Received: 3 July 2018 | Revised: 7 September 2018 | Accepted: 12 September 2018
DOI: 10.1002/ardp.201800194

FULL PAPER

DPhG ARCH PHARM
Archiv der Pharmazie

Donepezil–melatonin hybrids as butyrylcholinesterase inhibitors: Improving binding affinity through varying mode of linking fragments

Iwona Łozińska[1] | Aleksandra Świerczyńska[1] | Zuzanna Molęda[1] | Alwin M. Hartman[2] | Anna K. H. Hirsch[2,3] | Zbigniew Czarnocki[1]

[1] Faculty of Chemistry, University of Warsaw, Warsaw, Poland

[2] Department of Drug Design and Optimization, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) – Helmholtz Centre for Infection Research (HZI), Saarbrücken, Germany

[3] Department of Pharmacy, Medicinal Chemistry, Saarland University, Saarbrücken, Germany

Correspondence
Prof. Zbigniew Czarnocki, Faculty of Chemistry, University of Warsaw, ul. Pasteura 1, 02-093 Warsaw, Poland.
Email: czarnoz@chem.uw.edu.pl

Funding information
Helmholtz-Association's Initiative and Networking Fund

Abstract

Hybrid inhibitors of acetyl- and butyrylcholinesterase are compounds that combine structural motifs of two different classical inhibitors, leading to a dual binding ligand. A rapidly growing collection of those compounds involves a wide diversity of structural motifs, but the way of linking two active fragments and its impact on the affinity toward cholinesterases usually remains beyond the extent of investigation. We present hereby a detailed analysis of this aspect using melatonin–donepezil hybrids. A new series of compounds, in which two fragments are connected using a carbamate linker, exhibits excellent activity and selectivity toward butyrylcholinesterase.

KEYWORDS
biological activity, drug design, hydrolases, medicinal chemistry, structure–activity relationship

1 | INTRODUCTION

Cholinesterases are an important drug target. The corresponding inhibitors are promising agents in the therapy of Alzheimer's disease (AD), acting as modulators of acetylcholine levels and halting β-amyloid deposition.[1] From the two sister enzymes, acetylcholinesterase (AChE) was for years the better studied one as it functions in synapses hydrolyzing acetylcholine. Some of its inhibitors have been developed and introduced successfully to the market (rivastigmine, galantamine, and donepezil). Meanwhile, it has emerged that better effects can be reached by the inhibition of butyrylcholinesterase (BChE), which is much less known than its sister enzyme. It was shown that BChE also hydrolyzes acetylcholine, induces β-amyloid aggregation,[2] and enhances its toxicity. At the advanced stages of AD, the levels of AChE drop dramatically, which may explain the fact, that its inhibitors do not show therapeutic effects at this point. In contrast, levels of BChE remain very high. Those facts account for an intensive search for BChE inhibitors, as well as detailed studies of the structure and function of this enzyme.[3–5]

Both AChE and BChE contain a binding pocket, constituted of two binding sites – the catalytic active site (CAS) and the peripheral anionic site (PAS). The classical inhibitors can bind to either CAS or PAS. In recent years, so-called "hybrid compounds" as cholinesterase inhibitors have been developed.[6,7] These molecules are built from two motifs able to bind to CAS and PAS, linked with a bridge of diverse length and character. The effect is the dual binding to the enzyme which guarantees high efficacy of halting acetylcholine hydrolysis. Moreover, blocking the PAS is believed to hamper β-amyloid aggregation.[8] Therefore, it is expected that the new cholinesterase inhibitors will be designed to interact simultaneously with both binding sites of the enzyme.

Taking into account other factors connected with the development of AD, it is strongly desirable that the potential drug exhibits additional therapeutic effects, i.e., neuroprotective antioxidant

properties. In this respect, melatonin (**1**) seems to be a perfect candidate for inspiring the design of new hybrid molecules. This neurohormone plays a crucial role in the regulation of circadian rhythm, it is also an extremely potent free-radical scavenger and activator of antioxidant enzymes.[9] The indolyl moiety of the melatonin scaffold was shown to bind effectively to the PAS of cholinesterases,[10] and melatonin-derived fragments have been successfully incorporated into new hybrid compounds. Those involve hybrids of melatonin with tacrine,[11] berberine,[12] or (−)-meptazinol,[13] which, apart from dual binding to cholinesterases, maintain the antioxidant properties. Advances in the development of melatonin-derived multitarget hybrids have been recently reviewed.[14]

Despite the introduction of new methods in medicinal chemistry for more effective screening of series of compounds, hybrid cholinesterase inhibitors are usually developed by classical synthesis of a lead compound followed by laborious structural modifications aimed at optimizing the biological activity and/or properties. Those modifications usually concern substituents on the aromatic moieties and the length of the linker chain, while the type of the linker seems to be chosen arbitrarily.

In the case of tacrine–melatonin hybrids, a comparison between two different modes of linking active moieties is possible. The first compounds of this class connect a melatonin-derived moiety through an amide bond, using the aliphatic tail of the melatonin fragment (Figure 1a).[15] Previously, we demonstrated that the modification of the linker to a carbamate bond connected to the aromatic system of the melatonin moiety generates an excellent selectivity toward BChE (Figure 1b).[16]

Melatonin was recently connected to donepezil **2**, a molecule which is often successfully incorporated into multitarget hybrids,[17] creating a group of potent dual-binding cholinesterase inhibitors with strong antioxidant properties and impact on halting β-amyloid deposition.[18] We reasoned that changing the way melatonin and donepezil are linked may improve the affinity toward BChE, by analogy to melatonin–tacrine hybrids. To verify this hypothesis and understand the origin of the selectivity for BChE, we designed and synthesized a group of compounds possessing a melatonin moiety bridged to donepezil through a carbamate bond linked directly to the aromatic system (Figure 2).

2 | RESULTS

2.1 | Chemistry

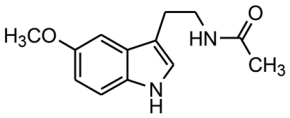
The compounds of interest were synthesized in a coupling reaction of alkylamine-substituted *N*-benzylpiperidine derivatives **10**–**14** with a carbonate derivative of *N*-acetylserotonin **15** (Scheme 1).

Melatonin-derived building block **15** was obtained according to a previously developed procedure from *N*-acetylserotonin and 4-nitrophenyl chloroformate, in the presence of *N*-methylmorpholine.[16] *N*-Benzyl piperidine derivatives **10** and **11** are commercially available. Compounds **12**–**14** were prepared as presented in Scheme 2.

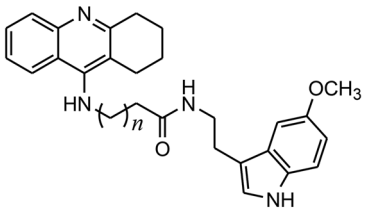
Starting with a diterminal bromo- or chloroalcohol (**16a**–**c**) possessing one carbon fewer than the side chain of the desired derivative, we protected the hydroxyl group with the THP-ether. The pyridine moiety was then introduced simultaneously with the side chain elongation in the reaction with the lithium salt of γ-picoline, leading to compounds **18a**–**c**. After coupling with benzyl bromide, the pyridinium salts were selectively reduced to *N*-benzylpiperidine derivatives **20a**–**c**. Deprotection under acidic conditions, azide formation, and Staudinger reaction allowed us to easily obtain the desired products **12**–**14**.

