Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *90* (5) 545–559 (2025)
JSCS–5405
Original scientific paper
Published 16 May, 2025

Hybrids of 4-aminoquinolines and adamantane as inhibitors of AChE

KATARINA KOMATOVIĆ[1], ANA MATOŠEVIĆ[2], MARIO ZLATOVIĆ[1], DUŠAN SLADIĆ[1], ANITA BOSAK[2] and DEJAN M. OPSENICA[3,4]*

[1]*University of Belgrade, Faculty of Chemistry, Studentski trg 12–16, 11158 Belgrade, Serbia,* [2]*Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10001 Zagreb, Croatia,* [3]*Institute of Chemistry Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Beograd, Serbia and* [4]*Centre of Excellence in Environmental Chemistry and Engineering, ICTM, 11000 Belgrade, Serbia*

(Received 31 January, revised 6 February, accepted 7 February 2025)

Abstract: Alzheimer's disease (AD) is an incurable and progressive neurodegenerative disorder that causes cognitive capabilities and memory loss and damage to brain functionality and structure. From diverse possibilities for drug development, the inhibition of acetylcholinesterase (AChE) remains as dominant treatment of symptoms. In our continued investigation of long-chain derivatives of 4-aminoquinoline containing an adamantyl (Ad) group, six new derivatives that differ in the substitution at the terminal amino group or the Ad moiety were synthesised. Their inhibition of AChE, *in silico* drug-likeness, the potential for passing through the blood-brain barrier (BBB) and possible binding modes with AChE for the most active compounds were investigated. It was shown that introducing OH, Br or acetamide group increases the inhibitory potency compared with less polar compounds containing the benzyl group as the second substituent at the amino group. Analysis of *in silico* obtained parameters defined by Lipinsky's rule showed that neither compound is likely to cross the BBB because of violation of at least one of the rules, in general, log *P* and a number of rotatable bonds (RB). Docking of the most active compounds to AChE suggests that compounds act as dual-binding site inhibitors since they have simultaneous interaction with catalytic and peripheral anionic sites of AChE. The substituents on the Ad could be the ones that determine the mode of binding into the enzyme and provide interactions that stabilise the complex between the compound and AChE.

Keywords: 4-aminoquinolines; adamantane; cholinesterase; AChE.

INTRODUCTION

Alzheimer's disease (AD) is an uncurable neurodegenerative disorder which progressively and irreversibly causes damage to the brain functionality and manif-

* Corresponding author. E-mail: dejan.opsenica@ihtm.bg.ac.rs
https://doi.org/10.2298/JSC250131014K

ests with a loss of cognitive capabilities, memory and communication ability of patients. According to the report of the World Health Organization, AD currently affects more than 60 million people worldwide with estimation of 10 million new cases annually. The aetiology of the disease is complex, manifested by the enhanced depletion of the neurotransmitter acetylcholine (ACh), the deposition of amyloid-β peptides (Aβ plaques), the accumulation of hyperphosphorylated τ-protein (neurofibrillary tangles, NFTs), the dyshomeostasis of biometal cations, the oxidative stress and the overstimulation of *N*-methyl-D-aspartate (NMDA) receptors.[1,2] Although such a complex aetiology usually offers multiple druggable targets, the current treatment of AD is based on partially restoring the patient's cognitive functions by alleviating the symptoms of the disease, which is mainly achieved by the drugs acting as inhibitors of acetylcholinesterase (AChE), the enzyme responsible for the hydrolysis of ACh.[3] Currently, four drugs are approved by the FDA for the treatment of cognitive symptoms: donepezil, rivastigmine, galantamine and benzgalantamine (memogain)[4] as galantamine prodrug, all of them inhibitors of acetylcholinesterase (AChE).[5] Although most developed inhibitors decrease the action of AChE by interacting with its catalytic active site (CAS), the inhibition of AChE can also occur through interactions with its peripheral anion site (PAS). Such a mode of inhibition has additional benefits for slowing the progression of AD as AChE PAS is involved in the formation of the stable AChE-Aβ complex that is even more toxic than Aβ peptide aggregates.[6] Also, the Aβ secretion is under cholinergic control, in the way that increasing the level of ACh causes suppression of amyloid production.[7] Finally, AChE contributes to the inflammation because of the interference with the cholinergic-based cascade in the response of the immune system.[8] For these reasons, developing compounds that are capable of interfering both with CAS and PAS and acting as multi-target directed ligands (MTDL) is a very promising strategy for developing new AChE inhibitors.

Recently, our group studied two series of 4-aminoquinoline derivatives as MTDLs whose primary mode of action is the inhibition of human AChE – 4-aminoquinolines (4AQ) structural hybrids with adamantyl (Ad) moiety as the substituent on the terminal amino group[9] and *N*-benzyl (Bn) substituted 4AQ with 1,8-octanediyl side chain.[10] Both series have low cytotoxicity and exhibited excellent inhibitory potency of AChE in the 75 nM to 9.4 μM range (4AQ-Ad compounds) and 3.2 nM to 1.2 μM range (4AQ-Bn) of the enzyme–inhibitor dissociation constant (K_i). According to the calculated drug-likeness properties, it was estimated that compounds have good blood-brain barrier (BBB) penetration potential, *via* passive transport. The obtained kinetic results paired with molecular modelling revealed that the examined compounds are capable of binding to the CAS and the PAS of AChE and as such can also affect the formation of amyloid clusters.

Encouraged by the promising results previously obtained for compounds **1** (K_i = 75 nM)[9] and **2** (K_i = 9.8 nM)[10] our idea is to examine how changes close to the terminal basic amino group or introduction of a substituent on the Ad moiety influence the inhibition of AChE. For that purpose, in this work, a series of six new compounds (Fig. 1) was designed and synthesised to investigate their potency to inhibit AChE. The rationalisation of the observed activity was performed by molecular docking. Besides that, their BBB parameters were calculated. The first set of changes on the encirclement of the terminal group was to extend the distance between the Ad group and the terminal amino group (**3**) and introduce the benzyl group, a simple aryl fragment as an additional substituent (**4** and **5**). The second set (**6**–**8**) has a hydroxyl group, bromine or an amide group, introduced with the aim to affect the physicochemical properties and to enable additional interactions with the protein chain corresponding to the predecessor **1**.

