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Research Article

In silico and *in vitro* screening of pyrrole-based Hydrazide-Hydrazones as novel acetylcholinesterase inhibitors

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Abstract

Virtual screening is emerging as a highly applied technique and gained prominence as widely used method for the search and identification of potential hits, significantly reducing the time needed to discover novel and effective compounds compared to high-throughput screening. Recently, the superiority of simulations with multiple programs compared to a single software docking has been discussed. The aim of this work was to apply consensus docking, molecular mechanics/generalized Born surface area (MM/GBSA) free binding energy recalculations, and *in vitro* evaluations on an in-house dataset of recently synthesized pyrrole-based hydrazide-hydrazones in the search for novel acetylcholinesterase (AChE) inhibitors. Two licensed softwares – GOLD 5.3 and Glide, were employed for the virtual screenings, and several chemotherapeutic potential hits were identified. Furthermore, MM/GBSA free binding energy recalculations were provided to enhance the robustness of the *in silico* results. The MM/GBSA scores of the top ten pyrrole-based hydrazide-hydrazones were ranging from -60.44 to -70.93 Kcal/mol. Subsequent, *in vitro* evaluations of the top ranked compounds revealed that **12d** exhibited the highest AChE inhibitory activity, with a **55%** inhibition rate at a concentration of 10 μM. Moreover, this prominent pyrrole-based AChE inhibitor formed stable complex with the active site of the enzyme. Interactions with the active amino residues Tyr72 and Tyr286 indicated that **12d** was located near the peripheral anionic site of the enzyme. Additionally, *in silico* ADME investigations using QikProp demonstrated that **12d** possesses optimal pharmacokinetic properties. In conclusion, this study identified a novel pyrrole-based AChE inhibitor **12d** through a combination of computational and experimental findings.

Keywords

Acetylcholinesterase inhibitor, pyrrole-hydrazide-hydrazones, consensus docking, MM/GBSA, biological evaluation, ADME

Introduction

The positive role of acetylcholine (ACh) in the memory processes has been examined in details (Marucci et al. 2021), which has led to the development of several registered effective drugs with inhibitory capacity against the acetylcholinesterase enzyme (AChE). The AChE blocks the nerve impulses after the hydrolyzation of the acetylcholine in the cholinergic pathway of the nervous system. Moreover, cells with increased expression of

AChE on their surface undergo facile apoptosis (Zhang and Greenberg 2012). At the present, the improvement of the cholinergic neurotransmission still portrays the main approach in the symptomatic treatment of cognitive and behavioral symptoms of mild and moderate stages Alzheimer's disease (AD). Therefore, AChE inhibitors are currently the most efficacious approach for the treatment of Alzheimer's disease (AD) (Merzoug et al. 2021). The first crystallographic structure of AChE was resolved in 1993, and it confirmed the presence of two distinct binding sites-peripheral and catalytic. The former is located deeper into the binding gorge, and it comprises two subsites – esteratic and anionic (Moghadam et al. 2021). The peripheral site of the enzyme does not comprise any anionic residues.

Pyrrole is a five-atom N-containing heterocycle introduced in many biological compounds, such as chlorophyll, heme, vitamin B12, bile pigments, and alkaloids. Among the N-containing heterocycles, the derivatives of pyrroles possess various biological activities, such as antituberculosis, antifungal, antioxidant, antidiabetic, anti-inflammatory, analgesic, and anticancer effects (Mateev et al. 2022a). In addition, pyrrole-3-one derivatives were reported as highly potent inhibitors for AChE (Gümüş et al. 2021). Recently, Pourtaher et al. (2022) synthesized and biologically evaluated 39 pyrrole derivatives as AChE inhibitors. The authors found that most of the compounds acted as moderate AChE blockers in the micromolar range.

Structure-based drug design (SBDD) is successfully applied when a 3D structure of the corresponding protein is available. The major purpose of the SBDD is to distinguish false-positives from true inhibitors by employing scoring and searching algorithms (Pencheva et al. 2010). The process is known as molecular docking, and it is commonly involved in the virtual screenings of large databases. Additionally, the rising computing power is further accelerating the process of hit discovery and lead optimizations implementing various computer techniques (Soriano-Correa et al. 2015; De Vivo and Cavalli 2017). However, the main shortcoming of the docking software programs is the lack of unified docking protocol that achieves prominent results in each investigated protein (Morris and Corte 2021). Consequently, to overcome the mentioned drawback, diverse set of approaches, such as consensus docking, flexible docking, free binding energy calculations, etc., are being employed (Ren et al. 2018; Tuccinardi 2021).

