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Acetylcholinesterase inhibitors selected by docking-based screening – proof-of-concept study

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The enzyme acetylcholinesterase (AChE) plays an important role in the pathogenesis of neurodegenerative diseases. Its inhibition improves the cholinergic function and moderately delays the disease progress. In the present study, we performed a docking-based virtual screening for novel hits binding to AChE on the standard lead-like set of ZINC database containing more than 6 million small molecules. Two of the top best best-scored hits were tested *in vitro* for AChE affinity and neurotoxicity. Both compounds bind to the enzyme with affinities in the micromolar range but are moderately toxic. They are promising for further lead optimization to increase affinity and reduce toxicity. The present study proves the concept that the virtual screening is a reliable technique for discovery of novel AChE inhibitors.

Keywords: acetylcholinesterase, molecular docking, virtual screening, isothermal titration calorimetry, neurotoxicity.

INTRODUCTION

The enzyme acetylcholinesterase (AChE) is available in the cholinergic chemical synapses in the central nervous system and in neuromuscular junctions. It is located on the post-synaptic membrane and its function is to terminate the neurotransmission by hydrolysing the acetylcholine (ACh). In neurodegenerative diseases, like Alzheimer's disease, the cholinergic neurons are extensively lost leading to decline in memory and cognition [1, 2]. The inhibition of AChE enhances the levels of ACh and improves the cholinergic transmission.

The binding site of AChE is a deep and narrow gorge (Fig. 1) [3]. At the bottom of this gorge is located the catalytic anionic site (CAS) where the quaternary trimethylammonium choline moiety of ACh binds and is hydrolysed. Along the gorge are situated additional binding domains like the acyl pocket determining the selective binding of ACh and the oxyanion hole hosting a molecule of structural water. At the entrance of the binding gorge is situated the peripheral anionic site (PAS) which modulates the catalysis allosterically and takes non-cholinergic functions like amyloid deposition [4], cell adhesion and neurite outgrowth [5].

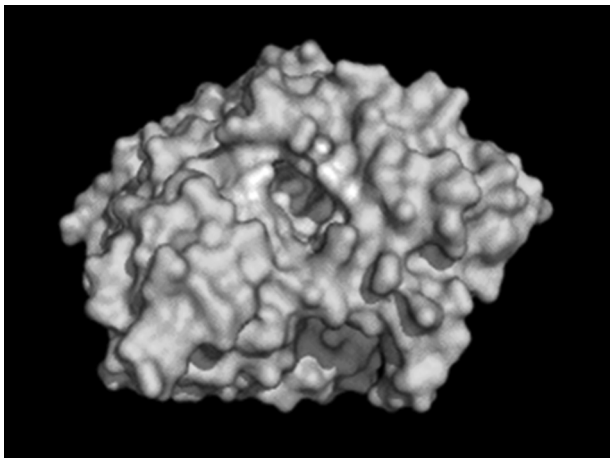

Fig. 1. Binding gorge (light grey) of *rh*AChE (pdb code: 4EY6).

Several AChE inhibitors (AChEIs) are approved as anti-Alzheimer's drugs and many others are under development [6–10]. In the present study, we describe a docking-based virtual screening on ZINC database leading to the identification of several new

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hits. Two of them (Fig. 2) were synthesized and tested for AChE binding affinity and neurotoxicity.

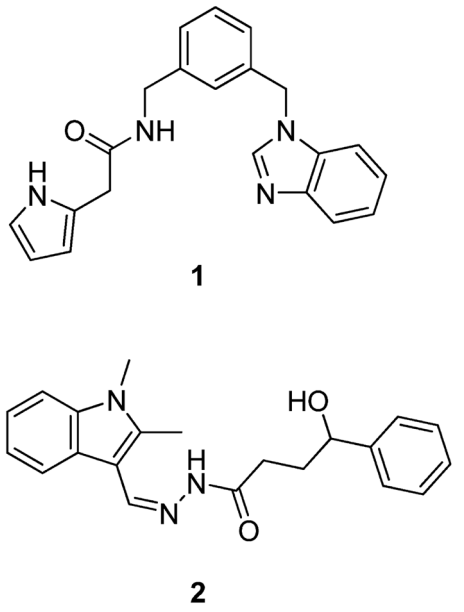


Fig. 2. Structures of the new hits derived by docking-based virtual screening.

EXPERIMENTAL

Database and docking protocol

ZINC (zinc.docking.org) contains several databases of biologically active structures. We selected the Standard Lead-like database which consists of 6,053,287 small molecules with molecular weights between 250 and 350 g/mol, *logP* up to 3,5 and up to 7 rotatable bonds. The set was downloaded in March 2015. The molecules were docked into the X-ray structure of human recombinant acetylcholinesterase (*rh*AChE, pdb id: 4EY6, R = 2.15 Å) [3]. The docking simulations were performed by GOLD v. 5.1.(CCDC Ltd., Cambridge, UK) using the following settings: scoring function ChemPLP, flexible ligand, rigid protein, radius of the binding site 6Å, no structural water molecules in the binding site, 10 runs for each compound. The AChEI galantamine (GAL) was used as a positive control.

