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Synthesis, *in vitro* and docking studies of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one derivatives as agents for the treatment of Alzheimer's disease

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Abstract

Alzheimer's disease is a chronic neurodegenerative disease, which is characterized mainly by a progressive decrease in intellectual abilities, memory impairment and a change in a person's personality. Unfortunately, there are practically no medicines that act on the pathogenesis of Alzheimer's disease. The development of new highly effective medicines for the treatment of this pathology is one of the crucial areas of pharmaceutical research. The aim of this work is to search among 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one effective compounds with anticholinesterase and antiamyloid activities. As a result, it was found that compounds **4d**, **4e** and **4f** have the highest anticholinesterase ability, containing in their structure the residues of hydroxy-methoxyphenyl fragments. Structures **4c**, **4g**, **4h**, **4j**, **4k**, **4m**, **4n** and **4p** showed slightly lower activity, the effect of which did not differ statistically from that of Donepezil. Compounds **4c**, **4e**, **4k** and **4m** have the greatest ability to inhibit the formation of the amyloid, comparable to GV-971. It should be noted that the molecular docking data are consistent with the results of the determination of the anticholinesterase activity of the studied compounds obtained *in vitro*. Thus, the prospects for future studies of these compounds concerning the possibility of creating a pharmaceutical active substance for the treatment of neurodegenerative diseases have been revealed.

Keywords

Alzheimer's disease
tetrahydrothienopyrimidine
synthesis
molecular docking
AChE
Acetylcholinesterase
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1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases in humans. Currently, there are practically no pathogenetic drugs that can cure the patient. Drug therapy is aimed only at eliminating the symptoms of the disease and slowing its progression. The most widely used anticholinesterase (AChE) drugs that can neutralize the symptoms of cholinergic insufficiency. Recently, the development of antiamyloid drugs that can directly affect the pathogenesis of the disease and thereby significantly improve the patient's well-being has been intensified. Thus, the search for new compounds with the above properties is a cutting-edge area of medicinal chemistry and neuropharmacology [1].

Research is actively underway to develop the new acetylcholinesterase inhibitors. Thus, new thiazolylhydrazone derivatives were designed and synthesized as acetylcholinesterase and butyrylcholinesterase (BChE) inhibitors. All compounds showed a weak inhibitory effect on BChE; meanwhile, most of the compounds had a certain AChE inhibitory activity [2]. Research was carried out to study the possibility of designing acetylcholinesterase inhibitors based on isoquinolone and azepanone derivatives. Overall, the compounds studied are weak AChE inhibitors, but, nonetheless, important insights were obtained on their mode of inhibition so that more potent analogues can be designed, prepared and tested [3]. There are literature data indicating that chalcone can be used as the scaffold for cholinesterase inhibitor [4]. Pharmacophore based 3D QSAR

models for human acetylcholinesterase inhibitors with good significance, statistical values were generated. Virtual screening experiments and subsequent *in vitro* evaluation of promising hits revealed a novel and selective AChE inhibitor [5]. A number of pyrimidine derivatives were synthesized, among which there are compounds that may be considered as leaders for investigations in neurodegenerative diseases [6]. Some of diversely functionalized pyrimidine fused thiazolino-2-pyridones have an ability to inhibit the formation of amyloid-β fibrils associated with Alzheimer's disease, while others bind to mature amyloid-β and α-synuclein fibrils [7]. A new series of pyrimidine and pyridine diamines was designed as dual binding site inhibitors of cholinesterases, characterized by two small aromatic moieties separated by a diaminoalkyl flexible linker [8]. To obtain a multipotent framework that can target simultaneously cyclooxygenase-2, arachidonate 5-lipoxygenase, acetylcholinesterase, and butyrylcholinesterase to treat neuroinflammation, a series of derivatives containing pyrimidine and pyrrolidine cores were rationally synthesized and evaluated. Tacrine–pyrrolidine hybrids and tacrine–pyrimidine hybrid emerged as the most potent AChE inhibitors [9]. A series of 2,4-phenylsulfonyl-pyrimidine carboxylate derivatives was designed and synthesized. Two compounds among them exhibited promising AChE inhibition and significantly inhibited Aβ aggregation, that is important for anti-Alzheimer's action [10]. 2-Arylidene derivatives of thiazolopyrimidine with different linker size and target-anchoring functional groups for the treatment of AD were synthesized. Some of them showed excellent to good AChE and BChE inhibition potential at nanomolar to low micromolar concentration [11]. A series of novel tetrahydropyrimidin-4-yl)pyridine derivatives was designed and synthesized as inhibitors of AChE and BChE. The *in vitro* studies showed that all the synthesized derivatives showed significant BChE inhibitory activity and were more potent than donepezil as the standard. All the target compounds demonstrated good AChE inhibitory effects, comparable with donepezil as the reference drug [12]. 4-(Pent-4-yn-1-yloxy)phenyl)-2-phenylpyrimidine derivatives were synthesized and screened for monoamine oxidase and AChE inhibitory activities [13]. New triazolopyridopyrimidine was easily prepared in good yields showing anticholinesterase inhibition and strong antioxidant power, which allows using new hit-triazolo pyridopyrimidine for AD therapy [14].

