtained from Sigma. The protein was directly re-suspended in 100 mM sodium acetate pH 7.0 with 1mM DTT obtaining a final concentration of 50 μM.

Aggregation studies: 80 µL of *tau*-441 at 50 µM was mixed with 2.5 µL of heparin 15 kDa at 200 µM (Millipore, USA), 1 µL of compound at 1 mM in DMSO (final concentration of compound of 10 µM) or DMSO without compound (for the control) and the appropriate amount of 100 mM sodium acetate pH 7.0 with 1mM DTT, obtaining samples with 20 µM *tau*-441, 10 µM heparin. The samples were placed in a Thermomixer (Eppendorf, Germany) at 37 ºC and stirred at 1500 rpm. After 48 h the aggregation was checked by addition of 12.5 µL of 250 µM ThT (final concentration of ThT of 25 µM), detecting ThT fluorescence (λex= 445 nm; λem = 485 nm) using an Aminco Bowman Series 2 luminescence spectrophotometer (Aminco-Bowman AB2, SLM Aminco, Rochester, NY, USA).

4.2.6.2 Truncated tau in vitro assays

Preparation of truncated *tau*: *Escherichia coli* competent cells BL21 (DE3) were transformed with the pET28a vector from Novagen carrying the DNA sequence of fragment *tau*(244-372). For expression of *tau*(244-372), 2 L DYT medium were inoculated with an overnight culture of BL21(DE3) bearing the plasmid to be expressed at 37 °C. When an OD_{600} of 0.5–0.6 was reached, the bacteria were induced with 1 mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 4 h at 37 °C, then the culture were centrifuged and the cell pellet were frozen at –80 °C. *Tau*(244-372) protein expressed as His-tagged construct in *E.coli* was purified under native conditions (50 mM Tris pH 7.2, 300 mM NaCl) by affinity chromatography on Talon His-tag resin (GE). Buffer was ex-changed by gel filtration on mini PD-10 column (GE) for 100 mM sodium acetate pH 7.0 with 1 mM DTT. The protein at 50 µM was stored at 4 °C.

Aggregation studies: 80 µL of *tau*-441 at 50 µM was mixed with 2.5 µL of heparin 15kDa at 200 µM (Millipore, USA), 1 µL of compound at 1 mM in DMSO (final concentration of compound of 10 µM) or DMSO without compound (for the control) and the appropriate amount of 100 mM sodium acetate pH 7.0 with 1 mM DTT, obtaining samples with 20 µM *tau*-441, 10 µM heparin. The samples were placed in a Thermomixer (Eppendorf, Germany)

at 37 ºC and stirred at 1500 rpm. After 48 h the aggregation was checked by addition of 12.5 µL of 250 µM ThT (final concentration of ThT of 25 µM), detecting ThT fluorescence (λex= 445 nm ; λem= 485 nm) using an Aminco Bowman Series 2 luminescence spectrophotometer (Aminco-Bowman AB2, SLM Aminco, Rochester, NY, USA).

4.2.7. PAMPA-BBB assay

In order to predict passive blood-brain penetration of compounds **26**, **29**, **32**, **36**, **37** modification of the parallel artificial membrane permeation assay (PAMPA) has been used based on reported protocol[74,75]. 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=0.28cm2) to the acceptor well. The concentration of the drug in both donor and the acceptor wells was assessed after 3, 4, 5 and 6 hours 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 the equation (1)[74,86]:

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

4.3. Molecular modeling

To identify the best modifications of the compounds we prepared library containing 3-dimentional structures of ligands. For this purpose building blocks from Sigma Aldrich compound repository containing 3578 molecules, primary and secondary amines with aliphatic and aromatic substituents were connected through the nitrogen atom to 1-(benzylamino)propan-2-ol core with Maestro CombiGlide (Schrödinger).[87] Final ligands were generated and minimized by LigPrep (Schrödinger).[88] Prepared compounds were docked with GOLD 5.3 (CCDC)[89] to AChE (PDB code 1EVE), BuChE (PDB code1P0I) complex and to BACE-1 (PDB code 4D8C). Before docking all proteins were prepared using Hermes 1.7 (CCDC).[89] All histidine residues were protonated at Nε, the hydrogen atoms were added, ligand and water molecules were removed. The binding sites were defined as: all amino acid residues within radius of 10 Å from the donepezil (E20) presented in AChE, all amino acid residues within radius of 20 Å from the glycerol molecule (GOL), present in the active center of BuChE and all amino acid residues within radius of 10 Å from ligand (BXD) in the BACE-1 complex. We applied the standard settings of the genetic algorithm with population size 100, number of operations 100000.[77,90] For each ligand we received 10 conformations sorted by ChemScore (for BChE) and GoldScore (for BuChE) function value. Results of docking were visualized with PyMOL 0.99rc6 (DeLano Scientific LLC).[91]

Acknowledgments

This work was supported by the National Science Center of Poland (grants UMO-2016/21/B/NZ7/01744 and UMO-2016/21/N/NZ7/03288), the European Cooperation in Science and Technology COST Action CA15135, and the Slovenian Research Agency (research program P1-0208 and research project L1-8157).