2.2 | Biological evaluation

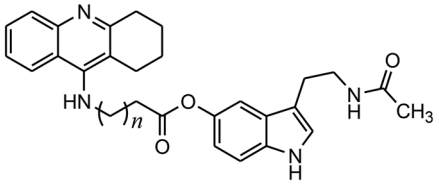
The inhibitory activity against human acetylcholinesterase (*h*AChE) and butyrylcholinesterase (*h*BChE) derived from erythrocytes and



1



a) $n = 6$
IC_{50} (*h*AChE) 0.65 nM ± 0.03 nM
IC_{50} (*h*BChE) 3.0 nM ± 0.2 nM



b) $n = 6$
IC_{50} (*h*AChE) 33.77 nM ± 2.74 nM
IC_{50} (*h*BChE) 0.25 nM ± 0.02 nM

FIGURE 1 Melatonin (**1**) and two different types of tacrine–melatonin heterodimers[15,16]

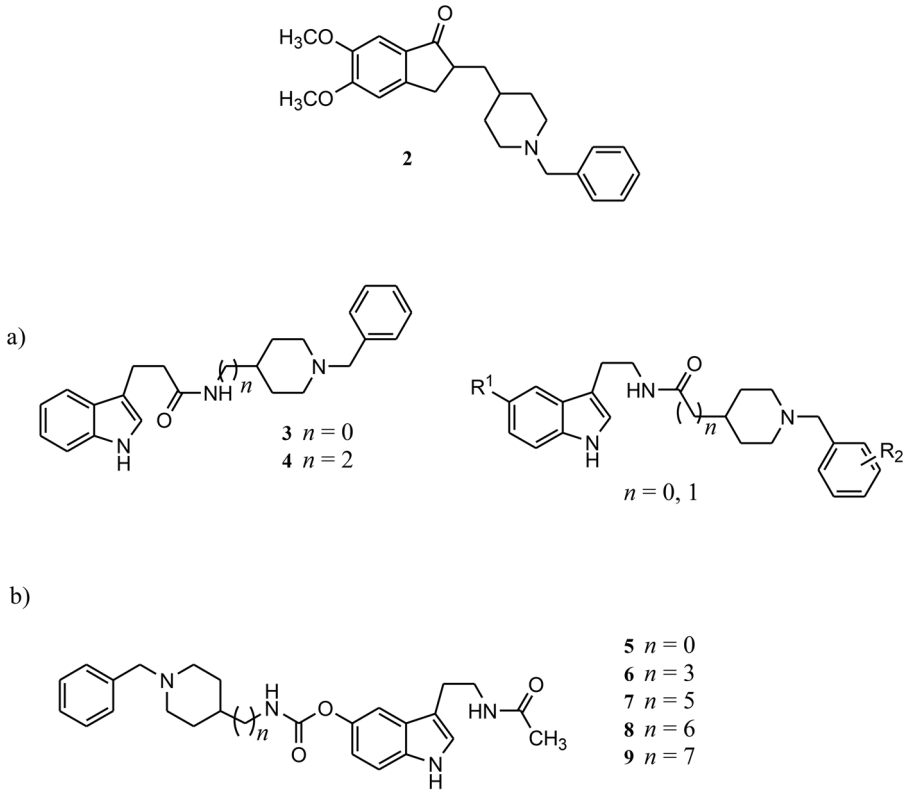
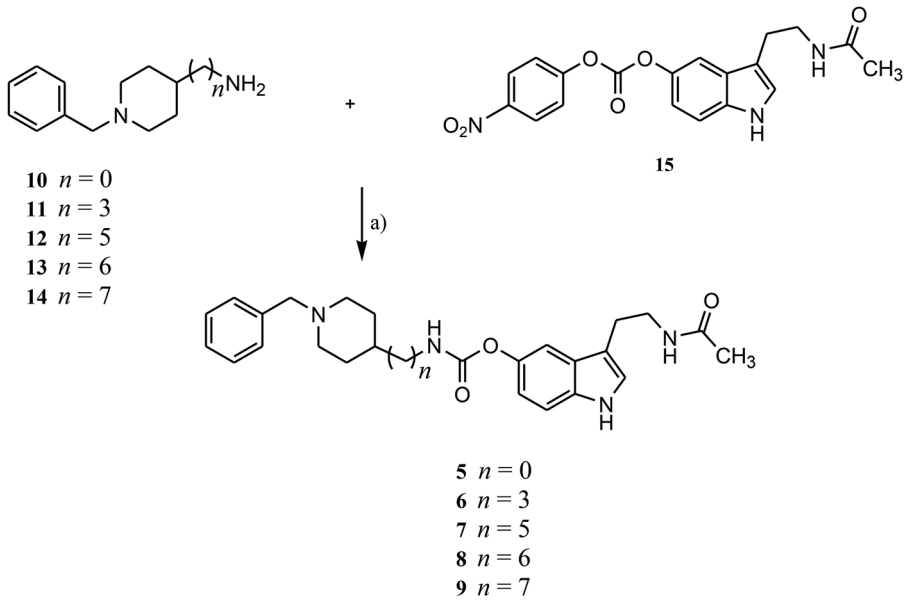


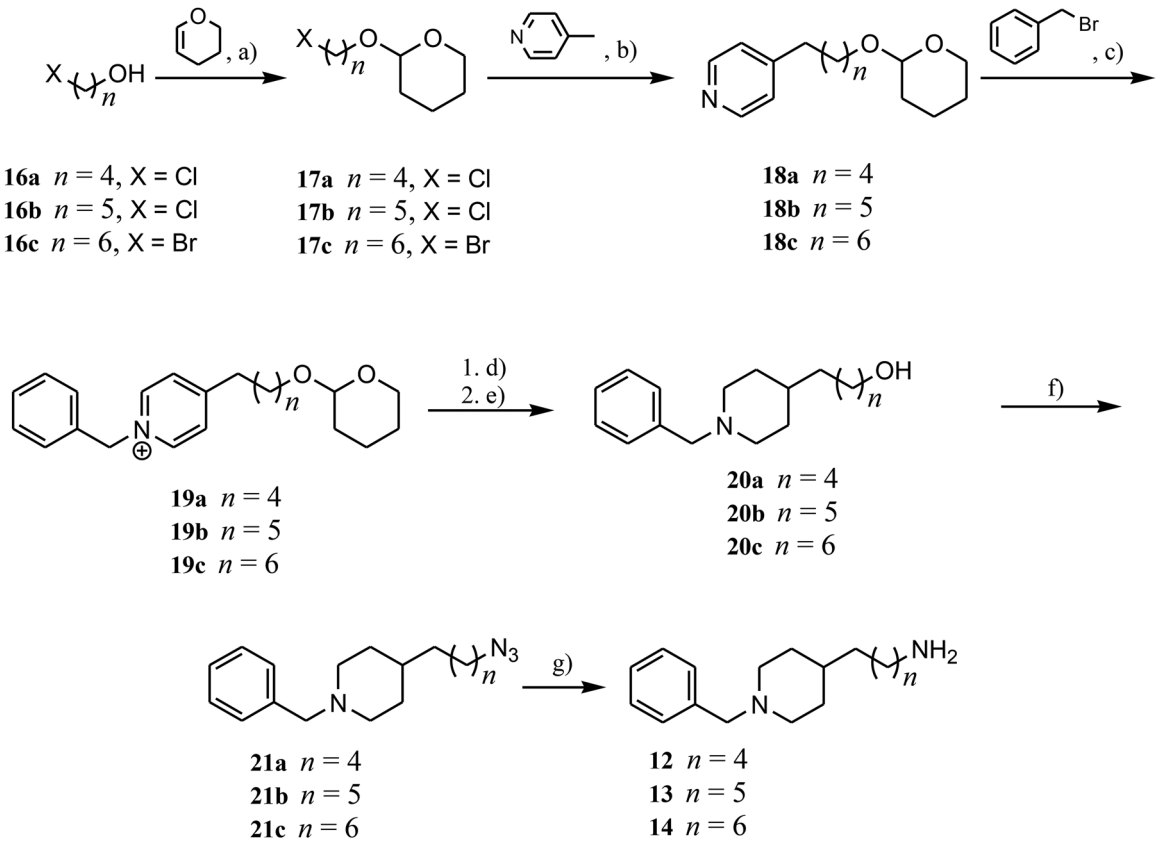
FIGURE 2 Donepezil (**2**) and donepezil–melatonin heterodimers. (a) Compounds synthesized by Wu et al.[18] (b) Linkage modification presented in this work

