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Synthesis and neuroprotective activity of 3-aryl-3-azetidinyl acetic acid methyl ester derivatives

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Abstract

A library of 3-aryl-3-azetidinyl acetic acid methyl ester derivatives was prepared from *N*-Boc-3-azetidinone employing the Horner-Wadsworth-Emmons reaction, rhodium(I)-catalyzed conjugate addition of arylboronic acids, and subsequent elaborations to obtain *N*-unprotected hydrochlorides, *N*-alkylated and *N*-acylated azetidine derivatives. The compounds were evaluated for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity, revealing several derivatives to possess AChE inhibition comparable to that of the AChE inhibitor rivastigmine. The binding mode of the AChE inhibitor donepezil and selected active compounds **26** and **27** within the active site of AChE was studied using molecular docking. Furthermore, the neuroprotective activity of the prepared compounds was evaluated in models associated with Parkinson's disease (salsolinol-induced) and aspects of Alzheimer's disease (glutamate-induced oxidative damage). Compound **28** showed the highest neuroprotective effect in both salsolinol- and glutamate-induced neurodegeneration models, and its protective effect in the glutamate model was revealed to be driven by a reduction in oxidative stress and caspase-3/7 activity.

KEYWORDS
acetylcholinesterase, Alzheimer disease, azetidine, neuroprotection, Parkinson disease

Urtė Šachlevičiūtė and Gabriel Gonzalez contributed equally to this study.

1 | INTRODUCTION

Alzheimer's (AD) and Parkinson's (PD) disorders are the leading neurodegenerative diseases affecting mostly elderly patients. AD is characterized by progressive cognitive decline, memory loss, personality impairment, and other symptoms resulting in patient death.[1] In the early stages of AD, patients suffer from the loss of neurons, that precedes the beta-amyloid accumulation or the formation of neurofibrillary tangles composed of tau proteins.[2,3] The loss of neurons is thought to correlate with the scale of clinical signs of AD patients.[4] PD, on the other hand, is primarily a motor-related neurodegeneration manifested by symptoms such as bradykinesia, tremor, postural instability, and frequent fall accidents.[5] Moreover, PD is also associated with cognitive impairment especially in case of Parkinson's disease dementia (PDD) or Lewy body dementia (LBD) forms.

Both disorders, AD and PD, share similar characteristics such as elevated oxidative stress and mitochondrial dysfunction leading to apoptosis and consequently to neuronal death, either cholinergic or nigral, respectively.[6,7] Moreover, iron homeostasis impairment is another factor contributing to ferroptosis or other form of cell death. Ongoing ferroptosis was reported in AD patients and cross-link between ferroptosis and oxidative stress was observed in Xc-antiporter inhibition. Xc-antiporter is responsible for cysteine supplies for cells, which is further utilized for glutathione biosynthesis. In connection to AD, Xc-antiporter was upregulated in AD patients as response to its inhibition.[8]

Current AD and PD treatment is limited to symptomatic therapy, which does not affect the ongoing degeneration processes. AChE inhibitors such as galantamine, donepezil, and rivastigmine are among the few therapeutics, namely memantine (NMDA antagonist) and aducanumab (antibody for clearance of amyloid beta-proteins), used in AD[9,10] and PD to slow down the cognitive decline and other symptoms.[11] More importantly, since the current therapeutics do not affect neuropathological processes, the field of disease-modifying therapy, that is, neuroprotection is worthwhile for further investigations.[12,13]

Nitrogen heterocycles can be found in many biologically active molecules and occupy a prominent place in drug design.[14] Azetidines have drawn a substantial attention due to their occurrence in natural compounds[15–20] and their wide spectrum of biological activities.[21] Azetidine ring has been employed in the preparation of rigidified glutamic acid analogs,[22–25] which could be used to search for new ligands for ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). Azetidines have also been demonstrated to be useful for the development of CNS-focused lead-like libraries[26] and have been incorporated in a number of CNS affecting compounds. Over the past decade, several azetidine structural unit-possessing sphingosine 1-phosphate receptor-1 (S1P1) agonists have been identified. For instance, AMG 369 delays the onset and reduces the severity of experimental autoimmune encephalomyelitis in rats,[27] while siponimod (Mayzent®) was recently approved for the treatment of secondary progressive multiple sclerosis (Figure 1).[28,29] KHG26792 was reported to significantly protect against MPP^+-induced neurotoxicity in SH-SY5Y cells,[30] possess neuroprotective effect on brain ischemia/reperfusion injury,[31] act as P2X7 receptor antagonist[32] and protect against amyloid-β-induced toxicity.[33] Thus, KHG26792 could have a potential use as a therapeutic agent

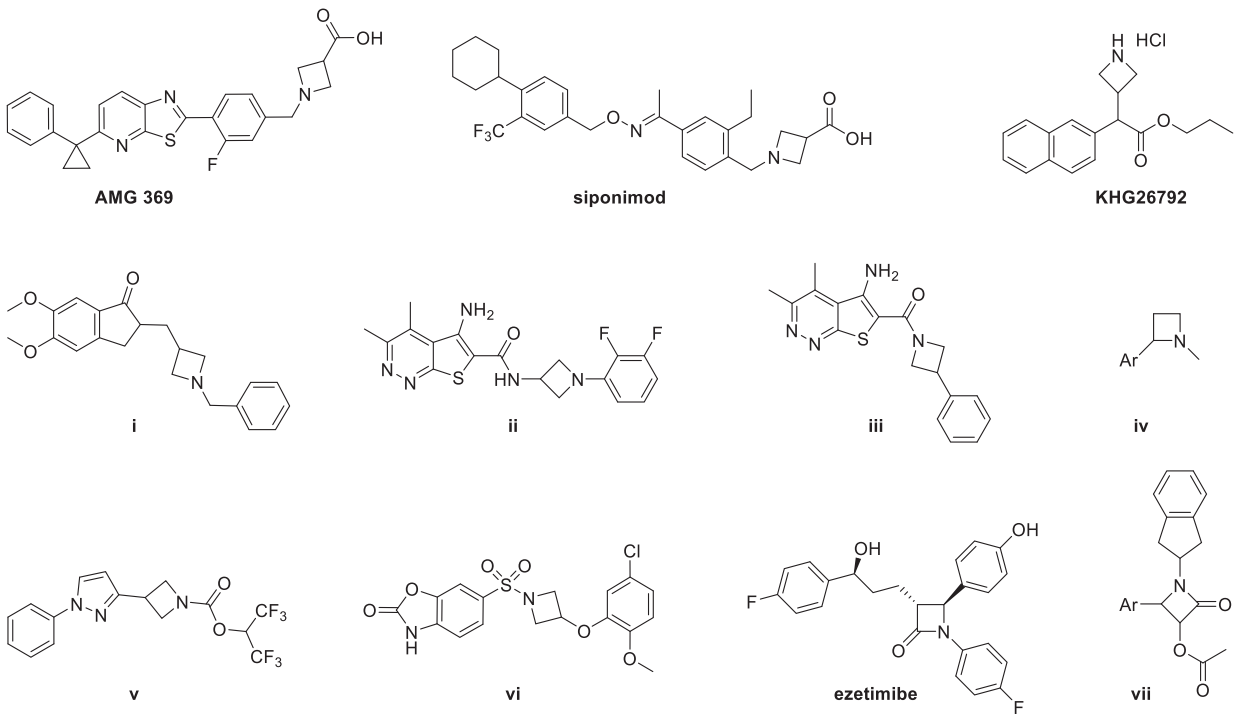


FIGURE 1 Azetidine derivatives with CNS activity.

in CNS diseases related to activated microglia, including various neurodegenerative disorders. Donepezil-inspired compound **i** was reported to possess AChE activity at nanomolar concentrations and no observable cytotoxicity.[34] Amides **ii** and **iii** were revealed as positive allosteric modulators (PAMs) of the muscarinic acetylcholine receptor (M_4).[35] New 2-arylazetidine derivatives **iv** were suggested as potential ligands for nicotinic acetylcholine receptors.[36] Azetidine carbamate **v** acts as an inhibitor of monoacylglycerol lipase (MAGL), which is the main enzyme responsible for the degradation of 2-arachidonoylglycerol (2-AG) to arachidonic acid (AA), a precursor to the pro-inflammatory eicosanoids.[37] Sulfonamide **vi** was reported as a potentiator of glycine receptor (GlyR) which plays an important role in CNS inhibition.[38] Hypocholesterolemic agent ezetimibe has been reported to attenuate oxidative stress and neuroinflammation.[39] Moreover, several β-lactam derivatives **vii** showed inhibitory activity against human carbonic anhydrase I, II, and AChE.[40]

In our recent work, we have been pursuing the construction of amino acid-like functionalized heterocyclic molecules possessing azetidine core.[41–44] In this study, we prepared a library of 3-aryl-3-azetidinyl acetic acid methyl ester derivatives, assessed their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity, and determined their neuroprotective effect in salsolinol (SAL)-induced model of PD and glutamate (Glu)-induced model of oxidative damage on neuron-like SH-SY5Y cells. Consequently, cholinesterase inhibition, alongside neuroprotective effect in Glu-induced Xc-antiporter inhibition models could offer a new strategy for disease-modifying AD and PD therapy.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

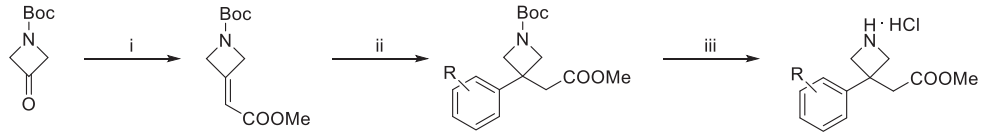
2.1.1 | Synthesis

The synthesis was started with the preparation of the azetidine ring bearing α,β-unsaturated ester **2**, which was accomplished by the condensation of *N*-Boc-3-azetidinone (**1**) with trimethyl phosphonacetate under the Horner-Wadsworth-Emmons reaction conditions (Scheme 1).[43,45] Subsequent treatment of **2** with arylboronic acids in the presence of rhodium(I)-catalyst[46] proceeded smoothly and provided the desired azetidines **3**–**9** as products of conjugate addition in 56%–71% yields. The efficiency of rhodium-catalyzed conjugate addition of arylboron species is strongly dependent on the competing side reaction, that is, protodeboronation.[46–48] To minimize arylboronic acid decomposition and improve yields, as an alternative, aqueous media was replaced with isopropanol, according to the methodology that has been developed by Parker et al.,[47] unfortunately, with no improvement. No significant changes were observed either by performing reactions for a shorter time under microwave-assisted conditions at 100°C for 5 min.[46] The addition of organoboronic acids in portions throughout the course of the reaction did not provide any advantages and the use of an excess (2–3 equivalents) of arylboronic acid turned out to be necessary.

Further deprotection of **3**–**9** with 4 N HCl in dioxane[49] furnished a set of *N*-deprotected analogs **10**–**16** in 79%–90% yields. Subsequently, based on preliminary screening (Supporting Information: Figure S1), *para*-trifluoromethyl derivative **15**, in which 4-trifluoromethylphenyl substituent resides in 3-position of the heterocyclic ring system, was selected for further elaborations.

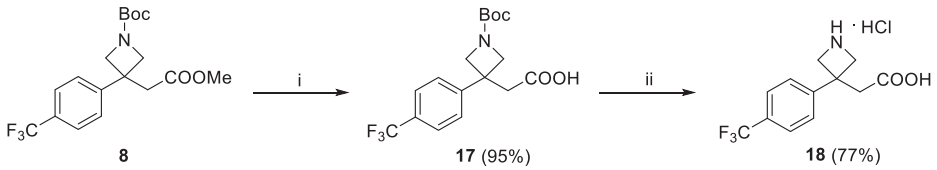
Various transformation reactions of compound **8** were performed to obtain amino acid **18**, *N*-alkylated **19**–**22**, and *N*-acylated **23**–**28** azetidine derivatives. Saponification of compound **8** by standard hydrolysis conditions[50] followed by the *N*-deprotection with 4 N HCl in dioxane[49] provided amino acid hydrochloride salt **18** (Scheme 2).

Subsequently, *N*-derivatisation reactions were investigated. After deprotection of the N-Boc group from compound **8** with TFA,[51] alkylation was performed with various iodoalkanes in the presence of K_2CO_3 in MeCN[52] to give compounds **20**–**22** in 35%–40% yields (Scheme 3). Unfortunately, amine methylation with iodomethane, which has previously been reported to suffer from ring cleavage[53] or dimethylazetidin-1-ium salt formation,[54] was not successful under these conditions. To our satisfaction, reductive amination of *N*-unprotected compound **8** with 37% aqueous formaldehyde by either using $NaBH_4$[55] or zinc dust[56] as reductive reagent led to the formation of compound **19** in 42% and 33% yield, respectively.

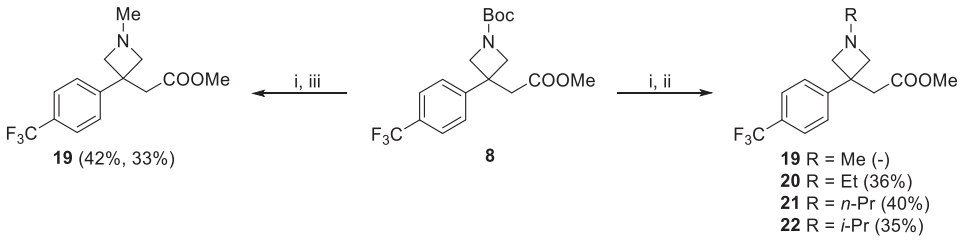


1
2 (78%)
3 (R = H, 71%)
4 (R = 4-OH, 63%)
5 (R = 4-OMe, 68%)
6 (R = 4-Cl, 69%)
7 (R = 4-F, 69%)
8 (R = 4-CF_3, 66%)
9 (R = 3-CF_3, 56%)
10 (R = H, 90%)
11 (R = 4-OH, 86%)
12 (R = 4-OMe, 79%)
13 (R = 4-Cl, 85%)
14 (R = 4-F, 87%)
15 (R = 4-CF_3, 88%)
16 (R = 3-CF_3, 90%)

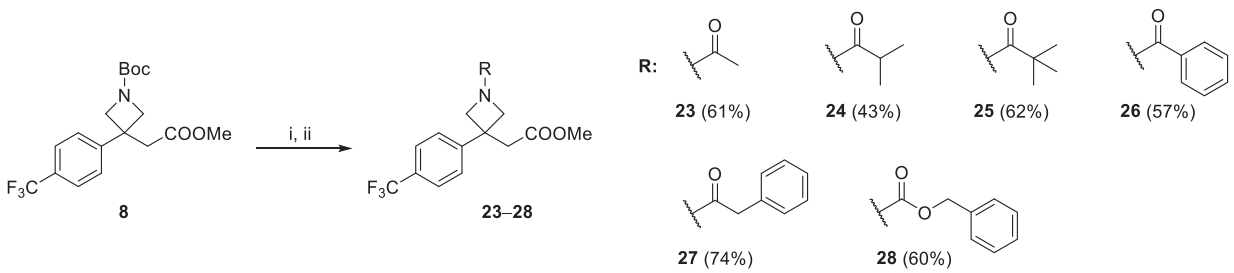
SCHEME 1 Synthesis of compounds **3**–**9** and **10**–**16**. Reagents and conditions: (i) methyl 2-(dimethoxyphosphoryl)acetate, NaH, THF, 0°C to rt; (ii) arylboronic acid, $[Rh(cod)Cl]_2$, KOH, 1,4-dioxane, THF, rt; (iii) 4 N HCl in dioxane, 1,4-dioxane, rt.



SCHEME 2 Synthesis of compound **18**. Reagents and conditions: (i) 2 N NaOH, MeOH, 0°C to rt; (ii) 4 N HCl in dioxane, 1,4-dioxane, rt.



SCHEME 3 Synthesis of compounds **19**–**22**. Reagents and conditions: (i) TFA, DCM, 0°C to rt; (ii) K_2CO_3, iodoalkane, MeCN, 0°C to rt; (iii) Method I: TEA, 37% aqueous formaldehyde, AcOH, $NaBH_4$, MeOH, rt; Method II: AcOH, 37% aqueous formaldehyde, zinc dust, 1,4-dioxane, rt.



SCHEME 4 Synthesis of compounds **23**–**28**. Reagents and conditions: (i) TFA, DCM, 0°C to rt; (ii) TEA, acyl chloride, 0°C to rt.

