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Novel deoxyvasicinone and tetrahydro-*beta*-carboline hybrids as inhibitors of acetylcholinesterase and amyloid beta aggregation

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ABSTRACT

A novel series of deoxyvasicinone-tetrahydro-*beta*-carboline hybrids were synthesized and evaluated as acetylcholinesterase (AChE) and β-amyloid peptide (Aβ) aggregation inhibitors for the treatment of Alzheimer's disease. The results revealed that the derivatives had multifunctional profiles, including AChE inhibition, A$\beta_{1\text{-}42}$ aggregation inhibition, and neuroprotective properties. Inspiringly, hybrids **8b** and **8d** displayed excellent inhibitory activities against *h*AChE (IC_{50} = 0.93 and 1.08 nM, respectively) and Aβ_{1-42} self-aggregation (IC_{50} = 19.71 and 2.05 μM, respectively). In addition, **8b** and **8d** showed low cytotoxicity and good neuroprotective activity against Aβ_{1-42}-induced damage in SH-SY5Y cells.

Alzheimer's disease (AD) is progressive, fatal, and the most common neurodegenerative disease. Alzheimer's Disease International (ADI) estimates that more than 50 million people suffered from AD in 2019, a figure set to increase to 152 million by 2050.[1] The causes of AD are not yet fully understood, but some factors, including neuron loss, Aβ deposits, τ-aggregation, neuroinflammation, and oxidative stress, have been suggested to play significant roles in AD.[2–6] Currently, drugs for the treatment of AD include four acetylcholinesterase inhibitors (tacrine, donepezil, rivastigmine and galantamine) and one *N*-methyl-D-aspartate (NMDA) receptor antagonist (memantine). Unfortunately, these drugs can only improve symptoms but not cure the disease. Furthermore, side effects, including nausea, vomiting, and liver damage, have been reported after the use of these drugs.[7] Thus, it is necessary to develop more effective drugs for the treatment of AD.

Due to the complexity of AD and the interconnections of various factors in its progression, the development of multitarget-directed ligands (MTDLs) with two or more disease targets has attracted much attention as a therapeutic method for the treatment of AD.[8,9] Among the diverse factors of AD, both the low levels of ACh and the deposition of Aβ play vital roles in AD pathogenesis.[10,11] AChE is a key enzyme in the hydrolysis of acetylcholine. Thus, acetylcholinesterase inhibitors (AChEIs) could increase the amount of ACh in AD patients. Furthermore, some researchers have identified that the peripheral anionic site (PAS) of AChE contributes to accelerating senile Aβ deposition.[12] The progressive deposition of Aβ plays a crucial role in AD pathogenesis, which can cause neuronal death and eventually dementia. Therefore, many series of compounds have been developed as inhibitors of AChE and Aβ aggregation in recent years.[13–15]

Natural products are important sources of novel lead compounds with bioactive properties.[16] Deoxyvasicinone (**A**) and β-carboline (**B**) alkaloids (Fig. 1) are the main active ingredients of *Peganum harmala,* which possesses many biological activities, including antitumor, antibacterial, and anti-inflammatory activities.[17] Recently, deoxyvasicinone and its derivatives have been researched as cholinesterase inhibitors (Fig. 1).[18–21] For instance, derivatives **C** and **D** show potent inhibitory activity against AChE with IC_{50} values of 50 and 23 nM, respectively.[22,23] Moreover, there is growing evidence that β-carboline derivatives demonstrate potential inhibitory activities against cholinesterase, monoamine oxidase, and Aβ aggregation.[24–26] For example, derivative **E** displays an excellent ability to inhibit cholinesterase (IC_{50}, 21.6 nM for AChE, and 39.8 nM for butyrylcholinesterase (BuChE)), good inhibition of Aβ aggregation (65.8% at 20 μM) and good antioxidant activity (1.57 Trolox equivalents).[27] In addition, Qu et al reported that a novel series of bivalent β-carboline derivatives (**F**) showed good potency for BuChE inhibition, A$\beta_{1\text{-}42}$ disaggregation and neuroprotection.[25] Thus, β-carboline is regarded as a useful framework for AD drug design. Additionally, a literature study reported that two naturally occurring dimers (**G**) of deoxyvasicinone and β-carboline