blood serum, respectively, was determined using spectrophotometric Ellman's assay.[19] The results are presented in Table 1 and compared to the most active known melatonin–donepezil hybrid, **4**.

As can be seen, the only compound lacking activity is compound **5**, in which the *N*-benzylpiperidinyl skeleton is connected directly to the carbamate bridge. In case of compounds **6–9**, elongation of the linker chain introduces additional aliphatic interactions, which improves the affinity toward both cholinesterases. All compounds with longer linkers exhibit significant activity.



SCHEME 1 Synthesis of compounds **5–9**. (a) DMAP, DCM, RT, 71–92%



SCHEME 2 Synthesis of compounds **12–14**. (a) PPTS, DCM, RT, 97–100%; (b) *n*BuLi, THF, RT, 61–91%; (c) toluene, 60°C; (d) H_2, PtO_2, EtOH, RT; (e) $HCl_{aq.}$, MeOH, EtOH, RT, 53–68%; (f) DPPA, DBU, NaN_3, DMF, RT, 76–80%; (g) PPh_3, H_2O, THF, RT, 80–95%

2.3 | Modeling studies

To be able to investigate the binding modes, for the different ways in which donepezil (**2**) and melatonin (**1**) can be linked, we used two computer programs. The X-ray crystal structures of *h*AChE (PDB code: 4EY7) and *h*BChE (PDB code: 4TPK) in complex with donepezil (**2**) and a naphthamide derivative, are used, respectively, for our docking studies. The docking module FlexX in the LeadIT suite was used to dock the compounds in the active site of *h*AChE and *h*BChE.[20] To evaluate the docking results, the program SeeSAR, with the in-built scoring function HYDE, was used.[21]

As compared to compound **4** reported by Wang et al.,[18] we observe a slightly different binding mode, showing a dependence of the pose on the way donepezil (**2**) and melatonin (**1**) are linked. Predominately for *h*AChE, the docked inhibitors show π–π stacking interactions of W286 and the indolyl part and W86 with the benzene ring, where W86 as well as Y337 are engaged in a cation–π interaction with the tertiary amine. Our compounds resemble more the melatonin structure to which donepezil is linked via a carbamate.

Figure 3 shows the overlap of the best poses for each of our dimers, in the pocket of *h*AChE with donepezil as a reference ligand (see also Figure 4). It can be seen that the melatonin part of our compounds is predicted to remain in the CAS site, having interactions with W86, E202, and S203. The donepezil part, the piperidinyl and benzyl moieties, are directed toward Y341 and W286. Due to the increased linker length, the donepezil part is gradually more solvent-exposed.

From the docking studies the structures can be ranked according to their increasing affinity: **5** ≈ **7** < **6** ≈ **8** ≈ **9**.

For *h*BChE it is expected that the benzene part predominately interacts with G117, V288, L286, W231, and F398, the indolyl moiety will most probably show lipophilic interactions with W82. It is also expected that the NH of the piperidinyl will form a hydrogen bond with

TABLE 1 Inhibitory activity of the melatonin–donepezil hybrids **5–9** against human acetyl- and butyrylcholinesterase

	IC_{50} [μM][a]		
	*h*AChE	*h*BChE	Selectivity index[b]
5	>10	9.034 ± 1.753	>0.9
6	2.222 ± 0.248	0.176 ± 0.017	0.079
7	0.753 ± 0.036	0.015 ± 0.002	0.020
8	0.725 ± 0.024	0.018 ± 0.001	0.025
9	0.621 ± 0.016	0.003 ± 0.0003	0.005
4[17]	0.273 ± 0.05	0.056 ± 0.01	0.21

[a]Values are the mean ± SD obtained from three experiments.
[b]IC_{50} (*h*BuChE)/IC_{50} (*h*AChE).

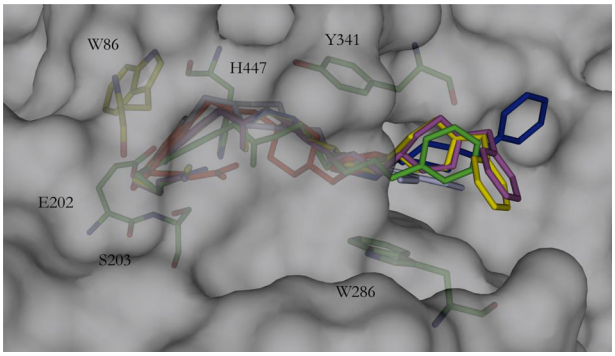


FIGURE 3 Cocrystal structure of *h*AChE with donepezil (PDB code: 4EY7) and the best pose of each dimer. Color code: protein surface: gray; interacting residues: sticks; inhibitor skeletons: donepezil: light blue; **5**: red; **6**: green; **7**: blue; **8**: yellow; **9**: magenta. Figures of this type were generated with PyMOL (Schrödinger)

the carbonyl oxygen of P285 (Figures 5 and 6). Compared to the amide in structure **4** of Wang et al.,[18] in *h*BChE, it is expected that the activity of the carbamate does not change much. The extra binding activity might arise from additional interactions stemming from the amide linker, from the melatonin moiety. This part is not present in the parent compound reported by Wu et al.[18] The rest of the molecule shows a similar pose, the indolyl is directed toward W82 and the benzene toward G117.

From the docking studies, the structures can be ranked according to their increasing affinity: **5** ≤ **6** < **9** ≤ **7** ≤ **8**.