Considering the vast pharmacological profile of the pyrrole-based compounds (Mateev et al. 2022a), as well as the reliability of the consensus docking as a drug design technique (Raka et al. 2019), the aim of this work was to identify potent AChE inhibitors out of an in-house database through consensus docking and MM/GBSA free binding energy recalculations (Fig. 1). The AChE inhibitory activities of the top ranked compounds were validated through *in vitro* assays. Moreover, the ADME properties of the most active AChE inhibitor were generated.

Materials and methods

Dataset

The employed dataset comprised recently synthesized pyrrole-based hydrazide-hydrazones with various biological activities (Fig. 2) (Bijev and Georgieva 2010; Tzankova et al. 2020; Mateev et al. 2022b).

The exact structures of the utilized database are provided in Supplementary Table 1. The compounds were generated with the 2D Sketcher (Maestro), and converted to the corresponding 3D structures with the LigPrep module in

Table 1. Scores obtained after consensus docking and MM/GBSA recalculations.

Compound	XP docking score (Kcal/mol)	ChemPLP (fitness score)	MM/GBSA (Kcal/mol)
11b	-8.159	132.51	-70.93
12d	-8.738	132.68	-69.29
11c	-7.152	131.50	-66.04
12b	-7.684	141.20	-64.06
12a	-8.259	132.51	-62.68
TZ4	**-20.189**	**122.92**	**-192.06**
Donepezil	**-18.41**	**114.06**	**-187.64**

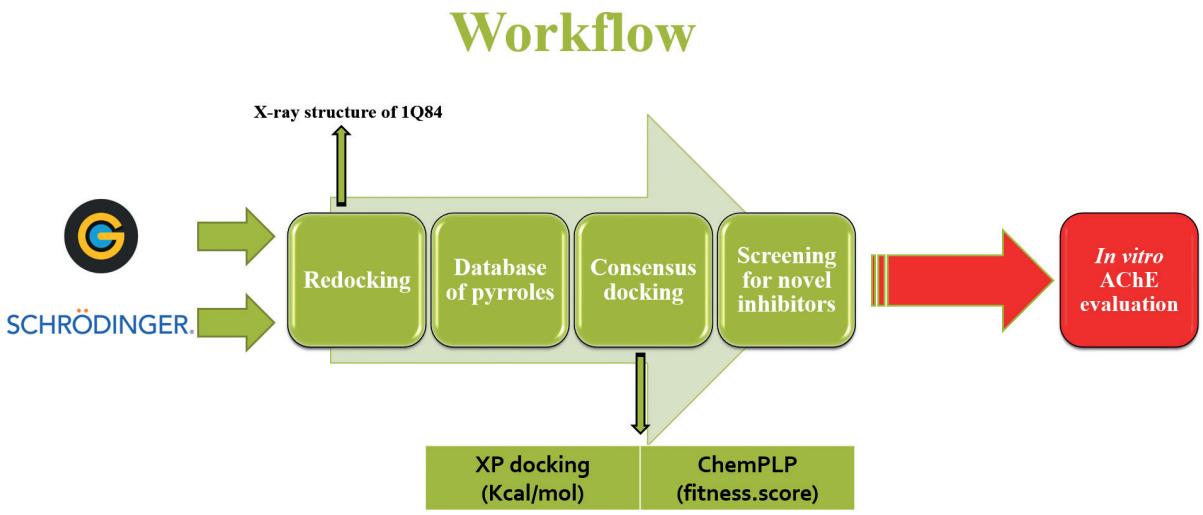

Figure 1. Workflow for identifying novel pyrrole-based acetylcholinesterase inhibitors.

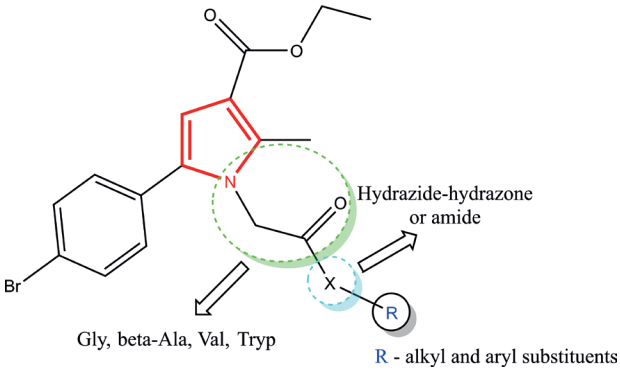

Figure 2. General core structure of the employed in-house database.