Calculation of drug-like properties

Six drug-like properties were calculated by ACD/LogD v.9.0 (Advanced Chemistry Development, Inc.): molecular weight *Mw*, distribution coefficient at pH 7.4 $logD_{7.4}$, polar surface area *PSA*, free rotatable bonds *FRB*, number of hydrogen-bond donors *HBD* and hydrogen-bond acceptors *HBA*. The ability of compounds to cross the blood-brain barrier (BBB) by passive diffusion was predicted by the BBB Predictor (http://www.cbligand.org/BBB/). The BBB predictor includes eight models for BBB permeability prediction.

Chemistry

Reagents were commercial grade and used without further purification. Thin layer chromatography (TLC) was performed on aluminium sheets pre-coated with Merck Kieselgel 60 F_{254} 0.25 mm (Merck). Flash column chromatography was carried out using Silica gel, for chromatography, 0.035–0.070 mm, 60 A (Acros) and Aluminum oxide for chromatography; basic; 0.05–0.15 mm; pH 9.5±0.5 (Fluka). Commercially available solvents for reactions, TLC and column chromatography were used after distillation (and were dried when needed). Melting points of the compounds were determined using "Electrothermal" MEL-TEMP apparatus (uncorrected). The NMR spectra were recorded on a Bruker Avance II+ 600 spectrometer (600.13 MHz for ^{1}H and 150.92 MHz for ^{13}C NMR) with TMS as internal standard for chemical shifts (δ, ppm). ^{1}H and ^{13}C NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), integration, identification. The assignment of the ^{1}H and ^{13}C NMR spectra was made on the basis of DEPT, COSY and HSQC, experiments. Elemental analyses were performed by Microanalytical Service Laboratory of Faculty of Pharmacy, Medical University of Sofia, using Vario EL*3* CHNS(O).

*Synthesis of 3-((1H-benzo[d]imidazol-1-yl)methyl)benzonitrile **3**.*

Benzimidazole (0.236 g, 2 mmol) was mixed with 0.5 ml 50% aq.NaOH and after 10 min at r.t. a clear solution was obtained. DMSO (0.2 ml) was added, followed by 3-(bromomethyl)benzonitrile (0.432 g. 2.2 mmol). The reaction mixture was stirred at 30 °C for 1 hour, diluted with water and extracted with CH_2Cl_2. The combined organic phases were washed with water, dried and concentrated. The product was purified by flash column chromatography on silica gel (CH_2Cl_2/EtOAc = 1:1) to give 0.461 g, 99% of the desired product as white crystals; m.p. 99–101 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 8.04 (s, 1H, benzimid.), 7.87 (d, *J* = 7.9 Hz, 1H, benzimid.), 7.62 (d, *J* = 7.6 Hz, 1H, arom.), 7.49 (s, 1H, arom.), 7.46 (t, *J* = 7.8 Hz, 1H, benzimid.), 7.37 (dd, *J* = 7.9, 0.7 Hz, 1H, benzimid.), 7.33 (t, *J* = 7.8 Hz, 1H, benzimid.), 7.29 (t, *J* = 7.6 Hz, 1H, arom.), 7.22 (d, *J* = 7.6 Hz, 1H, arom.), 5.45 (s, 2H, CH_2) ppm.

^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 142.92 (CH), 137.16 (C), 133.46 (C), 132.00 (CH), 131.09 (CH), 130.29 (CH), 130.00 (CH), 123.60 (CH), 122.77 (CH), 120.65 (CH), 118.09 (C), 113.30 (C), 109.67 (CH), 47.95 (CH_2) ppm.

*Synthesis of (3-((1H-benzo[d]imidazol-1-yl)methyl)phenyl)methanamine **4**.*

To a solution of nitrile **3** (0.111 g, 0.476 mmol) in dry THF (6 ml) was added portionwise at 0 °C $LiAlH_4$ (0.090g, 2.380 mmol). The mixture was refluxed for 3 hours and cooled on ice bath, diluted with ether and carefully quenched by adding dropwise 0.09 ml water, followed by 0.09 ml 10% aq.NaOH and 0.27 ml water (Fieser workup). After additional 30 min stirring at r.t. the mixture was filtered through a pad of Celite and the filtrate was concentrated. The product was purified by flash column chromatography on silica gel (CH_2Cl_2/CH_3OH/NH_4OH = 20:1:0.05) to give 0.063 g, 56% of the desired amine **4** as waxy solid. ^{1}H NMR ($CDCl_3$/CD_3OD = 6:1, 600 MHz) δ = 8.01 (s, 1H, benzimid.), 7.78 (d, *J* = 7.7 Hz, 1H, benzimid.), 7.34–7.26 (m, 3H, benzimid., 2H, arom.), 7.17 (s, 1H, arom.), 7.10 (d, *J* = 7.6 Hz, 1H, arom.), 5.38 (s, 2H, CH_2N), 3.81 (s, 2H, C*H*$_2$$NH_2$) ppm. ^{13}C NMR ($CDCl_3$/$CD_3OD$ = 6:1, 150.9 MHz) δ = 142.92 (CH), 142.66 (C), 135.58 (2C), 133.47 (C), 129.25 (CH), 127.12 (CH), 125.90 (CH), 125.83 (CH), 123.22 (CH), 122.49 (CH), 119.66 (CH), 110.07 (CH), 48.66 (CH_2), 45.35 (CH_2) ppm.