Author Contributions

Dawid Panek (D.P.) designed the research, synthesized most of the compounds, performed the hBACE-1 inhibitory test and drafted the manuscript.

Anna Więckowska (A.W.) designed the research, supervised part of the synthesis, wrote a part of the manuscript and corrected it.
Jakub Jończyk (J.J.) designed the novel compounds, performed molecular modelling studies, wrote a part of manuscript.
Justyna Godyń (J.G.) performed the inhibition potency on the cholinesterases and the kinetic study, performed the *h*BACE-1 inhibitory test and wrote a part of manuscript.
Dr. Marek W. Bajda (M.B.) supervised molecular modelling studies and corrected the manuscript.
Tomasz Wichur (T.W.) participated in the chemical synthesis.
Anna Pasieka (A.P.) performed the *h*BACE-1 inhibitory test.
Damijan Knez (D.K.) performed the Aβ test and corrected the manuscript.
Anja Pislar (A.P.) performed the Aβ test.
Jan Korabecny (J.K.) supervised the performed PAMPA-BBB assay, wrote a part of the manuscript and corrected it.
Ondrej Soukup (O.S.) and Vendula Sepsova (V.S.) performed the PAMPA-BBB assay and inhibitory tests on *h*BuChE.
Raimon Sabaté (R.S.) performed tau tests, wrote a part of the manuscript and corrected it.
Janko Kos (J.K.) supervised the Aβ tests.
Stanislav Gobec (S.G.) supervised the Aβ tests and corrected the manuscript.
Barbara Malawska (B.M.) supervised and coordinated all studies and corrected the manuscript.
All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Supporting information

Table S1. Calculations of essential physico-chemical characteristics of described ligands.

References

(1) Klimova, B.; Maresova, P.; Kuca, K. Non-Pharmacological Approaches to the Prevention and Treatment of Alzheimer's Disease with Respect to the Rising Treatment Costs. *Curr. Alzheimer Res.* **2016**, *13* (11), 1249–1258.

(2) Marešová, P.; Mohelská, H.; Dolejš, J.; Kuča, K. Socio-Economic Aspects of Alzheimer's Disease. *Curr. Alzheimer Res.* **2015**, *12* (9), 903–911.

(3) Rizzi, L.; Rosset, I.; Roriz-Cruz, M. Global Epidemiology of Dementia: Alzheimer's and Vascular Types. *Biomed Res. Int.* **2014**, *2014*, 1–8.

(4) Kepp, K. P. Alzheimer's Disease due to Loss of Function: A New Synthesis of the Available Data. *Prog. Neurobiol.* **2016**.

(5) Bolós, M.; Perea, J. R.; Avila, J. Alzheimer's Disease as an Inflammatory Disease. *Biomol. Concepts* **2017**, *8* (1), 37–43.

(6) Guo, T.; Noble, W.; Hanger, D. P. Roles of Tau Protein in Health and Disease. *Acta Neuropathol.* **2017**, *133* (5), 665–704.

(7) Sanabria-Castro, A.; Alvarado-Echeverría, I.; Monge-Bonilla, C. Molecular Pathogenesis of Alzheimer's Disease: An Update. *Ann. Neurosci.* **2017**, *24* (1), 46–54.

(8) Davies, P.; Maloney, A. J. F. Selective Loss of Central Cholinergic Neurons in Alzheimer's Disease. *Lancet* **1976**, *308* (8000), 1403.

(9) Bowen, D. M.; Smith, C. B.; White, P.; Davison, A. N. Neurotransmitter-Related Enzymes and Indices of Hypoxia in Senile Dementia and Other Abiotrophies. *Brain* **1976**, *99* (3), 459–496.

(10) Pinto, T.; Lanctôt, K. L.; Herrmann, N. Revisiting the Cholinergic Hypothesis of Behavioral and Psychological Symptoms in Dementia of the Alzheimer ' S Type. *Ageing Res. Rev.* **2011**, *10* (4), 404–412.

(11) Contestabile, A. The History of the Cholinergic Hypothesis. *Behav. Brain Res.* **2011**, *221* (2), 334–340.

