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SEARCH FOR POTENTIAL CHOLINESTERASE INHIBITORS FROM THE ZINC DATABASE BY VIRTUAL SCREENING METHOD

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Abstract: A virtual screening of the ZINC database was applied for the identification of novel cholinesterase inhibitors. The first step allowed to select compounds with favorable physicochemical properties. Then, the compounds were screened with the pharmacophore models built using crystal structures of donepezil, tacrine, decamethonium and *bis*-7-tacrine with acetylcholinesterase and well characterized interactions of *bis*-nor-meptazinol with butyrylcholinesterase. The selected compounds from the group of donepezil were docked to acetylcholinesterase giving 7 structures for further studies. These compounds were tested against cholinesterases and two of them, 1-[4-(1*H*-indol-3-ylmethyl)piperazin-1-yl]-2-phenoxyethanone **2** and 2-[(1-benzylpiperidine-4-yl)amino]-1-phenylethanol **4** displayed, respectively, 50.1% and 79.5% of inhibition against butyrylcholinesterase at the concentration of 100 μM.

Keywords: acetylcholinesterase inhibitors, butyrylcholinesterase inhibitors, drug design, pharmacophore model, virtual screening

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, 3.1.1.8) are enzymes belonging to a group of serine hydrolases. AChE is a key enzyme involved in the hydrolysis of acetylcholine (ACh) in cholinergic synapses, while BuChE is able to hydrolyze ACh as well as other esters. Both enzymes are differentiated on the basis of their structures, substrate specificities, tissue distribution and sensitivity to inhibitors (1). The inhibition of these enzymes causes an increase in the level of ACh in cholinergic synapses and can therefore influence different pathological processes. Defects in cholinergic neurotransmission are significant for the progression of Alzheimer's disease (AD) and other pathological processes. According to the cholinergic hypothesis of AD, many symptoms of dementia, especially learning and memory difficulties, were explicable by the lack of ACh (2). Thus, cholinesterase inhibitors that improve cholinergic neurotransmission in the synaptic cleft were developed as therapeutic agents for the treatment of AD. The first drug approved for the treatment of AD was tacrine. Currently, three other cholinesterase inhibitors: rivastigmine, donepezil and galantamine are available on the market and are used as symptomatic therapy of AD (3). In spite of limited efficacy of cholinergic therapy, both cholinesterases still remain valuable targets in search for new agents against AD (4, 5). That is due to their additional, non-hydrolytic properties associated with β-amyloid (Aβ) and neurofibrillary tangles formation, which are important for AD pathogenesis (6-8). In recent years, many inhibitors of AChE or both AChE and BuChE have been developed (9, 10). Among different approaches for the design of novel cholinesterase inhibitors virtual screening techniques have also been applied (11-13). Our studies focus on the design, synthesis and biological evaluation of potential cholinesterase inhibitors and multifunctional ligands with cholinesterase inhibitory activity (14-18). The aim of this study was to design new potential dual binding site cholinesterase inhibitors using a virtual screening method, synthesize the selected compounds, test their AChE and BuChE inhibitory activity and select hits for further development.

RESULTS AND DISCUSSION

Data set for virtual screening

A two-step virtual screening of the ZINC database was applied for the identification of new

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cholinesterase inhibitors. The ZINC database is a free data set of commercially available compounds, serving to virtual screening. The latest version of the database (ZINC 12) contains over 35 million compounds and is provided by the Shoichet Laboratory from the University of California (19). The ZINC database is a huge source of compounds to search for new active substances and therefore it was chosen to search for new cholinesterase inhibitors. ZINC 8, which was available at the time of the screening carried out for this study, contained 8.5 million compounds. The two-step virtual screening was preceded by a prefiltering of compounds, which was based on the physicochemical properties, important for proper absorption and distribution to the central nervous system (20, 21), as described in the Experimental part. This prefiltering left 65396 compounds for the subsequent virtual screening.

Creation of pharmacophore models

Wolber suggested application of crystal structures from PDB (22) for creation of pharmacophore models (23, 24). As similar methodology was applied in the presented studies. Different PDB files were used to assign pharmacophore features with the MOE program (25). In the case of acetylcholinesterase, for creation of pharmacophore models we utilized crystal structures of the enzyme complexes with four inhibitors: donepezil, *bis*-tacrine, decamethonium and tacrine (Fig. 1).