*Synthesis of methyl 2-(1H-pyrrol-2-yl)acetate **5**.*

A 50 ml Schlenk flask was loaded under argon atmosphere with pyrrole (0.201 g, 3 mmol) and dry THF (10 ml). The mixture was cooled to –10 °C and EtMgBr (3M in Et_2O, 1.2 ml, 3.6 mmol) was added dropwise. The reaction was allowed to reach r.t. and was stirred for 30 min. It was again cooled to –10 °C and methyl bromoacetate (0.550 g, 3.6 mmol) was added. After stirring for 30 min at r.t. the reaction was quenched with aq.NH_4Cl, extracted with EtOAc, dried and concentrated. The product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc = 10:1) to give 0.120 g, 29% of the desired ester **5** as yellowish oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 8.71 (br, 1H, NH), 6.76–6.75 (m, 1H, pyrrole), 6.15–6.14 (m, 1H, pyrrole), 6.03–6.02 (m, 1H, pyrrole), 3.72 (s, 3H, CH_3), 3.69 (s, 2H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 171.63 (CO), 123.09 (C), 117.76 (CH), 108.27 (CH), 107.34 (CH), 52.16 (CH_3), 32.99 (CH_2) ppm.

*Synthesis of N-(3-((1H-benzo[d]imidazol-1-yl)methyl)benzyl)-2-(1H-pyrrol-2-yl)acetamide **1**.*

A mixture of amine **4** (0.058 g, 0.244 mmol) and ester **5** (0.034 g, 0.244 mmol) was heated for 3 hours at 80 °C. The reaction was cooled to r.t. and directly subjected to flash column chromatography on basic aluminum oxide (EtOAc = 10:1) to give 0.019 g, 23% of the desired product **1** as white crystals; m.p. 146–148 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 9.14 (br, 1H, NH-pyrrole), 7.91 (s, 1H, benzimid.), 7.81 (d, *J* = 7.7 Hz, 1H, benzimid.), 7.30–7.26 (m, 3H, benzimid.), 7.23 (t, *J* = 7.6 Hz, 1H, arom.), 7.10 (d, *J* = 7.6 Hz, 1H, arom.), 7.06 (d, *J* = 7.6 Hz, 1H, arom.), 6.88 (s, 1H, arom.), 6.72–6.70 (m, 1H, pyrrole), 6.28 (t, *J* = 5.5 Hz, 1H, CONH), 6.11 (m, 1H, pyrrole), 5.98–5.97 (m, 1H, pyrrole), 5.26 (s, 2H, CH_2N), 4.29 (d, *J* = 6.1 Hz, 2H, C*H*$_2$NHCO), 3.51 (s, 2H, C*H*$_2$CONH) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 170.74 (CO), 143.45 (C), 143.23 (CH-benzimid.), 139.17 (C), 135.89 (C), 133.77 (C), 129.25 (CH-arom.), 127.22 (CH-arom.), 126.00 (CH-arom.), 125.44 (CH-arom.), 124.23 (C), 123.30 (CH-benzimid.), 122.52 (CH-benzimid.), 120.16 (CH-benzimid.), 118.26 (CH-pyrrole), 110.10 (CH-benzimid.), 108.50 (CH-pyrrole), 107.75 (CH-pyrrole), 48.54 (CH_2N), 42.88 (*C*H_2NHCO), 35.49 (*C*H_2CONH) ppm. $C_{21}H_{20}N_4O$ (344.41): calcd. C 73.23; H 5.85; N 16.27, found C 73.16, H 6.11, N 16.08.

*Synthesis of racemic 4-hydroxy-4-phenylbutanehydrazide **6*** [6].

To a solution of 5-phenyldihydrofuran-2(3*H*)-one (1.135 g, 7 mmol) in EtOH (14 ml) was added hydrazine hydrate (0.491 g, 9.8 mmol) and the mixture was refluxed for 2 hours. After cooling to rt, the product crystallized and was filtered. The crystals were washed with EtOH (4 ml) and dried under vacuum to give 0.987 g, 73% of the desired product as white crystals.

*Synthesis of racemic N'-((1,2-dimethyl-1H-indol-3-yl)methylene)-4-hydroxy-4-phenylbutanehydrazide **2**.*

A mixture of hydrazide **6** (0.056 g, 0.288 mmol), 1,2-dimethyl-1*H*-indole-3-carbaldehyde (0.050 g, 0.288 mmol) and a pinch of p-toluenesulfonic acid (PTSA) in abs.EtOH (4 ml) was refluxed for 30 min. The mixture was concentrated under vacuum till dry. Crystallization from Et_2O/MeOH = 10:1 followed by filtration gave 0.088 g, 88% of the desired product **2** as yellow crystals; m.p. 170–172 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 9.62 (s, 1H, NH), 8.11 (d, *J* = 7.7 Hz, 1H, indole), 8.08 (s, 1H, HC=N), 7.43 (d, *J* = 7.4 Hz, 2H, arom.), 7.34 (t, *J* = 7.5 Hz, 2H, arom.), 7.28–7.20 (m, 1H, arom., 3H, indole), 4.90 (t, *J* = 6.0 Hz, 1H, C*H*OH), 4.02 (br, 1H, OH), 3.64 (s, 3H, NCH_3), 3.09-3.04 (m, 1H, C*H*$_2$CHOH), 3.01-2.96 (m, 1H, C*H*$_2$CHOH), 2.48 (s, 3H, CH_3), 2.25–2.22 (m, 2H, CH_2CO) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 175.80 (CO), 144.80 (C), 140.57