Previously, we studied the biological activity of azomethine derivatives of 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxamide, which are acyclic precursors of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one. The results show that some representatives of the studied azomethines have pronounced anticholinesterase and antiamyloid activities [15]. In this study, we decided to continue our research on finding candidates for the treatment of Alzheimer's disease. We decided to take 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one as the objects of the study, since their azomethine precursors demonstrated the ability to inhibit the acetylcholinesterase enzyme and the formation of the amyloid.

The proposed class of organic compounds has various types of biological activity. It was found that 2-(4-Methoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one may be an efficacious compound for the treatment of prostate cancer in advanced stages [16, 17]. Some thieno[2,3-d]pyrimidine-4(1*H*)-one-based analogs inhibit the growth of human colon tumor cells [18]. Also, this class of organic compounds can suppress the production of inflammatory mediators [19]. Studies on thiophenpyrimidine derivatives with various conjugated cyclic systems showed that modification of 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one by replacing conjugated cyclohexane with 1-methylpiperidine can increase the ability of such compounds to be used in breast cancer therapy [20]. Derivatives of thieno[2,3-d]pyrimidine-4-one may have antioxidant properties [21]. Some new thieno[2,3-d]pyrimidine-4(3*H*)-one derivatives showed good analgesic activity by using Eddy´s hot plate method [22]. There are data indicating that tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine scaffolds may serve as models for the development of antimalarial agents [23]. Some of the 5-alkoxytetrazolo[1,5-c]thieno[2,3-e]pyrimidine derivatives may exhibit anticonvulsant and antidepressant effects, which makes it possible to design compounds based on them with an effect on the central nervous system [24].

A method was proposed for the synthesis of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one by suspending 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxamide in a small amount of butanol and the corresponding aldehyde with a catalytic amount of concentrated hydrochloric acid [17]. There is a technique for obtaining compounds of this series using ZnO-CeO_2 nanocomposite as a catalyst. ZnO-TiO_2 nanocomposites were added to the mixture of aminoamide and aldehyde [25]. It is possible to carry out the chemical interaction of 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxamide with aldehydes in a DMF and piperidine medium when heated [26] and in the environment of hydrochloric acid and methanol [18]. A method was proposed for the preparation of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one by adding 2-amino-3-carbethoxythiophene in anhydrous dioxane saturated with hydrogen chloride gas nitrile to a solution of 2-amino-3-carbethoxythiophene in anhydrous dioxane [20].

2. Experimental

2.1. Molecular modeling

A virtual model of human acetylcholinesterase from the RCSB Protein Data Bank database was taken as an object for molecular docking with the identification number 4EY7 [27]. The three-dimensional structures of the studied compounds were constructed in the HyperChem 8.0.4 pro-

gram and then geometrically optimized by the MM+ method. The final geometry optimization of the virtual structures was calculated in the ORCA 4.1 program using the density functional theory (UB3LYP) method and the 6-311G** basis set. The docking study was performed using the Autodock4 program. It was set to search for 200 energetically favorable conformations of the ligand-enzyme complex formation using the Lamarckian GA 4.2 scoring function for calculating the energy of the ligand-enzyme interaction. RMSD is 0.44 Å for donepezil. Molecular docking is presented in more detail in the following work [15].

2.2. Chemistry

All chemicals were acquired from Sigma-Aldrich (SigmaAldrich, St. Louis, MO, USA), Carl Roth (Carl Roth, Karlsruhe, Germany) and Merck Chemicals (MerckKGaA, Darmstadt, Germany). Melting points (m.p.) were recorded using the PMP-M1 melting point apparatus (Himlaborpribor, Klin, Russia). All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 F254 TLC plates (Merck, Darmstadt, Germany). Spectroscopic data were registered with the following instruments: IR, IR-Fourier FSM 1201 spectrophotometer (Spectrum, Moscow, Russia); UV, SF-2000 device (Spectrum, Moscow, Russia); ^{1}H NMR and ^{13}C NMR, Bruker Avance III 400 MHz spectrometer (Bruker, Germany) in DMSO-d6 using tetramethylsilane as the internal standard. Coupling constant (*J*) values are measured in hertz (Hz) and spin multiplets are given as follows: *s* (singlet), *d* (double), *t* (triplete), *q* (quartet), *m* (multiplet).