Finally, *N*-acylated derivatives **23**–**28** were successfully obtained, adopting standard acylation reaction conditions using various acyl chlorides and TEA as a base[57] in 43%–74% yields (Scheme 4).

2.1.2 | NMR spectroscopic investigations

The structures of all new 3-aryl-3-azetidinyl acetic acid methyl esters **3**–**9** and their derivatives **10**–**28** were confirmed by detailed analysis of their spectroscopic data and are given in Section 4. The lead compound **28** was subjected to an in-depth NMR analysis (Figure 2). The signal assignment was carried out by the combined application of standard and advanced NMR spectroscopic techniques including DEPT, HSQC, HMBC, H2BC, COSY, 1,1-ADEQUATE, and NOESY experiments. For instance, in the ^{1}H NMR spectrum of compound **28**, the azetidine ring signals of the diastereotopic methylene protons were observed as a broadened multiplet in the region of δ 4.27–4.32 ppm and a doublet at 4.35 ppm ($^2J_{Ha,\ Hb}$ = 8.7 Hz), while the methylene protons (singlet, δ 3.02 ppm) from acetate moiety appeared upfield. A comparison between the DEPT-90, DEPT-135, and ^{13}C-NMR spectra of compound **28** clearly indicated the characteristic signals of the 3-(4-trifluoromethylphenyl)-3-azetidinyl acetic acid methyl ester carbons, namely the aliphatic azetidine quaternary carbon C-3 (δ 40.1 ppm) and acetate moiety methylene carbon (δ 45.1 ppm). The latter methylene carbon shared a negative cross-peak with protons at δ 3.02 ppm in the multiplicity-edited ^{1}H-^{13}C HSQC spectrum. Direct connectivities of the aforementioned azetidine ring methylene protons were elucidated analogously and displayed negative HSQC cross-peaks with the methylene carbons C-2,4 at δ 60.0–60.2 ppm. With this information in hand, the connectivity of acetate moiety with the azetidine ring was unambiguously assigned from 1,1-ADEQUATE spectral data, where the protonated methylene carbon (δ 45.1 ppm) showed correlations with an adjacent quaternary carbon C-3 (δ 40.1 ppm) and carbonyl carbon (δ 170.4 ppm), respectively. The ^{19}F NMR spectrum revealed a chemical shift of CF_3 group at δ −62.6 ppm, which is in good agreement with the data reported in the literature.[58,59] Moreover, the ^{13}C NMR spectrum exhibited characteristic resonances of the 4-trifluoromethylphenyl moiety, where the CF_3 group was observed as a quartet at δ 124.0 ppm ($^1J_{C,F}$ = 272.1 Hz), while the C-4' and C-3',5' carbons appeared as quartets at δ 129.3 ppm ($^2J_{C,F}$ = 32.5 Hz) and δ 125.6 ppm ($^3J_{C,F}$ = 3.7 Hz), respectively. These assignments followed from the difference between the magnitudes of $J_{C,F}$ coupling constants. Then, 4-trifluoromethylphenyl ring protons 3',5'-H (δ 7.60 ppm) were easily resolved, as it shared an HSQC cross-peak with carbons C-3',5' (δ 125.6 ppm). The latter information allowed the assignment of adjacent protonated carbons C-2',6' from an appropriate correlation in the ^{1}H-^{13}C H2BC spectrum. As expected, the ^{1}H-^{13}C HMBC spectral data revealed

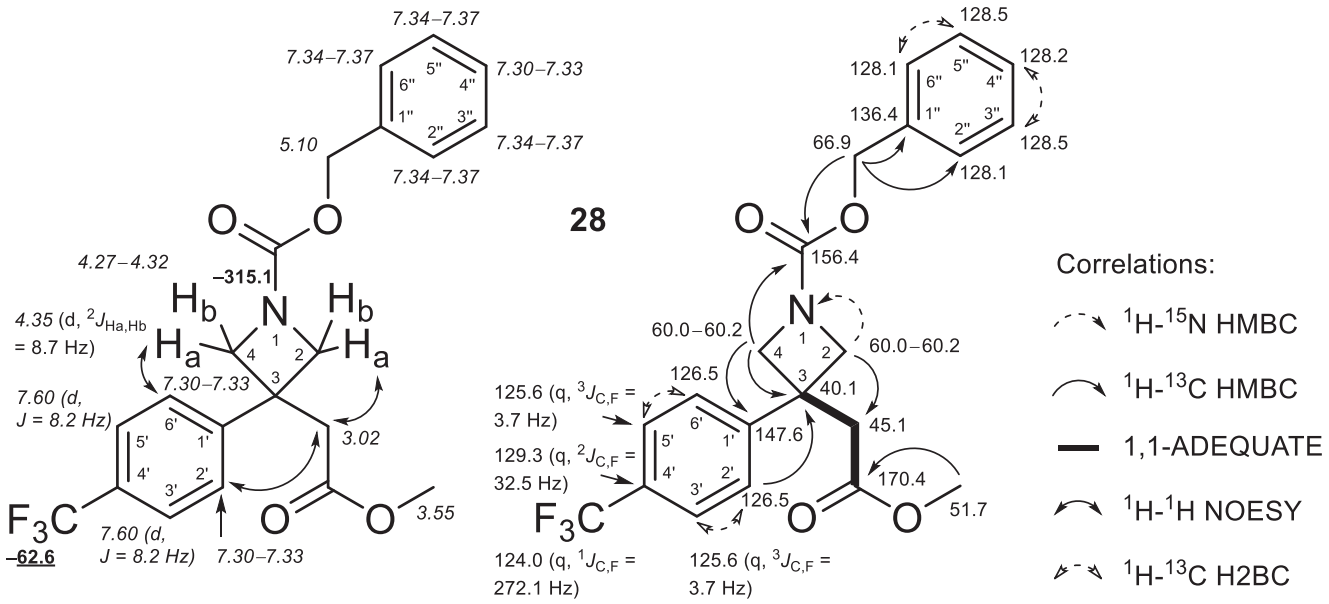


FIGURE 2 ^{1}H NMR (italics), ^{13}C NMR, ^{15}N NMR (bold), and ^{19}F NMR (bold, underlined) chemical shifts and relevant ^{1}H-^{15}N HMBC, ^{1}H-^{13}C HMBC, 1,1-ADEQUATE, ^{1}H-^{1}H NOESY, and ^{1}H-^{13}C H2BC correlations of compound **28**.

connectivities with the rest of the neighboring moieties, where the azetidine ring methylene protons (*δ* 4.27–4.32 and 4.35 ppm) exhibited long-range HMBC correlations with 4-trifluoromethylphenyl C-1' quaternary (*δ* 147.6 ppm), acetate moiety methylene (*δ* 45.1 ppm) and neighboring carbonyl (*δ* 156.4 ppm) carbons, respectively. The aforementioned carbonyl carbon shared an HMBC cross-peak with benzyl group methylene protons (*δ* 5.10 ppm) thus confirming their connectivity with 3,3-disubstituted azetidine moiety. Finally, the NOESY experimental data further elucidated the connectivities based on through-space correlations, thus confirming proximity in space of different structural fragments throughout 3,3-disubstituted azetidine ring system.

Analysis of the data showed that the chemical shift values were highly consistent within each series of compounds, thus validating the shifts for each position. In the case of 3-aryl-3-azetidinyl acetates **3**–**9** the characteristic azetidine quaternary carbon C-3 signal was in a range from *δ* 39.1 to 39.7 ppm, and acetate moiety methylene carbon was in a range from *δ* 45.2 to 45.7 ppm. The aforementioned distinct carbon signals of compounds **10**–**28** can be summarized as follows: *N*-unprotected derivatives (*δ* C-3, 41.5–42.0 and acetate CH_2, 43.2–43.7 ppm), *N*-alkylated derivatives (*δ* C-3, 39.3–40.8 and acetate CH_2, 44.4–45.0 ppm), *N*-acylated derivatives (*δ* C-3, 39.4–40.5 and acetate CH_2, 45.1–45.3 ppm), respectively.

2.2 | Biology

2.2.1 | Inhibition of AChE and BChE

AChE is one of the most clinically important enzymes for AD and other neurodegenerative disorders such as Parkinsonism with dementia since AChE inhibition is associated with elevated acetylcholine and consequently improved cognitive response.[60,61] Moreover, it has been proven, that in the later stages of AD BChE substitutes the function of AChE; thus, BChE inhibition can also contribute to the improvement of cognitive functions.[62–64] As discussed above, current AD and PD treatments are limited to symptomatic therapy, which does not affect the ongoing degeneration processes. Hence, we directed our efforts toward compounds targeting AChE inhibition and evaluating their neuroprotective or anti-inflammatory properties.

The inhibitory activity of newly synthesized methyl 2-(3-phenylazetidin-3-yl)acetates **10**–**16** and **18**–**28** was first studied against AChE from electric eel (eeAChE) and BChE from equine serum (eqBChE), as described previously,[65] and compared to that of galantamine (Table 1). The IC_{50} values for AChE ranged from 52.82 to 131.88 μM and for BChE from 54.47 to more than 500 μM. Apart from compound **15**, the rest of the studied compounds were more effective at inhibiting AChE than BChE. Moreover, some of the compounds, namely **11**, **14**, **16**, and **24**, proved to be highly selective for AChE with respect to BChE. The IC_{50} values obtained for the studied compounds were compared with those for galantamine, revealing lower effectiveness of our prepared compounds. However, to our satisfaction, benzamide **26** (IC_{50} = 52.82 μM) and phenylacetamide **27** (IC_{50} = 59.17 μM) showed comparable efficacy in inhibiting AChE as rivastigmine (IC_{50} = 56.1 μM),[66] a drug used for the treatment of Alzheimer's disease.

The type of inhibition was determined for these two most effective AChE inhibitors, **26** and **27**, using the Lineweaver–Burk plot[67] and the comparison of kinetic parameters K_M and V_m of uninhibited and inhibited reactions. The obtained result shows that K_M, V_m, and K_M/V_m for inhibited reactions are different from those for uninhibited reactions. Further, the intersection of the lines in the

TABLE 1 Biological evaluation of novel azetidine derivatives in inhibition of acetylcholinesterase (AChE)/butyrylcholinesterase (BChE), cytotoxicity and neuroprotective activity in salsolinol- and glutamate-induced models of cell death.

Compound	IC_{50} (μM)[a] AChE	BChE	SI[b]	Viability (%)[c] 10 μM	Cytotoxicity (%)[d] 10 μM	Viability salsolinol model (%)[c] 10 μM	Cell death glutamate model (%)[e] 10 μM
SAL 800 μM	n.d.	n.d.	n.d.	n.d.	n.d.	63.06 ± 0.39	n.d.
10	131.88 ± 0.36	161.84 ± 25.55	1.23	100.90 ± 5.06	9.28 ± 0.62	72.79 ± 5.29	99.98 ± 3.14
11	104.10 ± 1.68	>500	–	>100	10.09 ± 0.88	61.07 ± 2.07	80.51 ± 3.96
12	86.65 ± 0.52	160.33 ± 0.22	1.85	96.42 ± 2.96	8.41 ± 0.96	64.89 ± 2.73	86.48 ± 3.89
13	64.53 ± 1.51	95.57 ± 1.26	1.48	>100	9.63 ± 0.84	61.79 ± 3.56	90.79 ± 5.14
14	77.14 ± 1.58	>500	–	>100	8.38 ± 0.55	56.82 ± 3.38	89.45 ± 5.24
15	77.65 ± 1.40	54.47 ± 0.27	0.70	>100	8.71 ± 0.63	63.84 ± 1.44	108.5 ± 4.26
16	93.92 ± 6.41	>500	–	>100	7.39 ± 0.68	58.61 ± 3.41	94.16 ± 5.08
18	88.86 ± 0.05	212.51 ± 12.44	2.39	>100	8.61 ± 0.98	64.51 ± 2.17	86.09 ± 3.34
19	75.40 ± 3.19	165.30 ± 0.93	2.19	>100	9.21 ± 0.69	64.40 ± 2.18	100.8 ± 4.41
20	81.40 ± 0.25	204.82 ± 5.47	2.52	>100	9.39 ± 0.84	65.59 ± 2.15	103.6 ± 2.71
21	80.37 ± 1.06	150.15 ± 1.84	1.87	>100	8.58 ± 0.94	65.38 ± 1.95	92.02 ± 4.38
22	87.18 ± 3.19	203.45 ± 6.13	2.33	>100	9.35 ± 0.96	66.46 ± 1.90	97.97 ± 4.59
23	75.13 ± 0.91	132.58 ± 3.59	1.76	99.84 ± 5.06	8.18 ± 1.22	64.21 ± 1.77	98.22 ± 3.75
24	74.51 ± 1.14	357.44 ± 31.99	4.76	>100	7.93 ± 1.10	64.32 ± 1.62	96.47 ± 6.13
25	80.88 ± 0.97	201.15 ± 19.95	2.49	>100	7.73 ± 0.84	64.37 ± 1.59	92.83 ± 4.27
26	52.82 ± 1.16	225.22 ± 8.39	4.26	>100	6.38 ± 0.91	75.55 ± 2.67	99.00 ± 5.68
27	59.17 ± 1.94	171.24 ± 8.50	2.89	>100	12.54 ± 1.74	69.67 ± 1.32	100.30 ± 5.08
28	79.90 ± 0.46	165.92 ± 3.40	2.08	>100	6.24 ± 0.97	86.67 ± 3.79	78.68 ± 4.32
Galantamine	0.38 ± 0.06	2.21 ± 0.08	5.82	n.d.	n.d.	n.d.	n.d.
NAC 1000 μM	n.d.	n.d.	n.d.	n.d.	n.d.	85.57 ± 3.30	n.d.
R-LA 50 μM	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	76.17 ± 3.29

[a]IC_{50} values are expressed as mean ± standard error of mean (SEM) of at least two independent measurements.

[b]SI (selectivity index) = IC_{50} (AChE/BChE).

[c]Cell viability (including salsolinol model) is shown as percentage of means ± SEM normalized to DMSO control.

[d]Cytotoxicity is displayed as percentage of means ± SEM with normalization to Triton X-100 control (set to 100%).

[e]Cell death is expressed as percentage of means ± SEM normalized to glutamate control (100% of cell death). Compounds were tested in triplicate experiments in at least 3 independent days.

Lineweaver–Burk plot lies below the *x*-axis in the III quadrant (Figure 3a,b), revealing that both derivatives **26** and **27** act via mixed type of inhibition.

2.2.2 | Evaluation of the interactions of compounds **26** and **27** with AChE by molecular docking

As discussed above, several derivatives demonstrated moderate AChE inhibitory effect. To study the binding mode and interactions of the most active compounds **26** and **27** in the catalytic active site of AChE, the molecular docking into the crystal structure of human AChE (PDB: 4EY7) was used. As shown in Figure 4, original ligand donepezil (orange) has (1-benzylpiperidin-4-yl)methyl group pointed into the depth of AChE cavity, while 5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one part is headed outside of the binding site. Interestingly, similar binding was observed in case of both compounds **26** (yellow) and **27** (green), which directed 4-trifluorophenyl part towards the AChE binding cavity and polar azetidine amide part stayed outside.

In relation to binding energy and interactions with AChE, crystalized original ligand donepezil was in a narrow active site with one strong hydrogen bond to the main chain of Phe295 in a distance of 1.8 Å. The binding energy of donepezil with the protein was −12.2 kcal/mol. On the other hand, ligand **26**, with a binding energy −10.5 kcal/mol, had a hydrogen bond with Tyr337, however, due to the positions of the atoms, this bond was weaker, as also indicated by

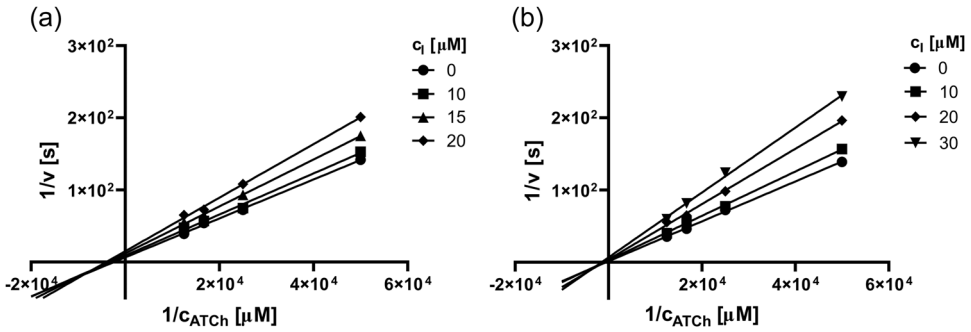


FIGURE 3 The Lineweaver–Burk plots for **26** (a) for **27** (b) inhibiting electric eel acetylcholinesterase (eeAChE).