(CH=N), 140.41 (C), 137.20 (C), 128.31 (2CH-arom.), 127.17 (CH-indole), 125.76 (2CH-arom.), 124.94 (C), 122.32 (CH-indole), 121.27 (CH-indole), 121.18 (CH-indole), 108.88 (CH-arom.), 107.35 (C), 73.86 (CHOH), 33.78 (CH_2CO), 29.64 (CH_2OH), 29.55 (CH_3N), 10.58 (CH_3) ppm. $C_{21}H_{23}N_3O_2$ (349.18): calcd. C 72.18; H 6.63; N 12.03, found C 72.40, H 6.89, N 12.07.

Isothermal titration calorimetry (ITC) protocol

The ITC measurements were performed on NanoITC tool (TA Instuments, Lindon, UT, USA) with 190 μL sample cell and 50 μL syringe. The lyophilized AChE from *Electrophorus electricus* (electric eel) (Sigma Aldrich, St. Luis, MO, USA) was reconstructed in 50 mM TRIS-HCl pH 7.4 buffer with the 0.1% addition of BSA as an enzyme stabilizing factor, according to the manifacturer's instuctions. The tested compounds were prepared in 5 mM stock solutions in DMSO or ethanol and diluted to 0.5 mM in 50 mM TRIS-HCl pH 7.4 buffer. All samples were degassed prior the experiments. The AChE solution was placed into the sample cell and titrated by the tested compounds in 25 steps of 2 μL at 5 min intervals at 25°C. The blank samples (buffer lacking AChE) were titrated at the same conditions. The corresponding K_d values were calculated using NanoAnalyze software (TA Instuments, Lindon, UT, USA).

Neurotoxicity test

Murine neuroblastoma NEURO-2A cells (German collection DSMZ, Braunschweig, Germany) were cultivated under standard conditions: complete medium (90% DMEM, 10% heat inactivated FBS and 1 × non-essential amino acids); 37°C and 5% CO_2 in fully humidified atmosphere. The cell line was kept in the logarithmic growth phase by splitting 1:4 once a week using trypsin/EDTA. About 30% of the cells grow like neuronal cells. For the experimental evaluation of the cytotoxicity NEURO-2A, cells were plated in 96-well flat bottomed cell culture plates at the recommended density of 1 × 106 cells/25 cm^2. After 24 hours the cells were treated with various concentrations of the investigational compounds and after 72-hr incubation, a MTT-dye reduction assay was performed [7]. Briefly, at the end of incubation, a MTT stock solution (10 mg/ml in PBS) was added (10 μl/well). Plates were further incubated at 37°C for 4 hr. Next, the formazan crystals were dissolved by the addition of 110 μl/well 5% formic acid in 2-propanol (v/v). Absorption was measured at 580 nm wavelength on an automated ELISA reader Labexim LMR1. At least six wells per concentration were used, and data were processed using the GraphPad Prism 5.0 software 2.

RESULTS

Docking-based screening of ZINC database on rhAChE

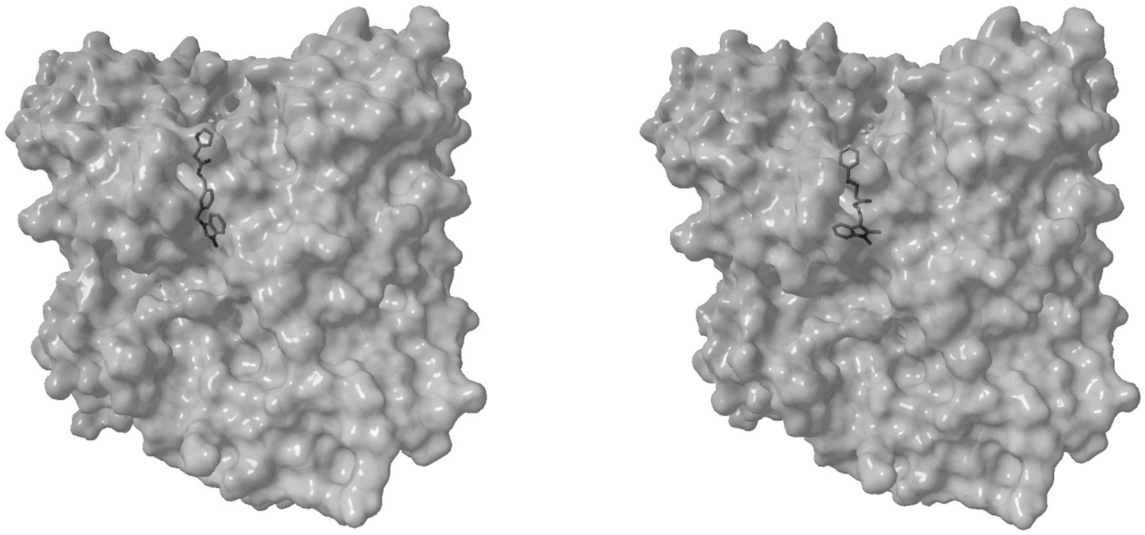
The dockings were performed with flexible ligands and rigid binding site lacking structural water molecules. The RMSD value for the docked pose of GAL was 0.204 Å. Among the top ten best-scored hits by ChemPLP were compounds **1** and **2**. (Table 1). Both compounds consisted of two aromatic moieties connected by a linker of 3-7 carbon chain containing NHCO group. Compound **1** has one additional phenyl ring. The docking poses showed that the first aromatic moiety binds in CAS, the aliphatic chain stretches along the binding gorge and the second aromatic ring binds in PAS (Figure 3).