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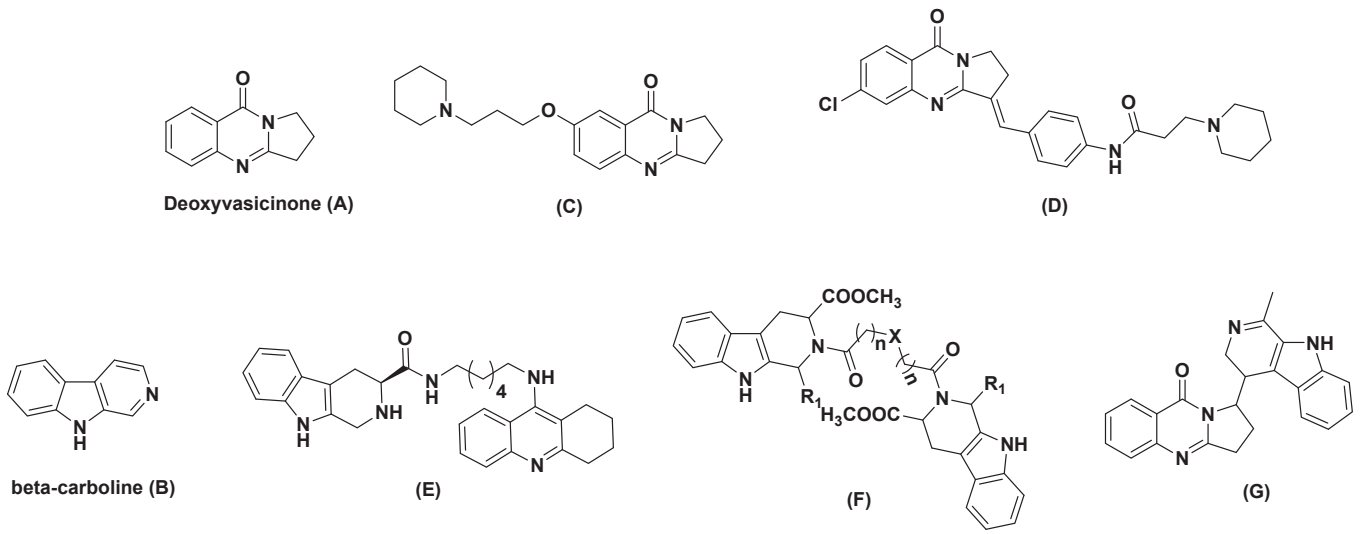


Fig. 1. The structures of deoxyvasicinone, β-carboline and their derivatives discussed in the text.