Maestro. Utilizing the former module, addition of hydrogen atoms, bond order assignment and energy minimization with the OPLS4 force field was performed. Moreover, the tautomerization and the stereoisomer forms for all compounds were generated. The ionization states were generated in physiological pH values. Consequently, the size of the final chemical library consisted of 139 molecules (including the generated tautomers and enantiomers). The major interactions between the most active pyrrole-based ligand and the active site the enzyme were visualized employing Discovery Studio Visualizer (BIOVIA Dassault Systèmes, Pharmacopeia, Inc) and Maestro (Schrödinger Release 2021-3: Maestro, Schrödinger, LLC, New York, NY, 2021).

Molecular docking

The docking simulations were performed with both Glide (Schrödinger Release 2021-3: Glide, Schrödinger, LLC, New York, NY, 2021) and GOLD 5.3 on an AMD Ryzen 9 5950× 16-core 4.0 GHz CPU, NVIDIA GeForce RTX 3060 12GB GPU, 64 GB RAM installed memory and 64-bit Operating system Windows 11 Pro. The default GOLD 5.3 docking protocol was constructed out of a 8 Å binding gorge around the co-crystallized ligand, ChemPLP as a scoring function, addition of active waters in the active site and no rotatable side chain residues. For Glide, we selected the Extra-Precision (XP) option and created the enzyme's active site using Receptor Grid Generation based on the conformation of the co-crystallized ligand. The crystal structure with PDB code 1Q84 was selected, which includes the highly active AChE co-crystallized ligand TZ4 (Bourne et al. 2004) due to the absence of a pyrrole-based co-crystallized AChE inhibitor. Moreover, the TZ4 inhibitor was suitable considering the bulkiness of the compounds included in our database. The latter structure was retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org) with a reliable resolution of 2.45 Å. The initial preparation of the crystallographic structure was carried out with the protein preparation module in Schrödinger (Schrödinger Release 2021-3: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2021; Impact, Schrödinger, LLC, New York, NY; Prime, Schrödinger, LLC, New York, NY, 2021).

Molecular Mechanics – Generalized Born Surface Area (MM-GBSA) calculations

In this study, Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) recalculations with Prime were employed to assess the free binding energies of the obtained through the docking studies complexes. The calculations were performed by the incorporation of the OPLS4 force field and VSGB dissolvable model (Sahakyan et al. 2021).

Chemicals

Donepezil, Dimethyl sulfoxide (DMSO), 5,5′-dithiobis-(2-nitrobenzoic acid (DTNB), acetylthiocholine iodide (ATChI), and acetylcholinesterase were purchased from Sigma-Aldrich in analytically pure or chemically pure grades. No further purifications were applied.

In vitro AChE assay

The inhibitory AChE potential of the top ranked pyrrole-based agents was measured according to a modified Ellman's method (Chigurupati et al. 2016). Stock solutions (1 mg/ml) of test agents were diluted in DMSO. Subsequently, working solutions (10 μM concentration) were prepared by dilutions. The compounds (10 μM) were incubated with sodium phosphate buffer (0.1 M; pH 8.0; 200 μL), and AChE solution (0.1 U/mL; 40 μL) for 10 min at 36.5 °C. The reaction was initiated by addition of DTNB (10 mM; 20 μL) and ATChI (14 mM; 20 μL). The absorbance was measured employing a microplate reader at 412 nm wavelength against blank DMSO probe. The % inhibition was calculated against blank probe. Donepezil was applied as a positive control.

Prediction of ADME properties

The significant physicochemical and pharmacokinetic properties of the most prominent AChE inhibitor in the current database were calculated with the QikProp module in Schrödinger (Schrödinger Release 2021-2: QikProp, Schrödinger, LLC, New York, NY, 2021.). The simulation provides ranges based on the properties of 95% of the known drugs and also evaluates outliners based on the Lipinski's rule of five.

Results and discussion

Re-docking simulation

The docking protocols of GOLD 5.3 and Glide were initially validated through re-docking simulations. The re-docking procedures are essential for the preliminary assessments of the softwares' reliability and robustness (Mateev et al. 2021). During the latter simulations, the co-crystallized ligand of the protein is removed, and without any minimizations, re-docked back into the original protein. A root-mean-square-deviation (RMSD) is

calculated, and values under 2 Å are considered as optimal (Mateev et al. 2022c).