The biologically active conformation, derived from the crystal complexes was used directly to generate the models. Based on how these four inhibitors bound with AChE, the specific interactions were included in the pharmacophore models. The model for donepezil took into account two aromatic features and one cation – donor, the model for *bis*-tacrine two aromatic and two cation – donor features, the model for decamethonium two cation features and the model for tacrine one aromatic and one cation – donor feature (Fig. 2).

To generate a pharmacophore model for butyrylcholinesterase inhibitors, a different method was used due to the lack of crystal structures of BuChE complexes with inhibitors interacting in a non-covalent manner at that time. The research was undertaken on the basis of *bis*-nor-meptazinol, which is a potent inhibitor of both cholinesterases (Fig. 1) (26). The possible interactions of this compound with both enzymes, determined by molecular modeling techniques were widely described in the literature (27). The structure of this inhibitor was optimized with the program MOE (27) using the MMFF94 force field and then used for creation of a pharma-

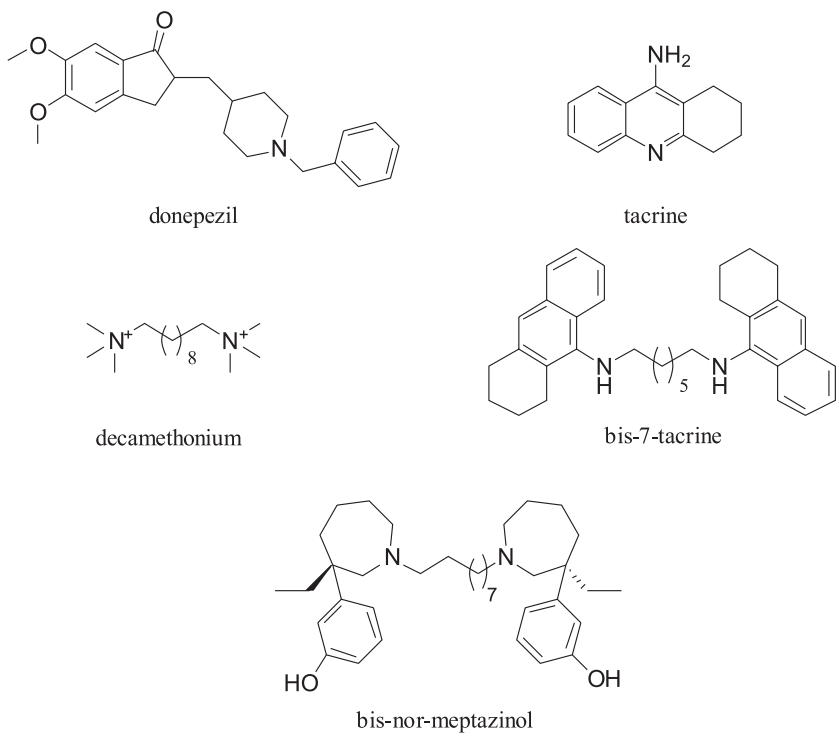


Figure 1. Chemical structure of the selected cholinesterase inhibitors used for the development of pharmacophore models