(12) Mohamed, T.; Rao, P. P. N. Alzheimer's Disease: Emerging Trends in Small Molecule Therapies. *Curr. Med. Chem.* **2011**, *18* (28), 4299–4320.

(13) Zemek, F.; Drtinova, L.; Nepovimova, E.; Sepsova, V.; Korabecny, J.; Klimes, J.; Kuca, K. Outcomes of Alzheimer's Disease Therapy with Acetylcholinesterase Inhibitors and Memantine. *Expert Opin. Drug Saf.* **2014**, *13* (6), 759–774.

(14) Casey, D. A.; Antimisiaris, D.; O'Brien, J. Drugs for Alzheimer's Disease: Are They Effective? *P T* **2010**, *35* (4), 208–211.

(15) Ibach, B.; Haen, E. Acetylcholinesterase Inhibition in Alzheimer's Disease. *Curr. Pharm. Des.* **2004**, *10* (3), 231–251.

(16) Selkoe, D. J.; Hardy, J. The Amyloid Hypothesis of Alzheimer's Disease at 25 Years. *EMBO Mol. Med.* **2016**, *8* (6), 595–608.

(17) Hardy, J.; Selkoe, D. J. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. *Science (80-.).* **2002**, *297* (5580), 353–356.

(18) Selkoe, D. J. Amyloid Protein and Alzheimer's Disease. *Sci. Am.* **1991**, *265* (5), 68–71, 74–76, 78.

(19) Barage, S. H.; Sonawane, K. D. Amyloid Cascade Hypothesis: Pathogenesis and Therapeutic Strategies in Alzheimer's Disease. *Neuropeptides* **2015**, *52*, 1–18.

(20) Mohamed, T.; Shakeri, A.; Rao, P. P. N. Amyloid Cascade in Alzheimer's Disease: Recent Advances in Medicinal Chemistry. *Eur. J. Med. Chem.* **2016**, *113*, 258–272.

(21) Multhaup, G.; Huber, O.; Buée, L.; Galas, M.-C. Amyloid Precursor Protein (APP) Metabolites APP Intracellular Fragment (AICD), Aβ42, and Tau in Nuclear Roles. *J. Biol. Chem.* **2015**, *290* (39), 23515–23522.

(22) Cacabelos, R.; Torrellas, C.; Carrera, I.; Cacabelos, P.; Corzo, L.; Fernández-Novoa, L.; Tellado, I.; Carril, J. C.; Aliev, G. Novel Therapeutic Strategies for Dementia. *CNS Neurol. Disord. Drug Targets* **2016**, *15* (2), 141–241.

(23) Bonham, L. W.; Desikan, R. S.; Yokoyama, J. S.; Alzheimer's Disease Neuroimaging Initiative. The Relationship between Complement Factor C3, APOE ε4, Amyloid and Tau in Alzheimer's Disease. *Acta Neuropathol. Commun.* **2016**, *4* (1), 65.

(24) Bulic, B.; Pickhardt, M.; Mandelkow, E. Progress and Developments in Tau Aggregation Inhibitors for Alzheimer Disease. *J. Med. Chem.* **2013**, *56* (11), 4135–4155.

(25) Pérez-Areales, F. J.; Di Pietro, O.; Espargaró, A.; Vallverdú-Queralt, A.; Galdeano, C.; Ragusa, I. M.; Viayna, E.; Guillou, C.; Clos, M. V.; Pérez, B.; Sabaté, R.; Lamuela-Raventós, R. M.; Luque, F. J.; Muñoz-Torrero, D. Shogaol-Huprine Hybrids: Dual Antioxidant and Anticholinesterase Agents with β-Amyloid and Tau Anti-Aggregating Properties. *Bioorg. Med. Chem.* **2014**, *22* (19), 5298–5307.

(26) Viayna, E.; Sola, I.; Bartolini, M.; De Simone, A.; Tapia-Rojas, C.; Serrano, F. G.; Sabaté, R.; Juárez-Jiménez, J.; Pérez, B.; Luque, F. J.; Andrisano, V.; Clos, M. V.; Inestrosa, N. C.; Muñoz-Torrero, D. Synthesis and Multitarget Biological Profiling of a Novel Family of Rhein Derivatives as Disease-Modifying Anti-Alzheimer Agents. *J. Med. Chem.* **2014**, *57* (6), 2549–2567.

(27) Bloom, G. S. Amyloid-β and Tau. *JAMA Neurol.* **2014**, *71* (4), 505.

(28) Giacobini, E.; Gold, G. Alzheimer Disease Therapy—moving from Amyloid-β to Tau. *Nat. Rev. Neurol.* **2013**, *9* (12), 677–686.