The molecular weights of both compounds are below 350 (Table 1). Compound **1** ($logD_{7.4}$ = 4.09) is more hydrophilic than compound **2** ($logD_{7.4}$ = 4.09) although the *PSA* of **1** is slightly less than that of **2**. Compound **1** has one rotatable bond less than compound **2** and both compounds have equal number of

Table 1. Docking score, *ee*AChE affinity and neurotoxicity of the tested compounds

ID	ZINC ID	ChemPLP score	K_d μM ITC	IC_{50} μM Neuro2A	*Mw*	$logD_{7.4}$	*PSA*	*FRB*	*HBD*	*HBA*	*BBB*
1	89571446	99.28	10.191	45.092 ± 3.526	344.41	1.91	62.71	6	2	5	Yes
2	83312851	99.29	2.872	69.722 ± 16.930	349.43	4.09	66.62	7	2	5	Yes
GAL	–	74.560*	388.2	> 50*	287.35	1.12	41.93	1	1	2	Yes

*Ref. [8].



a b

Fig. 3. Docking poses of **1** (a) and **2** (b) in the complexes with *rh*AChE.

HB donors and acceptors. The drug-like properties show that both compounds will have good permeability though the gastro-intestinal tract. They are BBB permeable by passive diffusion according to all 8 models included in the BBB Predictor.

Chemistry

The choice of synthetic strategy was based on theoretical fragmentation of the target structures to key building blocks. Thus, amide bonding between benzimidazole containing benzylamine **4** and 2-pyrroleacetate **5** was foreseen for the synthesis of compound **1** (Scheme 1). The first building block was synthesised *via* deprotonation of benzimidazole with aq.NaOH [9] and subsequent nucleophilic substitution of the commercially available 3-(bromomethyl)benzonitrile to give intermediate **3**, which was reduced with $LiAlH_4$ to the corresponding amine **4**. The ester **5** was synthesised by reacting the *in situ* prepared Grignard derivative of pyrrole [10] with methyl bromoacetate. Pyrolysis of the two building blocks resulted in the formation of the target compound **1**.

The synthesis of hydrazone **2** was based on the condensation of 1,2-dimethyl-1*H*-indole-3-carbaldehyde with initially prepared hydrazide **6** (Scheme 2). The latter was prepared *via* ring opening of racemic gamma-phenyl-gamma-butyrolactone with hydrazine hydrate according to literature procedure [6]. The target compound **2** was synthesised by heating of hydrazine **6** with the chosen aldehyde in absolute ethanol. The addition of catalytic amount of p-toluenesulfonic acid was crucial for the success of the condensation.