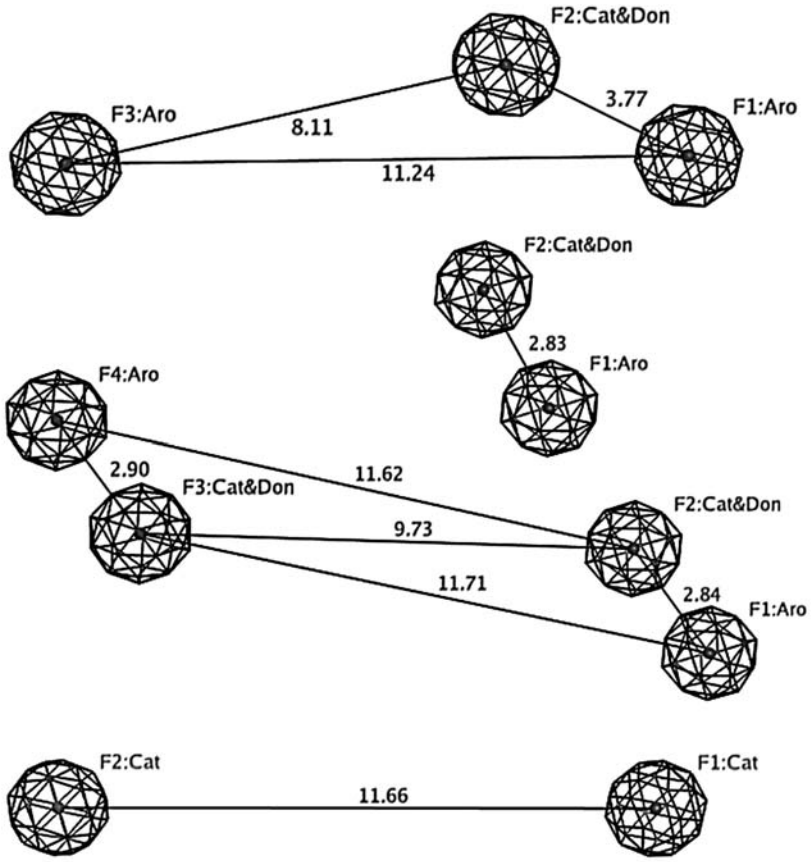


Figure 2. The pharmacophore models for donepezil, tacrine, *bis*-(7)-tacrine and decamethonium (the features were marked as follows: aromatic ring - Aro, cation and H-bond donor – Cat&Don, cation - Cat)

Table 1. The results of virtual screening with the pharmacophore models for the selected AChE and BuChE inhibitors.

Enzyme	Pharmacophore model	Number of hits	Rmsd value range [Å]
AChE	Donepezil	88	0.13 - 0.79
	bis-7-Tacrine	0	-
	Decamethonium	12	0.28 - 0.91
	Tacrine	5102	0.015 - 1.00
BuChE	*bis*-nor-Meptazinol	0	-

Rmsd = root-mean-square deviation

cophore model, taking into account two aromatic and two cationic / H-bond donors features (Fig. 3).

Screening with the pharmacophore models

The compounds from the ZINC database were optimized with MOE program, using the MMFF94 force field and then screened with the previously created pharmacophore models. As a measure of a fit quality of the compounds the root-mean-square deviation (rmsd) value was used with respect to the center of each feature of the model (Table 1).

The pharmacophore screening yielded 88 compounds for the donepezil model, 12 for decamethonium and 5102 for tacrine. Models for tacrine and decamethonium were two-point pharmacophores and screening with them resulted in too many compounds. Therefore, only compounds chosen with the donepezil model were used in further studies.

Screening with docking

The 88 compounds, which passed the pharmacophore screening with the donepezil model, were selected for docking to acetylcholinesterase. The aim of this procedure was an accurate selection of hits and search for additional interactions in the active gorge of the enzyme. The next criterion for compound selection was the score obtained with the scoring function ChemScore. Donepezil, used as a reference, obtained a ChemScore value of 49.48. Compounds with a scoree above 43 were chosen for further studies, and the results are summarized in Table 2. Comparison of the scoring function value for the selected hits with the result for donepezil suggested that all of them would probably be less active than the reference compound.

Structures of the selected hits **1**-**7** are shown in Figure 4. According to literature data, they have not been tested against cholinesterases so far, therefore the chosen compounds were a good starting point to search for new inhibitors. Compounds **2** and **4** were selected for synthesis, while the remaining compounds were purchased.

Chemistry

Scheme 1A describes the procedure used for the synthesis of compound **2**. At first, 1*H*-indole-3-carbaldehyde **8** was obtained by the formylation of 1*H*-indole, using $POCl_3$ and DMF as a reagent and a solvent (28). In the next step, the reductive condensation of compound **8** with piperazine was carried out in the presence of acetic acid and $NaBH_4$ in methanol (29). In the parallel pathway phenol was converted into sodium phenoxide using sodium hydroxide and then reacted with iodoacetic acid giving phenoxyacetic acid **10**. Compound **10** was subsequently converted into the acid chloride **11** in the reaction with thionyl chloride, and was used as an acylation agent. The final product was obtained in the reaction of 3-(piperazin-1-ylmethyl)-1*H*-indole **9** with the phenoxyacetic acid chloride **11**. The reaction was carried out in dry THF at room temperature followed by the purification by silica gel column chromatography. The final compound 1-[4-(1*H*-indol-3-ylmethyl)piperazin-1-yl]-2-phenoxyethanone **2** was isolated as a red oil and then converted into a hydrochloride salt. The synthesis of compound **4** was accomplished in two steps and it is presented in Scheme 1B. In the first step, 2-bromo-1-phenylethanol **12** was synthesized from styrene by bromination of the alkyl chain. The reaction was carried out using *N*-bromosuccinamide as a brominating agent in a mixture of water and acetonitrile at room temperature. In the next step, compound **12** was used for alkylation of 1-benzylpiperidine-4-amine. The reaction was performed in acetonitrile, in the presence of potassium carbonate under reflux (30). Then the crude product was purified by silica

Table 2. Compounds from the ZINC database selected in virtual screening with the donepezil pharmacophore model and docking.