3 | DISCUSSION

Derivatives **5**–**9** present a significantly lower affinity toward AChE than the previously described donepezil–melatonin hybrids.[18] This arises probably from the different orientation of the ligand – in this case the donepezil part of the hybrids is located at the end of the

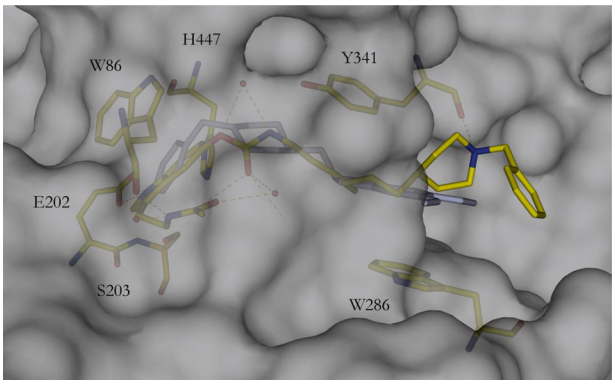


FIGURE 4 Cocrystal structure of *h*AChE with donepezil (PDB code: 4EY7) and the best pose of **9**. Color code: protein surface: gray; interacting residues: sticks; inhibitor skeletons: C: yellow; N: blue; O: red; donepezil: light blue; H-bonds, below 3.2 Å: dashed lines

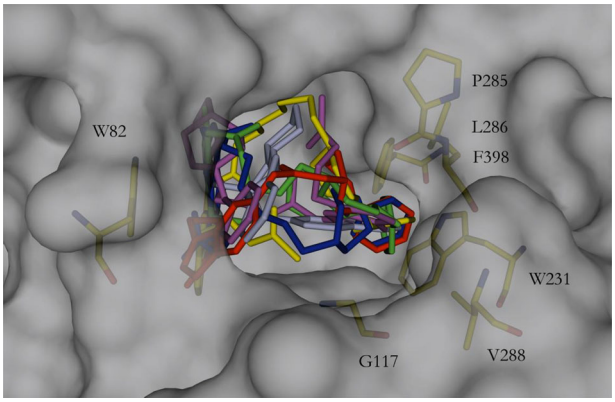


FIGURE 5 Cocrystal structure of *h*BChE with a naphthamide derivative (PDB code: 4TPK) and the best pose of each dimer. Color code: protein surface: gray; interacting residues: sticks; inhibitor skeletons: donepezil: light blue; **5**: red; **6**: green; **7**: blue; **8**: yellow; **9**: magenta

pocket, near the PAS site, while in the case of compound **4**, the PAS is occupied by the indolyl moiety and the donepezil part interacts with Y337 and W86 located in the pocket. A conclusion can be drawn that linking the melatonin fragment through its phenyl oxygen atom notably reduces possible interactions with AChE.

This effect is not observed in case of BChE. Compounds **6**–**9** exhibit very strong inhibitory activity, which can be explained by the fact that the interactions similar to those of compound **4** are enhanced by additional binding of the acetyl moiety, not present in the compounds of Wang et al.[18] The strongest inhibition of BChE was achieved in case of compound **9**, which additionally exhibits excellent selectivity for this type of enzyme.

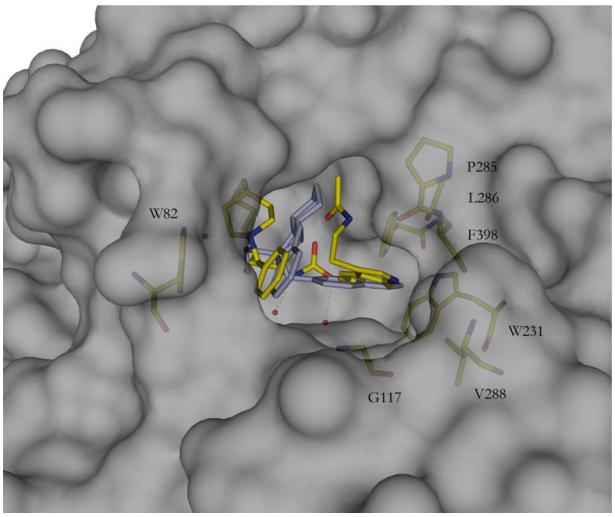


FIGURE 6 Cocrystal structure of *h*BChE with a naphthamide derivative (PDB code: 4TPK) and the best pose of **9**. Color code: protein surface: gray; interacting residues: sticks; inhibitor skeletons: C: yellow; N: blue; O: red; naphthamide: light blue; H-bonds, below 3.2 Å: dashed lines

4 | CONCLUSIONS

New potent and selective inhibitors of BChE have been developed. A detailed study of their binding mode allowed us to understand the origin of this phenomenon. It was demonstrated that in case of melatonin–donepezil hybrids, the modification of the linker to a carbamate bond connected to the aromatic system of the melatonin moiety improves the affinity toward BChE in the same way as in the case of melatonin–tacrine hybrids. These results open new promising perspectives for the search for new neurotherapeutics based on melatonin-derived fragments.

5 | EXPERIMENTAL

5.1 | Chemistry

5.1.1 | General

Serotonin hydrochloride was obtained from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). Other reagents were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE apparatus, operating at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) and VARIAN Unity Plus (respectively 200, 50 MHz) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in ppm. The following abbreviations were used to indicate the peak multiplicity and shape: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. High-resolution mass spectra were recorded on a Quatro LC AMD 604 apparatus using TOF MS ES+ method. Column chromatography was performed on silica gel MN-Kieselgel 100–200 mesh ASTM (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and aluminum oxide (Fluka, St. Louis, MO, USA) using the indicated eluents. The progress of the reactions was followed by thin-layer chromatography using plates with silica gel and aluminum oxide with methanol, methylene chloride, ethyl acetate, and 20% aqueous ammonia as eluents. Anhydrous magnesium sulfate or sodium sulfate was used as drying agent for the organic phases. Organic solvents were removed under reduced pressure at 40°C. The melting points were measured with Kofler's apparatus (Boetius HMK type).

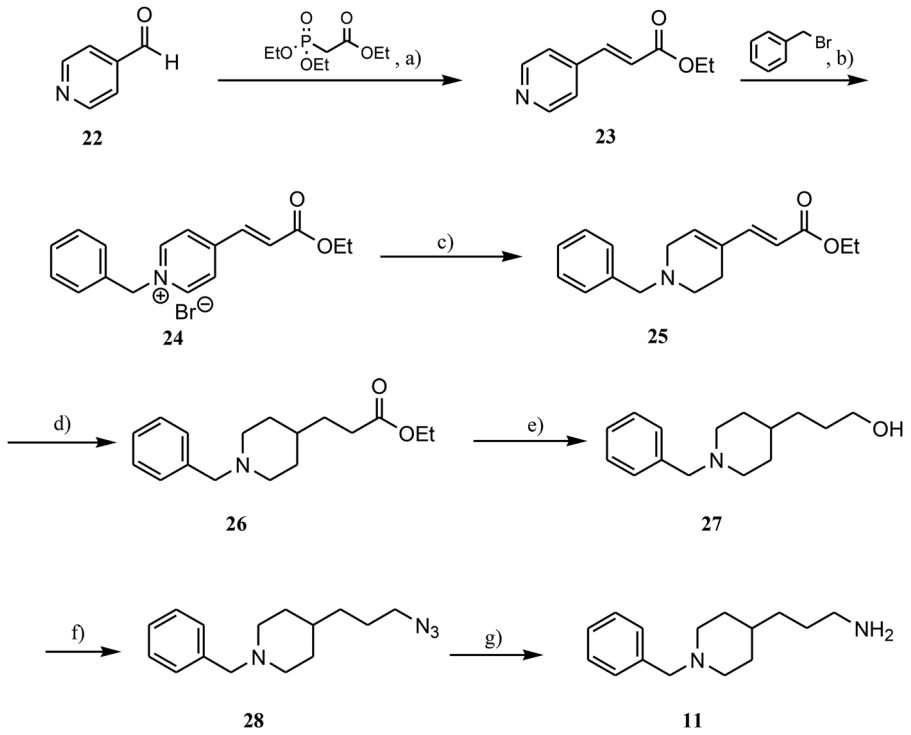
The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

5.1.2 | Synthesis of compounds 15, 11, and 23

3-(Acetamidomethyl)-1*H*-indol-5-yl 4-nitrophenylcarbonate (**15**) was obtained according to the procedure described in our previous work.[16] *N*-Benzylpiperidine derivative **11** was prepared according to Scheme 3. Ethyl (2*E*)-3-(pyridin-4-yl)prop-2-enoate (**23**) was prepared according to the methods reported in the literature, from the commercially available isonicotinaldehyde.[22]