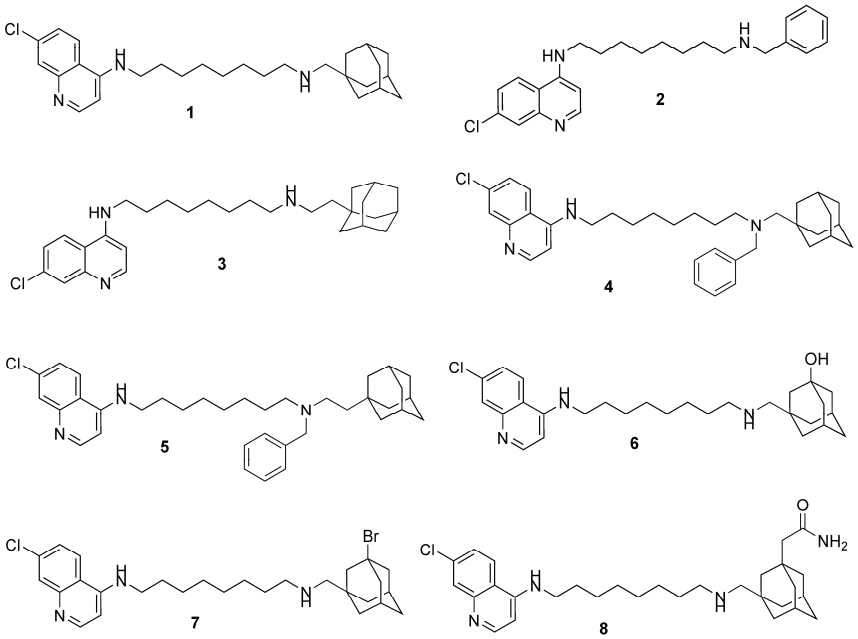


Fig. 1. Structures of **1**, **2** and new compounds examined in this study.

EXPERIMENTAL

Chemistry

All of the chemicals and reagents for the synthesis of 4-aminoquinolines and adamantane hybrid compounds were purchased from commercial sources and used without additional purification. Melting points were recorded on an electrothermal melting point apparatus (1428 6/4 457), England and Boetius PMHK, and were not corrected. IR spectra were recorded on a Perkin–Elmer spectrophotometer FT-IR 1725X (Perkin–Elmer, Waltham, MA 02451, USA).

The positions of absorbance band are expressed in cm^{-1} and intensity is labelled as *w* (weak), *m* (medium) or *s* (strong). ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian/Agilent spectrometer (Agilent Technology, Santa Clara, CA, USA, 95051) at 400 and 100 MHz, for a ^{1}H- and ^{13}C-NMR spectra, respectively, and on a Bruker Ultrashield Advance III spectrometer (Bruker Scientific Instruments, Billerica, MA, USA, 01821) at 500 and 125 MHz, for a ^{1}H- and ^{13}C--NMR spectra, respectively, employing indicated solvents, using TMS as the internal standard. The chemical shifts are expressed in ppm (δ) values and coupling constants (*J*) in Hz. The ESI--MS spectra were recorded on an Agilent Technologies 6210 time-of-flight LC–MS instrument with positive ion mode using MeCN/H_2O gradient with 0.2 % HCOOH as the carrying solvent solution. Samples were dissolved in MeOH (HPLC grade purity). GC/MS analysis: Agilent Technologies 7890A gas chromatograph 5975, MSD and FID detector and DB-5 MS column (30 m×0.25 mm×0.25 μm). LC/MS analysis: Acquity UPLC H-Class LC instrument, Acquity UPLC BEH C18 column (50 mm×2.1 mm×1.7 μm) and Waters ACQ-TQD MS instrument. Eluent: A = H_2O, B = MeCN. Gradient protocol: 0–1 min (5 % B), 1–10 min (5 → 95 % B), 10–11 min (95 % B), 11–12 min (95 → 5 % B), 12–13min (5 % B). Ionisation method: ESI. For the chromatography purification silica gel provided by Merck–Supelco was used, SiO_2 40––63 μm for the dry-flash chromatography and SiO_2 GF254 or SiO_2 PF254 for the preparative thin-layer chromatography.

HPLC purity

The compounds were analysed for purity using an Agilent 1260 Infinity HPLC system equipped with Quat pump (G1311B), injector (G1329B) 1260 ALS, TCC 1260 (G1316A) and detector 1260 DAD VL+ (G1315C) (Agilent, Santa Clara, CA, USA, 95051). The compounds were dissolved in MeOH, and their final concentrations were ~0.5 mg mL^{-1}. The flow rate was 0.5 mL min^{-1}. Compounds were eluted using gradient protocol: 0–1 min 95 % solvent A, 1–6 min 95 % solvent A → 5 % solvent A, 6–11 min 5 % solvent A, 11–14 min 5 % solvent A → 95 % solvent A, 14–15 min 95 % solvent A. The analysis was performed at the UV max of the compounds to maximise selectivity. Four different methods were used for HPLC analysis:

Method I – Zorbax Eclipse Plus C18 4.6 mm×150 mm, 1.8 μm, S.N. USWKY01594 was used as the stationary phase for the analysis of **3** and **5**. The eluent was made of solvent A = 0.2 % HCOOH in deionised water and solvent B = MeCN;

Method II – Zorbax Eclipse Plus C18 4.6 mm×150 mm, 1.8 μm, S.N. USWKY01594 was used as the stationary phase for the analysis of **3** and **5**. The eluent was made of solvent A = 0.2 % HCOOH in deionised water and solvent B = MeOH;

Method III – Zorbax Eclipse Plus C18 4.6 mm×100 mm, 1.8 μm was used as the stationary phase for the analysis of **4** and **6**–**8**. The eluent was made of solvent A = 0.2 % HCOOH in deionised water and solvent B = MeCN;

Method IV – Zorbax Eclipse Plus C18 4.6 mm×100 mm, 1.8 μm was used as the stationary phase for the analysis of **4** and **6**–**8**. The eluent was made of solvent A = 0.2 % HCOOH in deionised water and solvent B = MeOH.