(29) Tatarnikova, O. G.; Orlov, M. A.; Bobkova, N. V. Beta-Amyloid and Tau-Protein: Structure, Interaction, and Prion-Like Properties. *Biochem.* **2015**, *80* (13), 1800–1819.

(30) Guzior, N.; Wieckowska, A.; Panek, D.; Malawska, B. Recent Development of Multifunctional Agents as Potential Drug Candidates for the Treatment of Alzheimer's Disease. *Curr. Med. Chem.* **2015**, *22* (3), 373–404.

(31) Viayna, E.; Sabate, R.; Muñoz-Torrero, D. Dual Inhibitors of β-Amyloid Aggregation and Acetylcholinesterase as Multi-Target Anti-Alzheimer Drug Candidates. *Curr. Top. Med. Chem.* **2013**, *13* (15), 1820–1842.

(32) Ismaili, L.; Refouvelet, B.; Benchekroun, M.; Brogi, S.; Brindisi, M.; Gemma, S.; Campiani, G.; Filipic, S.; Agbaba, D.; Esteban, G.; Unzeta, M.; Nikolic, K.; Butini, S.; Marco-Contelles, J. Multitarget Compounds Bearing Tacrine- and Donepezil-like Structural and Functional Motifs for the Potential Treatment of Alzheimer's Disease. *Prog. Neurobiol.* **2017**, *151*, 4–34.

(33) Panek, D.; Więckowska, A.; Wichur, T.; Bajda, M.; Godyń, J.; Jończyk, J.; Mika, K.; Janockova, J.; Soukup, O.; Knez, D.; Korabecny, J.; Gobec, S.; Malawska, B. Design, Synthesis and Biological Evaluation of New Phthalimide and Saccharin Derivatives with Alicyclic Amines Targeting Cholinesterases, Beta-Secretase and Amyloid Beta Aggregation. *Eur. J. Med. Chem.* **2017**, *125*, 676–695.

(34) Rueeger, H.; Lueoend, R.; Rogel, O.; Rondeau, J.-M.; Möbitz, H.; Machauer, R.; Jacobson, L.; Staufenbiel, M.; Desrayaud, S.; Neumann, U. Discovery of Cyclic Sulfone Hydroxyethylamines as Potent and Selective β-Site APP-Cleaving Enzyme 1 (BACE1) Inhibitors: Structure-Based Design and in Vivo Reduction of Amyloid β-Peptides. *J. Med. Chem.* **2012**, *55* (7), 3364–3386.

(35) Kryger, G.; Silman, I.; Sussman, J. L. Structure of Acetylcholinesterase Complexed with E2020 (Aricept): Implications for the Design of New Anti-Alzheimer Drugs. *Structure* **1999**, *7* (3), 297–307.

(36) Rueeger, H.; Rondeau, J.-M.; McCarthy, C.; Möbitz, H.; Tintelnot-Blomley, M.; Neumann, U.; Desrayaud, S. Structure Based Design, Synthesis and SAR of Cyclic Hydroxyethylamine (HEA) BACE-1 Inhibitors. *Bioorg. Med. Chem. Lett.* **2011**, *21* (7), 1942–1947.

(37) Ghosh, A. K.; Kumaragurubaran, N.; Hong, L.; Kulkarni, S.; Xu, X.; Miller, H. B.; Reddy, D. S.; Weerasena, V.; Turner, R.; Chang, W.; Koelsch, G.; Tang, J. Potent

Memapsin 2 (Beta-Secretase) Inhibitors: Design, Synthesis, Protein-Ligand X-Ray Structure, and in Vivo Evaluation. *Bioorg. Med. Chem. Lett.* **2008**, *18* (3), 1031–1036.

(38) Chang, W.-P.; Huang, X.; Downs, D.; Cirrito, J. R.; Koelsch, G.; Holtzman, D. M.; Ghosh, A. K.; Tang, J. Beta-Secretase Inhibitor GRL-8234 Rescues Age-Related Cognitive Decline in APP Transgenic Mice. *FASEB J.* **2011**, *25* (2), 775–784.

(39) Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. Structure of the Protease Domain of Memapsin 2 (Beta-Secretase) Complexed with Inhibitor. *Science* **2000**, *290* (5489), 150–153.

(40) Guzior, N.; Bajda, M.; Skrok, M.; Kurpiewska, K.; Lewiński, K.; Brus, B.; Pišlar, A.; Kos, J.; Gobec, S.; Malawska, B. Development of Multifunctional, Heterodimeric Isoindoline-1,3-Dione Derivatives as Cholinesterase and β-Amyloid Aggregation Inhibitors with Neuroprotective Properties. *Eur. J. Med. Chem.* **2015**, *92*, 738–749.