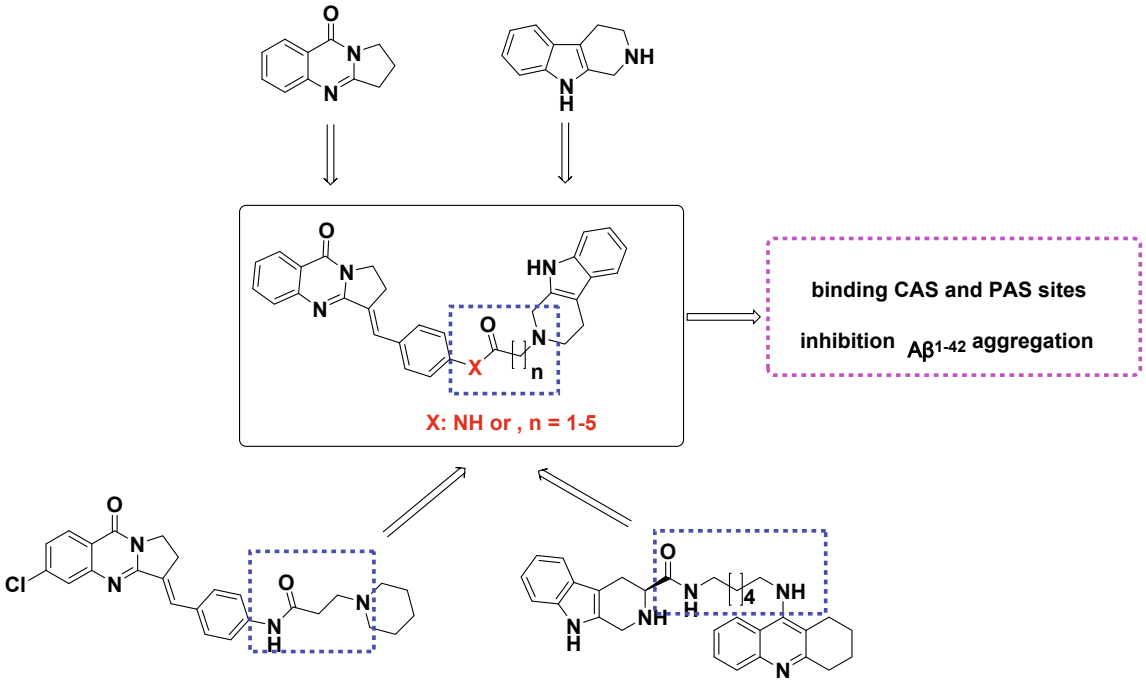


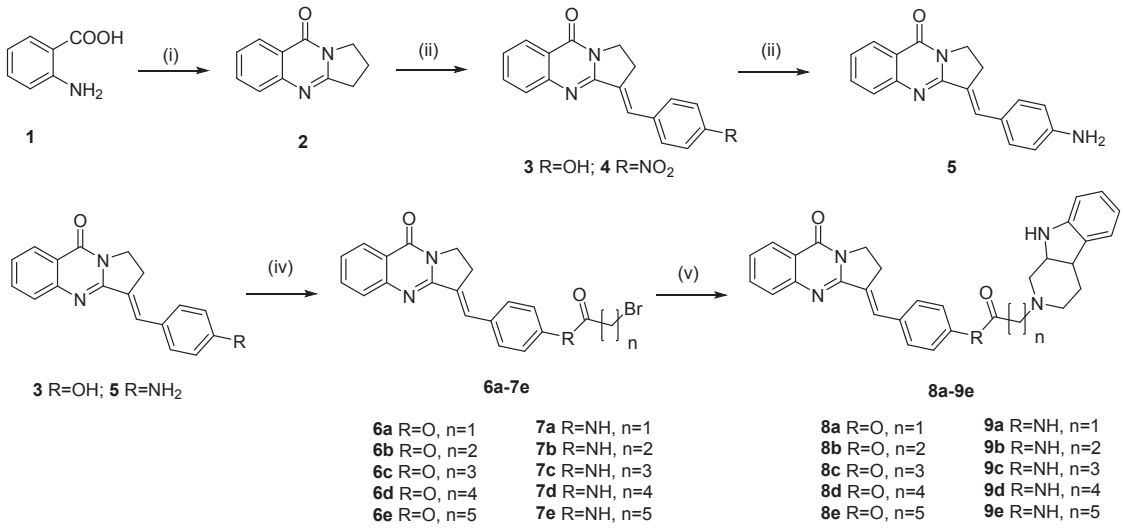
Fig. 2. Design strategy for the new hybrids of deoxyvasicinone and tetrahydro-β-carboline.

display inhibitory activity against AChE.[28]

Inspired by the above studies, we assumed that the rational combination of deoxyvasicinone and *β*-carboline may enhance inhibitory activity against AChE. Therefore, in this work, we designed, synthesized and evaluated a novel series of deoxyvasicinone-tetrahydro-*beta*-carboline hybrids as multifunctional agents for the treatment of AD (Fig. 2). These new hybrids could bind simultaneously to the catalytic active site (CAS) and peripheral anionic site (PAS) of AChE and show inhibitory activity against Aβ_{1-42} aggregation. Additionally, two potential compounds were investigated for their cytotoxicity and protective capacity for Aβ_{1-42}-induced SH-SY5Y cell death. Furthermore, molecular docking studies were performed to research binding modes in the active site.