Comp. No.	Symbol	ChemScore
1	ZINC00207679	45.57
2	ZINC0015483	45.22
3	ZINC00146717	44.17
4	ZINC00152443	44.04
5	ZINC00110981	43.99
6	ZINC00087269	43.81
7	ZINC00212881	43.26

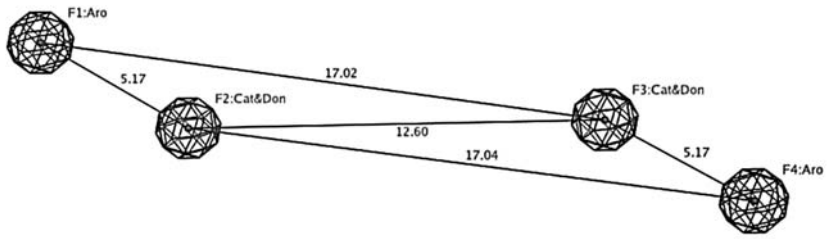


Figure 3. The pharmacophore model for *bis*-nor-meptazinol (aromatic ring - Aro, cation and H-bond donor – Cat&Don)

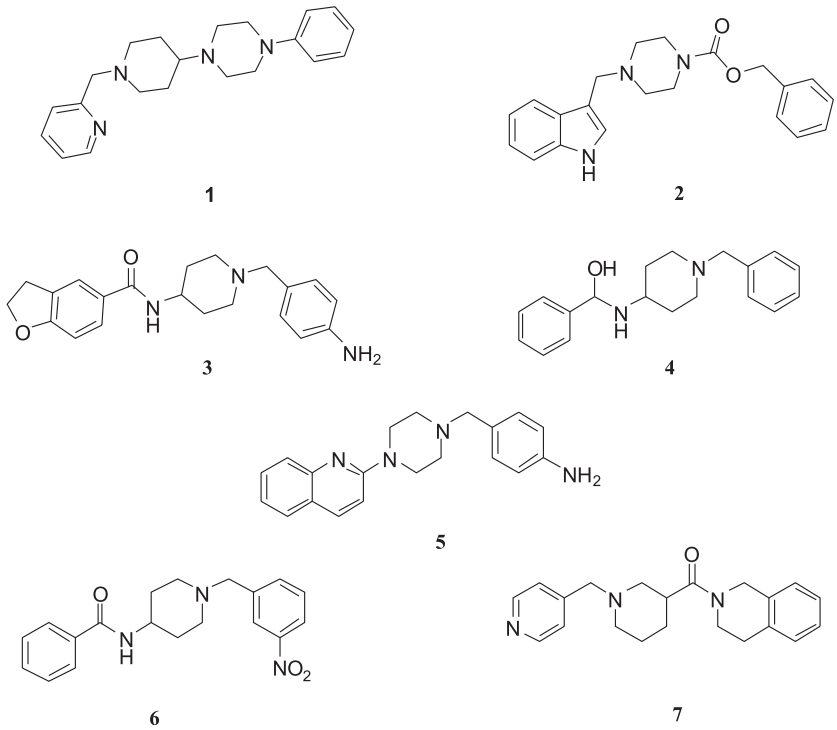


Figure 4. Structures of potential cholinesterase inhibitors selected from the ZINC database by virtual screening technique

Table 3. Inhibitory activity of the tested compounds against *Ee*AChE and *Eq*BuChE.

Compound	***Ee*AChE**		***Eq*BuChE**	
	% inhibition	IC_{50} [μM]	% inhibition	IC_{50} [μM]
1	8.47[a]	-	n. a.[a]	-
2	8.87[a]	-	50.09[a]	-
3	n. a.[b]	-	n. a.[b]	-
4	17.71[a]	-	79.48[a]	-
5	n. a.[a]	-	n. a.[a]	-
6	n. a.[b]	-	n. a.[b]	-
7	n. a.[a]	-	n. a.[a]	-
Donepezil	93.06[b]	0.031	24.36[b]	2.84
Tacrine	93.77[b]	0.050	99.44[b]	0.0054

[a]The screening concentration of 100 μM. [b]The screening concentration of 1 μM; n. a. – not active, the percentage of inhibition lower than 5%.

gel column chromatography. The final compound **4** was isolated as colorless oil, and then converted into a hydrochloride salt.