Binding affinity to AChE

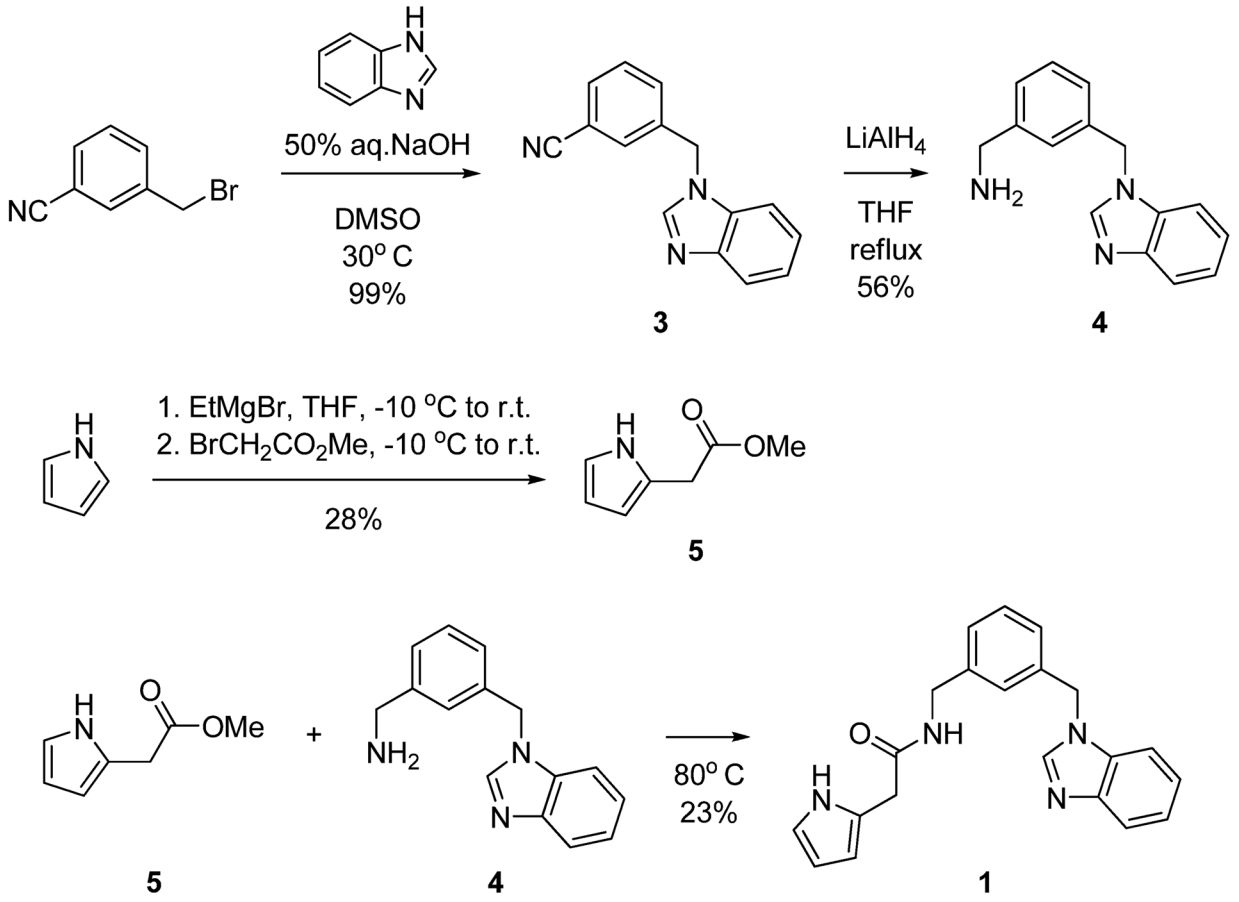
The binding affinity of the best-scored compounds was tested *in vitro* by ITC as described in Experimental. AChE from electric eel (*ee*AChE) was used in the measurements. The UniProt alignment of *rh*AChE (UniProt: P22303) and *ee*AChE (UniProt: O42275) have showed that all 17 residues forming the binding gorges are identical [11]. Thus, the target *ee*AChE is a good and cheaper alternative of *rh*AChE. The K_d values of the tested compounds are given in Table 1. The K_d values for compounds **1** and **2** are 10.191 μM and 2.872, respectively. Both of them have higher affinity than GAL (K_d = 388.2 μM).

Neurotoxicity on Neuro-2A cells

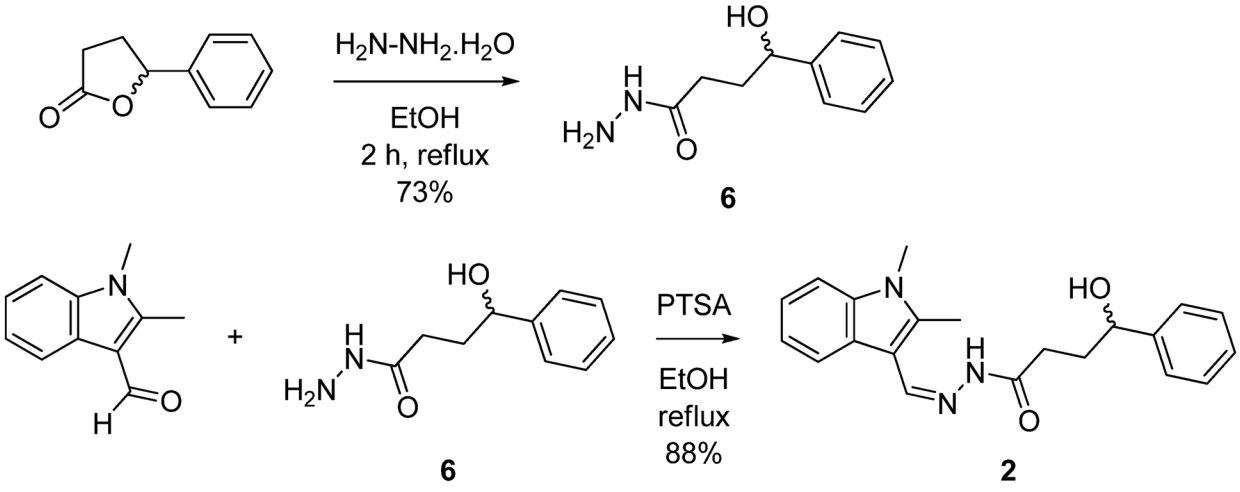
The neurotoxicity of the compounds was tested on NEURO-2A cells as described in Experimental. Both of them are moderately toxic with IC_{50} values of 45 μM for compound **1** and 70 μM for compound **2**.

DISCUSSION

The standard lead-like set of ZINC database was virtually screened by molecular docking on *rh*AChE and two of the best-scored structures were tested *in*



Scheme 1. Synthesis of compound **1**.