Synthetic procedures, spectral data, and copies of NMR spectra and HPLC chromatograms are given in the Supplementary material to this paper.

Inhibition of AChE

Acetylthiocholine (ATCh) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co., St. Louis, MO, USA. ATCh was dissolved in water, DTNB in 0.1 M sodium phosphate buffer (pH 7.4). Stock solutions of 4AQ hybrids with adamantane were dissolved in DMSO, and all further dilutions were made in water. The source of AChE

was human hemolysed erythrocytes. Blood from healthy donors was collected at the Institute for Medical Research and Occupational Health, Zagreb, Croatia, in accordance with the approval from the Ethics Committee of the Institute for Medical Research and Occupational Health. Erythrocytes were separated from the plasma by centrifugation, after which the sedimented erythrocytes were made up to the volume of whole blood by phosphate buffer. The AChE was used without further purification; all enzyme dilutions were done in phosphate buffer. Cholinesterase activity measurements were done following the procedures described previously.[11,12] Briefly, activities of AChE were measured spectrophotometrically at 0.10 mM ATCh in the absence and presence of a 4AQ hybrid with adamantane (the final concentrations 10 and 1 μM). At least two experiments for each substrate concentration were conducted.

Docking studies

The ligands for docking were prepared using Maestro from Schrödinger Suite 2021-4.[13] Ligands were docked as double protonated based on predicted pK_a calculations and the pH of the biological assay (pH 7.4). The enzyme structures were prepared as described previously,[14] starting from the crystal structures of free AChE (PDB ID: 4EY4).[15] Most active ligands were docked into the enzyme active site using the InducedFit docking protocol from Schrödinger Suite 2021-4.[16] The AChE binding site was defined as described in the Supplementary material. Figures were made using DiscoveryStudio Client v18.1. (Dassault Systèmes, Vélizy-Villacoublay, France).

*p*K$_a$ *Calculation*

The pK_a value of ionisable sites of the tested compounds was predicted *in silico* using the Chemicalize 2022 platform.[17]

In silico prediction of drug-likeness

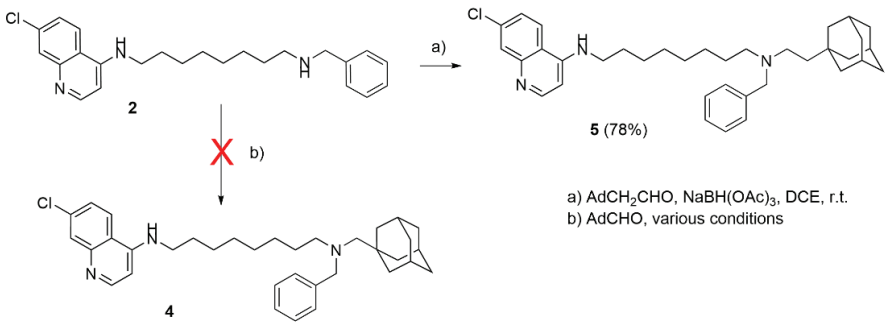
The drug-likeness was estimated for all synthesised compounds, according to the physicochemical properties important for oral bioavailability: molecular weight (*Mw*), number of rotatable bonds (*RB*), number of H-bond acceptors (*HBA*), number of H-bond donors (*HBD*), topological polar surface area (*TPSA*) and lipophilicity (log *P*). For an orally active drug in humans, it is allowed to violate only one of the listed criteria: $180 \leq Mw \leq 500$, $RB \leq 10$, $HBA < 10$, $HBD < 5$, $PSA < 120$ Å^2 and $3 < \log P < 5$.[18] *In silico* calculated data were obtained using Chemicalize 2022 platform.[17]

RESULTS AND DISCUSSION

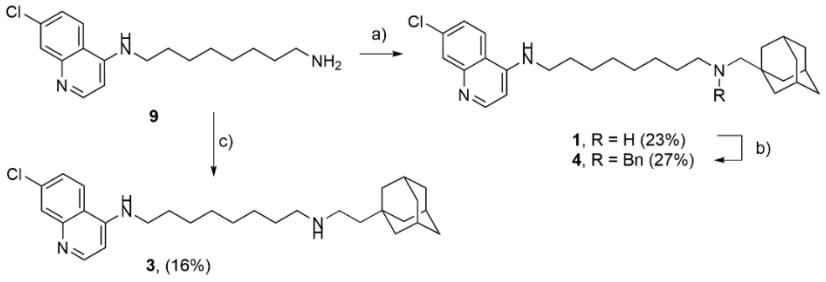
Synthesis of compounds

In our previous studies[9] a low yield of the reaction that attached Ad moiety to the terminal amino group was observed. For that reason, we thought that for the synthesis of the **4** and **5**, the most cost-effective route is to start from **2** and attach the Ad group later (Scheme 1), while **3** will be synthesised directly from **9** (Scheme 2).

The starting compound **2** was prepared following the previously described procedure[19] and derivative **5** was obtained using a corresponding 1-adamantylacetaldehyde and $NaBH(OAc)_3$ in 1,2-dichloroethane (DCE) in a very good yield. Unfortunately, **4** could not be obtained following the planned route. All attempts, which comprise various hydride reagents ($NaBH_4$, $NaBH(OAc)_3$, $NaBH_3CN$) and solvents (MeOH and DCE), including activating reagents such as $ZnCl_2$, were unsuccessful, probably due to high steric demands of Ad fragment.