Biological activity

The activity of the compounds selected from virtual screening (**1**–**7**) was tested against *Ee*AChE (AChE from *Electrophorus electricus*) and *Eq*BuChE (BuChE from horse serum) in the spectrometric method established by Ellman et al. (29). Ellman's test is based on the reaction of 5,5'-dithio*bis*-(2-nitrobenzoic) acid (DTNB, Ellman's reagent) with thiocholine, which is the product of hydrolysis of acetylthiocholine (ATC) or butyrylthiocholine (BTC), by AChE or BuChE, respectively. The screening concentration was 100 μM for the compounds soluble in water (**1**, **2**, **4, 5** and **7**) and 1 μM for the compounds tested with addition of DMSO (**3**, **6**). The obtained results for the

tested compounds and the references (tacrine and donepezil) are presented in Table 3.

Very low activity or complete lack of activity of the tested compounds in a relatively high concentration of 100 μM (except for **3** and **6**, which were tested at 1 μM due to solubility problems) makes us define them as inactive towards AChE. On the other hand, two of the compounds, **2** and **4**, exhibited above 50% of inhibition at the concentration of 100 μM against BuChE. Compounds **2** and **4** belong to different structural classes. Compound **2** is an indole derivative with a piperazine group and an additional phenyl ring, whereas compound **4** contains *N*-benzylpiperidine moiety, which is a characteristic pharmacophore fragment occurring in the cholinesterase inhibitors like donepezil. The obtained results indicate that compounds **2** and **4** could be described as relatively weak inhibitors of BuChE. However, structures of these compounds provide ample opportunities for modifications that can improve their activity so that they can become good lead structures for the search of new cholinesterase inhibitors.

SUMMARY

Virtual screening performed on the compounds from the ZINC database enabled us to select over 65 000 potential cholinesterase inhibitors with optimal physicochemical properties. An application of a pharmacophore model for donepezil and then docking to AChE led to a selection of 7 structures for further studies. The activity of the selected compounds against AChE and BuChE was tested using the Ellman's test. None of the compounds displayed activity against AChE and two of them, **2** and **4**, were weak inhibitors of BuChE. The lack of the activity against AChE is probably the result of a too tolerant selection criterion based on a scoring function value, which was lower than that for the reference compound - donepezil. Moreover, the number of the tested compounds was very limited. However, it is worthy to note that the applied method of docking to AChE has been previously used with success and led to the design of potent AChE inhibitors in the group of isoindoline-1,3 derivatives (17, 18).

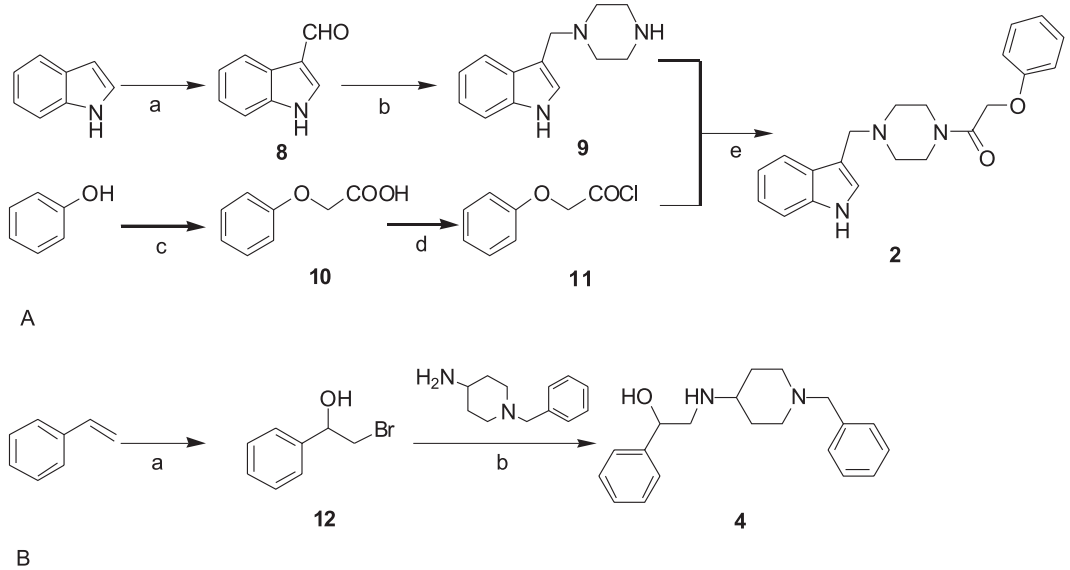
The identification of compounds **2** and **4** as BuChE inhibitors is an interesting result of this study. Even though their activity is rather weak when compared with the reference compounds, they can be subject to further modifications, which may increase their activity. Such methodology is accepted during a drug discovery project, where at the beginning, even a low activity of a hit compound is a good starting point for further development.