The re-docking of the co-crystallized ligand TZ4 back into the active site of **1Q84** was carried out with the ChemPLP docking score of GOLD 5.3 and the XP score of Glide. Notably, GOLD 5.3 demonstrated RMSD value of 1.21 Å, whereas Glide re-docked the native ligand with RMSD of 0.54 Å, which indicated the slightly better performance of Glide. The re-docking conformations obtained with both docking softwares are provided in Fig. 3.

However, the major concern is the immense gap of the binding free energy related to the native co-crystallized ligand – TZ4. In the former case the complex protein-ligand demonstrated drastically elevated stability (MM/GBSA score of -192.06). Thus, the expected *in vitro* experimental values of the title compounds may not achieve the IC_{50} value of TZ4. Nevertheless, the docking simulations suggest that pyrroles condensed with a short, non-bulky aminoacids could potentially be used as AChE inhibitors.

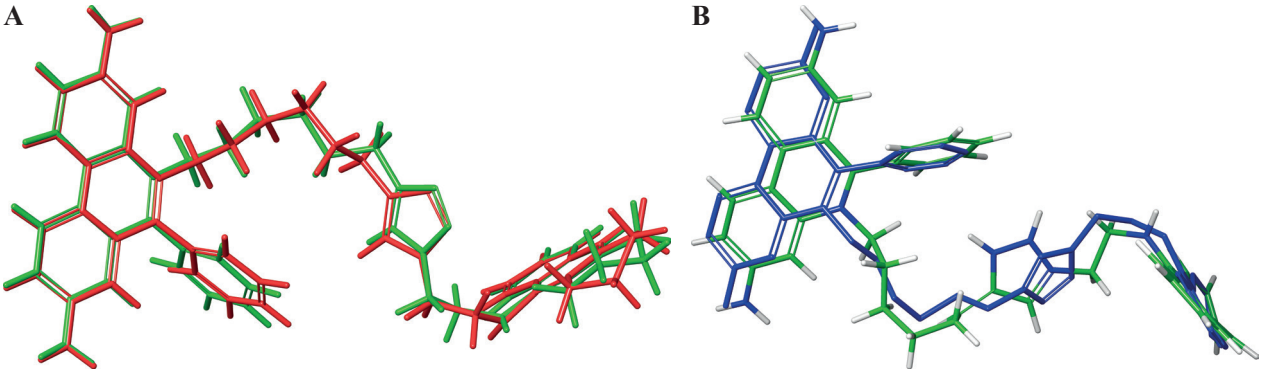

Figure. 3. Superimposed native conformation of TZ4 and the re-docking conformations acquired with Glide (**A**) and GOLD 5.3 (**B**).

Consensus docking and MM/GBSA rescoring

To increase the reliability of the structure-based drug design simulations, a consensus docking technique was implemented. Several studies have reported the superiority of simulations with multiple programs when compared to a single software docking (Houston et al. 2013; Ren et al. 2018). The main justification behind the process is that all docking softwares have limitations, and the simulations with various searching and scoring algorithms could improve the overall hit rate (Ren et al. 2018).

Initial molecular docking simulations were carried out with the XP option in Glide, and the ChemPLP scoring function in GOLD 5.3 for the whole dataset (Supplementary Table 1). The consensus docking acquired similar docking scores for the applied dataset, thus an increased experimental correlation could be expected. The docking scores of the co-crystallized ligand were dissimilar. Glide provided significantly lower docking scores of TZ4 and Donepezil (-20.18 Kcal/mol and -18.41 Kcal/mol, respectively), therefore, higher affinity towards the AChE enzyme. However, GOLD 5.3 identical fitness score of all employed ligands, including the native inhibitor **TZ4**, which should correspond to close experimental blocking capacities (Table 1).

The final recalculations of the binding free energies of the complexes were conducted with the MM/GBSA method considering the enhanced hit rate of the latter (Table 1) (Sahakyan et al. 2021). Moreover, focusing on the top-scored docking poses implies that significant computational costs could be saved (Sun et al. 2014). The MM/GBSA scores of the top ten pyrrole-based hydrazide-hydrazones were ranging from -60.44 to -70.93 Kcal/mol. The most prominent AChE inhibitor after the rescoring was **11b**.

In vitro AChE assay

Pharmacologically active drugs acting as acetylcholinesterase (AChE) inhibitors are frequently employed for patients suffering from AD. Drugs such as Donepezil, Rivastigmine and Galanthamine are registered as AChE inhibitors (Sitaram et al. 1978).