2.2.1. General procedure for synthesis of azomethine derivatives of 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxamide (3a–3s)

0.01 mol (1.92 g) of compound 1 and the equimolar amount of the corresponding aldehyde (2) were dissolved by heating in a minimum amount of ethanol. Then the solutions were combined. The reaction was carried out until a precipitate was formed. It took about 30 minutes. The precipitate was filtered and purified by recrystallization from ethanol [15, 28].

2.2.2. General procedure for synthesis of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one (4a–4s)

Azomethine 3 (0.08 mol) was refluxed for 30–60 min in the glacial acetic acid. Then 1 ml DMSO was added and the reaction mixture was refluxed for 60 min. After cooling, the formed precipitate was filtered. In the filtrate the remaining target product was precipitated with a 0.1 M cold water solution of sodium chloride. The precipitates were combined. Recrystallization of the obtained compounds was carried out from acetic acid. Compounds **4a–4j**, **4p**, **4q** were obtained earlier [29].

2.3. Pharmacological study

2.3.1. Evaluation of anti-amyloid activity *in vitro*

The fragments Aß 1-42 were obtained from Sigma-Aldrich (Germany). GV-971 was provided by Hunan warrant pharm. (China). The aggregation process of amyloid particles was evaluated in the reaction of the interaction of Aß with Congo red. 25 µl of a solution of the test compounds in dimethyl sulfoxide (the final concentration is 20 mg/ml, GV-971 in a similar concentration was used as a referent compound) was mixed with 225 µl of a 20 mM solution of congo red in phosphate buffer solution. The resulting mixture was incubated at room temperature. Then the absorbance of the samples was recorded at wavelengths of 540 nm and 405 nm. after nine days of incubation. The number of aggregates Aß was calculated by the following equation on the 3rd, 6th and 9th day of the experiment:

$$Aß = \frac{A540}{4780} - \frac{A405}{6380} - A405BL/8620 \quad (1)$$

where A405BL is the absorbance of the Congo red solution at a wavelength of 405 nm; A540 and A405 are the absorbances of the solution containing the test substances at a wavelength of 540 nm and 405 nm, respectively.

The difference between the compounds was evaluated by the ANOVA method with the Tukey post-test [30].

2.3.2. Evaluation of anticholinesterase activity *in vitro*

The activity of acetylcholinesterase was determined by the modified Ellman method. The analyzed medium contained 20 ml of acetylcholinesterase solution (3.2 U/l), 25 ml of a solution of the test compounds in various concentrations (30 mg/ml, 15 mg/ml, 7.5 mg/ml, 3.75 mg/ml and 1.875 mg/ml) and a potassium-phosphate buffer solution in a volume of up to 300 ml. Donepezil (KRKA, Slovenia) in similar concentrations was used as a reference substance. The mixture was incubated for 5 minutes. The reaction was started by adding the acetylcholine chloride (25 µl, 0.02 M solution) and 5.5'-dithiobis-2-nitrobenzoic acid (25 µl, 0.02 M solution). The absorbance of the mixture was recorded after 5 minutes at 412 nm using the Infinite F50 microplate reader (Tecan, Austria). The tests were performed in a triplet version. IC_{50} (mg/ml) was calculated by probit analysis. The data is presented in the form of M±SEM (mean ± standard error of the mean). Statistical differences were evaluated at a significance level of $p<0.05$ by the ANOVA method with post-processing by Tukey [31].

3. Results and Discussion

3.1. Synthesis

As shown in Scheme 1, 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxamide **1** and aldehydes **2** were refluxed in ethanol to obtain azomethine derivatives **3**. The reactions were performed in ethanol as a green solvent. Heterocyclization reaction was performed using glacial acetic acid and DMSO to afford the 2-substituted

5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one **4**. The products **4a-4s** were obtained with the high yields. The compounds were characterized by nuclear magnetic resonance and infrared spectroscopy.