(41) Szałaj, N.; Bajda, M.; Dudek, K.; Brus, B.; Gobec, S.; Malawska, B. Multiple Ligands Targeting Cholinesterases and β-Amyloid: Synthesis, Biological Evaluation of Heterodimeric Compounds with Benzylamine Pharmacophore. *Arch. Pharm. (Weinheim).* **2015**, *348* (8), 556–563.

(42) Hebda, M.; Bajda, M.; Więckowska, A.; Szałaj, N.; Pasieka, A.; Panek, D.; Godyń, J.; Wichur, T.; Knez, D.; Gobec, S.; Malawska, B. Synthesis, Molecular Modelling and Biological Evaluation of Novel Heterodimeric, Multiple Ligands Targeting Cholinesterases and Amyloid Beta. *Molecules* **2016**, *21* (4), 410.

(43) Guzior, N.; Bajda, M.; Rakoczy, J.; Brus, B.; Gobec, S.; Malawska, B. Isoindoline-1,3-Dione Derivatives Targeting Cholinesterases: Design, Synthesis and Biological Evaluation of Potential Anti-Alzheimer's Agents. *Bioorg. Med. Chem.* **2015**, *23* (7), 1629–1637.

(44) Bajda, M.; Filipek, S. Computational Approach for the Assessment of Inhibitory Potency against Beta-Amyloid Aggregation. *Bioorg. Med. Chem. Lett.* **2017**, *27* (2), 212–216.

(45) Pérez-Areales, F. J.; Di Pietro, O.; Espargaró, A.; Vallverdú-Queralt, A.; Galdeano, C.; Ragusa, I. M.; Viayna, E.; Guillou, C.; Clos, M. V.; Pérez, B.; Sabaté, R.; Lamuela-Raventós, R. M.; Luque, F. J.; Muñoz-Torrero, D. Shogaol-Huprine Hybrids: Dual Antioxidant and Anticholinesterase Agents with β-Amyloid and Tau Anti-Aggregating Properties. *Bioorg. Med. Chem.* **2014**, *22* (19), 5298–5307.

(46) Fuse, S.; Matsumura, K.; Fujita, Y.; Sugimoto, H.; Takahashi, T. Development of Dual Targeting Inhibitors against Aggregations of Amyloid-β and Tau Protein. *Eur. J. Med. Chem.* **2014**, *85*, 228–234.

(47) ChemAxon. MarvinSketch (Version 6.2.2). 2014.

(48) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Deliv. Rev.* **1997**, *23* (1–3), 3–25.

(49) Ellman, G. L.; Courtney, K. D.; Andres, V.; Feather-Stone, R. M. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95.

(50) Prati, F.; Bergamini, C.; Fato, R.; Soukup, O.; Korabecny, J.; Andrisano, V.; Bartolini, M.; Bolognesi, M. L. Novel 8-Hydroxyquinoline Derivatives as Multitarget Compounds for the Treatment of Alzheimer′s Disease. *ChemMedChem* **2016**, *11* (12), 1284–1295.

(51) Guzior, N.; Bajda, M.; Skrok, M.; Kurpiewska, K.; Lewiński, K.; Brus, B.; Pišlar, A.; Kos, J.; Gobec, S.; Malawska, B. Development of Multifunctional, Heterodimeric

Isoindoline-1,3-Dione Derivatives as Cholinesterase and β-Amyloid Aggregation Inhibitors with Neuroprotective Properties. *Eur. J. Med. Chem.* **2015**, *92*, 738–749.

(52) Greig, N. H.; Utsuki, T.; Ingram, D. K.; Wang, Y.; Pepeu, G.; Scali, C.; Yu, Q.-S.; Mamczarz, J.; Holloway, H. W.; Giordano, T.; Chen, D.; Furukawa, K.; Sambamurti, K.; Brossi, A.; Lahiri, D. K. Selective Butyrylcholinesterase Inhibition Elevates Brain Acetylcholine, Augments Learning and Lowers Alzheimer Beta-Amyloid Peptide in Rodent. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (47), 17213–17218.

(53) DeBay, D. R.; Reid, G. A.; Macdonald, I. R.; Mawko, G.; Burrell, S.; Martin, E.; Bowen, C. V.; Darvesh, S. Butyrylcholinesterase-Knockout Reduces Fibrillar β-Amyloid and Conserves 18 FDG Retention in 5XFAD Mouse Model of Alzheimer's Disease. *Brain Res.* **2017**, *1671*, 102–110.