The *in vitro* inhibitory capacity of the top ranked through docking compounds was measured against ee-AChE (electric eel acetylcholinesterase) according to the method of Ellman et al. (Chigurupati et al. 2016). Donepezil was used as a standard. The compounds were applied at 10 μM concentrations (Fig. 4).

The most active pyrrole-based compound was the hydrazide-hydrazone condensed with 2-nitrofuran **12d** which inhibited the enzyme with 55% at 10 μM. In comparison, the standard drug Donepezil revealed blocking capacity of 93% at the same concentration. The enhanced AChE inhibitory effects could be related to the nitro moiety (Parveen et al. 2016). The hydrazide-hydrazone substituted

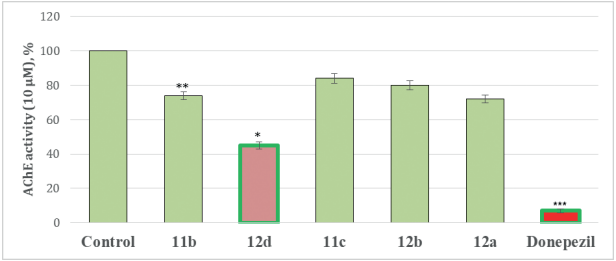

Figure 4. Inhibitory activity of the top ranked ligands against AChE (10 μM concentrations). * $P < 0.1$; *** $P < 0.001$ vs control (pure eeAChE). Data are presented as means from three independent experiments ± SD.

with a benzaldehyde moiety inhibited the enzyme with 28%. The top ranked compound **11b** showed only 26% blocking capacity, which underlines the current drawback of the structure-based drug design techniques – low capacity in differentiating true inhibitors from false-positive hits.

Visualizations of the 12d-AChE interactions

Subsequently, the major intermolecular interactions between **12d** and the active site of **1Q84** were examined (Fig. 5). One stable halogen bond between Ser293 and the bromo atom from p-bromophenyl fragment was formed. A hydrogen bond between the carbonyl moiety from the ester group and the active amino acid Thr75 was also detected. The amino acid Tyr72, which is included in the active loop in the substrate pocket of MAO-B, was interacting with the pyrrole structure through a hydrophobic π–π stacking. Moreover, the pyrrole and the benzene aromatic rings were involved in hydrophobic interactions with Trp286 and Tyr341 amino acids. Trp286 has been reported to be part of the quaternary ammonium binding locus (Geromichalos et al. 2021).

The molecular docking simulations showed that **12d** is located near the opening of the hydrophobic pocket. Hydrophobic interactions with the active amino residues Tyr286 and π–π bond with Tyr72 (5.25 Å) indicate that the most active pyrrole-base inhibitor was located near the PAS site (above the active site triad and near the gorge entrance) of the protein (Geromichalos et al. 2012). The probable reason for the incomplete binding to the active site of AChE might be the narrow entrance of the catalytic gorge (Lu et al. 2011). A hydrogen bond with Thr75 (2.13 Å) and the carbonyl moiety from the ethyl ester fragment was formed. A halogen bond between the bromine atom from the p-bromophenyl moiety and the active amino acid Ser293 was detected. Tyr72, Trp86, Trp286, Leu289, Ile294, Phe297, Tyr337, Phe338, and Tyr341 were involved in hydrophobic interactions with the active pyrrole-based AChE inhibitor **12d** (Table 2).

The residues Trp86 and Tyr341 were also found to stabilize the active conformation of recently synthesized pyrrole-based AChE inhibitor (Pourtaher et al. 2022). The visualizations of the docking simulations revealed that the future design of pharmacologically active pyrrole-based AChE inhibitors should be targeted against smaller pyrrole molecules. The former ligands will suite better the active pocket of AChE.

ADME investigation

As a final stage of our study, we carried out an *in silico* ADME analysis to examine the pharmaceutically relevant properties of the most prominent compound in our dataset – **12d**. The QikProp module in Maestro 11.8 was employed for the virtual determination of the absorption, distribution, metabolism and excretion (ADME) (Table 3).