The detailed synthetic route for the target compounds is outlined in Scheme 1. Deoxyvasicinone **(2)** was produced from 2-aminobenzoic acid **(1)** according to a previously described method.[21] Intermediates **3** and **4** were prepared *via* the Claisen-Schmidt condensation reaction between **2** and 4-hydroxybenzaldehyde/4-nitrobenzaldehyde in the presence of acetic acid and sodium acetate. Then, the reduction of compound **4** was carried out with sodium sulfide/nonahydrate sodium hydroxide to yield intermediate **5**. The condensation of intermediates **3** and **5** with different linear bromo-acids and EDCI in dry dichloromethane gave intermediates **6** and **7**. Their further amination with tetrahydro-*β*-carboline produced target compounds **8a-8e** and **9a-9e**. These deoxyvasicinone- tetrahydro-*beta*-carboline hybrids were characterized by NMR and HRMS (see supplementary material).

The inhibitory properties of the target compounds against *h*AChE (from human erythrocytes) and *h*BuChE (from human serum) were evaluated using Ellman's method,[29] with tacrine and donepezil as positive controls. The IC_{50} values and selectivity indices for AChE over BuChE are given in Table 1. The tested target compounds were potent



Scheme 1. Synthesis of deoxyvasicinone and tetrahydro-β-carboline hybrids. **Reagents and conditions:** (i) 2-pyrrolidinone, $POCl_3$, dry toluene; (ii) PNBD or PHBA, AcOH, AcONa, reflux; (iii) NaOH, $Na_2S_9{\cdot}H_2O$, EtOH, reflux; (iv) Different bromo-acids, EDCI, dry CH_2Cl_2, reflux; (v) Tetrahydro-β-carboline, K_2CO_3, EtOH, reflux.

Table 1
Inhibition of *h*AChE, *h*BuChE and A$\beta_{1\text{-}42}$ aggregation by the synthesized compounds.

Compound	IC_{50} (nM)[a] or inhibition % at 1 μM		S.I.[b]	IC_{50} (μM)[a] for A$\beta_{1\text{-}42}$ or inhibition % at 20 μM
	*h*AChE	*h*BuChE		
8a	216.34 ± 21.82	238.31 ± 18.28	1.1	38.8%
8b	0.93 ± 0.12	69.18 ± 25.56	74.4	19.71 ± 0.82
8c	16.54 ± 1.25	44.15 ± 2.47	2.7	41.5%
8d	1.08 ± 0.26	422.28 ± 29.52	391.0	2.05 ± 0.13
8e	10.72 ± 0.81	507.07 ± 32.29	47.3	15.12 ± 0.75
9a	21.6%	596.15 ± 48.15	< 0.6	37.5%
9b	9.75 ± 0.59	725.95 ± 60.58	74.5	31.6%
9c	49.9%	215.14 ± 10.83	<0.2	21.2%
9d	98.3 ± 7.97	32.75 ± 1.76	0.3	27.9%
9e	153.25 ± 13.58	485.53 ± 35.62	3.2	18.3 ± 0.62
Deoxyvasicinone and Tetrahydro-β-carboline	29.7%	40.5%	–	20.7%
Tacrine	72.38 ± 5.39	n.d.[c]	n.d.[c]	n.d.[c]
Donepezil	17.76 ± 1.26	n.d.[c]	n.d.[c]	n.d.[c]
Resveratrol	n.d.[c]	n.d.[c]	n.d.	13.43 ± 0.85
Curcumin	n.d.[c]	n.d.[c]	n.d.	16.25 ± 0.73

[a] Values are expressed as mean ± SD of five independent experiments.
[b] Selectivity index for AChE is defined as IC_{50} (*h*BuChE)/IC_{50} (*h*AChE).
[c] n.d. Not determined.

inhibitors of *h*AChE and *h*BuChE, with IC_{50} values <1 μM (except for compounds **9a** and **9c**). Most compounds showed selectivity for *h*AChE over *h*BuChE (except for compounds **9a**, and **9c**). Importantly, compounds **8b**, **8c**, **8d**, **8e** and **9b** exhibited *h*AChE inhibitory activity that was greater than that of tacrine (IC_{50} = 72.38 nM) and donepezil (IC_{50} = 17.76 nM). Compounds **8b** and **8d** displayed excellent inhibitory potential for *h*AChE, with IC_{50} values of 0.93 nM and 1.08 nM, respectively.