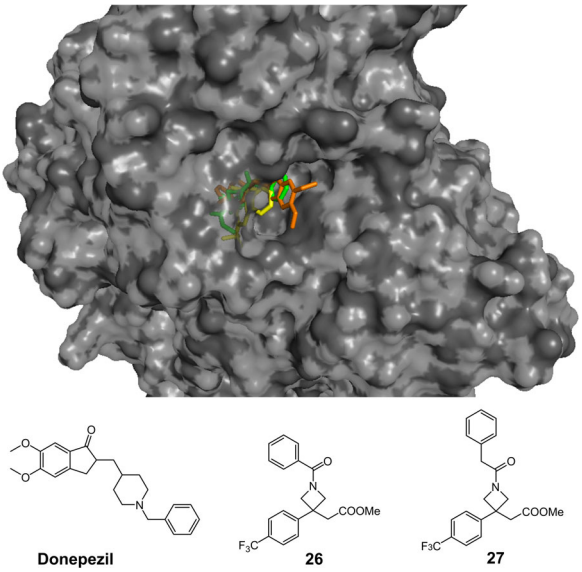


FIGURE 4 Best binding poses of all compounds in the catalytically active site of acetylcholinesterase (AChE) (PDB: 4ey7), below: structures of docked compounds; best pose of donepezil (orange), compound **26** (yellow) and **27** (green) in catalytically active site of AChE.

the distances between the atoms (2.1 Å). The weakest ligand of the three, derivative **27** with binding energy −9.3 kcal/mol, had a hydrogen bond with His477 with a distance between atoms of 2.3 Å (Figure 5).

2.2.3 | Safety evaluation on neuron-like SH-SY5Y cells after treatment with novel compounds

Before conducting neuroprotective assays, cytotoxicity of the novel compounds was evaluated on neuron-like cells. SH-SY5Y were differentiated into neuron-like phenotype by 10 µM all-*trans* retinoic acid (ATRA) for 48 h[68] and subsequently exposed to selected concentration of tested compounds for 24 h. The viability and cytotoxicity were quantified by calcein AM and propidium iodide (PI) staining assays.[69] As shown in Table 1, the majority of the derivatives did not decrease cell viability below 90%. Additionally, PI assay, in which Triton X-100 was used as a positive control (set as 100% cytotoxicity),[69] did not reveal any cytotoxic effect (10% additional cell death above DMSO control [7.55 ± 0.32%]) induced by novel azetidine derivatives.

2.2.4 | Neuroprotective activity of novel azetidine derivatives in SAL- and Glu-induced model of PD and oxidative damage

SAL is known to induce toxicity to dopaminergic SH-SY5Y via various effects such as inhibition of complex I and II of respiratory chain in mitochondria, elevation of oxidative stress by inhibition of catalase or Cu/Zn superoxide dismutase, and glutathione depletion with subsequent activation of caspase-3 resulting in apoptotic cell death. Additionally, the effect of SAL was associated with necrosis in SH-SY5Y cells.[70] The effect of Glu on neuron-like SH-SY5Y is characterized by the inhibition of cysteine/Glu antiporter system (Xc), rather than the activation of *N*-methyl-D-aspartate (NMDA) receptors. As a result, Glu induces elevation of oxidative stress by glutathione depletion and Rac-NADPH oxidase activity, lipoxidation, and impairment of iron homeostasis leading to ferroptosis, necroptosis, or apoptosis of neuronal cells.[71]

ATRA-differentiated SH-SY5Y cells were co-treated with SAL and the compounds (0.1; 1; 10 µM) or antioxidant *N*-acetylcysteine (NAC) (10; 100; 1000 µM) as a positive control.[66,72] After 24 h treatment, the protective effect of the studied derivatives was evaluated by the Calcein AM assay (SAL model) as described previously.[68] Cell viability was expressed as percentage of the DMSO control, set as 100%.

SAL (800 µM) caused a drop in cell viability down to 63.06 ± 0.40%, while NAC (1000 µM) increased cell viability by approximately 20% (Table 1). Effect of NAC was set as a threshold for identification of promising protective effect. From all tested compounds, at 10 µM concentration only derivative **28** demonstrated an effect comparable to that of NAC. Furthermore, compound **28**-mediated protective effect was validated by propidium iodide cell death assay within SAL model. Data were normalized to the toxic effect of SAL on cells, set as 100%, so that the protective

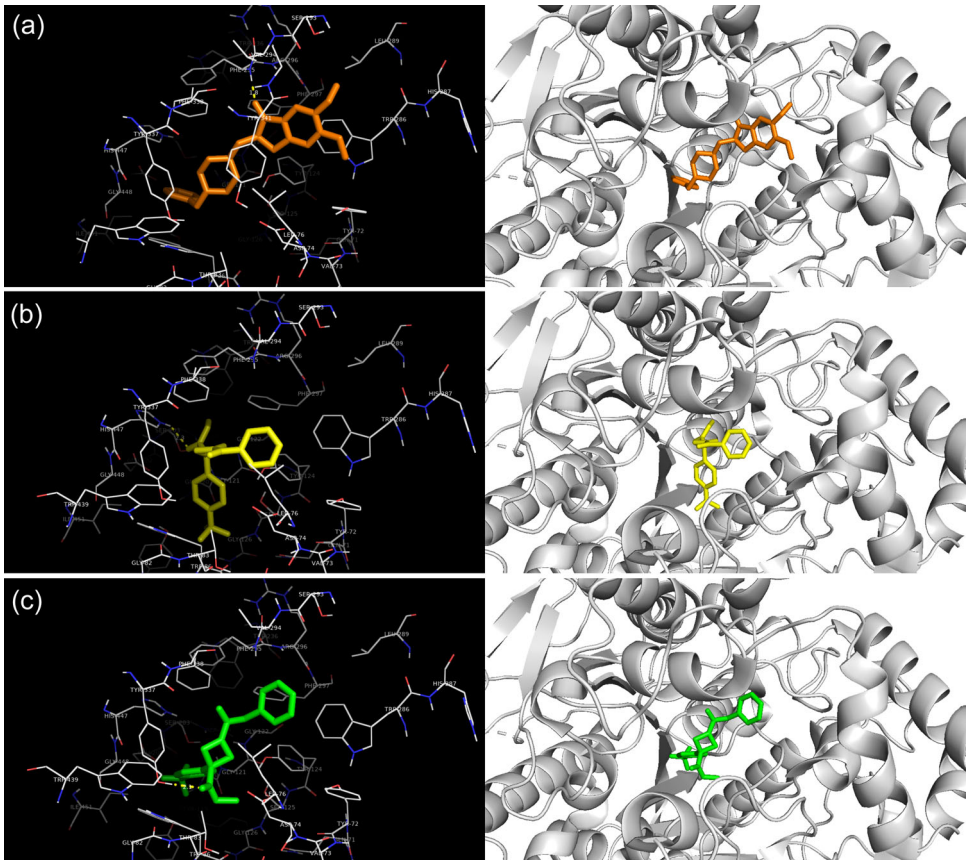


FIGURE 5 The binding poses of donepezil (orange, a), compound **26** (yellow, b), and **27** (green, c) with interactions in catalytically active site of (PDB: 4ey7).

effect induced by compounds was observed as the reduction in cell death relative to the threshold. Both NAC at 100 and 1000 μM, and **28** at 10 μM demonstrated significant dose-dependent decline of cell death accounting for 68.0 ± 6.97%, 52.8 ± 9.77%, and 66.0 ± 6.65%, respectively (Figure 6a). Overall, compound **28** showed high neuroprotective effect within SAL model at 10 to 100-times lower active concentration than the positive control.

Furthermore, compounds were evaluated in Glu-induced model of oxidative damage. ATRA-differentiated SH-SY5Y cells were co-treated with Glu and newly prepared compounds in selected concentrations alongside *R*-lipoic acid (*R*-LA) (0.5; 5; 50 μM) as a positive control[66] for 24 h, as described previously.[73] After 24 h treatment, the protective effect of studied derivatives was determined by propidium iodide cell death assay. Data were normalized to cell death induced by 160 mM Glu, set as 100%. At 10 μM concentration, derivatives **11** (80.5 ± 3.96%), **12** (86.5 ± 3.90%), **18** (86.09 ± 3.34%) and **28** (78.7 ± 4.32%) showed a moderate-to-comparable protective effect to that of 50 μM *R*-LA (Table 1). Likewise to SAL model of neurotoxicity, in Glu model benzylcarbamate **28** proved to be the most promising compound of the whole series. Subsequently, the most active derivate **28** was selected to confirm the protective activity by lactate dehydrogenase (LDH)-release assay[74] (Figure 6b). Glu increased LDH release more than five times compared to healthy controls (18.88 ± 0.73%), while treatment with 50 μM *R*-LA and 10 μM of compound **28** was associated with a significant decrease, accounting for 81.3 ± 4.39% and 79.3 ± 1.65%, respectively. These results confirm the neuroprotective effect of compound **28** and *R*-LA, which is in accordance with the literature.[73,75] Noteworthy, compound **28** outperformed positive control due to five-fold lower active concentration and slightly better drop in LDH release. As compound **28** was found to be active in both cellular models of neurodegeneration, it was further investigated for its activity under oxidative stress and caspase-3/7 activity within Glu model.

As summarized by Kritis et al.,[76] Glu is a strong oxidative stress (OS) inducer, which drives the toxicity within neuron-like SH-SY5Y cells. In our model, oxidative stress was induced with Glu (160 mM) as described above. After 4 h, the formation of superoxide radicals was evaluated by dihydroethidium (DHE) assay[68] with normalization of results to the effect of 160 mM Glu. Overall, Glu elevated ROS three times in comparison to healthy control, accounting for 31.4 ± 2.86% (Figure 7a). Interestingly, co-treatment with both compound **28** at 10 μM and *R*-LA at 50 μM caused significant and comparable decrease in Glu-mediated oxidative stress, accounting for 82.1 ± 3.02% and 82.1 ± 2.91%, respectively. Noteworthy, the effect of *R*-LA is in line with previously reported study using Glu model on HT4 cells[77] and our previous work.[73] Despite similar activity toward the oxidative stress, compound **28** demonstrated higher effectivity since the active concentration was five-fold lower than that of *R*-LA.

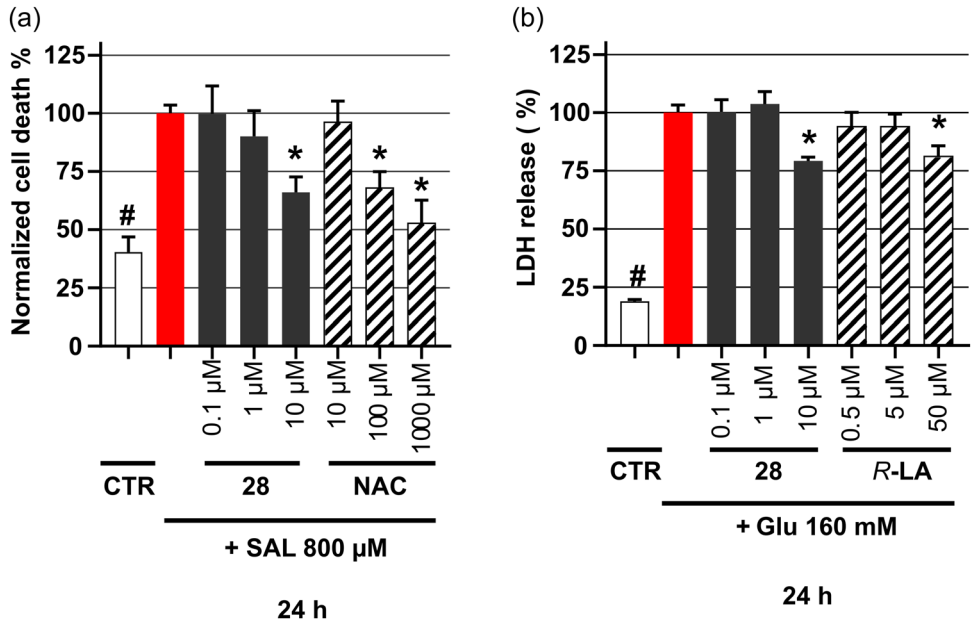


FIGURE 6 The activity of azetidine derivative **28** and *N*-acetylcystein (NAC) in SAL-induced model of Parkinson's disease (PD) on neuron-like SH-SY5Y cells (a). Data are presented as means ± standard error of the mean (SEM) from triplicates in three separated days. Analysis of variance (ANOVA), Tukey post hoc test. ⋆*p* compared with vehicle with salsolinol (SAL) 800 µM, #*p* compared with vehicle without SAL 800 µM. Neuroprotective effect of compound **28** and positive control *R*-lipoic acid was evaluated by lactate dehydrogenase (LDH) release assay (b) in Glu-induced oxidative damage on neuron-like SH-SY5Y cells. Data are presented as means ± standard error of the mean (SEM) from triplicates in four independent days. Kruskal–Wallis test, Mann–Whitney post hoc test with Bonferroni correction of *p*-values. ⋆*p* compared with vehicle with Glu 160 mM, #*p* compared with vehicle without Glu 160 mM.

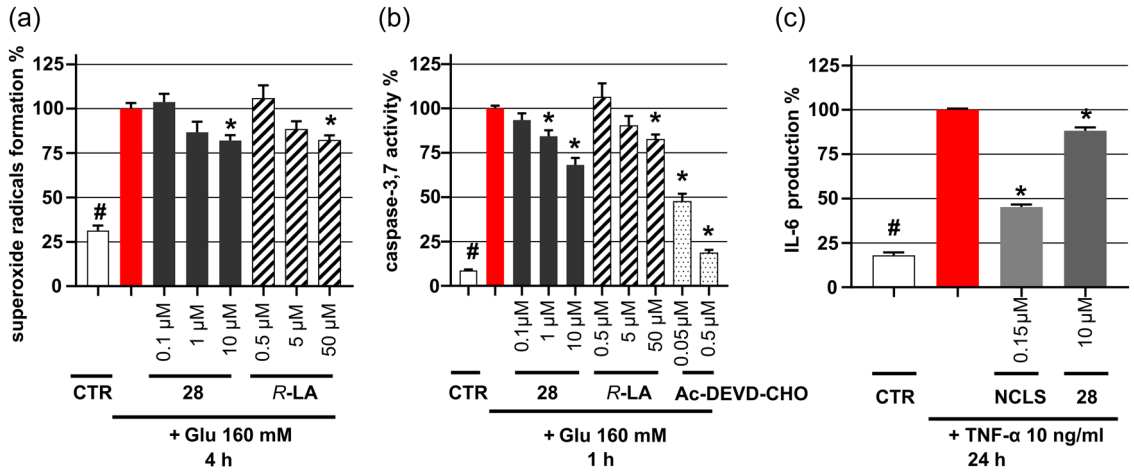


FIGURE 7 Glutamate-induced superoxide radical formation in neuron-like SH-SY5Y cells after 4 h (a). Data are presented as means ± standard error of the mean (SEM) from triplicates in four independent days. Analysis of variance (ANOVA), Tukey's multiple comparison test ⋆*p* compared with vehicle with Glu 160 mM, #*p* compared with vehicle without Glu 160 mM; caspase-3/7 activation in neuron-like SH-SY5Y cells after 1 h treatment by glutamate 160 mM and compounds (b). Data are presented as means ± standard error of the mean (SEM) from triplicates in at least four independent days. Kruskal–Wallis test, Mann–Whitney post hoc test with Bonferroni correction of *p*-values. ⋆*p* compared with vehicle with Glu 160 mM, #*p* compared with vehicle without Glu 160 mM. Anti-inflammatory effect of compound **28** and positive control narciclasine (NCLS) in tumor necrosis factor (TNF-α)-induced elevation of interleukin-6 (IL-6) in Human Umbilical Vein Endothelial cells (HUVEC) (c). Data are presented as means ± standard error of the mean (SEM) from duplicates in five independent days. ANOVA, Tukey's multiple comparison test ⋆*p* compared with vehicle with TNF-α 10 ng/mL, #*p* compared with vehicle without TNF-α 10 ng/mL.