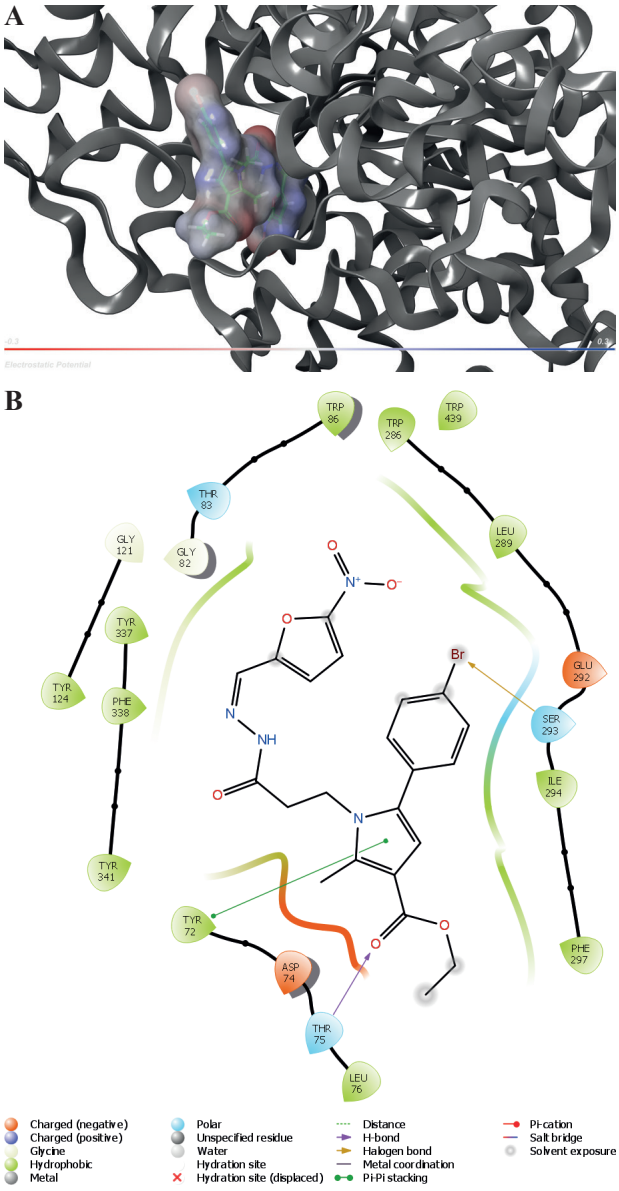

Figure 5. Major intermolecular interactions between **12d** and the active site of AChE (PDB: **1Q84**). The interactions are provided in 2D (**A**) and 3D (**B**) forms. The AChE enzyme is depicted in grey while the active inhibitor – **12d**, is presented as green sticks with its electrostatic potential.

Notably, **12d** exerted excellent physicochemical properties related to 95 % of the existing drugs. None of the calculated descriptors felt out of range during the conducted simulations. However, the observed molecular mass of 517.35 violated one of the Lipinski's Rule of 5. Importantly, the calculated brain/blood partition coefficient (QPlogBB) of the examined compound was in the optimal range of -3.0–1.2, which is essential for potential AChE inhibitors (Thomas 2000).

Table 2. *In silico* and *in vitro* evaluation of **12d**.

Compound	XP Glide	ChemPLP	MM/GBSA	Amino residues participating in stabilization	*In vitro* AChE activity
12d	-8.73	132.68	-69.29	Tyr72, Thr75 (H-bond), Trp86, Trp286, Leu289, Ser293, Ile294, Phe297, Tyr337, Phe338, and Tyr341	55% inhibition (10 μM concentration)

Table 3. ADME properties of the most promising pyrrole-based compound.

Compound	[a)]MW	[b)]Donor HB	[c)]Accept HB	[d)]QPLog Po/w	[e)]QPLog BB	[f)]Percent human oral absorption	[g)]PSA	[h)]Rule of five	[i)]Metab
12d	517.35	1	6	4.942	-2.445	77 %	141.39	1	3

[a)]Molecular weight of the molecule (Range:130.0–725.0); [b)]Number of hydrogen bond acceptors (Range: 2.0–20.0); [c)]Number of hydrogen bond donors (Range: 0.0–6.0); [d)]Predicted octanol/water partition coefficient. (Range: – 2.0–6.5); [e)]QPlogBB:Predicted brain/blood partition coefficient. Range from – 3.0 to 1.2; [f)]Percent Human Oral Absorption; [g)]PSA:Van der Waals surface area of polar nitrogen and oxygen atoms. Range from 7.0 to 200.0; [h)]Number of violations of Lipinski's rule of five (Range: maximum is 4); [i)]Number of likely metabolic reaction (Metab).