To investigate the inhibitory potency of the derivatives on Aβ_{1-42} self-aggregation, a thioflavin-T (ThT) fluorescence assay was carried out,[30] and resveratrol and curcumin were used as controls. As shown in Table 1, 4 compounds (**8b**, **8d**, **8e** and **9e**) displayed potent inhibition of Aβ_{1-42} aggregation that was similar to or greater than that of resveratrol (IC_{50} = 12.43 nM) and curcumin (IC_{50} = 15.37 nM). In particular, compound **8d** exhibited the strongest inhibitory activity, which was 6-fold better than that of resveratrol. Moreover, **8d** also acts as a potent *h*AChE inhibitor. Furthermore, it was interesting to observe that the esterified derivatives **8a**-**8e** have better *h*AChE and inhibitory activity than the corresponding amidated hybrids **9a**-**9e**.

To support the above experimental results, molecular docking simulations of compound **8d** with *h*AChE (PDB code: 4EY7), BuChE (PDB: 4TPK) and ***A***$\beta_{1\text{-}42}$ (PDB code: 1IYT) were performed using the Surflex-Dock program in Sybyl-X 2.0 software. The figures were prepared using PyMOL and PoseView (Fig. 3).[31,32] As shown in Fig. 3a-b, hybrid **8d** could simultaneously bind to both the *h*AChE catalytic active site (CAS) and the peripheral anionic site (PAS). The β-carboline moiety of **8d** occupied the catalytic active site (CAS), forming π–π stacking interactions with Trp86 (4.4 Å). The benzene group was able to bind to the PAS through π–π stacking interactions with Trp286 (3.6 Å) and Tyr341 (5.0 Å). Additionally, the long alkyl linker and the deoxyvasicinone moiety formed hydrophobic interactions with Gly120, Gly121, Tyr337, Phe338, Trp286, His287 and Tyr341, which could enhance the interaction with *h*AChE. These results indicated that π–π stacking interactions and hydrophobic interactions play important roles in the binding of **8d** with *h*AChE. It can be seen from Fig. 3c-3d that the β-carboline moiety interacts with the Trp82 residue of *h*BuChE *via* a π–π stacking interaction at a distance of 3.0 Å. Meanwhile, the amino group of the β-carboline formed a hydrogen bond with His438 at a distance of 2.2 Å. Additionally, hybrid **8d** showed hydrophobic interactions with Trp82, Ala328 and Tyr332. From Fig. 3e-f, a π–π stacking interaction was found between the Phe20 residue of Aβ_{1-42} and the benzene group of the linker (4.3 Å). In addition, the carbonyl group of the linker formed a hydrogen bond with Lys16 (2.0 Å). Moreover, hydrophobic interactions between hybrid **8d** and residues of Aβ_{1-42} (His13, Lys16, Leu17, Phe20, Ala21 Val24 and Gly25) were found, which could improve the Aβ_{1-42} self-aggregation inhibitory activity.

In order to evaluate safety, two potential compounds (**8b** and **8d**) were selected to investigate toxicity in the human neuronal cell line SH-SY5Y using the MTT assay.[31] As depicted in Fig. 4, compound **8b** did not