(54) Darvesh, S.; Reid, G. A. Reduced Fibrillar β-Amyloid in Subcortical Structures in a Butyrylcholinesterase-Knockout Alzheimer Disease Mouse Model. *Chem. Biol. Interact.* **2016**, *259* (Pt B), 307–312.

(55) Reid, G. A.; Darvesh, S. Butyrylcholinesterase-Knockout Reduces Brain Deposition of Fibrillar β-Amyloid in an Alzheimer Mouse Model. *Neuroscience* **2015**, *298*, 424–435.

(56) Gabriel, A. J.; Almeida, M. R.; Ribeiro, M. H.; Durães, J.; Tábuas-Pereira, M.; Pinheiro, A. C.; Pascoal, R.; Santana, I.; Baldeiras, I. Association between Butyrylcholinesterase and Cerebrospinal Fluid Biomarkers in Alzheimer's Disease Patients. *Neurosci. Lett.* **2017**, *641*, 101–106.

(57) Nordberg, A.; Ballard, C.; Bullock, R.; Darreh-Shori, T.; Somogyi, M. A Review of Butyrylcholinesterase as a Therapeutic Target in the Treatment of Alzheimer's Disease. *Prim. care companion CNS Disord.* **2013**, *15* (2).

(58) Kamal, M. A.; Qu, X.; Yu, Q.-S.; Tweedie, D.; Holloway, H. W.; Li, Y.; Tan, Y.;

Greig, N. H. Tetrahydrofurobenzofuran Cymserine, a Potent Butyrylcholinesterase Inhibitor and Experimental Alzheimer Drug Candidate, Enzyme Kinetic Analysis. *J. Neural Transm.* **2008**, *115* (6), 889–898.

(59) Carolan, C. G.; Dillon, G. P.; Khan, D.; Ryder, S. A.; Gaynor, J. M.; Reidy, S.; Marquez, J. F.; Jones, M.; Holland, V.; Gilmer, J. F. Isosorbide-2-Benzyl Carbamate-5-Salicylate, a Peripheral Anionic Site Binding Subnanomolar Selective Butyrylcholinesterase Inhibitor. *J. Med. Chem.* **2010**, *53* (3), 1190–1199.

(60) Copeland, R. A. Evaluation of Enzyme Inhibitors in Drug Discovery. A Guide for Medicinal Chemists and Pharmacologists. *Methods Biochem. Anal.* **2005**, *46*, 1–265.

(61) Singh, J.; Petter, R. C.; Baillie, T. A.; Whitty, A. The Resurgence of Covalent Drugs. *Nat. Rev. Drug Discov.* **2011**, *10* (4), 307–317.

(62) Brus, B.; Košak, U.; Turk, S.; Pišlar, A.; Coquelle, N.; Kos, J.; Stojan, J.; Colletier, J.-P.; Gobec, S. Discovery, Biological Evaluation, and Crystal Structure of a Novel Nanomolar Selective Butyrylcholinesterase Inhibitor. *J. Med. Chem.* **2014**, *57* (19), 8167–8179.

(63) Košak, U.; Brus, B.; Knez, D.; Šink, R.; Žakelj, S.; Trontelj, J.; Pišlar, A.; Šlenc, J.; Gobec, M.; Živin, M.; Tratnjek, L.; Perše, M.; Sałat, K.; Podkowa, A.; Filipek, B.; Nachon, F.; Brazzolotto, X.; Więckowska, A.; Malawska, B.; Stojan, J.; Raščan, I. M.; Kos, J.; Coquelle, N.; Colletier, J.-P.; Gobec, S. Development of an in-Vivo Active Reversible Butyrylcholinesterase Inhibitor. *Sci. Rep.* **2016**, *6* (1), 39495.

(64) Darvesh, S.; Darvesh, K. V; McDonald, R. S.; Mataija, D.; Walsh, R.; Mothana, S.; Lockridge, O.; Martin, E. Carbamates with Differential Mechanism of Inhibition toward Acetylcholinesterase and Butyrylcholinesterase. *J. Med. Chem.* **2008**, *51* (14), 4200–4212.

(65) Zha, X.; Lamba, D.; Zhang, L.; Lou, Y.; Xu, C.; Kang, D.; Chen, L.; Xu, Y.; Zhang, L.; De Simone, A.; Samez, S.; Pesaresi, A.; Stojan, J.; Lopez, M. G.; Egea, J.; Andrisano, V.; Bartolini, M. Novel Tacrine-Benzofuran Hybrids as Potent Multitarget-Directed Ligands for the Treatment of Alzheimer's Disease: Design, Synthesis, Biological Evaluation, and X-Ray Crystallography. *J. Med. Chem.* **2016**, *59* (1), 114–131.