3.1.1. 2-(3,5-di-tert-butyl-4-hydroxy-phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3d]pyrimidine-4(3H)-one (4k)

The beige crystals were obtained. Yield: 85%. M.p.: 293–294 °C. UV spectrum (ethanol), λ_{max}, nm: 207, 337. IR spectrum (KBr), ν, cm^{-1}: 3620 (NH), 3447 (OH, stretching), 2951 (Csp_3-H), 1649 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 1.44 (*s*, 18H, CH_3), 1.87–1.71 (*m*, 4H, CH_2), 2.72 (*t*, *J* = 6.0 Hz, 2H, CH_2), 2.90 (*t*, *J* = 5.9 Hz, 2H, CH_2), 7.60 (*s*, 1H, OH), 7.84 (*s*, 2H, ArH), 12.50 (*s*, 1H, NH). ^{13}C NMR spectrum (100,6 MHz, DMSO-d_6), δ, ppm: 22.26, 23.00, 24.96, 25.83, 35.21, 56.49, 120.46, 123.47, 131.14, 131.72, 139.13, 153.64, 157.60, 159.56, 164.00.

3.1.2. 2-(2-hydroxy-5-nitro-phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3d]pyrimidine-4(3H)-one (4l)

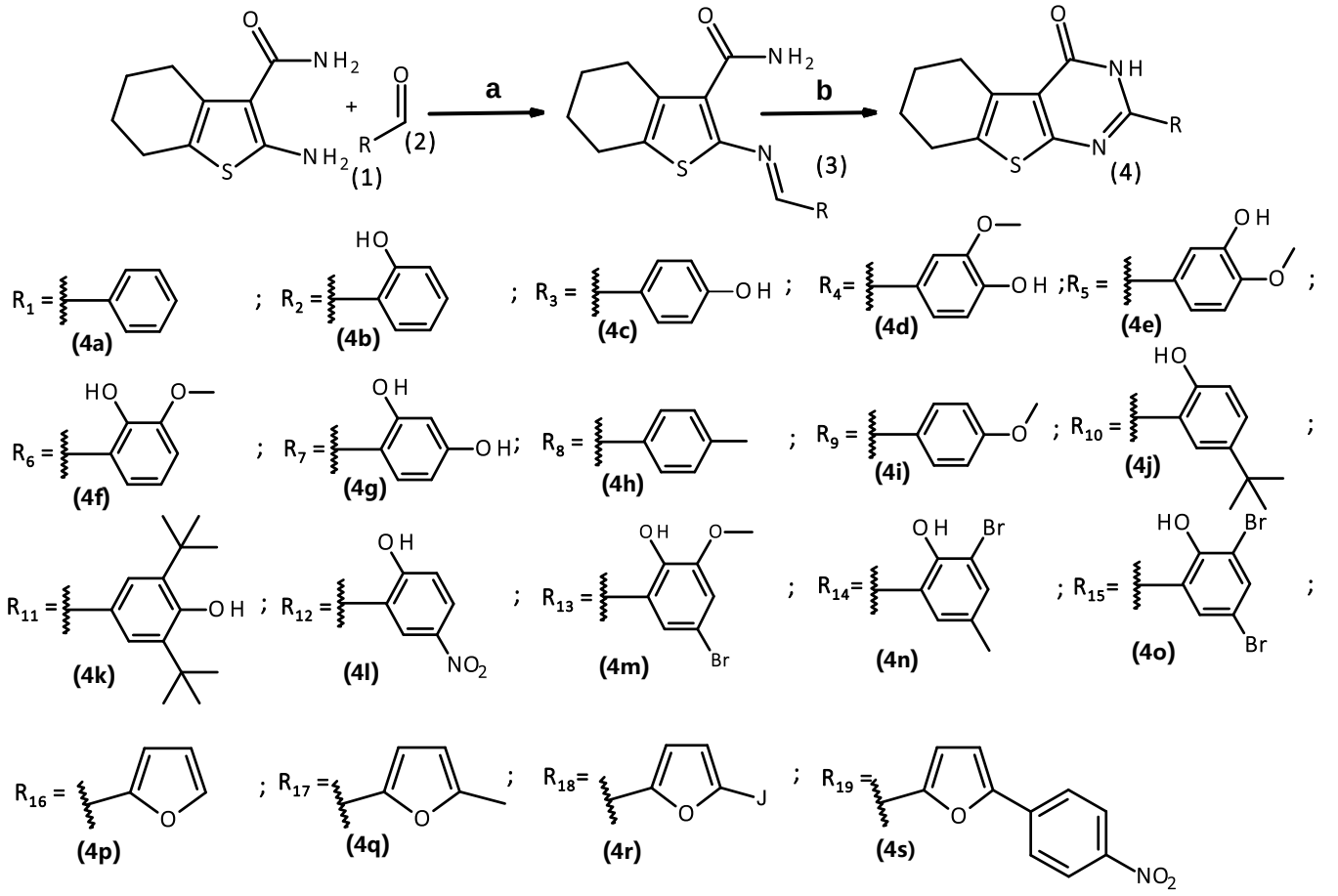
The yellow crystals were obtained. Yield: 88%. M.p.: 285–286 °C. UV spectrum (ethanol), λ_{max}, nm: 220, 370. IR spectrum (KBr), ν, cm^{-1}: 3466 (OH, stretching), 2939 (Csp_3-H), 1657 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 1.90–1.57 (*m*, 4H, CH_2), 2.78 (*t*, *J* = 5.9 Hz, 2H, CH_2), 2.91 (*t*, *J* = 6.0 Hz, 2H, CH_2), 7.12 (*d*, *J* = 9.1 Hz, 1H, ArH), 8.25 (*dd*, *J* = 9.2, 2.9 Hz, 1H, ArH), 8.92 (*d*, *J* = 2.9 Hz, 1H, ArH), 12.84 (*s*, 1H, NH).