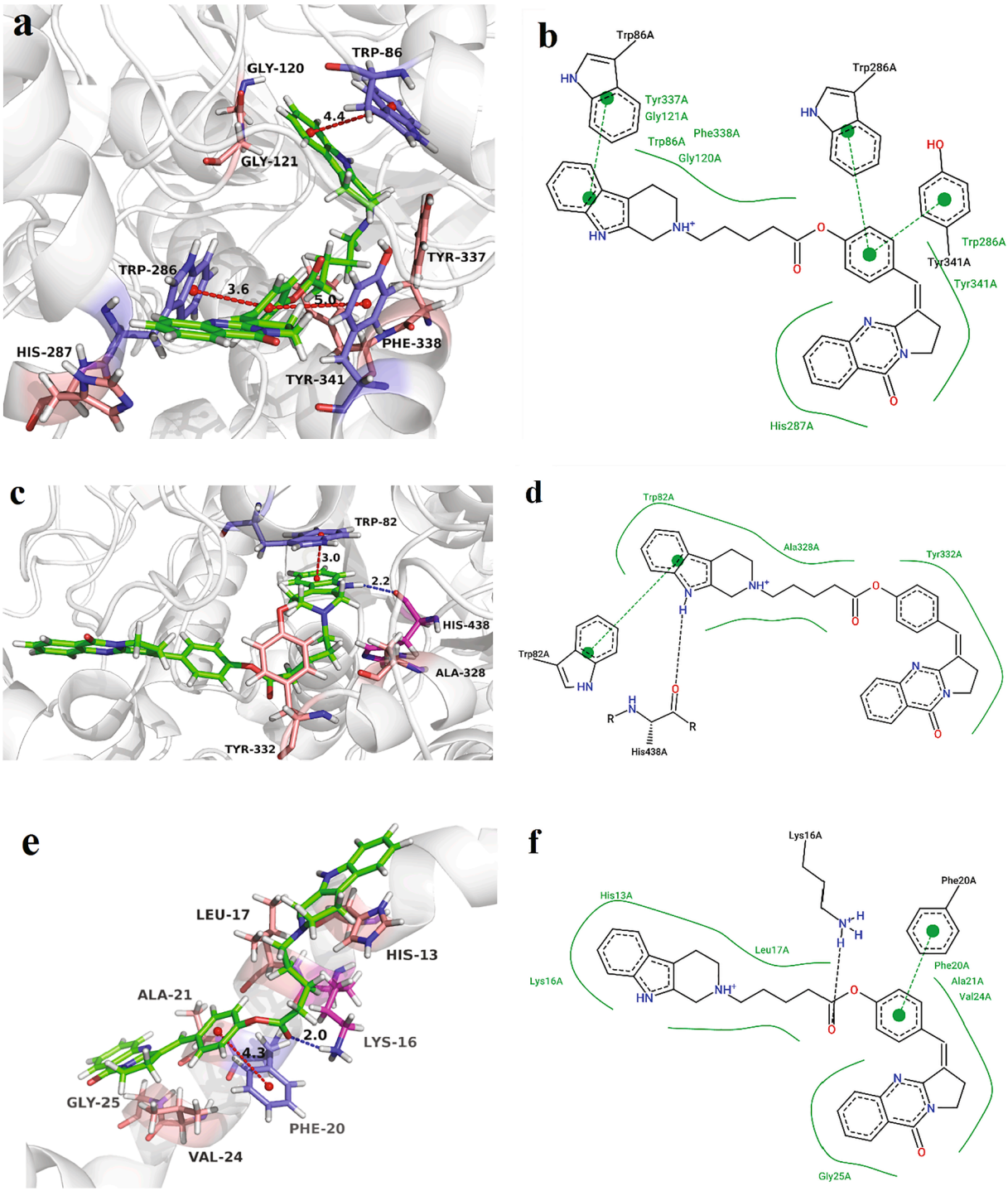


Fig. 3. 3D docking model of compound **8d** with (a) AChE (PDB: 4ey7), (c) BuChE (PDB: 4tpk) and (e) A$\beta_{1\text{-}42}$ (PDB: 1iyt). Dashed blue lines represent hydrogen bonds, and dashed red lines stand for π-π interaction. 2D schematic diagram of docking model of compound **8d** with (b) AChE, (d) AChE and (f) A$\beta_{1\text{-}42}$. Dashed dark lines represent hydrogen bonds, dashed green lines stand for π-π interaction, and solid green lines stand for hydrophobic interaction.

display a significant effect on cell viability at a concentration of 0.1 μM after incubation for 48 h. Compound **8d** did not show cytotoxicity at concentrations of 0.1–1 μM. When the concentrations increased to 10 μM, compounds **8b** and **8d** exhibited significant effects on SH-SY5Y cell viability, but the cell viability was still above 76%. These results demonstrated that compounds **8b** and **8d** exhibit very low toxicity to SH-SY5Y cells in the range of tested concentrations.