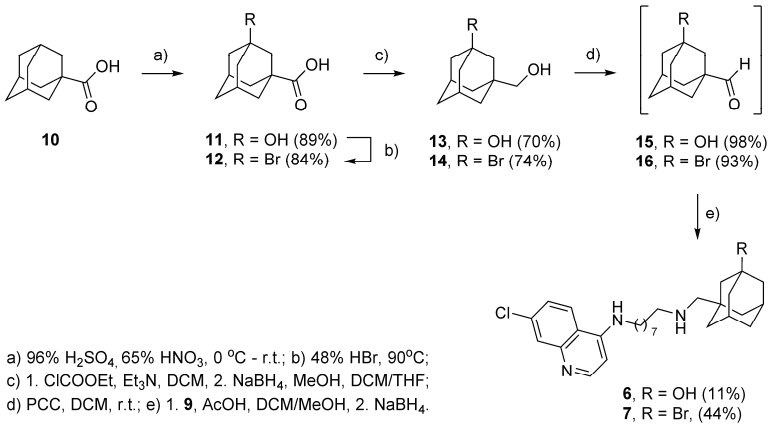
Scheme 1. Synthesis of derivative **5**.



Scheme 2. Synthesis of derivatives **3** and **4**.

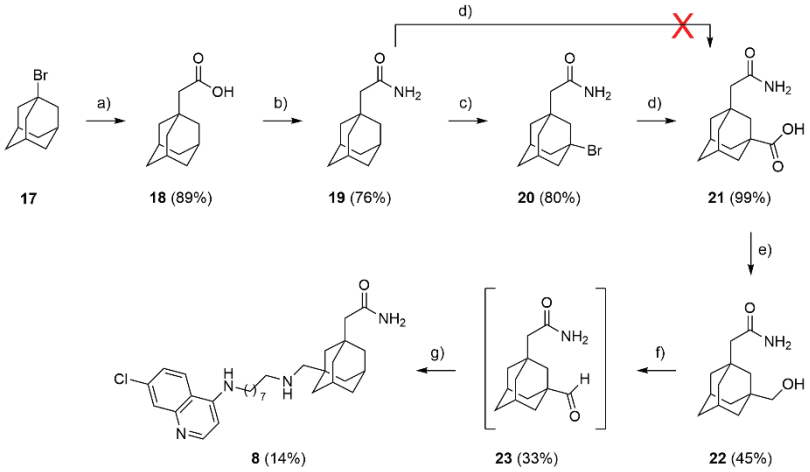
The derivative **4** was finally obtained by applying the reverse synthetic pathway (Scheme 2). The derivative **1** was obtained from **9** and 1-adamantanecarboxaldehyde using the above-described procedure, and it was transformed into **4** using benzaldehyde and $NaBH_3CN/ZnCl_2$ in MeOH, in a low yield as expected. The derivative **3** was obtained from **9** using 1-adamantylacetaldehyde and $NaBH(OAc)_3$ in dichloromethane (DCM), also in a low yield.

The synthesis of the derivatives **6** and **7** (Scheme 3) begins with the hydroxylation of 1-adamantanecarboxylic acid **10**, applying the described procedure[20,21] using the mixture of 96 % sulfuric and 65 % nitric acid. The obtained hydroxyacid **11** was transformed into **12**, using 48 % HBr.[22] The carboxylic acids **11** and **12** were transformed into the corresponding mixed anhydrides using ClCOOEt and Et_3N in DCM, which were reduced, without any additional purification, into corresponding alcohols **13** and **14**, using $NaBH_4$ and MeOH in DCM/THF (THF: tetrahydrofuran). The spectral data for the isolated alcohols **13** and **14** were in accordance with those published.[23,24] The diols **13** and **14** were oxidised into the corresponding aldehydes **15** and **16**, which were, *via* reductive amination using the amine **9**, transformed into **6** and **7** respectively.



Scheme 1. Synthesis of derivatives **6** and **7**.

Synthesis of the compound **8** (Scheme 4) started from 1-bromoadamantane **17** applying the described procedure,[25] using 1,1-dichloroethene in the presence of 96 % sulfuric acid. Obtained 1-adamantaneacetic acid **18** was transformed into **19**,[26] applying a modified procedure,[27] through intermediary mixed anhydride, using the solution of ammonia. The direct carboxylation of the amide **19** to the acid (**21**) failed. The product **21** was obtained indirectly, after bromination using *t*-BuBr,[28] followed by Koch–Haaf carboxylation of **20** in sulfuric acid in the presence of formic acid.[29] After the reduction of the carboxyl group in **21** to alcohol **22**, the



a) 96% H_2SO_4, CH_2=CCl_2, 10 - 20 °C; b) 1. ClCOOEt, Et_3N, DCM, 2. NH_3 (*aq*), DCM/THF; c) *t*-BuBr, 96% H_2SO_4, CCl_4, 10 °C - r.t.; d) 96% H_2SO_4, HCOOH, Hex, 0 °C; e) 1. ClCOOEt, Et_3N, DCM, 2. $NaBH_4$, MeOH, DCM/THF; f) PCC, DCM, r.t.; g) 1. **9**, AcOH, DCM/MeOH, 2. $NaBH_4$.

Scheme 4. Synthesis of derivative **8**.

obtained product was further oxidised to aldehyde **23**, and by the reductive amination with amine **9**, compound **8** was obtained.

Inhibition of acetylcholinesterase

The ability of compounds to inhibit the action of human AChE is expressed as the percent of the inhibition (*%Inh*) of enzyme activity (Table I). A reversible inhibition was determined for all of the tested derivatives, with the percent of inhibition in the 63–99 % range for 10 μM and 22–92 % range for 1 μM concentration of inhibitor.