3.1.3. 2-(5-bromo-2-hydroxy-3-methoxy-phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3d]pyrimidine-4(3H)-one (4m)

The brown crystals were obtained. Yield: 95%. M.p.: *T*>300 °C. UV spectrum (ethanol), λ_{max}, nm: 215, 235, 282. IR spectrum (KBr), ν, cm^{-1}: 3455 (OH, stretching), 2928 (Csp_3-H), 1657 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 1.86–1.72 (*m*, 4H, CH_2), 2.77 (*t*, *J* = 6.1 Hz, 2H, CH_2), 2.89 (*t*, *J* = 6.1 Hz, 2H, CH_2), 3.88 (*s*, 3H, CH_3), 7.30 (*s*, 1H, ArH), 7.84 (*s*, 1H, ArH), 11.84 (*s*, 1H, OH), 12.27 (*s*, 1H, NH).

3.1.4. 2-(3-bromo-2-hydroxy-5-methyl-phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3d]pyrimidine-4(3H)-one (4n)

The brown crystals were obtained. Yield: 93%. M.p.: 287–288 °C. UV spectrum (ethanol), λ_{max}, nm: 210, 390. IR spectrum (KBr), ν, cm^{-1}: 3458 (OH, stretching), 2928 (Csp_3-H), 1658 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 1.86–1.70 (*m*, 4H, CH_2), 2.28 (*s*, 3H, CH_3), 2.78 (*t*, *J* = 5.8 Hz, 2H, CH_2), 2.91 (*t*, *J* = 6.0 Hz, 2H, CH_2), 7.60 (*s*, 1H, ArH), 8.07 (*s*, 1H, ArH), 12.93 (*s*, 1H, NH), 13.24 (*s*, 1H, OH).



Scheme 1 Reagents and conditions: (a) ethanol, reflux; (b) glacial acetic acid, DMSO, reflux.

3.1.5. 2-(3,5-dibromo-2-hydroxy-phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3d]pyrimidine-4(3H)-one (4o)

The brown crystals were obtained. Yield: 92%. M.p.: 290–291 °C. UV spectrum (ethanol), λ_{max}, nm: 211, 389. IR spectrum (KBr), ν, cm^{-1}: 3429 (OH, stretching), 2928 (Csp_3-H), 1653 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 1.88–1.72 (*m*, 4H, CH_2), 2.78 (*t*, *J* = 5.9 Hz, 2H, CH_2), 2.89 (*t*, *J* = 5.8 Hz, 2H, CH_2), 7.94 (*d*, *J* = 2.4 Hz, 1H, ArH), 8.44 (*d*, *J* = 2.3 Hz, 1H, ArH), 13.04 (*s*, stretching, 2H, OH, NH).

3.1.6. 2-(5-iodo-2-furyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3d]pyrimidine-4(3H)-one (4r)

The brown crystals were obtained. Yield: 79%. M.p.: 297–298 °C. UV spectrum (ethanol), λ_{max}, nm: 218, 283, 353. IR spectrum (KBr), ν, cm^{-1}: 3436 (NH, stretching), 2928 (Csp_3-H), 1649 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 1.84–1.74 (*m*, 4H, CH_2), 2.75 (*t*, *J* = 6.1 Hz, 2H, CH_2), 2.88 (*t*, *J* = 6.2 Hz, 2H, CH_2), 6.97 (*dd*, *J* = 3.5, 1.6 Hz, 1H, ArH), 7.54–7.47 (*m*, 1H, ArH), 12.51 (*s*, 1H, NH). ^{13}C NMR spectrum (100,6 MHz, DMSO-d_6), δ, ppm: 22.20, 22.89, 25.02, 25.76, 98.87, 121.46, 131.53, 133.22, 143.14, 150.73, 158.55.