To further explore the neuroprotective effects of **8b** and **8d** on A$\beta_{1\text{-}42}$-induced damage, SH-SY5Y cells were exposed to **8b** and **8d** at different concentrations (0.1 μM, 1 μM, and 10 μM) for 48 h. The results (Fig. 4) showed that the cell viability significantly decreased (52.8%, $^{***}p < 0.001$) when SH-SY5Y cells were cultivated with A$\beta_{1\text{-}42}$. However, the two compounds showed significant neuroprotection at concentrations between 1 μM and 10 μM. Compared with the A$\beta_{1\text{-}42}$-treated group, the most promising compound, **8d**, induced marked cellular recovery at the lowest concentration of 0.1 μM ($^{\#}p < 0.05$). The results demonstrated that the two hybrids have neuroprotective activity against A$\beta_{1\text{-}42}$-induced neurotoxicity.

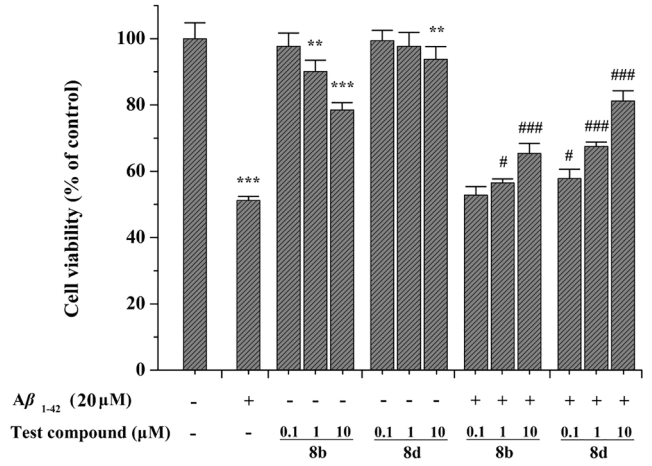


Fig. 4. Neurotoxicity and neuroprotective effects of compounds **8a** and **8d** in SH-SY5Y cells. Data are represented as mean ± SD of five independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs* control group (untreated cells); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs* A$\beta_{1\text{-}42}$-treated cells.

In this study, a series of novel deoxyvasicinone-tetrahydro-*beta*-carboline hybrids was designed, synthesized and evaluated as dual inhibitors for AChE and amyloid beta aggregation. The results showed that most compounds were potent inhibitors of *h*AChE and Aβ_{1-42} self-aggregation. Among them, 5 compounds had inhibitory activity against *h*AChE that was higher than that of the standard drugs tacrine (IC_{50} = 72.38 nM) and donepezil (IC_{50} = 17.76 nM). In addition, four compounds displayed potent inhibition of Aβ_{1-42} aggregation that was similar to or higher than that of resveratrol (IC_{50} = 12.43 nM) and curcumin (IC_{50} = 15.37 nM). In particular, hybrids **8b** and **8d** showed significant inhibition of *h*AChE (IC_{50} = 0.93 and 1.08 nM, respectively) and possessed strong Aβ_{1-42} self-aggregation inhibitory activities. Moreover, hybrids **8b** and **8d** exhibited good neuroprotective activity against Aβ_{1-42}-induced damage in SH-SY5Y cells and showed low toxicity. Thus, compounds **8b** and **8d** should be considered potent drug candidates for the treatment of AD and further investigation of their mechanisms of action.

Declaration of Competing Interest

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

Acknowledgments

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Appendix A. Supplementary data

Supplementary data (experimental procedures and spectroscopic characterizations of the compounds) to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127659.

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