1-Benzyl-4-[(1*E*)-3-ethoxy-3-oxoprop-1-en-1-yl]pyridin-1-ium bromide (24)

To ethyl (2*E*)-3-(pyridin-4-yl)prop-2-enoate (**23**) (2 g; 11.3 mmol) dissolved in toluene (30 mL), (bromomethyl)benzene (1.47 mL; 2.12 g; 12.4 mmol) was added. The reaction was left to stir for 14 h



SCHEME 3 Synthesis of compound **11**. (a) K_2CO_3, EtOH, RT, 90%; (b) toluene, RT, 77%; (c) $NaBH_4$, EtOH, RT, 56%; (d) H_2, PtO_2, EtOH, RT, 99%; (e) $LiAlH_4$, THF, RT, 99%; (f) DPPA, DBU, NaN_3, DMF, RT, 82%; (g) PPh_3, H_2O, THF, RT, 97%

at 60°C, under an inert atmosphere. The product precipitated and was recrystallized from a mixture of dichloromethane and ethyl acetate. Compound **24** was obtained as a yellow solid in 77% yield (2.3 g).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.74–9.60 (m, 2H, $2H_{arom}$); 8.17–8.07 (m, 2H, $2H_{arom}$); 7.82–7.68 (m, 2H, $2H_{arom}$); 7.60 (d, *J* = 16.0 Hz; 1H, *β*-C<u>H</u>═); 7.40–7.32 (m, 3H, $3H_{arom}$), 6.83 (d, *J* = 16.0 Hz, 1H, *α*-C<u>H</u>═), 6.31 (s, 2H, C<u>H</u>$_2$Ph), 4.28 (q, *J* = 7.1 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 1.34 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 164.68; 150.37; 145.64; 137.20; 133.17; 130.10; 130.05; 129.83; 129.75; 126.18; 63.78; 61.86; 14.29. MS EI(+) (*m/z*): 268 $[M]^+$.

Ethyl (2*E*)-3-(1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)prop-2-enoate (25)

To 1-benzyl-4-[(1*E*)-3-ethoxy-3-oxoprop-1-en-1-yl]pyridin-1-ium bromide **24** (0.83 g; 2.5 mmol) dissolved in ethanol (15 mL), $NaBH_4$ (0.19 g; 5.0 mmol) was added slowly (over 0.5 h) at 0°C. The reaction was left to stir for 90 min. The solvent was evaporated by half and DCM (30 mL) was added. The solution was washed with water and brine, then dried over anhydrous Na_2SO_4, and filtered. The product was purified by column chromatography (SiO_2, 15% EtOAc in hexane) to give **25** as a colorless oil in 56% yield (0.38 g).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.45–7.09 (m, 6H, $5H_{arom}$ + C<u>H</u>═); 6.04 (t, *J* = 3.5 Hz, 1H, C<u>H</u>═); 5.73 (d, *J* = 15.8 Hz, 1H, C<u>H</u>═); 4.16 (q, *J* = 7.2 Hz, 2H, OC<u>H</u>$_2$CH$_3$); 3.56 (s, 2H, C<u>H</u>$_2$Ph); 3.10 (br.d, *J* = 3.2 Hz, 2H); 2.60 (t, *J* = 5.7 Hz, 2H); 2.27–2.17 (m, 2H); 1.25 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 172.8; 146.41; 138.16; 135.03; 133.41; 129.34; 128.53; 127.41; 116.13; 62.81; 60.49; 53.57; 49.39; 25.38; 14.53. MS EI(+) (*m/z*): 272 $[M+H]^+$.

Ethyl 3-(1-benzylpiperidin-4-yl)propanoate (26)

Ethyl (2*E*)-3-(1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)prop-2-enoate **25** (310 mg; 1.29 mmol) was dissolved in ethanol (30 mL) and PtO_2 (50 mg) was added. Air was replaced by hydrogen and the reaction was left to stir overnight. The reaction mixture was filtered through Celite. The solvent was evaporated and the product was obtained as a colorless oil in 99% yield (351 mg). Analytical data are in agreement with the literature reports.[23]

5.1.3 | Synthesis of compounds 27, 28, and 11 and precursors 17a–c

Compounds **27**, **28**, and **11** were prepared according to the methods reported in the literature.[24] The precursors **17a–c** were prepared according to the methods reported in the literature.[25,26]

5.1.4 | General procedure for the synthesis of compounds 18a–c

Freshly distilled and dried over sodium carbonate γ-picoline (1.1 eq) was dissolved in anhydrous THF (25 mL), cooled down to −78°C under inert atmosphere (Ar) and *n*-BuLi (2.5 M in hexane, 1.5 eq) was added slowly. The reaction mixture was stirred for 30 min and the corresponding compound **17a–c** (10 mmol) dissolved in THF (5 mL) was added dropwise. The reaction was left to stir for 1 h at −78°C, it was allowed to reach room temperature. It was stirred for an additional 20 h. After that time, aqueous solution of NH_4Cl was added and the solution was extracted three times with ethyl acetate. The combined organic layers were dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (SiO_2, 20–60% EtOAc in hexane).

18a: The product was obtained as yellow oil in 70% yield (1.75 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.51–8.42 (m, 2H, $2H_{arom}$); 7.13–7.05 (m, 2H, $2H_{arom}$); 4.55 (t, *J* = 3.5 Hz, 1H); 3.91–3.66 (m, 2H); 3.55–3.30 (m, 2H); 2.62 (t, *J* = 7.5 Hz, 2H); 1.98–1.29 (m, 12H). MS EI (+) (*m/z*): 250 $[M+H]^+$.

18b: The product was obtained as yellow oil in 61% yield (1.61 g). Analytical data are in agreement with the literature reports.[27]

18c: The product was obtained as yellow oil in 91% yield (2.53 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.44 (d, *J* = 6.0 Hz, 1H, H_{arom}); 8.43 (d, *J* = 3.0 Hz, 1H, H_{arom}); 7.06 (d, *J* = 6.0 Hz, 1H, H_{arom}); 7.05 (d, *J* = 3.0 Hz, 1H, H_{arom}); 4.53 (t, *J* = 3.0 Hz, 1H); 3.87–3.78 (m, 1H); 3.74–3.64 (m, 1H); 3.51–3.41 (m, 1H); 3.39–3.29 (m, 1H); 2.55 (t, *J* = 6.0 Hz, 2H); 1.70–1.43 (m, 10H); 1.27–1.37 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 151.7; 149.5; 123.8; 98.8; 67.5; 62.3; 35.2; 30.7; 30.2; 29.6; 29.2; 29.0; 26.1; 25.4; 19.7.

5.1.5 | General procedure for the synthesis of compounds 19a–c

To the corresponding compound **18** (3.0 mmol), dissolved in toluene (10 mL), benzyl bromide (1.1 eq) was added. The reaction was left to stir overnight at 60°C. The solvent was evaporated and the product was used for the next step without purification.

5.1.6 | General procedure for the synthesis of compounds 20a–c

The corresponding crude compound **19a–c** (3.0 mmol) was dissolved in absolute ethanol (100 mL) and PtO_2 (300 mg) was added. Air was replaced with hydrogen and the reaction was left to stir for 3 h. The reaction mixture was filtered through Celite and the solvent was evaporated by half. Ten percent HCl_{aq} was added dropwise until pH = 3 was reached. After 1 h, $NaHCO_{3aq}$ was added, and the mixture was extracted four times with chloroform. The combined organic phases were washed with water, brine, dried over anhydrous $MgSO_4$, and filtered.