3.1.7. 2-[5-(4-nitrophenyl)-2-furyl]-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3d]pyrimidine-4(3H)-one (4s)

The brown crystals were obtained. Yield: 78%. M.p.: *T*>300 °C. UV spectrum (ethanol), λ_{max}, nm: 204, 219, 399. IR spectrum (KBr), ν, cm^{-1}: 3447 (NH, stretching), 2932 (Csp_3-H), 1645 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 1.85–1.71 (*m*, 4H, CH_2), 2.75 (*q*, *J* = 3.9, 2.3 Hz, 2H, CH_2), 2.91 (*t*, *J* = 5.6 Hz, 2H, CH_2), 7.53 (*d*, *J* = 3.7 Hz, 1H. ArH), 7.60 (*d*, *J* = 3.8 Hz, 1H, ArH), 8.35–8.25 (*m*, 4H, ArH), 12.84 (*s*, 1H, NH). ^{13}C NMR spectrum (100,6 MHz, DMSO-d_6), δ, ppm: 22.19, 22.88, 25.05, 25.80, 113.03, 116.85, 121.65, 124.70, 125.76, 131.65, 133.27, 135.32, 143.75, 147.07, 147.33, 154.00, 158.65, 162.98.

3.2. Docking studies

Based on the results of computational experiment, molecular complexes were selected, in which the simulated compounds occupy the most energetically advantageous location in the active site of the acetylcholinesterase enzyme. 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one mainly formed bonds with the following amino acid residues of the active site of AChE: Tyr 124, Trp 286, Val 294, Phe 295, Arg 296, Phe 297, Tyr 337, Phe 338, Tyr 341 and His 447.

Table 1 shows the minimum energies for the formation of ligand complexes with the active site of AChE and the hydrogen bonds. Figure 1 and 2 shows locations of **4a–4s** according to molecular docking.

Table 1 Results of molecular docking experiments for compounds **4a–4s**, donepezil and its hydrogen bonds.

Compound	AutoDock binding energy (kcal/mol)	Residue	Ligand atoms	Distance (Å)
4a	−9.05	–	–	–
4b	−10.16	Arg 296	O<u>H</u>	1.979
		Ser 293	<u>O</u>H	2.141
4c	−9.23	Ser 293	O<u>H</u>	2.041
4d	−9.55	Phe 295	<u>O</u>CH_3	2.199
4e	−10.29	Arg 296	<u>O</u>H	1.679
4f	−10.29	Phe 295	<u>O</u>H	1.895
		Arg 296	<u>O</u>CH_3	2.146
4g	−9.95	Ser 293	O<u>H</u>	1.804
		Arg 296	O<u>H</u>	1.898
		Arg 296	<u>O</u>H	1.895
4h	−9.55	–	–	–
4i	−9.48	–	–	–
4j	−10.86	Phe 295	C=<u>O</u>	2.225
4k	−10.30	–	–	–
4l	−9.67	Phe 295	C=<u>O</u>	2.155
		Arg 296	O<u>H</u>	2.064
		Arg 296	<u>O</u>H	2.188
4m	−11.64	Arg 296	<u>O</u>CH_3	2.211
		Phe 295	<u>O</u>H	2.153
4n	−10.52	Phe 295	C=<u>O</u>	2.128
		Ser 293	O<u>H</u>	1.822
4o	−10.84	Phe 295	<u>O</u>H	2.104
4p	−9.05	Arg 296	Fur<u>O</u>	1.891
4q	−8.93	Phe 295	C=<u>O</u>	1.700
4r	−9.71	Phe 295	–<u>S</u>–	2.222
4s	−10.67	Arg 296	N<u>O_2</u>	1.903
donepezil	−11.89	Phe 295	C=<u>O</u>	1.770

The compounds **4b**, **4c**, **4g** and **4n** form a hydrogen bond between their hydroxy groups and the amino acid residue Ser 293. The structures **4b**, **4e**, **4g** and **4l** by the same structural fragment can make a hydrogen bond with Arg 296. The compounds **4p** and **4s** form a hydrogen bond with Arg 296 by oxygen atoms of the furan heterocycle and the nitro group, respectively. It is often seen that the simulated compounds can form a hydrogen bond in a ligand-enzyme complex with Phe 295. **4j**, **4l**, **4n** and **4q** interact with Phe 295 with their carbonyl groups, and **4r** molecule forms a hydrogen bond between Phe 295 and the sulfur atom of the thiophene heterocycle. Three compounds among the simulated structures **4f**, **4m** and **4o** with the above amino acid residue interact with the oxygen atom of the hydroxy group of the aryl fragment of the molecule. It follows from the docking results that compounds **4f** and **4m** can form a hydrogen bond with the Arg 296 oxygen atom by the methoxy group, and in the structure of **4d** similarly structural fragments interacts with Phe 295 by forming a hydrogen bond with a length of 2.199 Å. According to the molecular docking data for **4a**, **4h**, **4i** and **4k,** the formation of hydrogen bonds is not observed. Donepezil makes a hydrogen bond between the oxygen atom of the carboxyl group of the five-membered cycle of the molecule and the amino acid Phe 295 of the active site of the en-

zyme. Many 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3H)-one form a hydrogen bond with Phe 295, as does the donepezil. There is no non-bonding interactions such as van der Waals, π–π, π–alkyl according to this docking protocol.