Further, the caspase-3/7 (casp-3,7) activity as key executor of cell death within Glu model on neuron-like SH-SY5Y cells was evaluated. Neuron-like cells were exposed to Glu (160 mM) for 1 h, which caused more than ten-fold elevation in casp-3,7 compared to healthy cells (8.75 ± 0.52%) (Figure 7b). Ac-DEVD-CHO, which in Glu-model was used as a positive control, showed strong to almost complete decrease in casp-3,7 activity at 0.05 μM (47.6 ± 4.38%) and 0.5 μM (18.5 ± 1.77%). The comparison of the treatment with compound **28** and *R*-LA on casp-3,7 activity within Glu-model showed an interesting difference: both compound **28** (1 μM) and *R*-LA (50 μM) showed nearly equal decrease in casp-3,7, accounting for 84.39 ± 3.30% and 82.59 ± 2.76%, respectively. Furthermore, 10 μM concentration of compound **28** outperformed positive control showing decline to 68.24 ± 3.81% of casp-3,7 activity.

Collectively, significant and stronger reducing effect on casp-3,7 present within Glu-model, as demonstrated by compound **28** compared to *R*-LA, was found to be a key contributor to overall neuroprotection in LDH assay (Figure 7b). Such correlation was also observed in our previous study using neuroprotective agent deferoxamine.[68]

2.2.5 | Compound **28** decreases levels of the inflammatory marker interleukin-6 (IL-6)

Inflammatory processes play many essential roles in neurodegeneration, including clearance of misfolded proteins and immune defence, as well as negative roles such as triggering degenerations like multiple sclerosis and deterioration in case of AD, PD, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). Interestingly, a strong correlation between cognitive impairment progression and elevation of IL-6 was found in AD.[78] In particular, AChE was found to be responsible for anti-inflammatory effect in reducing the levels of cytokines such as IL-6.[79] In connection with this work, it was hypothesized that the neuroprotective and AChE inhibitory activity-possessing compound **28** might show anti-inflammatory effect on tumor necrosis factor (TNF-α) induced IL-6 production. Endothelial cells are a key component of blood–brain barrier and one of the first types of cells to initiate inflammation. Therefore, HUVEC endothelial cells were used as a model of cells with inflammatory functions.

HUVEC cells were exposed to TNF-α (10 ng/mL) and compound **28** for 24 h, alongside anti-inflammatory agent narciclasine as a positive control. Data were normalized to IL-6 production of TNF-α treated cells (100%), as described previously.[80] At 10 μM concentration compound **28** reduced IL-6 production to 88.25 ± 1.84%, while the effect of narciclasine (0.15 μM) was superior, bringing IL-6 production to 45.22 ± 1.40% (Figure 7c).

3 | CONCLUSION

A small library of 3-aryl-3-azetidinyl acetic acid methyl ester derivatives was prepared from common starting material *N*-Boc-3-azetidinone, employing the Horner-Wadsworth-Emmons reaction, rhodium(I)-catalyzed conjugate addition of arylboronic acids, and subsequent elaborations to obtain *N*-unprotected hydrochlorides, *N*-alkylated and *N*-acylated azetidine derivatives. Compounds were evaluated for AChE and BChE inhibitory activity. Compounds **26** and **27** showed AChE inhibition comparable to that of the well-known AChE inhibitor rivastigmine. Additionally, the binding mode of compounds **26** and **27** and highly potent AChE inhibitor donepezil within active site of AChE was studied using molecular docking. Furthermore, neuroprotective activity of the prepared compounds was evaluated in models associated with Parkinson's disease (SAL-induced) and aspect of AD (Glu-induced oxidative damage). Compound **28** showed the highest neuroprotective effect in both SAL- and Glu-induced neurodegeneration models. Finally, **28**-mediated protective effect was found to be driven by a reduction of oxidative stress and caspase-3/7 activity in Glu model.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All chemicals and solvents were commercially available and were used as received. Anhydrous THF was purchased from Sigma Aldrich in Sure/Seal bottling and additionally sparged with argon before use. Reaction progress was monitored by thin-layer chromatography carried out on silica gel plates (Merck Kieselgel 60 F254) and visualized using UV light (254 nm) or $KMnO_4$ staining solution. Flash column chromatography was performed using silica gel (230–400 μm, 60 Å, Merck KGaA, Darmstadt, Germany). The ^{1}H, ^{13}C, and ^{15}N NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions at 25°C on a Bruker Avance III 700 (700 MHz for ^{1}H, 176 MHz for ^{13}C and 71 MHz for ^{15}N) spectrometer equipped with a 5 mm TCI ^{1}H-^{13}C/^{15}N/D z-gradient cryoprobe, a Bruker Avance III 400 (400 MHz for ^{1}H and 101 MHz for ^{13}C) or a Jeol ECA-500 (500 MHz for ^{1}H and 126 MHz for ^{13}C) spectrometer equipped with a 5 mm Royal probe. The chemical shifts expressed in parts per million (ppm), were relative to tetramethylsilane (TMS). The ^{15}N NMR spectra were referenced to neat, external nitromethane (coaxial capillary). ^{19}F NMR spectra (376 MHz, absolute referencing via Ξ ratio) were obtained on a Bruker Avance III 400 (400 MHz for ^{1}H and 101 MHz for ^{13}C) using a directly detecting BBO probe. Coupling constants (*J*) were reported in Hz and multiplicities of NMR signals are abbreviated as follows: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Fourier-transform infrared spectroscopy (FTIR) spectra were collected using the ATR method on a Bruker Vertex 70 v spectrometer with an integrated Platinum ATR accessory. The melting points of crystalline compounds were determined in open capillary tubes with a Buchi M—565 apparatus (temperature gradient—2°C/min) and are uncorrected. High-resolution mass spectrometry (HRMS) spectra were obtained in ESI mode on a Bruker MicrOTOF-Q III spectrometer.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | Procedure for the synthesis of compound 2

tert-Butyl 3-(2-methoxy-2-oxoethylidene)azetidine-1-carboxylate (**2**), previously reported in Gudelis et al. and Yang et al.[43,45]: To a solution of methyl 2-(dimethoxyphosphoryl)acetate (1.170 g, 6.44 mmol) in dry THF (20 mL), NaH (60% dispersion in mineral oil, 268 mg, 6.72 mmol) was added portionwise at 0°C under argon atmosphere and the resulting mixture was stirred at 0°C for 20 min. Subsequently, *N*-Boc-3-azetidinone (**1**) (1.000 g, 5.84 mmol), dissolved in dry THF (4 mL), was added and the reaction mixture was stirred at the room temperature for 30 min. Upon completion, THF was removed in vacuo, the residue was diluted with water (30 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:4, v/v) to give pure **2** as a colorless solid (1.035 g, 78%), mp 50–51°C. R_f = 0.38 (ethyl acetate/petroleum ether, 1:7, v/v). ^{1}H NMR (700 MHz, $CDCl_3$): δ 5.79–5.78 (m, 1H, CH), 4.82–4.80 (m, 2H, Az 2,4-H_a), 4.60–4.59 (m, 2H, Az 2,4-H_b), 3.72 (s, 3H, OCH_3), 1.46 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 165.8 ($\underline{C}OOCH_3$), 156.3($\underline{C}OOC(CH_3)_3$), 153.2 (Az C-3), 113.4 (CH), 80.3 ($\underline{C}(CH_3)_3$), 60.4 and 58.0 (Az C-2,4), 51.6 (OCH_3), 28.5 ($C(\underline{C}H_3)_3$). IR (ν_{max}, cm^{-1}): 3002, 2968, 2942, 2850 ($CH_{aliphatic}$), 1720, 1701, 1681 (C=O, C=C), 1400, 1278, 1151, 1121, 1024, 944, 865, 768. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{11}H_{17}NO_4Na$ 250.1050; Found 250.1050.

4.1.3 | General procedure A for the synthesis of compounds 3–9

To a solution of $[Rh(cod)Cl]_2$ (11 mg, 0.02 mmol) in 1,4-dioxane (1 mL), aqueous 1.5 M KOH (0.59 mL, 0.88 mmol) was added and the resulting mixture was stirred for 5 min. Subsequently, alkene **2** (100 mg, 0.44 mmol) and an appropriate arylboronic acid (0.88–1.32 mmol), dissolved in THF (1.5 mL), were added and the reaction mixture was stirred at the room temperature for 3 h. Upon completion, the mixture was diluted with water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude product was purified by column chromatography to obtain pure compounds **3**–**9**.

tert-Butyl 3-(2-methoxy-2-oxoethyl)-3-phenylazetidine-1-carboxylate (**3**): Prepared in accordance to general procedure (A) from **2** (100 mg, 0.44 mmol) and phenylboronic acid (107 mg, 0.88 mmol). The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:4, v/v) to give pure **3** as a light yellow solid (96 mg, 71%), mp 72–73°C. R_f = 0.38 (ethyl acetate/petroleum ether, 1:5, v/v). ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.34–7.32 (m, 2H, Ph 3',5'-H), 7.24–7.22 (m, 1H, Ph 4'-H), 7.19–7.18 (m, 2H, Ph 2',6'-H), 4.27 (d, $^2J_{Ha,\ Hb}$ = 8.6 Hz, 2H, Az 2,4-H_a), 4.23–4.16 (m, 2H, Az 2,4-H_b), 3.53 (s, 3H, OCH_3), 2.97 (s, 2H, CH_2CO), 1.44 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 170.9 ($\underline{C}OOCH_3$), 156.4 ($\underline{C}OOC(CH_3)_3$), 143.9 (Ph C-1'), 128.5 (Ph C-3',5'), 126.9 (Ph C-4'), 126.0 (Ph C-2',6'), 79.6 ($\underline{C}(CH_3)_3$), 60.4–59.6 (Az C-2,4), 51.5 (OCH_3), 45.6 ($\underline{C}H_2CO$), 39.7 (Az C-3), 28.4 ($C(\underline{C}H_3)_3$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −313.7 (N-1). IR (ν_{max}, cm^{-1}): 3060, 3026, 2974, 2886 ($CH_{aromatic,\ aliphatic}$), 1737, 1697 (C=O), 1390, 1365, 1144, 1118, 765, 700. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{17}H_{23}NO_4Na$ 328.1519; Found 328.1519.

tert-Butyl 3-(4-hydroxyphenyl)-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate (**4**): Prepared in accordance to general procedure (A) from **2** (100 mg, 0.44 mmol) and 4-hydroxyphenylboronic acid (182 mg, 1.32 mmol). The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:4, v/v) to give pure **4** as a colorless solid (89 mg, 63%), mp 120–121°C. R_f = 0.25 (ethyl acetate/petroleum ether, 1:2, v/v). ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.03–6.98 (m, 3H, ArH, OH), 6.78–6.76 (m, 2H, ArH), 4.23–4.14 (m, 4H, Az 2,4-H), 3.54 (s, 3H, OCH_3), 2.92 (s, 2H, CH_2CO), 1.45 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 171.2 (C=O), 156.7 (C=O), 155.2 (C), 135.0 (C), 127.2 (2 × CH), 115.4 (2 × CH), 80.2 ($\underline{C}(CH_3)_3$), 60.6–59.8 (Az C-2,4), 51.6 (OCH_3), 45.6 ($\underline{C}H_2CO$), 39.1 (Az C-3), 28.4 ($C(\underline{C}H_3)_3$). IR (ν_{max}, cm^{-1}): 3239 (OH), 3006, 2980, 2889 ($CH_{aromatic,\ aliphatic}$), 1737, 1720, 1650 (C=O), 1433, 1220, 1164, 1142, 996, 832, 756. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{17}H_{23}NO_5Na$ 344.1468; Found 344.1468.

tert-Butyl 3-(2-methoxy-2-oxoethyl)-3-(4-methoxyphenyl)azetidine-1-carboxylate (**5**): Prepared in accordance to general procedure (A) from **2** (100 mg, 0.44 mmol) and 4-methoxyphenylboronic acid (200 mg, 1.32 mmol). The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:5, v/v) to give pure **5** as a colorless solid (100 mg, 68%), mp 47–48°C. R_f = 0.38 (ethyl acetate/petroleum ether, 1:5, v/v). ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.11–7.09 (m, 2H, ArH), 6.86–6.84 (m, 2H, ArH), 4.22 (d, $^2J_{Ha,\ Hb}$ = 8.5 Hz, 2H, Az 2,4-H_a), 4.19–4.13 (m, 2H, Az 2,4-H_b), 3.78 (s, 3H, $PhOCH_3$), 3.54 (s, 3H, OCH_3), 2.94 (s, 2H, CH_2CO), 1.43 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 171.1 (C=O), 158.4 (C), 156.5 (C=O), 136.0 (C), 127.2 (2 × CH), 114.0 (2 × CH), 79.7 ($\underline{C}(CH_3)_3$), 60.7–59.9 (Az C-2,4), 55.4 ($PhOCH_3$), 51.6 (OCH_3), 45.7 ($\underline{C}H_2CO$), 39.2 (Az C-3), 28.5 ($C(\underline{C}H_3)_3$). IR (ν_{max}, cm^{-1}): 3002, 2970, 2889 ($CH_{aromatic,\ aliphatic}$), 1738, 1696 (C=O), 1515, 1392, 1366, 1247, 1160, 1118, 1020, 841. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{18}H_{25}NO_5Na$ 358.1625; Found 358.1625.

tert-Butyl 3-(4-chlorophenyl)-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate (**6**): Prepared in accordance to general procedure (A) from **2** (100 mg, 0.44 mmol) and 4-chlorophenylboronic acid (137 mg, 0.88 mmol). The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:6, v/v) to give pure **6** as a colorless oil, (103 mg, 69%). R_f = 0.38 (ethyl acetate/petroleum ether, 1:5, v/v). ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.31–7.29 (m, 2H, ArH), 7.15–7.13 (m, 2H, ArH), 4.22 (d, $^2J_{Ha,\ Hb}$ = 8.6 Hz, 2H, Az 2,4-H_a), 4.19–4.15 (m, 2H, Az 2,4-H_b), 3.55 (s, 3H, OCH_3), 2.97 (s, 2H, CH_2CO), 1.44 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 170.7 (C=O), 156.4 (C=O), 142.4 (C), 132.8 (C), 128.7 (2 × CH), 127.6 (2 × CH), 79.9 ($\underline{C}(CH_3)_3$), 60.4–59.7 (Az C-2,4), 51.7 (OCH_3), 45.4 ($\underline{C}H_2CO$), 39.3 (Az

C-3), 28.4 (C($\underline{C}H_3)_3$). IR (ν_{max}, cm^{-1}): 2974, 2931, 2887 ($CH_{aromatic, aliphatic}$), 1737, 1698 (C=O), 1390, 1365, 1145, 1092, 827, 759, 700. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{17}H_{22}ClNO_4Na$ 362.1130; Found 362.1130.

tert-Butyl 3-(4-fluorophenyl)-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate (**7**): Prepared in accordance to general procedure (A) from **2** (100 mg, 0.44 mmol) and 4-fluorophenylboronic acid (123 mg, 0.88 mmol). The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:6, v/v) to give pure **7** as a colorless oil (99 mg, 69%). R_f = 0.35 (ethyl acetate/petroleum ether, 1:5, v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 7.17–7.15 (m, 2H, ArH), 7.04–7.00 (m, 2H, ArH), 4.23 (d, $^2J_{Ha,\ Hb}$ = 8.6 Hz, 2H, Az 2,4-H_a), 4.20–4.15 (m, 2H, Az 2,4-H_b), 3.55 (s, 3H, OCH_3), 2.96 (s, 2H, CH_2CO), 1.44 (s, 9H, C($CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 170.9 (C=O), 161.7 (d, $^1J_{C,F}$ = 246.2 Hz, C), 156.5 (C=O), 139.7 (d, $^4J_{C,F}$ = 3.2 Hz, C), 127.9 (d, $^3J_{C,F}$ = 7.9 Hz, 2 × CH), 115.5 (d, $^2J_{C,F}$ = 21.4 Hz, 2 × CH), 79.9 ($\underline{C}(CH_3)_3$), 60.5–59.8 (Az C-2,4), 45.6 ($\underline{C}H_2CO$), 51.7 (OCH_3), 39.3 (Az C-3), 28.5 (C($\underline{C}H_3)_3$). IR (ν_{max}, cm^{-1}): 3071, 2975, 2887 ($CH_{aromatic, aliphatic}$), 1736, 1697 (C=O), 1512, 1390, 1365, 1156, 835. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{17}H_{22}FNO_4Na$ 346.1425; Found 346.1425.