TABLE I. Inhibition (*%Inh*) of hAChE by 4AQ hybrids with adamantane. All measurements were performed at least in duplicate, and *%Inh* with standard error values were determined from at least two experiments

Compound	Concentration, μM[a]	
	10	1
1	–	47.0 (0.1 μM)[b]
2	–	85.0 (0.1 μM)[b]; 42.7(0.01 μM)[c]
3	98.2	82.4
4	84.2	43.8
5	62.7	22.1
6	98.6	87.6
7	99.4	91.9
8	99.0	88.6
Tacrine	–	32±3 (0.1 μM)[b]

[a]Concentration of the tested compounds **3**–**8**; [b]concentration of the tested compound was 0.1 μM; [c]concentration of the tested compound was 0.01 μM

The newly synthesised compounds inhibited AChE activity by more than 80 % when the concentration of inhibitors was 10 μM, except for the derivative **5**. Reducing inhibitor concentration to 1 μM, the derivatives with the unsubstituted adamantane moiety exhibited a decrease of inhibition potency, while the derivatives with substituents on adamantane – **6**–**8** retained a high percentage of inhibition, 99 % at 10 μM of the inhibitor *vs.* 88–92 % at 1 μM. Although the study was carried out at different concentrations for **3** and **1**, it could be seen that the elongation of the linker between adamantane and the terminal amino group led to a lowering of *%Inh*. Derivative **5** proved to be the least active, less than its structural analogue **4**, indicating that replacing the adamantylethyl group in **5** with the adamantylmethyl group in **4** leads to a decrease in enzyme inhibition. Although the presence of one hydrophobic group, like Ad or Bn on the terminal amino group is shown to increase the inhibition potency compared with the corresponding precursor **9** (see Supplementary material) – K_i (**9**) = 0.61 μM *vs.* K_i (**1**) = 75 nM *vs.* K_i (**2**) = 9.8 nM,[9,10] the observed results suggest that introducing an additional nonpolar moiety decreased inhibitory potency. All three derivatives with a substituent on the Ad group showed high inhibition of AChE activity, with a minor

mutual difference. This indicates that an additional functional group does not disrupt the binding of inhibitors with the enzyme active site and/or can enable new interactions. Although compound **1** is the most active within this set of inhibitors, derivatives **6**–**8** are also promising candidates for the design of new more potent inhibitors.

Docking results

To rationalise the experimentally determined inhibition potency of the selected compounds and suggest a binding mode, the most active ligands (**6**–**8**) were docked into the enzyme active site using InducedFit docking procedure. Although the examined compounds showed very similar docking scores (Table S-I, Supplementary material), they have some specific differences in binding.

All docked compounds interacted with the amino acids from CAS and PAS and bound to AChE as dual-binding site inhibitors. The compounds **6** and **8**, are bonded similarly, with the aminoquinoline ring oriented to PAS and forming multiple hydrophobic T-shaped interactions with amino acids Tyr72 and Trp286 [Fig. 2B and C and Figs. S-2 and S-3 of the Supplementary material). The **8** interacted with Asp74, Tyr 124 and Tyr341, further stabilising the AChE-**8** complex. The Ad moiety, in both compounds, was oriented toward inside of the enzyme, forming interaction with some of the amino acids from CAS and choline-binding sites – Ser203, His447, Trp86 and Tyr337. The **7** has a different orientation, in which the protonated aminoquinoline ring was oriented toward CAS, while the adamantane group was directed toward PAS (Fig. 2 and Fig. S-1). The aminoquinoline ring is engaged in multiple π–π stacked and T-shaped interactions (Trp86, Tyr337 and His447), together with electrostatic ones and a conventional H-bond with His447. Bromine atom on Ad moiety directed the orientation of the ligand by forming multiple hydrophobic-stacked interactions with residues of Tyr72, Trp286 and His287 in the PAS. Different orientations between compounds may be accounted to following interactions: a) multiple H-bonds between OH-group with Gly121, Gly122, Ser203 for **6** and amide group with Tyr133 and Glu202 for **8**, b) multiple hydrophobic-stacked interactions of Br-atom with PAS and c) and a set of interaction of unprotonated quinoline moiety of **8** with the PAS. All identified interactions are listed in Tables S-II–S-IV of the Supplementary material.

*p*K$_a$ *and distribution of protonated species*

The ionisation constants of the tested aminoquinolines at physiological pH 7.4 are given in Table S-I. Compounds had pK_{a1} (quinoline nitrogen) = 7.7 and pK_{a2} (terminal amino group) in the 9.65–11.46 range. Considering the dynamic equilibrium between monoprotonated and diprotonated forms of the examined compounds at pH 7.4, 67 % is in deprotonated form.