3.3. Pharmacological studies

The results of the antiamyloid activity evaluation of the test substances are presented in Table 3.

As can be seen from the data in Table 3, the compounds **4c**, **4e**, **4k** and **4m** have the highest ability to inhibit the formation of β-amyloid in the model mixture. At the same time, the compounds **4c**, **4e** significantly suppressed the process of amyloidogenesis after 3 days of incubation. It is worth noting that on the 9th day of the experiment, all the leading compounds showed a comparable level of pharmacological efficacy, which, however, was lower than that of GV-971.

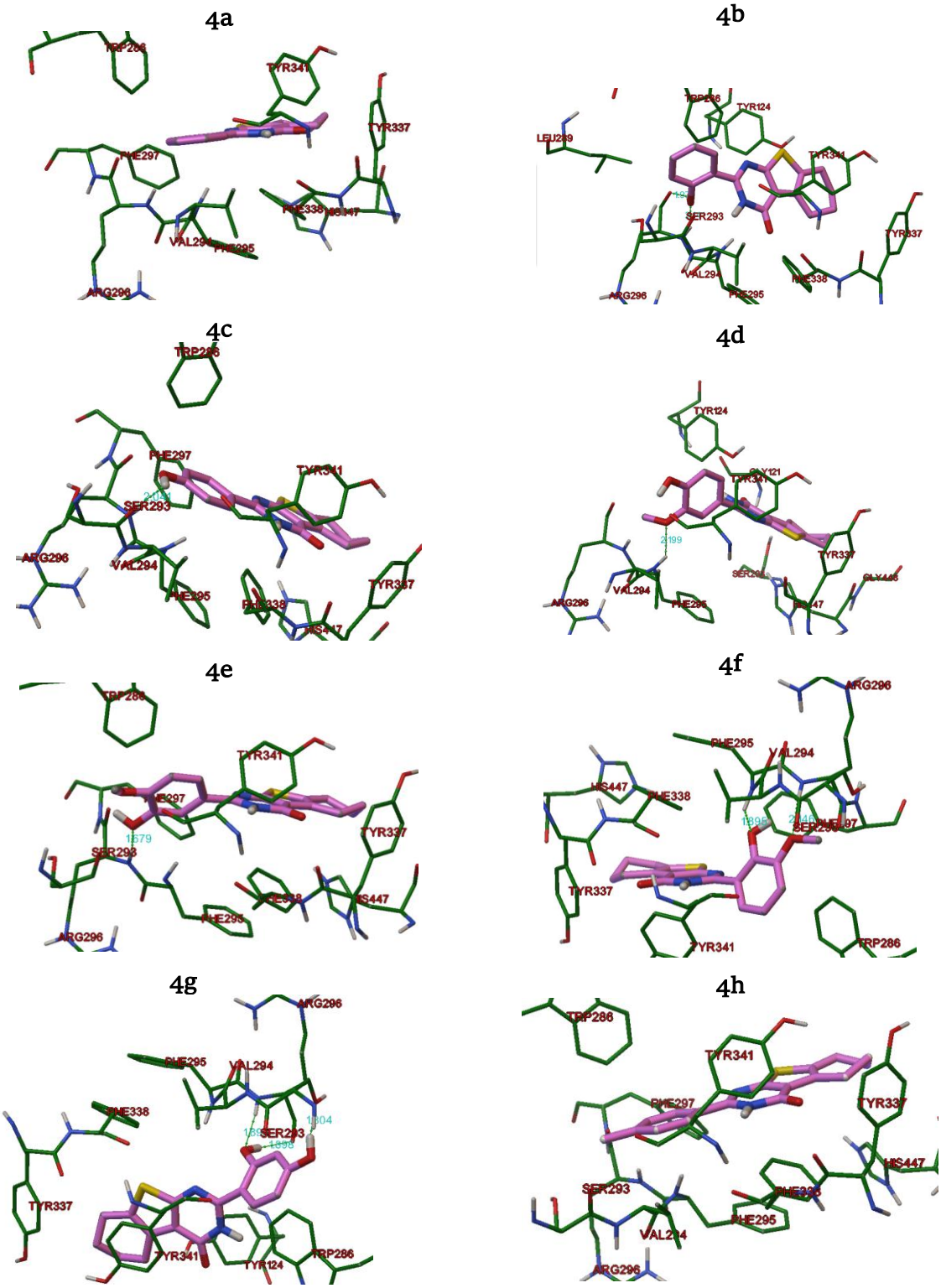


Figure 1 The location of **4a–4h** according to molecular docking.