20a: The product was obtained as a yellowish oil in 60% yield (470 mg) over three steps. Analytical data are in agreement with the literature reports.[28]

20b: The product was obtained as a yellowish oil in 68% yield (561 mg) over three steps. Analytical data are in agreement with the literature reports.[28]

20c: The product was obtained as in 53% yield (460 mg) over three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.31–7.27 (m, 5H, $5H_{arom}$); 3.60 (t, *J* = 6.0 Hz, 1H, C<u>H</u>$_2$OH); 3.47 (s, 2H, C<u>H</u>$_2$Ph); 2.85 (d,

J = 12.0 Hz, 2H); 1.90 (t, *J* = 12.0 Hz, 2H); 1.67–1.47 (m, 4H); 1.36–1.13 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 138.26; 129.33; 128.11; 126.91; 63.51; 68.98; 53.95; 36.57; 35.68; 32.80; 32.32; 29.82; 29.43; 26.72; 25.74. MS EI(+) (*m/z*): 290 $[M+H]^+$.

5.1.7 | General procedure for the synthesis of compounds 21a–c

To the corresponding compound **20** (0.5 mmol) dissolved in DMF (10 mL) and stirred at 0°C, the other reagents were added in sequence: DPPA (3 eq), DBU (3 eq) and after 30 min, NaN_3 (3 eq) were added. The reaction was let to stir for 4 h at 90°C. After that time, the solvent was evaporated, DCM was added, and the solution was washed with water and brine. The product was purified by column chromatography (SiO_2, 10–50% EtOAc in hexane).

21a: The product was obtained as a colorless oil in 80% yield (114 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.31–7.26 (m, 5H, $5H_{arom}$); 3.48 (s, 2H, $C\underline{H}_2Ph$); 3.25 (t, *J* = 7.0 Hz, 2H, $C\underline{H}_2N_3$); 2.86 (br.d, J_1 = 9.0 Hz, 2H); 1.91 (br.t, *J* = 9.0 Hz, 2H); 1.64–1.54 (m, 4H); 1.33–1.21 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 138.35; 129.34; 128.10; 126.89; 63.58; 62.80; 53.96; 36.58; 35.66; 32.80; 32.38; 26.62; 26.02. MS EI(+) (*m/z*): 287 $[M+H]^+$.

21b: The product was obtained as a yellowish oil in 78% yield (117 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.37–7.16 (m, 5H, $5H_{arom}$); 3.47 (br.s, 2H, $C\underline{H}_2Ph$); 3.23 (t, *J* = 6.0 Hz, 2H, $C\underline{H}_2N_3$); 2.85 (d, *J* = 9.0 Hz, 2H); 1.90 (t, *J* = 9.0 Hz, 2H); 1.65–1.52 (m, 4H); 1.36–1.15 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 138.06; 129.87; 128.11; 125.62; 63.58; 54.00; 51.49; 36.51; 35.72; 32.43; 29.40; 28.84; 26.73; 26.65. MS EI(+) (*m/z*): 301 $[M+H]^+$.

21c: The product was obtained as a yellowish oil in 76% yield (119 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.32–7.27 (m, 5H, $5H_{arom}$); 3.46 (s, 2H, $C\underline{H}_2Ph$); 3.23 (t, *J* = 6.0 Hz, 2H, $C\underline{H}_2N_3$); 2.84 (d, *J* = 9.0 Hz, 2H); 1.86 (t, *J* = 9.0 Hz, 2H); 1.66–1.52 (m, 4H); 1.35–1.15 (m, 13H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 138.64; 129.23; 128.08; 126.8; 63.59; 54.02; 51.47; 36.59; 35.74; 32.46; 29.70; 29.15; 28.83; 26.70; 26.68. MS EI(+) (*m/z*): 315 $[M+H]^+$.

5.1.8 | General procedure for the synthesis of compounds 12–14

To the corresponding azide **21** (0.4 mmol) dissolved in THF (10 mL), PPh_3 (1.5 eq) and water (4 eq) were added, the reaction was left to stir for 2.5 h under reflux. The solvent was evaporated and the product was purified by column chromatography (SiO_2, 0–20% MeOH in $CHCl_3$ to 20% MeOH in $CHCl_3$ saturated with NH_3).

12: The product was obtained as a yellowish oil in 95% yield (99 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.37–7.18 (m, 5H, $5H_{arom}$); 3.48 (s, 2H, $C\underline{H}_2Ph$); 2.87 (d, *J* = 11.1 Hz, 2H); 2.69 (t, *J* = 7.0 Hz, 2H, $C\underline{H}_2N_3$); 2.00 (br.s, 2H, $N\underline{H}_2$); 1.92 (t, *J* = 10.8 Hz, 2H); 1.84–1.57 (m, 2H); 1.35–1.37 (m, 2H); 1.36–1.16 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 138.61; 129.22; 128.08; 126.82; 63.58; 54.00; 42.03; 36.60; 35.70; 33.42; 32.45; 27.11; 26.64. MS EI(+) (*m/z*): 261 $[M+H]^+$, 283 $[M+Na]^+$.

13: The product was obtained as a yellowish oil in 80% yield (88 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.33–7.23 (m, 5H, $5H_{arom}$); 3.49 (s, 2H, $C\underline{H}_2Ph$); 2.87 (d, *J* = 12.0 Hz, 2H); 2.71 (br.s, 2H, $C\underline{H}_2N_3$); 2.60 (br.s, 2H, $N\underline{H}_2$); 1.92 (t, *J* = 12.0 Hz, 2H); 1.63 (m, 2H); 1.48 (m, 2H); 1.35–1.15 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 138.17; 129.38; 128.12; 126.95; 63.53; 53.94; 50.59; 41.50; 36.50; 35.66; 32.28; 29.58; 26.78; 26.70. MS EI(+) (*m/z*): 275 $[M+H]^+$.

14: The product was obtained as a yellowish oil in 82% yield (94 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.31–7.22 (m, 5H, $5H_{arom}$); 3.48 (s, 2H, $C\underline{H}_2Ph$); 2.86 (d, *J* = 12.0 Hz, 2H); 2.67 (t, *J* = 7.0 Hz, 2H, $C\underline{H}_2N_3$); 2.02 (br.s, 2H, $N\underline{H}_2$); 1.91 (t, *J* = 12.0 Hz, 2H); 1.61 (m, 2H); 1.43 (m, 2H); 1.27–1.20 (m, 13H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 138.43; 129.31; 128.09; 126.87; 63.58; 53.99; 42.07; 36.60; 35.73; 32.39; 29.84; 29.48; 26.87; 26.75; 18.45. MS EI(+) (*m/z*): 289 $[M+H]^+$.

5.1.9 | General procedure for the synthesis of compounds 5–9

To the corresponding amine **10–14** (0.25 mmol) dissolved in anhydrous THF (10 mL), DMAP (1.2 eq) was added. To this mixture ester **15** (1 eq) was added. The reaction was let to stir in anhydrous conditions, under an inert atmosphere (Ar), at room temperature, for 12 h. The product was purified by column chromatography (SiO_2, 20% MeOH in $CHCl_3$, saturated by NH_3 for **5–7**, 5% MeOH in DCM for **8** and **9**).