tert-Butyl 3-(2-methoxy-2-oxoethyl)-3-[4-(trifluoromethyl)phenyl] azetidine-1-carboxylate (**8**): Prepared in accordance to general procedure (A) from **2** (100 mg, 0.44 mmol) and 4-(trifluoromethyl) phenylboronic acid (250 mg, 1.32 mmol). The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:6, v/v) to give pure **8** as a colorless oil (108 mg, 66%). R_f = 0.28 (ethyl acetate/petroleum ether, 1:5, v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 7.59 (d, *J* = 8.1 Hz, 2H, ArH), 7.33 (d, *J* = 8.0 Hz, 2H, ArH), 4.25 (d, $^2J_{Ha,\ Hb}$ = 8.6 Hz, 2H, Az 2,4-H_a), 4.22–4.17 (m, 2H, Az 2,4-H_b), 3.55 (s, 3H, OCH_3), 3.01 (s, 2H, CH_2CO), 1.44 (s, 9H, C($CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 170.7 (C=O), 156.4 (C=O), 148.0 (C), 129.3 (q, $^2J_{C,F}$ = 32.6 Hz, $\underline{C}$-CF_3), 126.7 (2 × CH), 125.6 (q, $^3J_{C,F}$ = 3.7 Hz, 2 × CH), 124.1 (q, $^1J_{C,F}$ = 269.9 Hz, CF_3), 80.1 ($\underline{C}(CH_3)_3$), 60.4–59.7 (Az C-2,4), 51.8 (OCH_3), 45.2 ($\underline{C}H_2CO$), 39.7 (Az C-3), 28.5 (C($\underline{C}H_3)_3$). IR (ν_{max}, cm^{-1}): 3073, 2976, 2958 ($CH_{aromatic, aliphatic}$), 1737, 1698 (C=O), 1392, 1323, 1161, 1112, 1068, 1015, 840. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{18}H_{22}F_3NO_4Na$ 396.1393; Found 396.1393.

tert-Butyl 3-(2-methoxy-2-oxoethyl)-3-[3-(trifluoromethyl)phenyl] azetidine-1-carboxylate (**9**): Prepared in accordance to general procedure (A) from **2** (100 mg, 0.44 mmol) and 3-(trifluoromethyl) phenylboronic acid (250 mg, 1.32 mmol). The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:6, v/v) to give pure **9** as a colorless oil (92 mg, 56%). R_f = 0.28 (ethyl acetate/petroleum ether, 1:5, v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 7.52 (d, *J* = 7.8 Hz, 1H, ArH), 7.47 (t, *J* = 7.7 Hz, 1H, ArH), 7.43 (s, 1H, ArH), 7.41 (d, *J* = 7.8 Hz, 1H, ArH), 4.27 (d, $^2J_{Ha,\ Hb}$ = 8.6 Hz, 2H, Az 2,4-H_a), 4.24–4.18 (m, 2H, Az 2,4-H_b), 3.55 (s, 3H, OCH_3), 3.01 (s, 2H, CH_2CO), 1.45 (s, 9H, C($CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 170.6 (C=O), 156.4 (C=O), 145.0 (C), 131.0 (q, $^2J_{C,F}$ = 32.3 Hz, $\underline{C}$-CF_3), 129.8 (CH), 129.2 (CH), 124.1 (q, $^1J_{C,F}$ = 269.9 Hz, CF_3), 124.0 (q, $^3J_{C,F}$ = 3.8 Hz, CH), 123.1 (q, $^3J_{C,F}$ = 3.8 Hz, CH), 80.1 ($\underline{C}(CH_3)_3$), 60.3–59.7 (Az C-2,4), 51.7 (OCH_3), 45.5 ($\underline{C}H_2CO$), 39.7 (Az C-3), 28.5 (C($\underline{C}H_3)_3$). IR (ν_{max}, cm^{-1}): 2976, 2937, 2889 ($CH_{aromatic, aliphatic}$), 1738, 1699 (C=O), 1395, 1337, 1163, 1119, 1060, 703. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{18}H_{22}F_3NO_4Na$ 396.1393; Found 396.1393.

4.1.4 | General procedure B for the synthesis of compounds **10–16**

To a solution of an appropriate *tert*-butyl 3-(2-methoxy-2-oxoethyl)-azetidine-1-carboxylate **3**–**9** (0.10 mmol) in 1,4-dioxane (1 mL), 4 N HCl in dioxane (2 mL) was added dropwise under argon and the resulting mixture was stirred at the room temperature for 3 h. Upon completion, the solvent was removed in vacuo. The residue was dissolved in Et_2O (3 × 4 mL) and the solvent was removed in vacuo affording pure compounds **10**–**16**.

Methyl (3-phenylazetidin-3-yl)acetate hydrochloride salt (**10**): Prepared in accordance to general procedure (B) from *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-phenylazetidine-1-carboxylate (**3**) (30 mg, 0.10 mmol). Colorless solid (18 mg, 90%), mp 106–107°C. ^1H NMR (700 MHz, DMSO-d_6): δ 9.86 (br s, 1H), 9.20 (br s, 1H), 7.38–7.36 (m, 2H, ArH), 7.29–7.27 (m, 3H, ArH), 4.27–4.19 (m, 4H, Az 2,4-H), 3.45 (s, 3H, OCH_3), 3.26 (s, 2H, CH_2CO). ^{13}C NMR (176 MHz, DMSO-d_6): δ 170.4 (C=O), 142.8 (C), 128.4 (2 × CH), 127.0 (CH), 125.8 (2 × CH), 54.8 (Az C-2,4), 51.3 (OCH_3), 43.5 ($\underline{C}H_2CO$), 42.0 (Az C-3). IR (ν_{max}, cm^{-1}): 3000, 2916, 2787, 2630, 2612 ($CH_{aromatic, aliphatic}$, NH), 1735 (C=O), 1433, 1251, 1155, 783, 710. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{16}NO_2$ 206.1176; Found 206.1176.

Methyl [3-(4-hydroxyphenyl)azetidin-3-yl]acetate hydrochloride salt (**11**): Prepared in accordance to general procedure (B) from *tert*-butyl 3-(4-hydroxyphenyl)-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate (**4**) (32 mg, 0.10 mmol). Colorless solid (19 mg, 86%), mp 224–225°C. ^1H NMR (700 MHz, DMSO-d_6): δ 9.51–9.39 (m, 3H), 7.06–7.04 (m, 2H, ArH), 6.76–6.73 (m, 2H, ArH), 4.18–4.15 (m, 4H, Az 2,4-H), 3.46 (s, 3H, OCH_3), 3.16 (s, 2H, CH_2CO). ^{13}C NMR (176 MHz, DMSO-d_6): δ 170.5 (C=O), 156.3 (C), 132.8 (C), 126.9 (2 × CH), 115.1 (2 × CH), 55.1 (Az C-2,4), 51.3 (OCH_3), 43.7 ($\underline{C}H_2CO$), 41.5 (Az C-3). IR (ν_{max}, cm^{-1}): 3227 (OH), 2919, 2810, 2623 ($CH_{aromatic, aliphatic}$, NH), 1737 (C=O), 1517, 1440, 1266, 1191, 847. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{16}NO_3$ 222.1125; Found 222.1125.

Methyl [3-(4-methoxyphenyl)azetidin-3-yl]acetate hydrochloride salt (**12**): Prepared in accordance to general procedure (B) from *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-(4-methoxyphenyl)azetidine-1-carboxylate (**5**) (33 mg, 0.10 mmol). Colorless solid (17 mg, 79%), mp 103–104°C. ^1H NMR (700 MHz, DMSO-d_6): δ 9.86 (br s, 1H), 9.23 (br s, 1H), 7.20–7.18 (m, 2H, ArH), 6.93–6.90 (m, 2H, ArH), 4.20–4.15 (m, 4H, Az 2,4-H), 3.74 (s, 3H, $PhOCH_3$), 3.46 (s, 3H, OCH_3), 3.22 (s, 2H, CH_2CO). ^{13}C NMR (176 MHz, DMSO-d_6): δ 170.5 (C=O), 158.1 (C), 134.7 (C), 127.0 (2 × CH), 113.7 (2 × CH), 55.1 (Az C-2,4), 54.9 ($PhOCH_3$), 51.3 (OCH_3), 43.6 ($\underline{C}H_2CO$), 41.5 (Az C-3). IR (ν_{max}, cm^{-1}): 3369, 2916, 2849, 2638 ($CH_{aromatic, aliphatic}$, NH), 1732 (C=O), 1516, 1436, 1244, 1190, 1027, 832. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{18}NO_3$ 236.1281; Found 236.1281.

Methyl [3-(4-chlorophenyl)azetidin-3-yl]acetate hydrochloride salt (**13**): Prepared in accordance to general procedure (B) from

tert-butyl 3-(4-chlorophenyl)-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate (**6**) (34 mg, 0.10 mmol). Colorless solid (20 mg, 85%), mp 100–101°C. ^{1}H NMR (700 MHz, DMSO-d_6): δ 9.90 (br s, 1H), 9.25 (br s, 1H), 7.44–7.42 (m, 2H, ArH), 7.32–7.30 (m, 2H, ArH), 4.24–4.22 (m, 2H, Az 2,4-H_a), 4.18–4.17 (m, 2H, Az 2,4-H_b), 3.46 (s, 3H, OCH_3), 3.27 (s, 2H, CH_2CO). ^{13}C NMR (176 MHz, DMSO-d_6): δ 170.3 (C=O), 141.7 (C), 131.8 (C), 128.3 (2 × CH), 128.0 (2 × CH), 54.6 (Az C-2,4), 51.4 (OCH_3), 43.3 ($\underline{C}H_2CO$), 41.7 (Az C-3). IR (ν_{max}, cm^{-1}): 3406, 3335, 2967, 2841 ($CH_{aromatic,\ aliphatic}$, NH), 1723 (C=O), 1402, 1273, 1230, 1009, 844, 818. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{15}ClNO_2$ 240.0786; Found 240.0786.

Methyl [3-(4-fluorophenyl)azetidin-3-yl]acetate hydrochloride salt (**14**): Prepared in accordance to general procedure (B) from *tert*-butyl 3-(4-fluorophenyl)-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate (**7**) (32 mg, 0.10 mmol). Colorless oil (19 mg, 87%). ^{1}H NMR (700 MHz, DMSO-d_6): δ 9.89 (br s, 1H), 9.25 (br s, 1H), 7.35–7.32 (m, 2H, ArH), 7.22–7.19 (m, 2H, ArH), 4.25–4.22 (m, 2H, Az 2,4-H_a), 4.20–4.17 (m, 2H, Az 2,4-H_b), 3.46 (s, 3H, OCH_3), 3.27 (s, 2H, CH_2CO). ^{13}C NMR (176 MHz, DMSO-d_6): δ 170.4 (C=O), 161.1 (d, $^1J_{C,F}$ = 243.7 Hz, C), 139.0 (d, $^4J_{C,F}$ = 3.1 Hz, C), 128.1 (d, $^3J_{C,F}$ = 8.2 Hz, 2 × CH), 115.1 (d, $^2J_{C,F}$ = 21.4 Hz, 2 × CH), 54.8 (Az C-2,4), 51.4 (OCH_3), 43.5 ($\underline{C}H_2CO$), 41.6 (Az C-3). IR (ν_{max}, cm^{-1}): 3427, 2952, 2901, 2632 ($CH_{aromatic,\ aliphatic}$, NH), 1729 (C=O), 1513, 1348, 1223, 1175, 1001, 836, 814. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{15}FNO_2$ 224.1081; Found 224.1081.

Methyl {3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetate hydrochloride salt (**15**): Prepared in accordance with general procedure (B) from *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-[4-(trifluoromethyl)phenyl]azetidine-1-carboxylate (**8**) (37 mg, 0.10 mmol). Colorless solid (23 mg, 88%), mp 56–57°C. ^{1}H NMR (700 MHz, DMSO-d_6): δ 9.58 (br s, 1H), 9.04 (br s, 1H), 7.76 (d, *J* = 8.2 Hz, 2H, ArH), 7.54 (d, *J* = 8.1 Hz, 2H, ArH), 4.31–4.29 (m, 2H, Az 2,4-H_a), 4.24–4.23 (m, 2H, Az 2,4-H_b), 3.47 (s, 3H, OCH_3), 3.29 (s, 2H, CH_2CO). ^{13}C NMR (176 MHz, DMSO-d_6): δ 170.2 (C=O), 147.4 (C), 127.7 (q, $^2J_{C,F}$ = 31.9 Hz, $\underline{C}$-CF_3), 127.1 (2 × CH), 125.3 (q, $^3J_{C,F}$ = 3.6 Hz, 2 × CH), 124.2 (q, $^1J_{C,F}$ = 272.1 Hz, CF_3), 54.5 (Az C-2,4), 51.4 (OCH_3), 43.2 ($\underline{C}H_2CO$), 42.0 (Az C-3). IR (ν_{max}, cm^{-1}): 3391, 2955, 2919, 2626 ($CH_{aromatic,\ aliphatic}$, NH), 1731 (C=O), 1437, 1327, 1197, 1158, 1112, 1076, 840. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{15}F_3NO_2$ 274.1049; Found 274.1049.

Methyl {3-[3-(trifluoromethyl)phenyl]azetidin-3-yl}acetate hydrochloride salt (**16**): Prepared in accordance to general procedure (B) from *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-[3-(trifluoromethyl)phenyl]azetidine-1-carboxylate (**9**) (37 mg, 0.10 mmol). Colorless oil (24 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.81 (br s, 1H), 9.15 (br s, 1H), 7.66–7.61 (m, 4H, ArH), 4.32–4.21 (m, 4H, Az 2,4-H), 3.45 (s, 3H, OCH_3), 3.31 (s, 2H, CH_2CO). ^{13}C NMR (126 MHz, DMSO-d_6): δ 170.3 (C=O), 144.1 (C), 130.4 (CH), 129.5 (CH), 129.1 (q, $^2J_{C,F}$ = 31.4 Hz, $\underline{C}$-CF_3), 124.1 (q, $^1J_{C,F}$ = 272.8 Hz, CF_3), 124.0 ($^3J_{C,F}$ = 3.6 Hz, CH), 122.8 ($^3J_{C,F}$ = 3.2 Hz, CH), 54.6 (Az C-2,4), 51.4 (OCH_3), 43.4 ($\underline{C}H_2CO$), 42.0 (Az C-3). IR (ν_{max}, cm^{-1}): 3409, 2954, 2903, 2633 ($CH_{aromatic,\ aliphatic}$, NH), 1729 (C=O), 1438, 1321, 1164, 1119, 1067, 897, 703. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{15}F_3NO_2$ 274.1049; Found 274.1050.

4.1.5 | Procedure for the synthesis of compound **17**

{1-(*tert*-Butoxycarbonyl)-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetic acid (**17**): To a solution of *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-[4-(trifluoromethyl)phenyl]azetidine-1-carboxylate (**8**) (112 mg, 0.27 mmol) in MeOH (1 mL), 2 N NaOH (0.386 mL) was added dropwise at 0°C and the resulting mixture was stirred at the room temperature for 24 h. Upon completion, MeOH was removed in vacuo, the residue was dissolved in water (10 mL) and acidified with 1 M $NaHSO_4$ solution to pH 6–7. The resulting mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:4, v/v) to give pure **17** as a colorless oil (101 mg, 95%). R_f = 0.32 (DCM/MeOH, 100:3, v/v). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.41 (br s, 1H), 7.58 (d, *J* = 8.1 Hz, 2H, ArH), 7.34 (d, *J* = 8.1 Hz, 2H, ArH), 4.23–4.22 (m, 2H, Az 2,4-H_a), 4.19–4.17 (m, 2H, Az 2,4-H_b), 3.02 (s, 2H, CH_2CO), 1.42 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 174.6 (C=O), 156.6 (C=O), 147.8 (C), 129.3 (q, $^2J_{C,F}$ = 32.5 Hz, $\underline{C}$-CF_3), 126.8 (2 × CH), 125.6 (q, $^3J_{C,F}$ = 3.6 Hz, 2 × CH), 124.1 (q, $^1J_{C,F}$ = 272.1 Hz, CF_3), 80.5 ($\underline{C}(CH_3)_3$), 60.5–59.7 (Az C-2,4), 45.0 ($\underline{C}H_2CO$), 39.4 (Az C-3), 28.4 ($C(\underline{C}H_3)_3$). IR (ν_{max}, cm^{-1}): 3072, 2977, 2892 ($CH_{aromatic,\ aliphatic}$, OH), 1730, 1700 (C=O), 1410, 1323, 1160, 1112, 1069, 1015, 840. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{17}H_{20}F_3NO_4Na$ 382.1237; Found 382.1239.