EXPERIMENTAL

Molecular modeling

Virtual screening

The initial selection of compounds was based on physicochemical properties, important for proper



Scheme 1. The synthesis of compounds **2** and **4**. Reagents and conditions: A. (a) $POCl_3$, DMF, 0-35°C, 2 h; (b) piperazine, $NaBH_4$, CH_3COOH, CH_3OH, 65°C, r.t., 24 h; (c) ICH_2COOH, NaOH, H_2O, 10-100°C, 1.5 h; (d) $SOCl_2$, 75°C, 4 h; (e) THF, r.t., 2 h. B. (a) *N*-bromosuccinimide, CH_3CN/H_2O, r.t., 20 h; (b) K_2CO_3, CH_3CN, 81°C, 48 h

absorption and distribution to the central nervous system (20, 21). Accordingly, structures were chosen for which:

$1 \leq$ charge after ionization ≤ 3

$1 \leq \log P \leq 3$

number of rotatable bonds ≤ 8

$1 \leq$ number of hydrogen bond donors ≤ 5

$1 \leq$ number of hydrogen bond acceptors ≤ 10

$100 \leq$ molecular weight (MW) ≤ 500.

This led to 65396 compounds, including their isomers. They were downloaded from the ZINC database (19) in SMILES format, which further served to build 3D structures by the Corina standalone version (32). Finally, the compounds were saved in the mol2 format. At this step of the study, a two-stage screening methodology was applied. The first stage used pharmacophore models and the second one was based on the docking process of the selected molecules. Pharmacophore models were generated with the MOE 2008.10 program (25). Features were assigned based on the interactions pattern of the reference inhibitors, some features were assigned. The active conformations of AChE ligands were obtained directly from PDB crystal complexes (22), and in case of butyrylcholinesterase inhibitor it was drawn and optimized in MMFF94 force field with MOE 2008.10. Structures of the compounds selected for screening were also optimized in the same way. As a measure of fit quality of the compounds, the rmsd value with respect to the center of each model feature was used. Dockings to acetylcholinesterase were performed according the procedure described previously (18). During the protein preparation (PDB: 1EVE) (22) all the histidine residues were protonated at Nε, hydrogen atoms were added, ligands were removed, the binding site was defined as all amino acid residues within 10 Å from donepezil and three water molecules (1159, 1249, 1254) were included. Dockings were performed with Gold 4.0 (33). A standard setting of the genetic algorithm with a population size 100, a number of operations of 100000 and a clustering with a tolerance of 1Å was applied. ChemScore was used for evaluation of the docking results. For each ligand the final results involved 10 poses, arranged on the ranking list according to the scoring function values.

Chemistry

All solvents and reagents were purchased from commercial suppliers and were used without further purification. Compound **1** was purchased from Vitas-M Laboratory Ltd., compounds **3**, **5**, **6** and **7** were purchased from Molport. Column chromatography was performed on Merck silica gel 60 (63–200 μm). Analytical thin layer chromatography was done using aluminum sheets precoated with silica gel 60 F_{254}. Analytical RPLC-MS was performed on Waters Acquity TQD with a mass spectrometer (Waters TQD, Milford, MA, USA) with detection by UV (DAD) using an Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm). CH_3CN/H_2O gradient with 0.1% HCOOH was used as the mobile phase at a flow rate of 0.3 mL/min. ^{1}H NMR spectra were recorded on Varian Mercury 300 at 300 MHz. The chemical shifts for ^{1}H NMR are referenced to TMS *via* residual solvent signals (1H, $CDCl_3$ at 7.26 ppm, $(CD_3)_2SO$ at 2.50 ppm). All the compounds showed purity above 95%. Compounds **4**, **8** and **9** were previously reported (28–30).