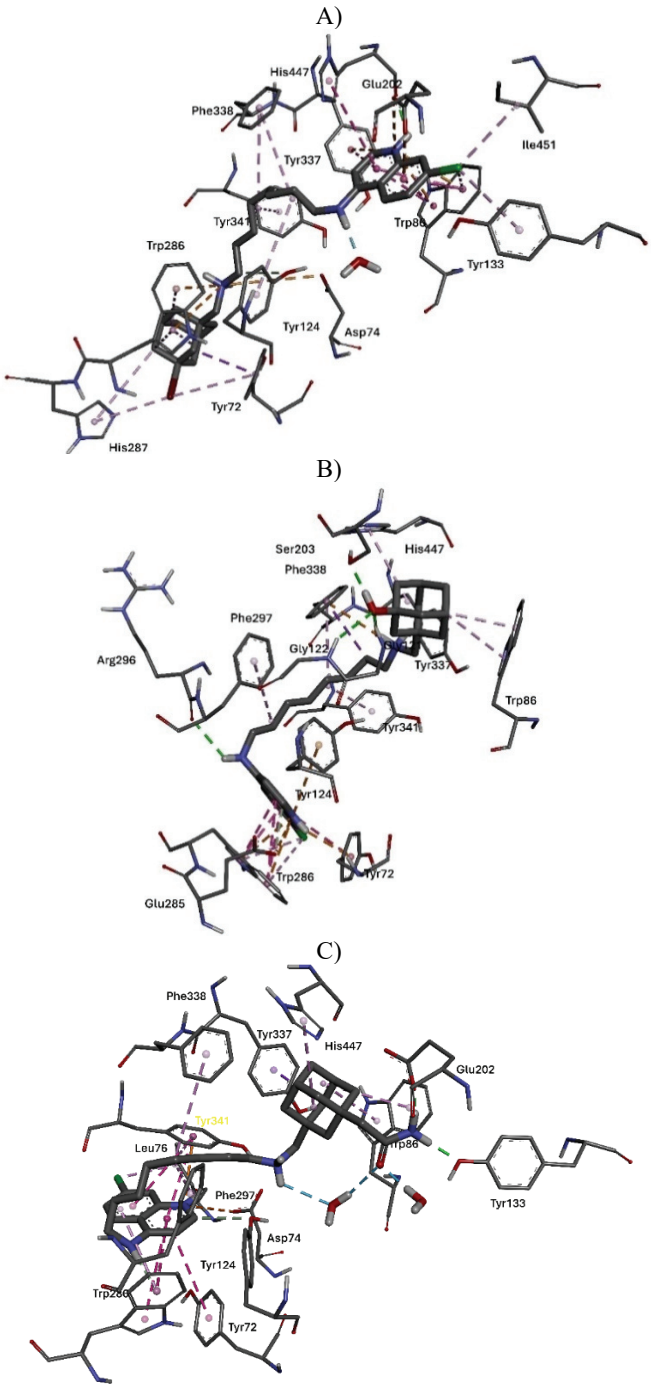


Fig. 2. Binding modes for complexes between compound **7** (A), **6** (B) and **8** (C) and AChE.

In silico *prediction of drug-likeness*

The important physicochemical properties that influence the passive transport of compounds into the central nervous system (CNS) after oral administration are: *Mw*, 1-octanol/water log *P*, *HBD*, *HBA*, *RB* and *TPSA*. Although the key parameters are not experimentally determined, we applied two *in silico* platforms, Chemicalize 2022 and CNS multiparameter optimised algorithm (CNS MPO),[18b,30] to calculate key parameters and to estimate drug-likeness properties of the examined compounds. The corresponding *in silico* data are given in Table S-I. From the calculated values of physicochemical properties, all tested compounds violate two of the six recommended values (Fig. 3). The first is log *P* and the second is RB. The largest deviation from the recommended log *P* values has been shown for **4** and **5**, which at the same time have the lowest activity. Further, the same compounds also have the highest values of Mw, and in combination with other parameters, they could be excluded as potential CNS-active compounds. The deviation in RB from the recommended value cannot be discussed without considering the necessity for conformational flexibility of compounds to attain the optimum occupation of CAS and interaction with PAS so that the most effective inhibition of AChE for the hydrolysis of ACh and interference with Aβ peptide aggregates could be achieved. All the tested compounds met the HBA, HBD, and TPSA requirements.

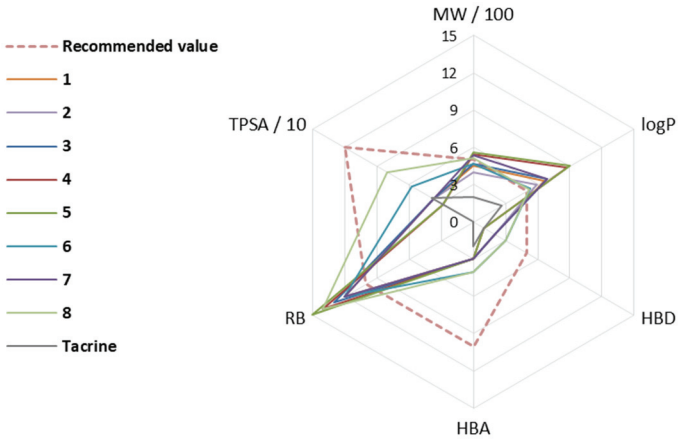


Fig. 3. Radar plot with calculated values of physicochemical properties of the tested compounds. The dashed red line represents the recommended values of *Mw*, log *P*, *HBD*, *HBA*, *RB* and *TPSA*.

Since compounds have two basic groups, which will be ionised in the blood (pH 7.4), the contribution of the pH-dependent partition coefficient (log *D*) and pK_a the values of ionisable groups should not be excluded from the evaluation of the drug-likeness properties. In order to additionally estimate the potential of the

compounds to be CNS-active, we analysed them using central nervous system multiparameter optimization (CNS MPO) algorithm, which is based on a set of six interrelated fundamental physicochemical parameters (*c*log *P*, *c*log *D*, *Mw*, *TPSA*, *HBD* and pK_a (the most basic group in the molecule)) and a variation of Harrington's optimisation method. All physicochemical properties were weighted equally, with a desirability score ranging from 0.0 to 1.0 for each property and a total CNS MPO desirability score ranging from 0.0 to 6.0. A higher score denoted more desirable overall parameters and for the majority of CNS-drugs or candidates, this score is in the $3 < CNS\ MPO \leq 5$ range. We used the parameters calculated on the Chemicalize 2022 platform for the prediction of *CNS MPO* score (Table II). The compounds have low scores which are in the 1.2–2.4 range, which are lower than for the starting compound **2**. Compounds **6** and **8** have the highest scores among synthesised compounds, 2.4 and 2.2, respectively. Tacrine, the drug used in AD therapy expectedly has the highest score, 5.1.