Scheme 2. Synthesis of compound **2**.

vitro for binding affinity to the enzyme and neurotoxicity. The drug-like properties were calculated and showed that both structures are able to permeate through the intestinal mucosa. The compounds bind well to the enzyme with K_d in the micromolar range and have higher affinity than that of GAL.

Compound **1** is a neutral molecule with $logD_{7.4}$ of 4.09 and micromolar affinity to AChE (K_d =

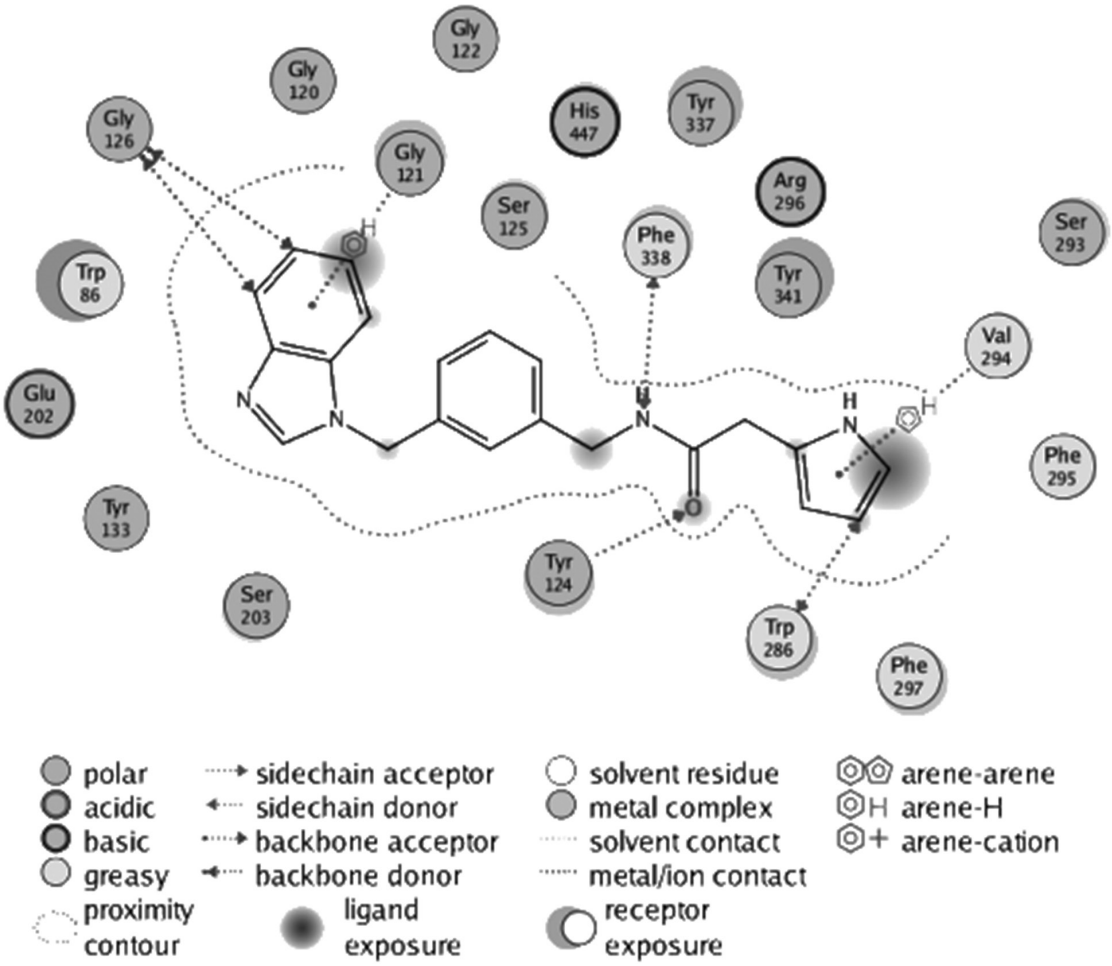


Fig. 4. 2D interaction plot of the complex **1** – *rh*AChE derived by MOE 2016.0801 (Chemical Computing Group, Montreal, Canada).

10.191 μM). It is able to cross the GIT and BBB but is more toxic on Neuro-2A cells (IC_{50} = 45 μM) than GAL. The docked pose of **1** into *rh*AChE shows that the benzimidazole fragment binds in CAS, while the pyrrole ring is placed in PAS (Figure 4). Hydrogen bonds are formed between Phe338 and NH and between Tyr124 and carbonyl oxygen atom from the linker. Gly121 and Gly126 from CAS interact with the benzimidazole fragment while Trp286 and Val294 from PAS – with the pyrrole ring.

Compound **2** is a weak base with $logD_{7.4}$ of 1.91, micromolar affinity to AChE (K_d = 2.872 μM), good intestinal and BBB permeability and moderate toxicity (IC_{50} = 70 μM). The indole moiety stacks with Trp86 in CAS, while the phenyl ring is positioned in PAS making interactions with Trp286, Val294, Phe295 and Tyr341 (Figure 5). A hydrogen bond is formed between Tyr124 and the carbonyl oxygen atom from the linker. Phe297 and Phe338 are involved in hydrophobic interactions with the linker.