1*H*-Indole-3-carbaldehyde (8)

To a solution of $POCl_3$ (5.00 mmol, 767 mg) in DMF (8.5 mL) at 10°C, a solution of indole (5.00 mmol, 586 mg) in 5 mL of DMF was added. The reaction mixture was stirred at this temperature for 1 h and then for 1 h at 35°C. To the resulting mixture 20 g of ice was added and then it was alkalized by aqueous solution of NaOH (5.65 g in 15 mL). The precipitate was filtered, washed and recrystallized from water to give 1*H*-indole-3-carbaldehyde **8** as a pink solid (yield: 71%). R_f (chloroform/methanol/NH_3; 8 : 1 : 0.1, v/v/v) 0.51; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 9.93 (s, 1H, CHO), 8.25-8.31 (m, 1H, H2), 8.03-8.13 (m, 1H, H9), 7.45-7.57 (m, 1H, H6), 7.16-7.33 (m, 2H, H7,8), 1NH - not detected; MS: m/z 146.03 [M + H$^+$]; purity: 100% (by HPLC); Mol. form. C_9H_7NO.

3-(Piperazin-1-ylmethyl)-1*H*-indole (9)

To a solution of 1*H*-indole-3-carbaldehyde **8** (1.03 mmol, 150 mg) and piperazine (5.17 mmol, 445 mg) in 7.0 mL of methanol, acetic acid (2.07 mmol, 118 μL) was added. The reaction mixture was stirred under reflux for 1 h. In the next step, after cooling the reaction mixture to room temperature, sodium borohydride (1.55 mmol, 58 mg) was added and then the mixture was stirred at room temperature overnight. Subsequently, the solvent was evaporated and the residue was purified by column chromatography on silica gel (chloroform/methanol/NH_3; 8 : 1 : 0.1, v/v/v) to give the desired product as a red oil (yield: 52%). R_f (chloroform/methanol/NH_3; 8 : 1 : 0.1, v/v/v) 0.21; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.25 (s, 1H, 1NH), 7.74 (qd, J = 0.73, 7.82 Hz, 1H, H4), 7.31-7.44 (m, 1H, H7), 7.04-7.23 (m, 3H, H2,5,6), 3.73 (d, J = 0.77 Hz, 2H, $CH_2\alpha$), 2.83-3.00 (t, 4H, $3CH_2$', $5CH_2$'), 2.52 (br. s.,

4H, 2CH_2', 6CH_2'), 4'NH - not detected; MS: m/z 216.22 [M + H^+]; purity: 98% (by HPLC); Mol. form. $C_{13}H_{17}N_3$.

Phenoxyacetic acid (10)

To a solution of iodoacetic acid (12.50 mmol, 2328 mg) in 15.0 mL of water an aqueous solution of NaOH (535 mg in 2.0 mL) was added dropwise at 10°C. Subsequently, phenol (12.50 mmol, 1178 mg) was added and then, again the same amount of an aqueous solution of NaOH was added dropwise. The reaction mixture was refluxed for 1-2 h. After the reaction was finished, the mixture was cooled by the addition of 10.0 mL of water and then the resulting precipitate was filtered. The crude residue was recrystallized from water to give phenoxyacetic acid **10** as a white solid (yield: 28%); R_f (DCM/methanol/acetic acid; 9 : 1 : 0.1, v/v/v) 0.41; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.27-7.37 (t, 2H, H2,3), 6.99-7.08 (t, 1H, H4), 6.88-6.97 (d, 2H, H3,5), 4.69 (s, 2H, CH_2α), COOH - not detected; MS: m/z 153.07 [M + H^+]; purity: 100% (by HPLC); Mol. form. $C_8H_8O_3$.

1-[4-(1*H*-Indol-3-ylmethyl)piperazin-1-yl]-2-phenoxyethanone (2)

A solution of phenoxyacetic acid **10** (0.99 mmol, 150 mg) in 1.0 mL of thionyl chloride was refluxed for 3 h under argon atmosphere. Then, the solvent was removed and the residue was dissolved in 1.0 mL of THF. The resulting solution of the obtained 2-phenoxyacetyl chloride **11** was added dropwise to a solution of 3-(piperazin-1-ylmethyl)-1*H*-indole **9** (1.00 mmol, 215 mg) in 5.0 mL of THF. Subsequently, the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was purified by column chromatography (DCM/methanol, gradient from 1 : 0 to 0.9 : 0.1, v/v) to afford the pure product as a red oil (yield: 89%); R_f (DCM/methanol 0.9 : 1, v/v) 0.37; ^{1}H NMR (300 MHz, methanol-d_4, δ, ppm): 7.65 (dt, *J* = 0.93, 7.89 Hz, 1H, H4''), 7.33-7.43 (d, 1H, H7''), 7.03-7.27 (m, 4H, H3,5,2",6"), 6.78-6.98 (m, 4H, H2,4,6,5''), 4.75 (s, 2H, CH_2α), 4.21 (s, 2H, NCH_2), 3.64-3,77 (br. s., 4H, H2',6'), 2.86-3.10 (br. s., 4H, H3',5'), 1''NH - not detected; MS: m/z 350.30 [M + H^+]; purity: 97% (by HPLC); Mol. form. $C_{21}H_{23}N_3O_2$.The final product was converted into a hydrochloride salt.