TABLE II. CNS MPO score of the evaluated compounds

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Tacrine
CNS MPO	2.2	2.6	1.9	1.2	1.4	2.4	1.7	2.2	5.1

CONCLUSION

In this study, the influence of changes close to the terminal amino group or the introduction of an additional substituent on the AD moiety on the inhibitory potential of AChE, the drug-likeness properties and the capability of compounds to penetrate BBB was investigated. The introduction of an additional hydrophobic benzyl group, or methylene group in the side chain leads to a decrease in the inhibitory activity of the compounds. On the other hand, the introduction of hydroxyl, bromine or amide group enhanced the potency for the inhibition of AChE, to more than 99 % inhibition at 10 μM or 92–88 % range at 1 μM concentration of the compound. *In silico* obtained indices that determine the drug-likeness of compounds revealed that all synthesised compounds have higher values of log *P* and an acceptable number of RB. Considering a new parameter, *CNS MPO*, that estimates the similarity of compounds with drugs or drug candidates, it was shown that new compounds have low scores which are in the 1.2–2.4 range, compared with tacrine with $CNS\ MPO = 5.1$. Docking of the most active compounds to AChE revealed their capacity to act as dual-binding site inhibitors due to simultaneous interaction with the amino acids from CAS and PAS. Further, our expectations regarding additional substituents on the adamantane moiety were justified, since it was observed that new compounds **6**–**8** have additional interactions with amino acid residues in comparison to **1**. These interactions determine the binding mode of compounds into the enzyme and provide additional stabilisation of the complex with AChE. Although the reported results of *in silico* parameters and drug-likeness

properties of the tested compounds suggest the low potential to be CNS candidates and to penetrate BBB, at the same time these results give the guidelines for the improvements of the structures of the compounds. They should be improved in terms of increasing compound polarity, which would result in increased TPSA and lowering log *P* and log *D*. At the same time, having in mind that the criteria for CNS drugs are more restrictive than for other orally bioavailable drugs, careful selection of potential structural changes, such as the introduction of polar functional groups (ethers, amides, esters, CF_3, *etc*.) should be made to avoid violation of additional parameters such as pK_a or HBD.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13230, or from the corresponding author on request.

Acknowledgements. This work was supported by the Croatian Science Foundation (Grant no. HrZZ-IP-2020-02-9343, to A.B.), the Next Generation EU-BioMolTox (Class: 643-02/23--01/00016, Reg. no. 533-03-23-0006), the MESTD of the Republic of Serbia (Grants No. 451--03-66/2025-03/200026 and 451-03-66/2025-03/200168). We thank Dr. Nataša Terzić-Jovanović for consulting on preparing the key precursor for the synthesis of tested compounds, and Dr. Milka Jadranin for help in the mass spectra analysis of the synthesised compounds. This research is aligned with the Sustainable Development Goals by the United Nations (https:////sdgs.un.org/2030agenda, The 2030 Agenda for Sustainable Development), namely with SDG 3 (Targets 3.4 and 3.8) and SDG 5 (Targets 5.4 and 5.5).

ИЗВОД

ХИБРИДИ 4-АМИНОХИНОЛИНА И АДАМАНТАНА КАО ИНХИБИТОРИ AChE

КАТАРИНА КОМАТОВИЋ[1], АНА МАТОШЕВИЋ[2], МАРИО ЗЛАТОВИЋ[1], ДУШАН САДИЋ[1], АНИТА БОСАК[2] и ДЕЈАН М. ОПСЕНИЦА[3,4]

[1]Универзитет у Београду, Хемијски факултет, Студентски трг 12–16, 11158 Београд, [2] Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10001 Zagreb, Croatia, [3]Институт за хемију, технологију и металургију Универзитет у Београду, Његошева 12, 11000 Београд и [4]Центар изврсности у хемији и инжењерству животне средине, ИХТМ, 11000 Београд

Алцхајмерова болест (AD) је неизлечиви и прогресивни неуродегенеративни поремећај који узрокује губитак когнитивних способности и памћења и оштећење функционалности и структуре мозга. Од различитих могућности за развој лекова, инхибиција ацетилхолин-естеразе (AChE) остаје доминантан третман симптома. У наставку наших истраживања дуголанчаних деривата 4-аминохинолина који садрже адамантил (Ad) групу као инхибитор AChE, истражили смо шест нових деривата који се разликују по супституцији терминалне амино-групе или по Ад остатку. Истражена је њихова способност инхибиције AChE, *in silico* сличност лековима, потенцијал за пролазак кроз БББ и могући начини везивања са AChE код најактивнијих једињења. Показало се да увођење хидроксилне групе, брома или ацетамидне групе повећава инхибиторну моћ у поређењу са мање поларним једињењима која садрже бензил групу као други супституент на амино--групи. Анализа *in silico* добијених параметара дефинисаних правилом Липинског показала је да сва једињења нарушавају log *P* и број веза око којих је могућа ротација (*RB*). Докинг најактивнијих једињења на AChE сугерише да једињења делују као двоструки

инхибитори због истовремених интеракција са каталитичким и периферним местом везивања. Супституенти на Ad одређују начин везивања за ензиме и обезбеђују интеракције које стабилизују комплекс између једињења и AChE.

(Примљено 31. јануара, ревидирано 6. фебруара, прихваћено 7. фебруара 2025)