In conclusion, both structures identified as hits by docking-based virtual screening showed high affinity to AChE and moderate neurotoxicity. They could be considered for further lead optimization to increase affinity and reduce toxicity. The present study was a preliminary proof-of-concept.

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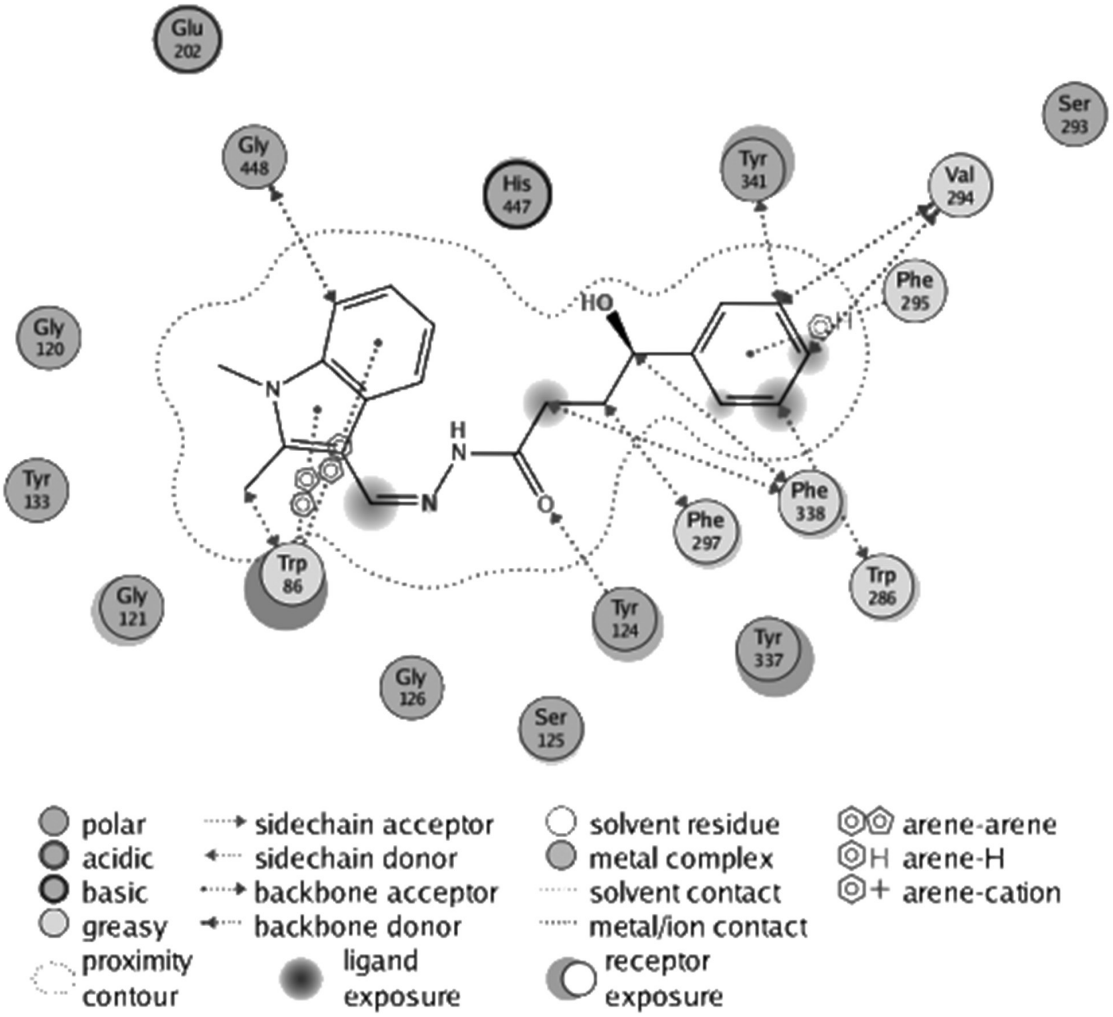

Fig. 5. 2D interaction plot of the complex **2** – *rh*AChE derived by MOE 2016.0801 (Chemical Computing Group, Montreal, Canada).

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ИНХИБИТОРИ НА АЦЕТИЛХОЛИНЕСТЕРАЗАТА, ИЗБРАНИ ЧРЕЗ ДОКИНГ-БАЗИРАН СКРИНИНГ – ДОКАЗВАНЕ НА КОНЦЕПЦИЯТА

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(Резюме)

Ензимът ацетилхолинестераза (AChE) играе важна роля в патогенезата на невродегенеративните заболявания. Неговото инхибиране подобрява холинергичната функция и умерено забавя напредъка на болестта. В настоящото изследване проведохме виртуален скрининг, базиран на молекулен докинг на 6 053 287 съединения от базата данни ZINC върху AChE. Две от съединенията с най-добър резултат бяха синтезирани и тествани *in vitro* за афинитет към AChE и невротоксичност. И двете съединения се свързват с ензима с афинитет в микромоларния диапазон, но са умерено токсични. Те са подходящи за по-нататъшна оптимизация с цел увеличаване на афинитета и намаляване на токсичността. Настоящото изследване доказва, че виртуалният скрининг е надежден метод за откриване на нови AChE инхибитори.