Conclusions

In the current study, the utilization of two widely employed docking software programs, namely GOLD 5.3 and Glide, coupled with MM/GBSA recalculations, a set of pyrrole-based scaffolds with potential binding affinity was identified. *In vitro* evaluations demonstrated a moderate correlation between the results of theoretical predictions and experimental tests. Notably, among the pyrrole-hydrazide-hydrazone tested compounds, the scaffold **12d** emerged as the most prominent AChE inhibitor, forming a stable complex with the active site of AChE. *In silico* ADME investigations using the QikProp module in Maestro revealed that **12d** exhibits favorable pharmacokinetic properties. To validate the findings of this study, further *in vivo* tests could be conducted.

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References

Bijev A, Georgieva M (2010) Pyrrole-based hydrazones synthesized and evaluated in vitro as potential tuberculostatics. Letters in Drug Design & Discovery 7: 430–437. https://doi.org/10.2174/157018010791306588

Bourne Y, Kolb HC, Radić Z, Sharpless KB, Taylor P, Marchot P (2004) Freeze-frame inhibitor captures acetylcholinesterase in a unique conformation. Proceedings of the National Academy of Sciences of the United States of America 101(6): 1449–1454. https://doi.org/10.1073/pnas.0308206100

Chigurupati S, Selvaraj M, Mani V, Selvarajan KK, Mohammad JI, Kaveti B, Bera H, Palanimuthu VR, Teh LK, Salleh M (2016) Identification of novel acetylcholinesterase inhibitors: Indolopyrazoline derivatives and molecular docking studies. Bioorganic Chemistry 67: 9–17. https://doi.org/10.1016/j.bioorg.2016.05.002

De Vivo M, Cavalli A (2017) Recent advances in dynamic docking for drug discovery. WIREs Computational Molecular Science 7(6): e1320. https://doi.org/10.1002/wcms.1320

Geromichalos GD, Lamari FN, Papandreou MA, Trafalis DT, Margarity M, Papageorgiou A, Sinakos Z (2012) Saffron as a source of novel acetylcholinesterase inhibitors: molecular docking and in vitro enzymatic studies. Journal of Agricultural and Food Chemistry 60(24): 6131–6138. https://doi.org/10.1021/jf300589c

Gümüş M, Babacan ŞN, Demir Y, Sert Y, Koca İ, Gülçin İ (2022) Discovery of sulfadrug-pyrrole conjugates as carbonic anhydrase and acetylcholinesterase inhibitors. Archiv der Pharmazie 355: e2100242. https://doi.org/10.1002/ardp.202100242

Houston DR, Walkinshaw MD (2013) Consensus docking: improving the reliability of docking in a virtual screening context. Journal of Chemical Information and Modeling 53(2): 384–390. https://doi.org/10.1021/ci300399w

Lu SH, Wu JW, Liu HL, Zhao JH, Liu KT, Chuang CK, Lin HY, Tsai WB, Ho Y (2011) The discovery of potential acetylcholinesterase inhibitors: a combination of pharmacophore modeling, virtual screening, and molecular docking studies. Journal of Biomedical Science 18(1): 1–8. https://doi.org/10.1186/1423-0127-18-8

Marucci G, Buccioni M, Ben DD, Lambertucci C, Volpini R, Amenta F (2021) Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. Neuropharmacology 190: e108352. https://doi.org/10.1016/j.neuropharm.2020.108352

Mateev E, Valkova I, Angelov B, Georgieva M, Zlatkov A (2021) Validation Through Re-Docking, Cross-Docking And Ligand Enrichment In Various Well-Resoluted Mao-B Receptors. International Journal of Pharmaceutical Science and Research 13: 1099–1107.

Mateev E, Angelov B, Kondeva-Burdina M, Valkova I, Georgieva M, Zlatkov A (2022) Design, Synthesis, biological evaluation and molecular docking of pyrrole-based compounds as antioxidant and MAO-B inhibitory agents. Farmacia 70: 344–354. https://doi.org/10.31925/farmacia.2022.2.21

Mateev E, Georgieva M, Zlatkov A (2022) Pyrrole as an Important Scaffold of Anticancer Drugs: Recent Advances. Journal of Pharmaceutical Sciences 25: 24–40. https://doi.org/10.18433/jpps32417

Mateev E, Georgieva M, Zlatkov A (2023) Improved molecular docking of MAO-B inhibitors with glide. Biointerface Research in Applied Chemistry 13: e159. https://doi.org/10.33263/BRIAC132.159

Merzoug A, Boucherit H, Khaled R, Chefiri A, Chikhi A, Bensegueni A (2021) Molecular docking study of the acetylcholinesterase inhibition. Current Issues in Pharmacy and Medical Sciences 34: 20–27. https://doi.org/10.2478/cipms-2021-0005