REFERENCES

1. Y. Xu, H. Wang, X. Li, S. Dong, W. Liu, Q. Gong, T. Wang, Y. Tang, J. Zhu, J. Li, H. Zhang, F. Mao, *Eur. J. Med. Chem.* **143** (2018) 33 (https://doi.org/10.1016/j.ejmech.2017.08.025)
2. C. X. Gong, F. Liu, K. Iqbal, *J. Alzheimer's Dis.* **64** (2018) S107 (https://doi.org/10.3233/JAD-17992)
3. J. Y. Y. Szeto, S. J. G. Lewis, *Curr. Neuropharmacol.* **14** (2016) 326 (https://doi.org/10.2174/1570159x14666151208112754)
4. C. Bakker, J. v. d. Aart, E. P. Hart, E. S. Klaassen, K. R. Bergmann, M. J. v. Esdonk, D. G. Kay, G. J. Groeneveld, *Alzheimer's Dement.* **6** (2020) e12093. (https://doi.org/10.1002/trc2.12093)
5. J. Cummings, *Drugs* **83** (2023) 569 (https://doi.org/10.1007/s40265-023-01858-9)
6. N. N. Nalivaeva, A. J. Turner, *Chem.-Biol. Interact.* **259** (2016) (301https://doi.org/10.1016/j.cbi.2016.04.009)
7. A. Fisher, *J. Neurochem.* **120** (2012) (Suppl. 1) 22 (https://doi.org/10.1111/j.1471-4159.2011.07507.x)
8. M. Mlakić, I. Odak, I. Faraho, S. Talić, M. Bosnar, K. Lasić, D. Barić, I. Škorić, *Eur. J. Med. Chem.* **241** (2022) 114616 (https://doi.org/10.1016/j.ejmech.2022.114616)
9. K. Komatović, A. Matošević, N. Terzić-Jovanović, S. Žunec, S. Šegan, M. Zlatović, N. Maraković, A. Bosak**,** D. M. Opsenica**,** *Pharmaceutics* **14** (2022) 1305 **(**https://doi.org/10.3390/pharmaceutics14061305)
10. A. Matošević, D. M. Opsenica, M. Spasić, N. Maraković, A. Zandona, S. Žunec, M. Bartolić, Z. Kovarik, A. Bosak, *Chem.-Biol. Interact.* **382** (2023) 110620 (https://doi.org/10.1016/j.cbi.2023.110620)
11. G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, *Biochem. Pharmacol.* **7** (1961) 88 (https://doi.org/10.1016/0006-2952(61)90145–9)
12. V. Simeon-Rudolf, G. Šinko, A. Štuglin, E. Reiner, *Croat. Chem. Acta* **74** (2001) 173 (https://hrcak.srce.hr/clanak/194637)
13. *Schrödinger release 2021–4: Maestro*, Schrodinger, LLC, New York
14. K. Komatović, A. Matošević, N. Terzić-Jovanović, S. Žunec, S. Šegan, M. Zlatović, N. Maraković, A. Bosak, D. M. Opsenica, *Pharmaceutics* **14** (2022) 1305. (https://doi.org/10.3390/pharmaceutics14061305)
15. J. Cheung, M. J. Rudolph, F. Burshteyn, M. S. Cassidy, E. N. Gary, J. Love, M. C. Franklin, J. J. Height, *J. Med. Chem.* **55** (2012) 10282 https://doi.org/10.1021/jm300871x)
16. *Schrödinger release 2021–4: InducedFit protocol*, Glide, Schröodinger, LLC, New York 2021
17. *Chemaxon Calculation Module*, 2022, available online: https://chemicalize.com/ (accessed on 14th January 2025)
18. a) C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev*. **46** (2001) 3 (https://doi.org/10.1016/S0169-409X(00)00129-0); b) T. T. Wager, X. Hou, P. R. Verhoest, A. Villalobos, *ACS Chem. Neurosci.* **7** (2016) 767 (https://doi.org/10.1021/acschemneuro.6b00029)

19. I. Aleksic, J. Jeremic, D. Milivojevic, T. Ilic-Tomic, S. Šegan, M. Zlatović, D. M. Opsenica, L. Senerovic, *ACS Chem. Biol.* **14** (2019) 2800 (https://doi.org/10.1021/acschembio.9b00682)
20. J. Li, X. Jiang, R. Gan, M. Zhang, X. Pan, X. Hu, *Res. Chem. Intermed.* **42** (2016) 5709 (https://doi.org/10.1007/s11164-015-2398-2)
21. G. L. Anderson, W. A. Burks, I. I. Harruna, *Synth. Commun.* **18** (1988) 1967 (https://doi.org/10.1080/00397918808068263)
22. D. G. Harman, S. J. Blanksby, *Org. Biomol. Chem.* **5** (2007) 3495 (https://doi.org/10.1039/B711156H)
23. L. Wang, G. A. Doherty, A. S. Judd, Z-F. Tao, T. M. Hansen, R. R. Frey, X. Song, M. Bruncko, A. R. Kunzer, X. Wang, M. D. Wendt, J. A. Flygare, N. D. Catron, R. A. Judge, C. H. Park, S. Shekhar, D. C. Phillips, P. Nimmer, M. L. Smith, S. K. Tahir, Y. Xiao, J. Xue, H. Zhang, P. N. Le, M. J. Mitten, E. R. Boghaert, W. Gao, P. Kovar, E. F. Choo, D. Diaz, W. J. Fairbrother, S. W. Elmore, D. Sampath, J. D. Leverson, A. J. Souers, *ACS Med. Chem. Lett.* **11** (2020) 1829 (https://doi.org/10.1021/acsmedchemlett.9b00568)
24. T. Kawamoto, T. Fukuyama, I. Ryu, *J. Am. Chem. Soc.* **134** (2012) 875 (https://doi.org/10.1021/ja210585n)
25. H. Yamane, Y. Okada, N. Matsumoto, JP2007153803A·2007-06-21 (https://worldwide.espacenet.com/patent/search/family/038238606/publication/JP2007153803A?q=pn%3DJP2007153803A)
26. H. Bae, J. Park, R. Yoon, S. Lee, J. Son, *RSC Adv.* **14** (2024) 9440 (https://doi.org/10.1039/D4RA00320A)
27. I. Opsenica, N. Terzić, D. Opsenica, G. Angelovski, M. Lehnig, P. Eilbracht, B. Tinant, Z. Juranić, K. S. Smith, Y. S. Yang, D. S. Diaz, P. L. Smith, W. K. Milhous, D. Doković, B. A. Šolaja, *J. Med. Chem*. **49** (2006) 3790 (https://doi.org/10.1021/jm050966r)
28. P. Šilhár, N. R. Silvaggi, S. Pellett, K. Čapková, E. A. Johnson, K. N. Allen, K. D. Janda, *Bioorg. Med. Chem*. **21** (2013) 1344 (https://doi.org/10.1016/j.bmc.2012.12.001)
29. M. Liu, Y. Chen, N. Fu, *Synth. Commun.* **43** (2013) 105 (https://doi.org/10.1080/00397911.2011.622061)
30. T. T. Wager, X. Hou, P. R. Verhoest, A. Villalobos, *ACS Chem. Neurosci*. **1** (2010) 435 (https://doi.org/10.1021/cn100008c).