4.1.6 | Procedure for the synthesis of compound **18**

{3-[4-(Trifluoromethyl)phenyl]azetidin-3-yl}acetic acid hydrogen chloride salt (**18**): To a solution of {1-(*tert*-butoxycarbonyl)-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetic acid (**17**) (36 mg, 0.1 mmol) in 1,4-dioxane (1 mL), 4 N HCl in dioxane (2 mL) was added dropwise under argon and the resulting mixture was stirred at the room temperature for 3 h. Upon completion, the solvent was removed in vacuo. The residue was dissolved in Et_2O (3 × 4 mL) and solvent was removed again in vacuo and afforded pure compound **18** as a colorless solid (26 mg, 77%), mp 183–184°C. ^{1}H NMR (700 MHz, DMSO-d_6): δ 12.43 (br s, 1H, COOH), 9.65 and 9.22 (overlapping br s, 2H, NH, HCl), 7.75 (d, *J* = 8.2 Hz, 2H, ArH), 7.56 (d, *J* = 8.2 Hz, 2H, ArH), 4.28–4.27 (m, 2H, Az 2,4-H_a), 4.22–4.21 (m, 2H, Az 2,4-H_b), 3.21 (s, 2H, CH_2CO). ^{13}C NMR (176 MHz, DMSO-d_6): δ 171.3 (C=O), 147.7 (C), 127.6 (q, $^2J_{C,F}$ = 31.9 Hz, $\underline{C}$-CF_3), 127.2 (2 × CH), 125.2 (q, $^3J_{C,F}$ = 3.7 Hz, 2 × CH), 124.2 (q, $^1J_{C,F}$ = 271.9 Hz, CF_3), 54.7 (Az C-2,4), 43.6 ($\underline{C}H_2CO$), 41.9 (Az C-3). ^{19}F NMR (376 MHz, $CDCl_3$): δ −60.9 (CF_3). IR (ν_{max}, cm^{-1}): 3414, 3185, 2947, 2592 ($CH_{aromatic,\ aliphatic}$, OH, NH), 1716 (C=O), 1549, 1325, 1108, 1074, 840. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_{13}F_3NO_2$ 260.0893; Found 260.0892.

4.1.7 | Procedure for the synthesis of compound **19**

Methyl {1-methyl-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetate (**19**): Method I: To a solution of *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-[4-

(trifluoromethyl)phenyl]azetidine-1-carboxylate (**8**) (70 mg, 0.18 mmol) in dry DCM (4 mL), TFA (0.8 mL) was added dropwise at 0°C and the resulting mixture was warmed to room temperature. After 30 min, reaction mixture was cooled to 0°C, quenched by slow addition of aqueous saturated $NaHCO_3$ solution until pH 7 and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated. Subsequently, obtained methyl {3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetate was dissolved in MeOH (2.2 mL) to which triethylamine (37 mg, 0.36 mmol) was added dropwise. After 10 min 37% aqueous formaldehyde (0.05 mL, 0.91 mmol), AcOH (1.1 mg, 0.02 mmol, 10 mol%) and $NaBH_4$ (69 mg, 1.82 mmol) were added and the reaction mixture was stirred at room temperature for 16 h. Upon completion, the solvent was removed in vacuo and the residue was dissolved in ethyl acetate (10 mL), washed with saturated aqueous $NaHCO_3$ solution (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **19** as a colorless oil (22 mg, 42%). Method II: To a solution of *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-[4-(trifluoromethyl)phenyl]azetidine-1-carboxylate (**8**) (70 mg, 0.18 mmol) in dry DCM (4 mL), TFA (0.8 mL) was added dropwise at 0°C and the resulting mixture was warmed to room temperature. After 30 min, reaction mixture was cooled to 0°C, quenched by slow addition of aqueous saturated $NaHCO_3$ solution until pH 7 and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated. Subsequently, to a solution of obtained methyl {3-[4-(trifluoromethyl)phenyl]azetidin-3-yl} acetate in 1,4-dioxane (2 mL) was added AcOH (0.08 mL, 1.44 mmol), 37% aqueous formaldehyde (0.03 mL, 0.27 mmol), zinc dust (24 mg, 0.36 mmol) and the reaction mixture was stirred at room temperature for 16 h. Upon completion, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **19** as a colorless oil (17 mg, 33%). R_f = 0.32 (DCM/MeOH, 100:5, v/v). ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.56 (d, J = 8.0 Hz, 2H, ArH), 7.27 (d, J = 8.0 Hz, 2H, ArH), 3.70–3.69 (m, 2H, Az 2,4-H_a), 3.52 (s, 3H, OCH_3), 3.38–3.37 (m, 2H, Az 2,4-H_b), 3.06 (s, 2H, CH_2CO), 2.34 (s, 3H, CH_3). ^{13}C NMR (176 MHz, $CDCl_3$): δ 171.4 (C=O), 149.7 (C), 128.8 (q, $^2J_{C,F}$ = 32.5 Hz, C-CF_3), 126.6 (2 × CH), 125.5 (q, $^3J_{C,F}$ = 3.8 Hz, 2 × CH), 124.3 (q, $^1J_{C,F}$ = 271.8 Hz, CF_3), 66.4 (Az C-2,4), 51.5 (OCH_3), 45.8 (CH_3), 45.0 (CH_2CO), 40.7 (Az C-3). ^{19}F NMR (376 MHz, $CDCl_3$): δ −62.5 (CF_3). IR (ν_{max}, cm^{-1}): 2938, 2832 2773 ($CH_{aromatic,\ aliphatic}$), 1735 (C=O), 1323, 1161, 1111, 1068, 839. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{14}H_{17}F_3NO_2$ 288.1206; Found 288.1206.

4.1.8 | General procedure C for the synthesis of compounds 20–22

To a solution *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-[4-(trifluoromethyl) phenyl]azetidine-1-carboxylate (**8**) (136 mg, 0.36 mmol) in dry DCM (4 mL), TFA (0.8 mL) was added drop-wise at 0°C and the resulting mixture was warmed up to room temperature. After 30 min, reaction mixture was cooled to 0°C, quenched by slow addition of aqueous saturated $NaHCO_3$ solution until pH 7 and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated. The obtained crude methyl {3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetate was dissolved in MeCN (3 mL) and cooled to 0°C. Then K_2CO_3 (126 mg, 0.91 mmol) and appropriate iodoalkane (0.73 mmol) were added. The reaction mixture was warmed up to room temperature and stirred for 24 h. Upon completion, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude product was purified by column chromatography to give pure compounds **20**–**22**.

Methyl {1-ethyl-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetate (**20**): Prepared in accordance to general procedure (C) using iodoethane (0.059 mL, 0.73 mmol). The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure compound **20** as a colorless oil (40 mg, 36%). R_f = 0.25 (DCM/MeOH, 100:3, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57 (d, J = 8.0 Hz, 2H, ArH), 7.28 (d, J = 7.8 Hz, 2H, ArH), 3.90–3.81 (m, 2H, Az 2,4-H_a), 3.52 (s, 3H, OCH_3), 3.45–3.44 (m, 2H, Az 2,4-H_b), 3.14 (s, 2H, CH_2CO), 2.58 (q, J = 7.1 Hz, 2H, CH_3CH_2), 1.03 (t, J = 7.2 Hz, 3H, CH_3CH_2). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.9 (C=O), 148.3 (C), 129.4 (q, $^2J_{C,F}$ = 32.7 Hz, C-CF_3), 126.5 (2 × CH), 125.8 ($^3J_{C,F}$ = 3.8 Hz, 2 × CH), 124.1 (q, $^1J_{C,F}$ = 272.1 Hz, CF_3), 63.3 (Az C-2,4), 52.7 (CH_3CH_2), 51.8 (OCH_3), 44.4 (CH_2CO), 40.5 (Az C-3), 11.3 (CH_3CH_2). ^{19}F NMR (376 MHz, $CDCl_3$): δ −62.7 (CF_3). IR (ν_{max}, cm^{-1}): 2956, 2936, 2831 ($CH_{aromatic,\ aliphatic}$), 1734 (C=O), 1323, 1163, 1113, 1068, 840. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{15}H_{19}F_3NO_2$ 302.1362; Found 302.1362.

Methyl {1-propyl-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetate (**21**): Prepared in accordance to general procedure (C) using 1-iodopropane (0.073 mL, 0.73 mmol). The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **21** as a colorless oil (46 mg, 40%). R_f = 0.28 (DCM/MeOH, 100:3, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57 (d, J = 8.1 Hz, 2H, ArH), 7.27 (d, J = 8.2, 2H, ArH), 3.89–3.87 (m, 2H, Az 2,4-H_a), 3.52 (s, 3H, OCH_3), 3.46–3.44 (m, 2H, Az 2,4-H_b), 3.14 (s, 2H, CH_2CO), 2.50 (t, J = 7.6, 2H, $CH_3CH_2CH_2$), 1.44 (h, J = 7.5, 2H, CH_3CH_2), 0.90 (t, J = 7.4, 3H, CH_3CH_2). ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.3 (C=O), 149.3 (C), 129.0 (q, $^2J_{C,F}$ = 32.6 Hz, C-CF_3), 126.5 (2 × CH), 125.6 ($^3J_{C,F}$ = 3.6 Hz, 2 × CH), 124.2 (q, $^1J_{C,F}$ = 272.2 Hz, CF_3), 64.3 (Az C-2,4), 61.1 ($CH_3CH_2CH_2$), 51.6 (OCH_3), 44.8 (CH_2CO), 40.8 (Az C-3), 20.5 (CH_3CH_2), 11.8 (CH_3CH_2). ^{19}F NMR (376 MHz, $CDCl_3$): δ −62.5 (CF_3). IR (ν_{max}, cm^{-1}): 2957, 2932, 2823 ($CH_{aromatic,\ aliphatic}$), 1737 (C=O), 1323, 1162, 1112, 1068, 840. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{16}H_{21}F_3NO_2$ 316.1519; Found 316.1519.

Methyl {1-isopropyl-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl} acetate (**22**): Prepared in accordance to general procedure (C) using 2-iodopropane (0.073 mL, 0.73 mmol). The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **22** as a colorless oil (40 mg, 35%). R_f = 0.28 (DCM/MeOH, 100:3, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57–7.56

(m, 2H, ArH), 7.28–7.27 (m, 2H, ArH), 3.88–3.75 (m, 2H, Az 2,4-H_a), 3.52 (s, 3H, OCH_3), 3.45–3.43 (m, 2H, Az 2,4-H_b), 3.13 (s, 2H, CH_2CO), 2.45–2.36 (m, 1H, CH), 0.99 (d, J = 6.3, 6H, $CH(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.4 (C=O), 149.5 (C), 128.9 (q, $^2J_{C,F}$ = 32.0 Hz, C-CF_3), 126.6 (2 × CH), 125.5 ($^3J_{C,F}$ = 3.5 Hz, 2 × CH), 124.2 (q, $^1J_{C,F}$ = 271.8 Hz, CF_3), 62.9 (Az C-2,4), 58.8 ($CH(CH_3)_2$), 51.6 (OCH_3), 45.0 (CH_2CO), 39.3 (Az C-3), 19.3 ($(CH_3)_2$). ^{19}F NMR (376 MHz, $CDCl_3$): δ −62.6 (CF_3). IR (ν_{max}, cm^{-1}): 2965, 2938, 2825 ($CH_{aromatic,\ aliphatic}$), 1734 (C=O), 1323, 1163, 1113, 1073, 839. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{16}H_{21}F_3NO_2$ 316.1519; Found 316.1519.

4.1.9 | General procedure D for the synthesis of compounds 23–28

To a solution of *tert*-butyl 3-(2-methoxy-2-oxoethyl)-3-[4-(trifluoromethyl)phenyl]azetidine-1-carboxylate (**8**) (50 mg, 0.14 mmol) in dry DCM (4 mL), TFA (0.8 mL) was added dropwise at 0°C and the resulting mixture was warmed up to room temperature. After 30 min, reaction mixture was cooled to 0°C, quenched by slow addition of aqueous saturated $NaHCO_3$ solution until pH 7 and extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated. Subsequently, to a solution of obtained methyl {3-[4-(trifluoromethyl)phenyl]azetidin-3-yl} acetate in DCM (1 mL), triethylamine (0.048 mL, 0.35 mmol) and appropriate acyl chloride (0.21 mmol) were added and the reaction mixture was stirred at room temperature for 2 h. Upon completion, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude product was purified by column chromatography to give pure compounds **23**–**28**.

Methyl {1-acetyl-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetate (**23**): Prepared in accordance to general procedure (D) using acetyl chloride (0.007 mL, 0.21 mmol). The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **23** as a colorless oil (26 mg, 61%). R_f = 0.27 (DCM/MeOH, 100:3, v/v). ^1H NMR (400 MHz, $CDCl_3$): δ 7.59 (d, J = 8.2 Hz, 2H, ArH), 7.32 (d, J = 8.1 Hz, 2H, ArH), 4.47–4.43 (m, 2H, Az 2,4-H_a), 4.29–4.22 (m, 2H, Az 2,4-H_b), 3.54 (s, 3H, OCH_3), 3.04–2.94 (m, 2H, CH_2CO), 1.88 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.9 (C=O), 170.4 (C=O), 147.4 (C), 129.5 (q, $^2J_{C,F}$ = 32.6 Hz, C-CF_3), 126.6 (2 × CH), 125.7 (q, $^3J_{C,F}$ = 3.8 Hz, 2 × CH), 124.0 (q, $^1J_{C,F}$ = 272.1 Hz, CF_3), 60.8 and 58.9 (Az C-2,4), 51.8 (OCH_3), 45.1 (CH_2CO), 39.4 (Az C-3), 18.9 (CH_3). IR (ν_{max}, cm^{-1}): 2955, 2885 ($CH_{aromatic,\ aliphatic}$), 1734, 1648 (C=O), 1436, 1323, 1163, 1112, 1067, 840. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{15}H_{16}F_3NO_3Na$ 338.0974; Found 338.0975.

Methyl {1-(2-methylpropanoyl)-3-[4-(trifluoromethyl)phenyl] azetidin-3-yl}acetate (**24**): Prepared in accordance to general procedure (D) using isobutyryl chloride (0.021 mL, 0.21 mmol). The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **24** as a colorless oil (20 mg, 43%). R_f = 0.30 (DCM/MeOH, 100:3, v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 7.61 (d, J = 8.2 Hz, 2H, ArH), 7.34 (d, J = 8.2 Hz, 2H, ArH), 4.50–4.48 (m, 2H, Az 2,4-H_a), 4.30–4.24 (m, 2H, Az 2,4-H_b), 3.56 (s, 3H, OCH_3), 3.04–2.96 (m, 2H, CH_2CO), 2.47 (hept, J = 6.8 Hz, 1H, CH), 1.13 (d, J = 6.8 Hz, 3H, $CH(CH_3)$), 1.07 (d, J = 6.9 Hz, 3H, $CH(CH_3)$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 177.4 (C=O), 170.5 (C=O), 147.6 (C), 129.6 (q, $^2J_{C,F}$ = 32.6 Hz, C-CF_3), 126.7 (2 × CH), 125.8 (q, $^3J_{C,F}$ = 3.7 Hz, 2 × CH), 124.1 (q, $^1J_{C,F}$ = 272.0 Hz, CF_3), 60.6 and 58.9 (Az C-2,4), 51.9 (OCH_3), 45.3 (CH_2CO), 39.8 (Az C-3), 30.1 ($CH(CH_3)_2$), 19.0 (CH_3), 18.9 (CH_3). IR (ν_{max}, cm^{-1}): 2970, 2881 ($CH_{aromatic,\ aliphatic}$), 1734, 1646 (C=O), 1436, 1322, 1163, 1113, 1071, 840. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{17}H_{20}F_3NO_3Na$ 366.1287; Found 366.1286.