2-Bromo-1-phenylethanol (12)

To a stirred solution of styrene (8.70 mmol, 906 mg) in 18.0 mL of acetonitrile the solution of *N*-bromopyrrolidine-2,5-dione (8.70 mmol, 1550 mg) in 18.0 mL of water was added dropwise at room temperature. After stirring at room temperature for 20 h, acetonitrile was evaporated and then 20.0 mL of water was added. The resulting mixture was washed with DCM (1 × 20.0 mL, 2 × 15.0 mL) and ethyl acetate (1 × 20.0 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. The obtained residue was purified on a silica gel by column chromatography (petroleum ether/ethyl acetate 9.6 : 0.4, v/v) to provide the pure product as a colorless oil (yield: 37%); R_f (petroleum ether/ethyl acetate 9.6 : 0.4, v/v) 0.23; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.28-7.44 (m, 5H, H2,3,4,5,6), 4.93 (dd, *J* = 3.33, 8.98 Hz, 1H, CHα), 3.61-3.70 (m, 1H, CHβ), 3.46-3.60 (m, 1H, CH'β), 2.66 (s, 1H, OH); MS: m/z 183.05 [M - 18 + H^+]; purity: 95% (by HPLC); Mol. form. C_8H_9BrO.

2-[(1-Benzylpiperidine-4-yl)amino]-1-phenylethanol (4)

A solution of 2-bromo-1-phenylethanol **12** (1.49 mmol, 300 mg), 1-benzylpiperidine-4-amine (1.49 mmol, 284 mg) and potassium carbonate (4.47 mmol, 615 mg) in 20.0 mL of acetonitrile was stirred under reflux for 20 h. After the reaction was finished, acetonitrile was evaporated and the resulting mixture was dissolved in DCM and washed with water (1 × 15.0 mL, 2 × 10.0 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. The obtained crude product was purified by column chromatography (DCM/methanol 0.9 : 0.1, v/v) to provide the pure product as a colorless oil (yield: 58%); R_f (DCM/ methanol 0.9 : 0.1, v/v) 0.26; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.16-7.43 (m, 10H, H2,3,4,5,6,2'',3'',4'', 5'',6''), 4.75 (dd, *J* = 3.33, 9.23 Hz, 1H, CHα), 3.48-3.51 (s, 2H, NCH_2), 2.66-3.15 (m, 7H, CH_2 2',6',β, OH), 2.49-2.65 (m, 1H, CH 4'), 1.81-2.11 (m, 4H, CH_2 3',5'), NH - not detected; MS: m/z 311.25 [M + H^+]; purity: 99% (by HPLC); Mol. form. $C_{20}H_{26}N_2O$. The final product was converter into a hydrochloride salt.

Biological activity - Ellman's test (31)

Reagents and chemicals: 5,5'-dithiobis(2-nitrobenzoic) acid (DTNB, Ellman's reagent), acetylthiocholine iodide (ATCh), butyrylthiocholine iodide (BTCh), AChE from *Electrophorus electricus* (425.96 U/mg solid), and BuChE from horse serum (2.5 units/1 mL) were purchased from Sigma-Aldrich. The measurement of absorbance was performed using the Perkin Elmer Lambda 12 appliance or EnSpire Multimode Microplate Reader PerkinElmer. The detection wavelength was 412 nm. Due to different solubility, compounds **1**, **2**, **4, 5**

and **7** were dissolved in water and measured at 100 μM concentration, while compounds **3** and **6** were dissolved in 1% solution of DMSO and experiments were carried out in a 1 μM concentration. The tested compounds were incubated with an enzyme at 25°C for 5 min before starting the reaction. The experiment was performed in 100 mM phosphate buffer (pH 8.0) containing 0.25 units of AChE or BuChE using 0.45 mmol ATCh or BTCh and 0.2 μM DTNB as substrates. The experiments were performed in triplicate.

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