Moghadam B, Ashouri M, Roohi H, Karimi-Jafari M (2021) Computational evidence of new putative allosteric sites in the acetylcholinesterase receptor. Journal of Molecular Graphics and Modelling 107: e107981. https://doi.org/10.1016/j.jmgm.2021.107981

Morris C, Corte D (2021) Using molecular docking and molecular dynamics to investigate protein-ligand interactions. Modern Physics Letters B 35(08): e2130002. https://doi.org/10.1142/S0217984921300027

Parveen M, Aslam A, Nami S, Malla A, Alam M, Lee D, Rehman S, Silva P, Silva M (2016) Potent acetylcholinesterase inhibitors: Synthesis, biological assay and docking study of nitro acridone derivatives. Journal of Photochemistry and Photobiology B: Biology 161: 304–311. https://doi.org/10.1016/j.jphotobiol.2016.05.028

Pencheva T, Soumana O, Pajeva I, Miteva M (2010) Post-docking virtual screening of diverse binding pockets: Comparative study using DOCK, AMMOS, X-Score and FRED scoring functions. European Journal of Medicinal Chemistry 45(6): 2622–2628. https://doi.org/10.1016/j.ejmech.2009.12.025

Pourtaher H, Hasaninejad A, Iraji A (2022) Design, synthesis, in silico and biological evaluations of novel polysubstituted pyrroles as selective acetylcholinesterase inhibitors against Alzheimer's disease. Scientific Reports 12(1): e15236. https://doi.org/10.1038/s41598-022-18224-6

Raka S, Ahamed R, Rahman A, Momen A (2019) In silico discovery of noteworthy multi-targeted acetylcholinesterase inhibitors for the treatment of Alzheimer's disease. Advances in Traditional Medicine 20: 351–366. https://doi.org/10.1007/s13596-019-00407-8

Ren X, Shi Y-S, Zhang Y, Liu B, Zhang L-H, Peng Y-B, Zeng R (2018) Novel consensus docking strategy to improve ligand pose prediction. Journal of Chemical Information and Modeling 58(8): 1662–1668. https://doi.org/10.1021/acs.jcim.8b00329

Sahakyan H (2021) Improving virtual screening results with MM/GBSA and MM/PBSA rescoring. Journal of Computer-Aided Molecular Design 35(6): 731–736. https://doi.org/10.1007/s10822-021-00389-3

Sitaram N, Weingartner H, Caine ED, Gillin JC (1978) Choline: Selective enhancement of serial learning and encoding of low imagery words in man. Life Sciences 22(17): 1555–1560. https://doi.org/10.1016/0024-3205(78)90011-5

Soriano-Correa C, Barrientos-Salcedo C, Campos-Fernández L, Alvarado-Salazar A, Esquivel R (2015) Importance of asparagine on the conformational stability and chemical reactivity of selected anti-inflammatory peptides. Chemical Physics 457: 180–187. https://doi.org/10.1016/j.chemphys.2015.06.005

Sun H, Li Y, Shen M, Tian S, Xu L, Pan P, Guan Y, Hou T (2014) Assessing the performance of MM/PBSA and MM/GBSA methods. 5. Improved docking performance using high solute dielectric constant MM/GBSA and MM/PBSA rescoring. Physical Chemistry Chemical Physics 16(40): 22035–22045. https://doi.org/10.1039/C4CP03179B

Thomas T (2000) Monoamine oxidase-B inhibitors in the treatment of Alzheimer's disease. Neurobiology of Aging 21(2): 343–348. https://doi.org/10.1016/S0197-4580(00)00100-7

Tuccinardi T (2021) What is the current value of MM/PBSA and MM/GBSA methods in drug discovery? Expert Opinion on Drug Discovery 16(11): 1233–1237. https://doi.org/10.1080/17460441.2021.1942836

Tzankova D, Vladimirova S, Aluani D, Yordanov Y, Peikova L, Georgieva M (2020) Synthesis, in vitro safety and antioxidant activity of new pyrrole hydrazones. Acta Pharmaceutica 70: 303–324. https://doi.org/10.2478/acph-2020-0026

Zhang XJ, Greenberg DS (2012) Acetylcholinesterase involvement in apoptosis. Frontiers in Molecular Neuroscience 5: 1–40. https://doi.org/10.3389/fnmol.2012.00040

Supplementary material 1

Docking scores and MM/GBSA recalculation of the applied dataset

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