Methyl {1-(2,2-dimethylpropanoyl)-3-[4-(trifluoromethyl)phenyl] azetidin-3-yl}acetate (**25**): Prepared in accordance to general procedure (D) using trimethylacetyl chloride (0.025 mL, 0.21 mmol). The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **25** as a colorless oil (30 mg, 62%). R_f = 0.35 (DCM/MeOH, 100:2, v/v). ^1H NMR (500 MHz, $CDCl_3$): δ 7.60 (d, J = 8.1 Hz, 2H, ArH), 7.33 (d, J = 8.1 Hz, 2H, ArH), 4.70–4.29 (m, 4H, Az 2,4-H), 3.55 (s, 3H, OCH_3), 2.99 (m, 2H, CH_2CO), 1.19 (s, 9H, $(CH_3)_3$). ^{13}C NMR (126 MHz, $CDCl_3$): δ 178.3 (C=O), 170.7 (C=O), 147.8 (C), 129.5 (q, $^2J_{C,F}$ = 32.6 Hz, C-CF_3), 126.7 (2 × CH), 125.8 ($^3J_{C,F}$ = 3.5 Hz, 2 × CH), 124.1 (q, $^1J_{C,F}$ = 272.1 Hz, CF_3), 61.8 (Az C-2,4), 51.9 (OCH_3), 45.3 (CH_2CO), 40.0 (Az C-3), 38.8 (C), 27.3 ($(CH_3)_3$). IR (ν_{max}, cm^{-1}): 2959, 2882 ($CH_{aromatic,\ aliphatic}$), 1735, 1618 (C=O), 1412, 1323, 1162, 1113, 1071, 840. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{18}H_{22}F_3NO_3Na$ 380.1444; Found 380.1444.

Methyl {1-benzoyl-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl} acetate (**26**): Prepared in accordance to general procedure (D) using benzoyl chloride (0.025 mL, 0.21 mmol). The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **26** as a colorless oil (29 mg, 57%). R_f = 0.32 (DCM/MeOH, 100:3, v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 7.66–7.64 (m, 2H, ArH), 7.62–7.61 (m, 2H, ArH), 7.49–7.47 (m, 1H, ArH), 7.43–7.41 (m, 2H, ArH), 7.36–7.35 (m, 2H, ArH), 4.66–4.61 (m, 2H, Az 2,4-H_a), 4.52–4.47 (m, 2H, Az 2,4-H_b), 3.56 (s, 3H, OCH_3), 3.08–3.01 (m, 2H, CH_2CO). ^{13}C NMR (176 MHz, $CDCl_3$): δ 170.7 (C=O), 170.5 (C=O), 147.6 (C), 132.9 (C), 131.4 (CH), 129.6 (q, $^2J_{C,F}$ = 32.6 Hz, C-CF_3), 128.6 (2 × CH), 128.1 (2 × CH), 126.7 (2 × CH), 125.8 (q, $^3J_{C,F}$ = 3.7 Hz, 2 × CH), 124.1 (q, $^1J_{C,F}$ = 271.9 Hz, CF_3), 63.7 and 59.8 (Az C-2,4), 51.9 (OCH_3), 45.3 (CH_2CO), 40.5 (Az C-3). ^{19}F NMR (376 MHz, $CDCl_3$): δ −62.6 (CF_3). IR (ν_{max}, cm^{-1}): 2954, 2888 ($CH_{aromatic,\ aliphatic}$), 1734, 1631 (C=O), 1410, 1323, 1164, 1111, 1070, 840, 706. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{20}H_{18}F_3NO_3Na$ 400.1131; Found 400.1132.

Methyl {1-(phenylacetyl)-3-[4-(trifluoromethyl)phenyl]azetidin-3-yl}acetate (**27**): Prepared in accordance to general procedure (D) using phenylacetyl chloride (0.028 mL, 0.21 mmol). The crude product was purified by column chromatography (SiO_2, eluent: DCM/MeOH, 100:3, v/v) to give pure **27** as a colorless solid (38 mg, 74%), mp 90–91°C. R_f = 0.33 (DCM/MeOH, 100:2, v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 7.60–7.59 (m, 2H, ArH), 7.33–7.24 (m, 7H, ArH), 4.44–4.41 (m, 2H, Az 2,4-H_a), 4.32–4.27 (m, 2H, Az 2,4-H_b),

3.54 (s, 3H, OCH_3), 3.52 (s, 2H, CH_2Ph), 2.99–2.93 (m, 2H, CH_2CO). ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.2 (C=O), 170.4 (C=O), 147.4 (C), 134.3 (C), 129.6 (q, $^2J_{C,F}$ = 32.6 Hz, C-CF_3), 129.1 (2 × CH), 128.9 (2 × CH), 127.1 (2 × CH), 126.6 (CH), 125.8 (q, $^3J_{C,F}$ = 3.7 Hz, 2 × CH), 124.0 (q, $^1J_{C,F}$ = 271.9 Hz, CF_3), 61.0 and 59.2 (Az C-2,4), 51.9 (OCH_3), 45.1 (CH_2CO), 39.8 (Az C-3), 39.5 (CH_2Ph). ^{19}F NMR (376 MHz, $CDCl_3$): δ −62.6 (CF_3). IR (ν_{max}, cm^{-1}): 3056, 3030, 2954, 2888 ($CH_{aromatic,\ aliphatic}$), 1726, 1631 (C=O), 1467, 1439, 1329, 1158, 1124, 1111, 838, 720. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{21}H_{20}F_3NO_3Na$ 414.1287; Found 414.1288.

Benzyl 3-(2-methoxy-2-oxoethyl)-3-[4-(trifluoromethyl)phenyl] azetidine-1-carboxylate (**28**): Prepared in accordance to general procedure (D) using benzyl chloroformate (0.030 mL, 0.21 mmol). The crude product was purified by column chromatography (SiO_2, eluent: ethyl acetate/*n*-hexane, 1:2, v/v) to give pure **28** as a colorless oil (33 mg, 60%). R_f = 0.27 (ethyl acetate/*n*-hexane, 1:2, v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 7.60 (d, *J* = 8.2 Hz, 2H, 4-CF_3Ph 3',5'-H), 7.37–7.34 (m, 4H, Ph 2",3",5",6"-H), 7.33–7.30 (m, 3H, 4-CF_3Ph 2',6'-H, Ph 4"-H), 5.10 (s, 2H, CH_2Ph), 4.35 (d, $^2J_{Ha,\ Hb}$ = 8.7 Hz, 2H, Az 2,4-H_a), 4.32–4.27 (m, 2H, Az 2,4-H_b), 3.55 (s, 3H, OCH_3), 3.02 (s, 2H, CH_2CO). ^{13}C NMR (176 MHz, $CDCl_3$): δ 170.4 (CH_2C=O), 156.4 (NC=O), 147.6 (4-CF_3Ph C-1'), 136.4 (Ph C-1"), 129.3 (q, $^2J_{C,F}$ = 32.5 Hz, 4-CF_3Ph C-4'), 128.5 (Ph C-3",5"), 128.2 (Ph C-4"), 128.1 (Ph C-2",6"), 126.5 (4-CF_3Ph C-2',6'), 125.6 (q, $^3J_{C,F}$ = 3.7 Hz, 4-CF_3Ph C-3',5'), 124.0 (q, $^1J_{C,F}$ = 272.1 Hz, CF_3), 66.9 (CH_2Ph), 60.2–60.0 (Az C-2,4), 51.7 (OCH_3), 45.1 (CH_2CO), 40.1 (Az C-3). ^{15}N NMR (71 MHz, $CDCl_3$): δ −315.1 (N-1). ^{19}F NMR (376 MHz, $CDCl_3$): δ −62.6 (CF_3). IR (ν_{max}, cm^{-1}): 3066, 3034, 2955, 2890 ($CH_{aromatic,\ aliphatic}$), 1734, 1706 (C=O), 1411, 1323, 1163, 1111, 1068, 840, 696. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{21}H_{20}F_3NO_4Na$ 430.1237; Found 430.1239.

4.2 | Pharmacological/biological assays

4.2.1 | Drugs and reagents

Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12), penicillin, streptomycin, fetal bovine serum, trypsin, propidium iodide, dihydroethidium, all-*trans* retinoic acid, *N*-acetylcysteine, deferoxamin, Glu monosodium salt, buffer components for One-step caspase 3,7 assay, and caspase-3,7 substrate (Ac-DEVD-AFC), lactate dehydrogenase (LDH) cytotoxicity kit were purchased from Sigma-Aldrich Merck. Calcein AM solution were obtained from ThermoFisher. SAL hydrochloride was obtained from Santiago Chemicals.

4.2.2 | AChE and BChE inhibition studies

Inhibitory activity of AChE from electric eel (eeAChE) and BChE from equine serum (eqBChE) using modified Ellman's method. The procedure was described in detail in.[65] The inhibitory activity of studied compounds was expressed as IC_{50} (i.e., 50% inhibiting concentration). We also calculated selectivity indices (SI) as a ratio of IC_{50} value for BChE/IC_{50} or AChE to measure the preference for inhibition of both enzymes. The value of SI over 1 indicates more potent inhibition of AChE. Galantamine, a drug used to treat Alzheimer's disease, was used as a standard.

4.2.3 | Kinetic studies

The modified Ellman's method was also used to determine the type of inhibition. The measuring procedure was similar to that for the determination of IC_{50}. The AChE activity in the final reaction mixture was 0.2 U/mL and the concentration of DTNB 0.1 mM. For the measurement, following concentrations of the substrate (i.e., acetylthiocholine, ATCh) were used: 20, 40, 60 and 80 μM. For each ATCh concentration four different concentrations of inhibitor were chosen. The measurement of dependence absorbance (412 nm) versus time was performed in duplicate at least for each combination of substrate and inhibitor concentration. The Lineweaver–Burk plot was constructed, the values of Michaelis constant (K_M) and maximum velocity (V_m) were calculated and the type of inhibition was elucidated.

4.2.4 | Molecular docking

Molecular docking was performed into the crystal structure of human AChE (PDB: 4EY7). The 3D structure of compounds **26** and **27** were prepared in Molecular Operating Environmet 2010.12 (Chemical computing group). Additionally, the addition polar hydrogens and energy minimization according to force field MMFF94x with gradient 0.0001 were performed for each ligand. Before the docking, the preparation of protein included water and solvents removing and addition of hydrogens. Ligands and protein were preprepared for docking using AutoDock Tools program.[48] The docking study was performed by AutoDock Vina 1.05[49] with subsequent figure generation in Pymol ver. 2.6.0a0 (Schrödinger, LLC).

4.2.5 | Cell culture

The cell line SH-SY5Y was purchased from European Collection of Authenticated Cell Cultures (ECACC) and was cultivated in DMEM/Ham's Nutrient mix (1:1) and maintained in humidified CO_2 incubator at 37°C with trypsin passaging procedure twice or thrice a week. Up to passage 20, cells were used for biological evaluations. Before the cytotoxicity or neuroprotection tests cells underwent all-*trans* retinoic acid-mediated differentiation procedure as described previously.[68] Endothelial cell proliferation medium (Provitro) with recommended supplements and 10% of heat inactivated fetal bovine serum (Sigma-Aldrich, Merck) was used for Human Umbilical Vein Endothelial cells (HUVEC) cultivation under standard conditions. HUVECs were a kind gift from Prof. Jitka Ulrichová from Faculty of Medicine and Dentistry of Palacký University in Olomouc.

4.2.6 | Cell treatment, safety and neuroprotective activity evaluation

Neuron-like SH-SY5Y cells were seeded to 96-multiwell plates and differentiated in densities according to assays: 7000 cells/well (calcein AM viability assay) and 20,000 cells/well (propidium iodide/lactate dehydrogenase toxicity assays). For safety tests, cells were tested in 0.1; 1; 10 μM for 24 h. The neuroprotectivity tests were performed in co-treatments in concentration range 0.1; 1; 10 μM with toxins SAL (800 μM) or Glu (160 mM) for 24 h. Resulting activity was measured by Calcein AM (viability), propidium iodide and commercial lactate dehydrogenase release assay (LDH kit) (toxicity) as previously described.[68]

4.2.7 | Measurement of superoxide radical formation (oxidative stress)

Neuron-like SH-SY5Y cells were co-treated with compounds and Glu (160 mM) for 4 h and evaluated by dihydroethidium assay as described previously.[68]

4.2.8 | Measurement of caspase-3/7 (oxidative stress)

Caspase-3/7 activation in neuron-like SH-SY5Y was evaluated after 1 h co-treatment with compounds and Glu (160 mM) by ONE-STEP cellular caspase-3/7 assay using caspase-3/7 substrate Ac-DEVD-AFC (ex./em. 400/505 nm) according to previously described procedure.[81]

4.2.9 | Measurement of IL-6 production by sandwich ELISA

HUVECs were seeded into 24 well-plates and grown to confluence. After 24 h, cells were pretreated with compound **28** (10 μM), narciclasine (150 nM) as positive control or DMSO as a vehicle control for 30 min and immediately stimulated with pro-inflammatory cytokine TNF-α (10 ng/mL) for 24 h. Level of IL-6 was determined by ELISA development Kit (PeproTech) in culture medium as described previously.[80] Cytotoxicity of compound **28** (10 μM) was evaluated in HUVECs for 24 h by resazurin as published before[82] and was found as nontoxic (Supporting Information: Figure S2).

4.2.10 | Statistical analysis

All data are shown as means ± SEM and visualized in bar graphs prepared by GraphPad Prism 9.3.1 (GraphPad software). Data were tested for the normality of distribution by Levene's test. Data with normal distribution were evaluated by analysis of variance with Tukey multiple comparison test, while nonnormal distributed data were assessed by nonparametric Kruskal-Wallis test followed by post-hoc Mann–Whitney test with sequential Bonferroni correction of *p*-values in PAST software (version 1.97).[83] p-value < 0.05 was considered statistically significant.

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CONFLICTS OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

[1] M. A. DeTure, D. W. Dickson, *Mol. Neurodegener.* **2019**, *14*, 32.
[2] T. Gómez-Isla, J. L. Price, D. W. McKeel Jr., J. C. Morris, J. H. Growdon, B. T. Hyman, *J. Neurosci.* **1996**, *16*, 4491.
[3] C. G. Rasool, C. N. Svendsen, D. J. Selkoe, *Ann. Neurol.* **1986**, *20*, 482.
[4] C. H. Andrade-Moraes, A. V. Oliveira-Pinto, E. Castro-Fonseca, C. G. da Silva, D. M. Guimaraes, D. Szczupak, D. R. Parente-Bruno, L. R. B. Carvalho, L. Polichiso, B. V. Gomes, L. M. Oliveira, R. D. Rodriguez, R. E. P. Leite, R. E. L. Ferretti-Rebustini, W. Jacob-Filho, C. A. Pasqualucci, L. T. Grinberg, R. Lent, *Brain* **2013**, *136*, 3738.
[5] R. B. Postuma, D. Berg, M. Stern, W. Poewe, C. W. Olanow, W. Oertel, J. Obeso, K. Marek, I. Litvan, A. E. Lang, G. Halliday, C. G. Goetz, T. Gasser, B. Dubois, P. Chan, B. R. Bloem, C. H. Adler, G. Deuschl, *Mov. Disord.* **2015**, *30*, 1591.
[6] E. Tönnies, E. Trushina, *J. Alzheimer's Dis.* **2017**, *57*, 1105.
[7] P. M. A. Antony, N. J. Diederich, R. Krüger, R. Balling, *FEBS J.* **2013**, *280*, 5981.
[8] H. Yu, P. Guo, X. Xie, Y. Wang, G. Chen, *J. Cell. Mol. Med.* **2017**, *21*, 648.
[9] K. G. Yiannopoulou, S. G. Papageorgiou, *Ther. Adv. Neurol. Disord.* **2013**, *6*, 19.
[10] J. Cummings, G. Lee, A. Ritter, M. Sabbagh, K. Zhong, *Alzheimer's Dement. Transl. Res. Clin. Intervent.* **2019**, *5*, 272.
[11] J.-H. Chen, T.-W. Huang, C.-T. Hong, *npj Park. Dis.* **2021**, *7*, 103.
[12] V. L. Dawson, T. M. Dawson, *Sci. Transl. Med.* **2019**, *11*, eaba1659. https://doi.org/10.1126/scitranslmed.aba1659
[13] D. Galimberti, E. Scarpini, *Ther. Adv. Neurol. Disord.* **2011**, *4*, 203.
[14] E. Vitaku, D. T. Smith, J. T. Njardarson, *J. Med. Chem.* **2014**, *57*, 10257.