(66) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. Beta-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. *Science* **1999**, *286* (5440), 735–741.

(67) Stachel, S. J.; Coburn, C. A.; Steele, T. G.; Jones, K. G.; Loutzenhiser, E. F.; Gregro, A. R.; Rajapakse, H. A.; Lai, M.-T.; Crouthamel, M.-C.; Xu, M.; Tugusheva, K.; Lineberger, J. E.; Pietrak, B. L.; Espeseth, A. S.; Shi, X.-P.; Chen-Dodson, E.; Holloway, M. K.; Munshi, S.; Simon, A. J.; Kuo, L.; Vacca, J. P. Structure-Based Design of Potent and Selective Cell-Permeable Inhibitors of Human Beta-Secretase (BACE-1). *J. Med. Chem.* **2004**, *47* (26), 6447–6450.

(68) LeVine, H. Thioflavine T Interaction with Synthetic Alzheimer's Disease Beta-Amyloid Peptides: Detection of Amyloid Aggregation in Solution. *Protein Sci.* **1993**, *2* (3), 404–410.

(69) Okuyama, K.; Nishiura, C.; Mizushima, F.; Minoura, K.; Sumida, M.; Taniguchi, T.; Tomoo, K.; Ishida, T. Linkage-Dependent Contribution of Repeat Peptides to Self-Aggregation of Three- or Four-Repeat Microtubule-Binding Domains in Tau Protein. *FEBS J.* **2008**, *275* (7), 1529–1539.

(70) Pérez-Areales, F. J.; Betari, N.; Viayna, A.; Pont, C.; Espargaró, A.; Bartolini, M.; De Simone, A.; Rinaldi Alvarenga, J. F.; Pérez, B.; Sabate, R.; Lamuela-Raventós, R. M.; Andrisano, V.; Luque, F. J.; Muñoz-Torrero, D. Design, Synthesis and Multitarget Biological Profiling of Second-Generation Anti-Alzheimer Rhein–huprine Hybrids. *Future Med. Chem.* **2017**, *9* (10), 965–981.

(71) Sola, I.; Aso, E.; Frattini, D.; López-González, I.; Espargaró, A.; Sabaté, R.; Di Pietro, O.; Luque, F. J.; Clos, M. V.; Ferrer, I.; Muñoz-Torrero, D. Novel Levetiracetam Derivatives That Are Effective against the Alzheimer-like Phenotype in Mice: Synthesis, in Vitro, Ex Vivo, and in Vivo Efficacy Studies. *J. Med. Chem.* **2015**, *58* (15), 6018–6032.

(72) Bulic, B.; Pickhardt, M.; Schmidt, B.; Mandelkow, E.-M.; Waldmann, H.; Mandelkow, E. Development of Tau Aggregation Inhibitors for Alzheimer's Disease. *Angew. Chemie Int. Ed.* **2009**, *48* (10), 1740–1752.

(73) Baell, J. B.; Holloway, G. A. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. *J. Med. Chem.* **2010**, *53* (7), 2719–2740.

(74) Wohnsland, F.; Faller, B. High-Throughput Permeability pH Profile and High-Throughput Alkane/water Log P with Artificial Membranes. *J. Med. Chem.* **2001**, *44* (6), 923–930.

(75) Di, L.; Kerns, E. H.; Fan, K.; McConnell, O. J.; Carter, G. T. High Throughput Artificial Membrane Permeability Assay for Blood–brain Barrier. *Eur. J. Med. Chem.* **2003**, *38* (3), 223–232.

(76) Rueeger, H.; Lueoend, R.; Rogel, O.; Rondeau, J.-M.; Möbitz, H.; Machauer, R.; Jacobson, L.; Staufenbiel, M.; Desrayaud, S.; Neumann, U. Discovery of Cyclic

Sulfone Hydroxyethylamines as Potent and Selective β-Site APP-Cleaving Enzyme 1 (BACE1) Inhibitors: Structure-Based Design and in Vivo Reduction of Amyloid β-Peptides. *J. Med. Chem.* **2012**, *55* (7), 3364–3386.

(77) Bajda, M.; Jończyk, J.; Malawska, B.; Filipek, S. Application of Computational Methods for the Design of BACE-1 Inhibitors: Validation of in Silico Modelling. *Int. J. Mol. Sci.* **2014**, *15* (3), 5128–5139.

(78) Bajda, M.; Więckowska, A.; Hebda, M.; Guzior, N.; Sotriffer, C. A.; Malawska, B. Structure-Based Search for New Inhibitors of Cholinesterases. *Int. J. Mol. Sci.* **2013**, *14* (3), 5608–5632.