5: The product was obtained as white glass-like solid in 92% yield (100 mg); ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 7.45–7.20 (m, 7H, $5H_{benzyl} + 2H_{indolyl}$); 7.13 (d, *J* = 1.0 Hz, 1H, $H_{indolyl}$); 6.84 (dd, J_1 = 8.6 Hz, J_2 = 2.2 Hz, 1H, $H_{indolyl}$); 3.57 (s, 2H, $C\underline{H}_2Ph$); 3.56–3.48 (m, 1H, $C\underline{H}$); 3.45 (t, *J* = 7.2 Hz, 2H, $C\underline{H}_2NHCOCH_3$); 3.04–2.82 (m, 4H), 2.19 (t, *J* = 11.6 Hz, 2H); 2.03–1.93 (m, 2H); 1.92 (s, 3H, $C\underline{H}_3$); 1.75–1.51 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 171.88; 156.39; 144.03; 136.88; 134.37; 129.40; 127.94; 127.58; 127.12; 123.54; 115.50; 112.24; 111.01; 110.30; 62.51; 51.89; 51.89; 40.16; 31.18; 24.72; 21.20. HR MS ES(+) (*m/z*) calculated for $C_{25}H_{30}N_4O_3Na$ ($[M+Na]^+$) 457.2216. Found: 457.2233.

6: The product was obtained as white glass-like solid in 83% yield (99 mg); ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 7.44–7.22 (m, 7H, $5H_{benzyl} + 2H_{indolyl}$); 7.12 (s, 1H, $H_{indolyl}$); 6.84 (dd, J_1 = 8.7 Hz, J_2 = 2.3 Hz, 1H, $H_{indolyl}$); 3.63 (s, 2H, $C\underline{H}_2Ph$); 3.44 (t, *J* = 7.2 Hz, 2H, $C\underline{H}_2NHCOCH_3$); 3.17 (t, *J* = 7.0 Hz, 2H); 2.98 (br.d, *J* = 11.4 Hz, 2H); 2.90 (t, *J* = 7.30 Hz, 2H); 2.16 (t, *J* = 11.3 Hz, 2H); 1.91 (s, 3H, $C\underline{H}_3$); 1.85–1.50 (m, 4H); 1.50–1.10 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 171.86; 157.12; 144.12; 135.54; 134.35; 129.80; 128.05; 128.05; 127.49; 123.58; 115.53; 112.24; 111.09; 110.32; 62.51; 53.15; 40.75; 40.17; 34.75; 33.11; 31.06; 26.65; 24.76; 21.29. HR MS ES(+) (*m/z*) calculated for $C_{28}H_{36}N_4O_3H$ ($[M+H]^+$) 477.2860. Found: 477.2859.

7: The product was obtained as a colorless oil in 79% yield (100 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.48 (s, 1H, $N\underline{H}_{indolyl}$); 7.44–7.17 (m, 7H, $5H_{benzyl} + 2H_{indolyl}$); 7.01–6.83 (m, 2H, $2H_{indolyl}$); 5.69 (br.s, 1H, $N\underline{H}$); 5.13 (t, *J* = 5.9 Hz; 1H, $N\underline{H}_{carbam}$); 3.62 (s, 2H, $C\underline{H}_2Ph$); 3.52–3.40 (m, 2H); 3.29–3.22 (m, 2H); 3.04–2.71 (m, 4H);

1.96 (t, *J* = 10.7 Hz, 2H); 1.90 (s, 3H, C<u>H</u>$_3$); 1.75–1.54 (m, 4H); 1.46–1.06 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.29; 162.31; 155.81; 144.44; 134.08; 129.44; 128.20; 127.65; 127.10; 123.40; 116.58; 113.09; 111.59; 111.03; 63.37; 53.85; 39.79; 36.41; 35.55; 32.14; 32.14; 29.89; 26.99; 26.43; 25.16; 23.32. HR MS ES(+) (*m/z*) calculated for $C_{30}H_{40}N_4O_3H$ ([M+H]$^+$) 505.3179. Found: 505.3172.

8: The product was obtained as a colorless oil in 79% yield (102 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.66 (s, 1H, N<u>H</u>$_{indolyl}$); 7.31–7.18 (m, 7H, 5H$_{benzyl}$ + 2H$_{indolyl}$); 6.91–6.85 (m, 2H, 2H$_{indolyl}$); 5.76 (br.s, 1H, N<u>H</u>); 5.19 (t, *J* = 5.19 Hz; 1H, N<u>H</u>$_{carbam}$); 3.48 (s, 2H, C<u>H</u>$_2$Ph); 3.47–3.43 (m, 2H); 3.28–3.22 (m, 2H); 2.91–2.77 (m, 4H); 1.92 (t, *J* = 10.7 Hz, 2H); 1.88 (s, 3H, C<u>H</u>$_3$); 1.67–1.51 (m, 4H); 1.40–1.15 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.55; 156.08; 144.54; 138.63; 134.28; 129.49; 128.31; 127.80; 127.08; 123.64; 116.65; 113.11; 111.80; 111.15; 63.74; 54.17; 41.51; 39.96; 36.72; 35.88; 32.56; 30.06; 29.70; 26.97; 26.88; 25.28; 23.45. HR MS ES(+) (*m/z*) calcd. for $C_{31}H_{42}N_4O_3H$ ([M+H]$^+$) 519.3330. Found: 519.3327.

9: The product was obtained as a colorless oil in 71% yield (95 mg); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.94 (s, 1H, N<u>H</u>$_{indolyl}$); 7.29–7.17 (m, 7H, 5H$_{benzyl}$ + 2H$_{indolyl}$); 6.92–6.85 (m, 2H, 2H$_{indolyl}$); 5.82 (br.s, 1H, N<u>H</u>); 5.24 (t, *J* = 5.9 Hz; 1H, N<u>H</u>$_{carbam}$); 3.50 (s, 2H, C<u>H</u>$_2$Ph); 3.50–3.41 (m, 2H); 3.23 (q, *J* = 6.0 Hz, 2H); 2.89–2.77 (m, 4H); 1.93 (m, 2H); 1.91 (s, 3H, C<u>H</u>$_3$); 1.65–1.49 (m, 4H); 1.37–1.13 (m, 13H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.30; 155.84; 144.37; 138.55; 134.17; 129.28; 128.10; 127.63; 126.85; 123.52; 116.45; 112.90; 111.63; 110.96; 63.59; 54.02; 41.33; 39.03; 36.61; 35.74; 32.44; 29.90; 29.79; 29.2; 26.79; 26.74; 25.15; 23.30. HR MS ES(+) (*m/z*) calcd. for $C_{32}H_{44}N_4O_3H$ ([M+H]$^+$) 533.3486. Found: 533.3490.

5.2 | Inhibitory activity assays

Samples of *h*AChE and *h*BChE were derived from human whole red blood cells and plasma, respectively, according to the known procedure.[29]

Ten percent of EDTA buffer was added to freshly collected blood (20 µL of buffer/1 mL of blood). Blood was centrifuged for 10 min (10000×*g*, 4°C). The plasma was diluted 1:125 with the 0.1 M Na_3PO_4 buffer (pH 7.4). Erythrocytes were washed three times with isotonic saline, lysed in 9 volumes of the 0.1 M Na_3PO_4 buffer (pH 7.4) containing 0.5% Triton-X, and diluted with additional 19 volumes of buffer.