[15] E. Rubenstein, H. Zhou, K. M. Krasinska, A. Chien, C. H. Becker, *Phytochemistry* **2006**, *67*, 898.
[16] Y. Aoyagi, *Phytochemistry* **2006**, *67*, 618.
[17] C.-Y. Lai, I.-W. Lo, R. T. Hewage, Y.-C. Chen, C.-T. Chen, C.-F. Lee, S. Lin, M.-C. Tang, H.-C. Lin, *Angew. Chem. Int. Ed.* **2017**, *56*, 9478.
[18] F. Yan, D. Auerbach, Y. Chai, L. Keller, Q. Tu, S. Hüttel, A. Glemser, H. A. Grab, T. Bach, Y. Zhang, R. Müller, *Angew. Chem. Int. Ed.* **2018**, *57*, 8754.
[19] K. Higuchi, K. Suzuki, H. Nakanishi, H. Yamaguchi, N.-K. Nishizawa, S. Mori, *Plant Physiol.* **1999**, *119*, 471.
[20] J. Kobayashi, J.-F. Cheng, M. Ishibashi, M. R. Wälchli, S. Yamamura, Y. Ohizumi, *J. Chem. Soc. Perkin Trans.* **1991** (5), 1135. https://doi.org/10.1039/P19910001135
[21] D. R. Parmar, J. Y. Soni, R. Guduru, R. H. Rayani, R. V. Kusurkar, A. G. Vala, *Arch. Pharm.* **2021**, *354*, e2100062. https://doi.org/10.1002/ardp.202100062
[22] B. Bechi, D. Amantini, C. Tintori, M. Botta, R. Fabio, *Beilstein J. Org. Chem.* **2014**, *10*, 1114.
[23] A. P. Kozikowski, W. Tuckmantel, Y. Liao, H. Manev, S. Ikonomovic, J. T. Wroblewski, *J. Med. Chem.* **1993**, *36*, 2706.
[24] A. P. Kozikowski, Y. Liao, W. Tückmantel, S. Wang, S. Pshenichkin, A. Surin, C. Thomsen, J. T. Wroblewski, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2559.
[25] A. P. Kozikowski, W. Tuckmantel, I. J. Reynolds, J. T. Wroblewski, *J. Med. Chem.* **1990**, *33*, 1561.
[26] J. T. Lowe, M. D. Lee, L. B. Akella, E. Davoine, E. J. Donckele, L. Durak, J. R. Duvall, B. Gerard, E. B. Holson, A. Joliton, S. Kesavan, B. C. Lemercier, H. Liu, J. C. Marié, C. A. Mulrooney, G. Muncipinto, M. Welzel-O'Shea, L. M. Panko, A. Rowley, B. C. Suh, M. Thomas, F. F. Wagner, J. Wei, M. A. Foley, L. A. Marcaurelle, *J. Org. Chem.* **2012**, *77*, 7187.
[27] V. J. Cee, M. Frohn, B. A. Lanman, J. Golden, K. Muller, S. Neira, A. Pickrell, H. Arnett, J. Buys, A. Gore, M. Fiorino, M. Horner, A. Itano, M. R. Lee, M. McElvain, S. Middleton, M. Schrag, D. Rivenzon-Segal, H. M. Vargas, H. Xu, Y. Xu, X. Zhang, J. Siu, M. Wong, R. W. Bürli, *ACS Med. Chem. Lett.* **2011**, *2*, 107.
[28] S. Pan, N. S. Gray, W. Gao, Y. Mi, Y. Fan, X. Wang, T. Tuntland, J. Che, S. Lefebvre, Y. Chen, A. Chu, K. Hinterding, A. Gardin, P. End, P. Heining, C. Bruns, N. G. Cooke, B. Nuesslein-Hildesheim, *ACS Med. Chem. Lett.* **2013**, *4*, 333.
[29] L. J. Scott, *CNS Drugs* **2020**, *34*, 1191.
[30] S. J. Yang, J. W. Yang, J. M. Na, J. S. Ha, S. Y. Choi, S. W. Cho, *BMB Rep.* **2018**, *51*, 590.
[31] E.-A. Kim, J.-M. Na, J. Kim, S. Y. Choi, J.-Y. Ahn, S.-W. Cho, *J. Neuroimmune Pharmacol.* **2017**, *12*, 447.
[32] E.-A. Kim, C. H. Cho, J. Kim, H.-G. Hahn, S. Y. Choi, S.-J. Yang, S.-W. Cho, *Neurotoxicology* **2015**, *51*, 198.
[33] S.-J. Yang, J. Kim, S. E. Lee, J.-Y. Ahn, S. Y. Choi, S.-W. Cho, *BMB Rep.* **2017**, *50*, 634.
[34] D. G. van Greunen, W. Cordier, M. Nell, C. van der Westhuyzen, V. Steenkamp, J.-L. Panayides, D. L. Riley, *Eur. J. Med. Chem.* **2017**, *127*, 671.
[35] J. C. Tarr, M. R. Wood, M. J. Noetzel, J. L. Bertron, R. L. Weiner, A. L. Rodriguez, A. Lamsal, F. W. Byers, S. Chang, H. P. Cho, C. K. Jones, C. M. Niswender, M. W. Wood, N. J. Brandon, M. E. Duggan, P. J. Conn, T. M. Bridges, C. W. Lindsley, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2990.
[36] L. Degennaro, M. Zenzola, A. Laurino, M. M. Cavalluzzi, C. Franchini, S. Habtemariam, R. Matucci, R. Luisi, G. Lentini, *Chem. Heterocycl. Comp.* **2017**, *53*, 329.
[37] C. R. Butler, E. M. Beck, A. Harris, Z. Huang, L. A. McAllister, C. W. am Ende, K. Fennell, T. L. Foley, K. Fonseca, S. J. Hawrylik, D. S. Johnson, J. D. Knafels, S. Mente, G. S. Noell, J. Pandit, T. B. Phillips, J. R. Piro, B. N. Rogers, T. A. Samad, J. Wang, S. Wan, M. A. Brodney, *J. Med. Chem.* **2017**, *60*, 9860.
[38] N. Chakka, K. L. Andrews, L. M. Berry, H. Bregman, H. Gunaydin, L. Huang, A. Guzman-Perez, M. H. Plant, J. R. Simard, J. Gingras, E. F. DiMauro, *Eur. J. Med. Chem.* **2017**, *137*, 63.
[39] J. Yu, W. Wang, N. Matei, X. Li, J. Pang, J. Mo, S. Chen, J. Tang, M. Yan, J. H. Zhang, *Oxid. Med. Cell. Longev.* **2020**, *2020*, 1.
[40] H. Genç, R. Kalin, Z. Köksal, N. Sadeghian, U. Kocyigit, M. Zengin, İ. Gülçin, H. Özdemir, *Int. J. Mol. Sci.* **2016**, *17*, 1736.
[41] M. Iškauskienė, G. Ragaitė, F. A. Sløk, A. Šačkus, *Mol. Divers.* **2020**, *24*, 1235.
[42] J. Bruzgulienė, G. Račkauskienė, A. Bieliauskas, V. Milišiūnaitė, M. Dagilienė, G. Matulevičiūtė, V. Martynaitis, S. Krikštolaitytė, F. A. Sløk, A. Šačkus, *Beilstein J. Org. Chem.* **2022**, *18*, 102.
[43] E. Gudelis, S. Krikštolaitytė, M. Stančiauskaitė, U. Šachlevičiūtė, A. Bieliauskas, V. Milišiūnaitė, R. Jankauskas, N. Kleizienė, F. A. Sløk, A. Šačkus, *Molecules* **2023**, *28*, 1091.
[44] K. Dzedulionytė, P. Voznikaitė, A. Bieliauskas, V. Malinauskienė, F. Sløk, A. Šačkus, *Molbank* **2021**, *2021*, M1207.
[45] X. Yang, W. Y. Kong, J. N. Gao, L. Cheng, N. N. Li, M. Li, H. T. Li, J. Fan, J. M. Gao, Q. Ouyang, J. B. Xie, *Chem. Commun.* **2019**, *55*, 12707.
[46] P. N. Collier, *Tetrahedron Lett.* **2009**, *50*, 3909.
[47] S. Brock, D. R. J. Hose, J. D. Moseley, A. J. Parker, I. Patel, A. J. Williams, *Org. Process Res. Dev.* **2008**, *12*, 496.
[48] T. Senda, M. Ogasawara, T. Hayashi, *J. Org. Chem.* **2001**, *66*, 6852.
[49] M. Pizzonero, S. Dupont, M. Babel, S. Beaumont, N. Bienvenu, R. Blanqué, L. Cherel, T. Christophe, B. Crescenzi, E. De Lemos, P. Delerive, P. Deprez, S. De Vos, F. Djata, S. Fletcher, S. Kopiejewski, C. L'Ebraly, J.-M. Lefrançois, S. Lavazais, M. Manioc, L. Nelles, L. Oste, D. Polancec, V. Quénéhen, F. Soulas, N. Triballeau, E. M. van der Aar, N. Vandeghinste, E. Wakselman, R. Brys, L. Saniere, *J. Med. Chem.* **2014**, *57*, 10044.
[50] E. Semina, A. Žukauskaitė, A. Šačkus, N. De Kimpe, S. Mangelinckx, *Eur. J. Org. Chem.* **2016**, *2016*, 1720. https://doi.org/10.1002/ejoc.201600036
[51] M. L. Go, J. L. Leow, S. K. Gorla, A. P. Schüller, M. Wang, P. J. Casey, *J. Med. Chem.* **2010**, *53*, 6838.
[52] C. M. N. Allerton, C. G. Barber, K. C. Beaumont, D. G. Brown, S. M. Cole, D. Ellis, C. A. L. Lane, G. N. Maw, N. M. Mount, D. J. Rawson, C. M. Robinson, S. D. A. Street, N. W. Summerhill, *J. Med. Chem.* **2006**, *49*, 3581.
[53] J. Xiao, S. W. Wright, *Tetrahedron Lett.* **2013**, *54*, 2502.
[54] J. Kim, H. Hwang, H. Yoon, J.-E. Lee, J. M. Oh, H. An, H. D. Ji, S. Lee, E. Cha, M. J. Ma, D.-S. Kim, S.-J. Lee, T. M. Kadayat, J. Song, S. W. Lee, J.-H. Jeon, K.-G. Park, I.-K. Lee, Y. H. Jeon, J. Chin, S. J. Cho, *Eur. J. Med. Chem.* **2020**, *205*, 112501.
[55] S. M. Bronner, K. A. Merrick, J. Murray, L. Salphati, J. G. Moffat, J. Pang, C. J. Sneeringer, N. Dompe, P. Cyr, H. Purkey, G. L. Boenig, J. Li, A. Kolesnikov, R. Larouche-Gauthier, K. W. Lai, X. Shen, S. Aubert-Nicol, Y. C. Chen, J. Cheong, J. J. Crawford, M. Hafner, P. Haghshenas, A. Jakalian, J. P. Leclerc, N. K. Lim, T. O'Brien, E. G. Plise, H. Shalan, C. Sturino, J. Wai, Y. Xiao, J. Yin, L. Zhao, S. Gould, A. Olivero, T. P. Heffron, *Bioorg. Med. Chem. Lett.* **2019**, *29*, 2294.
[56] R. A. da Silva, I. H. S. Estevam, L. W. Bieber, *Tetrahedron Lett.* **2007**, *48*, 7680.
[57] T. Suzuki, J. Kusakabe, K. Kitazawa, T. Nakagawa, S. Kawauchi, T. Ishizone, *Macromolecules* **2010**, *43*, 107.
[58] W. R. Dolbier, *Guide to Fluorine NMR for Organic Chemists*, John Wiley & Sons, Inc, Hoboken, NJ, USA **2016**.
[59] B. Razmienė, E. Řezníčková, V. Dambrauskienė, R. Ostruszka, M. Kubala, A. Žukauskaitė, V. Kryštof, A. Šačkus, E. Arbačiauskienė, *Molecules* **2021**, *26*, 6747.
[60] J. Pasquini, D. J. Brooks, N. Pavese, *Mov. Disord. Clin. Pract.* **2021**, *8*, 1012.
[61] T. van Laar, P. P. De Deyn, D. Aarsland, P. Barone, J. E. Galvin, *CNS Neurosci. Therap.* **2011**, *17*, 428.

[62] C. G. Ballard, *Eur. Neurol.* **2002**, *47*, 64.
[63] G. Mushtaq, N. Greig, J. Khan, M. Kamal, *CNS Neurol. Disord. Drug Targets* **2014**, *13*, 1432.
[64] N. H. Greig, T. Utsuki, Q. S. Yu, X. Zhu, H. W. Holloway, T. Perry, B. Lee, D. K. Ingram, D. K. Lahiri, *Curr. Med. Res. Opin.* **2001**, *17*, 159.
[65] U. Kiełczewska, R. Jorda, G. Gonzalez, J. W. Morzycki, H. Ajani, K. Svrčková, Š. Štěpánková, A. Wojtkielewicz, *J. Steroid Biochem. Mol. Biol.* **2021**, *205*, 105776.
[66] G. Gonzalez, M. Kvasnica, K. Svrčková, Š. Štěpánková, J. R. C. Santos, M. Peřina, R. Jorda, S. M. M. Lopes, T. M. V. D. P. Melo, *J. Steroid Biochem. Mol. Biol.* **2023**, *225*, 106194.
[67] H. Lineweaver, D. Burk, *J. Am. Chem. Soc.* **1934**, *56*, 658.
[68] G. Gonzalez, J. Grúz, C. W. D'Acunto, P. Kaňovský, M. Strnad, *Molecules* **2021**, *26*, 361.
[69] S. Castellani, A. Trapani, A. Spagnoletta, L. di Toma, T. Magrone, S. Di Gioia, D. Mandracchia, G. Trapani, E. Jirillo, M. Conese, *J. Transl. Med.* **2018**, *16*, 140.
[70] M. Kurnik-Łucka, P. Panula, A. Bugajski, K. Gil, *Neurotoxic. Res.* **2018**, *33*, 485.
[71] R. J. Bridges, N. R. Natale, S. A. Patel, *Br. J. Pharmacol.* **2012**, *165*, 20.
[72] M. A. Tammam, L. Rárová, M. Kvasnicová, G. Gonzalez, A. M. Emam, A. Mahdy, M. Strnad, E. Ioannou, V. Roussis, *Mar. Drugs* **2020**, *18*, 632.
[73] B. Maková, V. Mik, B. Lišková, G. Gonzalez, D. Vítek, M. Medvedíková, B. Monfort, V. Ručilová, A. Kadlecová, P. Khirsariya, Z. Gándara Barreiro, L. Havlíček, M. Zatloukal, M. Soural, K. Paruch, B. D'Autréaux, M. Hajdúch, M. Strnad, J. Voller, *Bioorg. Med. Chem.* **2021**, *33*, 115993.
[74] Z.-W. Sun, L. Zhang, S.-J. Zhu, W.-C. Chen, B. Mei, *Neurosci. Bull.* **2010**, *26*, 8.
[75] E. Park, J. Gim, D. K. Kim, C.-S. Kim, H. S. Chun, *Biol. Pharmaceut. Bull.* **2019**, *42*, 94.
[76] A. A. Kritis, E. G. Stamoula, K. A. Paniskaki, T. D. Vavilis, *Front. Cell. Neurosci.* **2015**, *9*, 91. https://doi.org/10.3389/fncel.2015.00091
[77] O. Tirosh, C. K. Sen, S. Roy, M. S. Kobayashi, L. Packer, *Free Rad. Biol. Med.* **1999**, *26*, 1418.
[78] N. M. Lyra e Silva, R. A. Gonçalves, T. A. Pascoal, R. A. S. Lima-Filho, E. P. F. Resende, E. L. M. Vieira, A. L. Teixeira, L. C. de Souza, J. A. Peny, J. T. S. Fortuna, I. C. Furigo, D. Hashiguchi, V. S. Miya-Coreixas, J. R. Clarke, J. F. Abisambra, B. M. Longo, J. Donato, P. E. Fraser, P. Rosa-Neto, P. Caramelli, S. T. Ferreira, F. G. De Felice, *Transl. Psychiatry* **2021**, *11*, 251.
[79] J.-M. Waldburger, D. L. Boyle, V. A. Pavlov, K. J. Tracey, G. S. Firestein, *Arthritis Rheum.* **2008**, *58*, 3439.
[80] H. C. Morrogh-Bernard, I. Foitová, Z. Yeen, P. Wilkin, R. de Martin, L. Rárová, K. Doležal, W. Nurcahyo, M. Olšanský, *Sci. Rep.* **2017**, *7*, 16653.
[81] R. A. Carrasco, N. B. Stamm, B. K. R. Patel, *Biotechniques* **2003**, *34*, 1064.
[82] L. Rárová, Z. Pakulski, M. Strnad, M. Kvasnicová, T. Štenclová, P. Cmoch, *The J. Steroid Biochem. Mol. Biol.* **2022**, *224*, 106161.
[83] Ø. Hammer, D. A. T. Harper, P. D. Ryan, *Palaeontol. Electron.* **2001**, *4*, 1.

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