(79) Panek, D.; Więckowska, A.; Wichur, T.; Bajda, M.; Godyń, J.; Jończyk, J.; Mika, K.; Janockova, J.; Soukup, O.; Knez, D.; Korabecny, J.; Gobec, S.; Malawska, B. Design, Synthesis and Biological Evaluation of New Phthalimide and Saccharin Derivatives with Alicyclic Amines Targeting Cholinesterases, Beta-Secretase and Amyloid Beta Aggregation. *Eur. J. Med. Chem.* **2017**, *125*, 676–695.

(80) Takeda, Y.; Okumura, S.; Tone, S.; Sasaki, I.; Minakata, S. Cyclizative Atmospheric CO 2 Fixation by Unsaturated Amines with t‑BuOI Leading to Cyclic Carbamates. *Org. Lett.* **2012**, *14* (18), 4874–4877.

(81) Bertini, S.; Ghilardi, E.; Asso, V.; Granchi, C.; Minutolo, F.; Pineschi, M.; Di Bussolo, V.; Bortolato, A.; Moro, S.; Saba, A.; Macchia, M. BACE1 Inhibitory Activities of Enantiomerically Pure, Variously Substituted N-(3-(4-Benzhydrylpiperazin-1-Yl)-2-Hydroxypropyl) Arylsulfonamides. *Bioorg. Med. Chem.* **2010**, *18* (22), 7991–7996.

(82) Albert, B. J.; Koide, K. Zirconium-Promoted Epoxide Rearrangement-Alkynylation Sequence. *J. Org. Chem.* **2008**, *73* (3), 1093–1098.

(83) Kennedy, M. E.; Wang, W.; Song, L.; Lee, J.; Zhang, L.; Wong, G.; Wang, L.; Parker,

E. Measuring Human β-Secretase (BACE1) Activity Using Homogeneous Time-Resolved Fluorescence. *Anal. Biochem.* **2003**, *319* (1), 49–55.

(84) Mancini, F.; De Simone, A.; Andrisano, V. Beta-Secretase as a Target for Alzheimer's Disease Drug Discovery: An Overview of in Vitro Methods for Characterization of Inhibitors. *Anal. Bioanal. Chem.* **2011**, *400* (7), 1979–1996.

(85) LeVine, H. Thioflavine T Interaction with Synthetic Alzheimer's Disease Beta-Amyloid Peptides: Detection of Amyloid Aggregation in Solution. *Protein Sci.* **1993**, *2* (3), 404–410.

(86) Sugano, K.; Hamada, H.; Machida, M.; Ushio, H. High Throughput Prediction of Oral Absorption: Improvement of the Composition of the Lipid Solution Used in Parallel Artificial Membrane Permeation Assay. *J. Biomol. Screen.* **2001**, *6* (3), 189–196.

(87) Schrödinger Release. CombiGlide. Schrödinger, LLC: New York 2016.

(88) Schrödinger Release. LigPrep. Schrödinger, LLC: New York 2016.

(89) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and Validation of a Genetic Algorithm for Flexible Docking 1 1Edited by F. E. Cohen. *J. Mol. Biol.* **1997**, *267* (3), 727–748.

(90) Bajda, M.; Więckowska, A.; Hebda, M.; Guzior, N.; Sotriffer, C. A.; Malawska, B. Structure-Based Search for New Inhibitors of Cholinesterases. *Int. J. Mol. Sci.* **2013**, *14* (3), 5608–5632.

(91) PyMOL 0.99rc6. DeLano Scientific LLC. Palo Alto 2006.

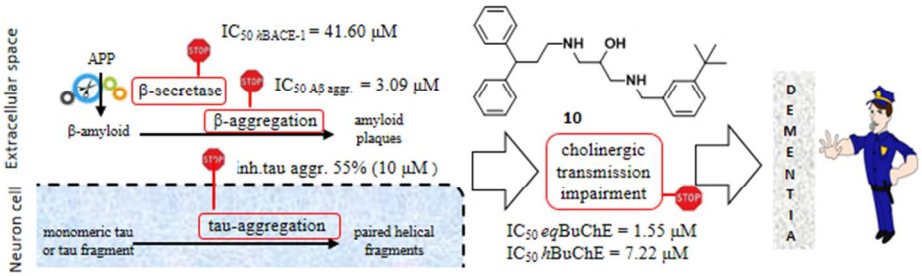
Abbreviations (nonstandard)

MeOH – methanol

MeCN – acetonitrile

NFT - neurofibrillary tangles

MTDL – multi-target-directed-ligand



189x55mm (96 x 96 DPI)