Stock solutions of test compounds were prepared in Tween 80/EtOH 3:1 (v/v). Final dilutions were prepared in the 0.1 M Na_3PO_4 buffer (pH 8.0). Samples of enzymes were preincubated with increasing concentrations of the test compound ranging from 0.3 nM to 10 mM (30 min, room temperature) and then incubated with their respective substrates (0.5 mM) and DTNB (25 min, 37°C). The production of a yellow anion was measured by a spectrophotometer. Each tested compound was analyzed in triplicate on three separate occasions.

The enzyme activity at each concentration of the tested compound was expressed as a percentage of the activity in the absence of the compound and plotted as a function of its log concentration. The inhibitory activity was calculated as IC_{50} values.

5.3 | Docking experiments

For the docking, the binding site in the protein was restricted to 6.5 Å around the co-crystallized ligand, and the 30 top-scored FlexX solutions were retained, subsequently post-scored with the program SeeSAR.[20]

ACKNOWLEDGMENT

A.K.H.H. gratefully acknowledges funding from the Helmholtz-Association's Initiative and Networking Fund.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Zuzanna Molęda http://orcid.org/0000-0002-0868-2216
Alwin M. Hartman http://orcid.org/0000-0002-0987-9088
Anna K. H. Hirsch http://orcid.org/0000-0001-8734-4663
Zbigniew Czarnocki http://orcid.org/0000-0002-6581-9282

REFERENCES

[1] M. B. Čolovićž, D. Z. Krtić, T. D Lazarević-Pašti, A. M. Bondžić, V. M. Vasić, *Curr. Neuropharmacol.* **2013**, *11*, 315.
[2] G. A. Reid, S. Darvesh, *Curr. Neurosci.* **2015**, *298*, 424.
[3] O. Lockridge, *Pharmacol. Ther.* **2015**, *148*, 34.
[4] N. H. Greig, T. Utsuki, D. K. Ingram, Y. Wang, G. Pepeu, C. Scali, Q-S. Yu, J. Mamczarz, H. W. Holloway, T. Giordano, D. M. Chen, K. Furukawa, K. Sambamurti, A. Brossi, D. K. Lahiri, *PNAS* **2005**, *102*, 17213.
[5] Q. Li, H. Yang, Y. Chen, H. Sun, *Eur. J. Med. Chem.* **2017**, *132*, 294.
[6] A. Muller-Schiffmann, H. Sticht, C. Korth, *BioDrugs* **2012**, *26*, 21.
[7] A. Agis-Torres, M. Söllhuber, M. Fernandez, J. M. Sanchez-Montero, *Curr. Neuropharmacol.* **2014**, *12*, 2.
[8] N. C. Inestrosa, A. Alvarez, C. A. Perez, R. D. Moreno, M. Vincente, C. Linker, O. I. Casanueva, C. Soto, J. Garrido, *Neuron* **1996**, *16*, 881.
[9] S. A. Rosales-Corral, D. Acuña-Castroviejo, A. Coto-Montes, J. A. Boga, L. C. Manchester, L. Fuentes-Broto, A. Korkmaz, S. Ma, D. X. Tan, R. J. Reiter, *J. Pineal Res.* **2012**, *52*, 167.
[10] M. Atanasova, G. Stavrakov, I. Philipova, D. Zheleva, N. Yordanov, I. Doytchinova, *Bioorg. Med. Chem.* **2015**, *23*, 5382.
[11] M. I. Fernandez-Bachiller, C. Perez, N. E. Campillo, J. A. Paez, G. C. Gonzalez-Munoz, P. Usan, E. Garcia-Palomero, M. G. Lopez, M. Villarroya, A . G. Garcia, A. Martinez, M. I. Rodriguez-Franco, *Chem. Med. Chem.* **2009**, *4*, 828.
[12] H. Jiang, X. Wang, L. Huang, Z. Luo, T. Su, K. Ding, X. Li, *Bioorg. Med. Chem.* **2011**, *19*, 7228.
[13] S. Cheng, W. Zheng, P. Gong, Q. Zhou, Q. Xie, L. Yu, P. Zhang, L. Chen, J. Li, J. Chen, H. Chen, *Bioorg. Med. Chem.* **2015**, *23*, 3110.
[14] E. Ramos, J. Egea, C. de Los Ríos, J. Marco-Contelles, A. Romero, *Future Med. Chem.* **2017**, *9*, 765.

[15] M. I. Rodriguez-Franco, M. I. Fernandez-Bachiller, C. Perez, B. Hernandez-Ledesma, B. Bartolome, *J. Med. Chem*. **2006**, *49*, 459.
[16] A. Zawadzka, I. Łozińska, Z. Molęda, M. Panasiewicz, Z. Czarnocki, *J. Pineal Res*. **2013**, *54*, 435.
[17] E. Mezeiova, K. Spilovska, E. Nepovimova, L. Gorecki, O. Soukup, R. Dolezal, Da. Malinak, J. Janockova, D. Jun, K. Kuca, J. Korabecny, *J. Enzyme Inhib. Med. Chem*. **2018**, *33*, 583.
[18] J. Wang, Z. M. Wang, X.-M. Li, F. Li, J.-J. Wu, L.-Y. Kong, X.-B. Wang, *Bioorg. Med. Chem*. **2016**, *24*, 4324.
[19] G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, *Biochem. Pharmacol*. **1961**, *7*, 88.
[20] BioSolveIT GmbH, SeeSAR 5.5.
[21] BioSolveIT GmbH, LeadIT 2.2.0.
[22] R. Ragno, G. R. Marshall, R. Di Santo, R. Costi, S. Massa, R. Rompei, M. Artico, *Bioorg. Med. Chem*. **2000**, *8*, 1423.
[23] M.-Y. Chang, Y.-C. Lin, S.-T. Chen, N.-C. Chang, *J. Chin. Chem. Soc*. **2002**, *49*, 1079.
[24] A. Samadi, M. Chioua, I. Bolea, G. Esteban, M. Unzeta, E. Gálvez, J. Marco-Contelles, *Eur. J. Med. Chem*. **2011**, *46*, 4665.
[25] J. Guan, D. E. Kyle, L. Gerena, *J. Med. Chem*. **2002**, *45*, 2741.
[26] J. Chang, S.-J. Zhang, Y.-W. Jiang, L. Xu, J. M. Yu, W. J. Zhou, X. Sun, *Chem. Med. Chem*. **2013**, *8*, 976.
[27] D. Brundish, A. Bull, V. Donovan, J. D. Fullerton, S. M. Garman, J. F. Hayler, D. Janus, P. D. Kane, M. McDonnell, G. P. Smith, R. Wakeford, C. V. Walker, G. Howarth, W. Hoyle, M. C. Allen, J. Ambler, K. Butler, M. D. Talbot, *J. Med. Chem*. **1999**, *42*, 4584.
[28] H. G. Bazin, Y. Li, J. K. Khalaf, S. Mwakwari, M. T. Livesay, J. T. Evans, D. A. Johnson, *Bioorg. Med. Chem. Lett*. **2015**, *25*, 1318.
[29] J. R. Atact, Q. S. Yu, T. T. Soncrant, A. Brossi, S. I. Rapoport, *J. Pharmacol. Exp. Ther*. **1989**, *249*, 194.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Łozińska I, Świerczyńska A, Molęda Z, Hartman AM, Hirsch AKH, Czarnocki Z. Donepezil–melatonin hybrids as butyrylcholinesterase inhibitors: Improving binding affinity through varying mode of linking fragments. *Arch Pharm Chem Life Sci*. 2018;1–10. https://doi.org/10.1002/ardp.201800194