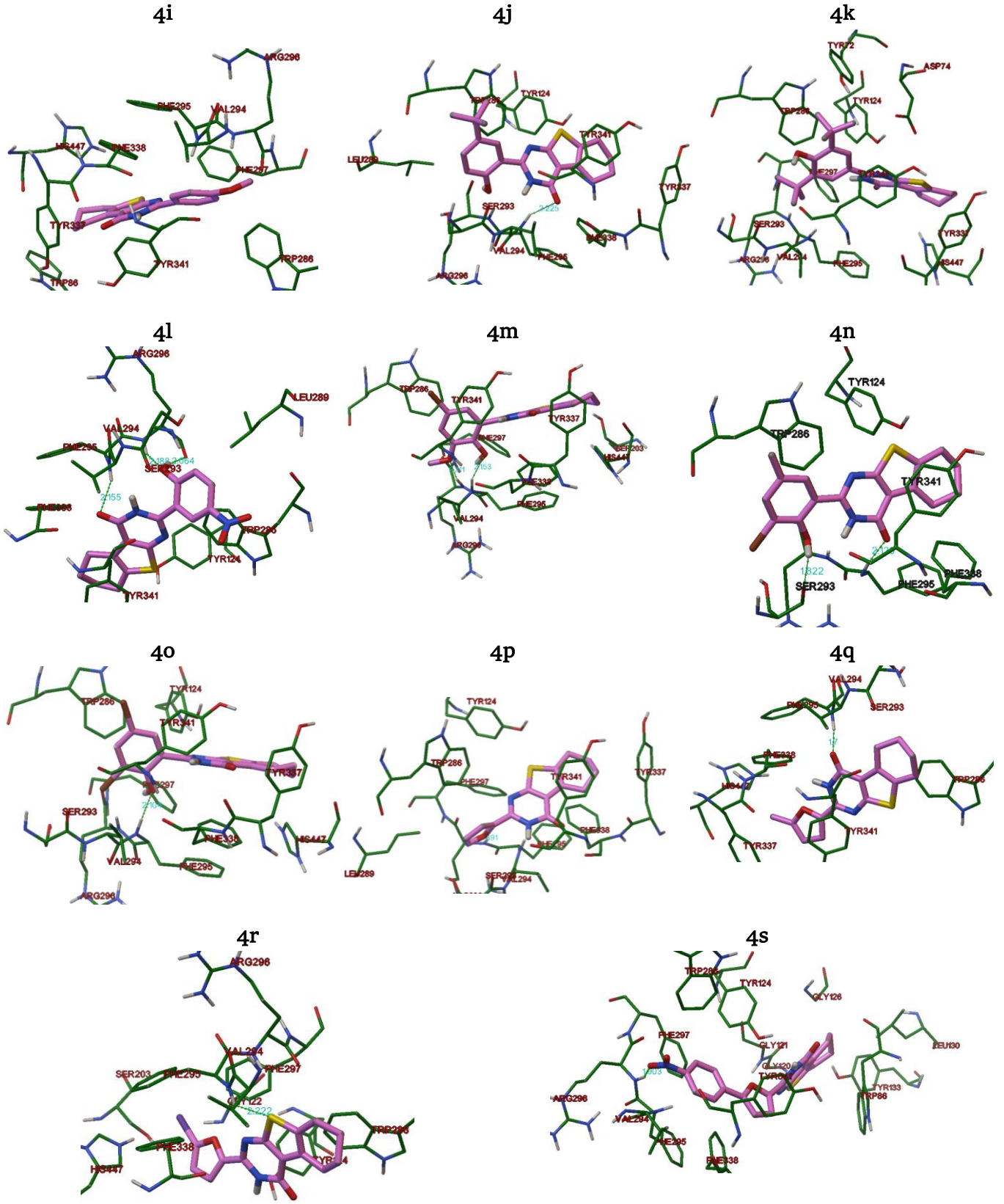


Figure 2 The location of **4i–4s** according to molecular docking.

As can be seen from the data obtained, the highest anticholinesterase activity was established for the compounds **4d**, **4e** and **4f**, surpassing that of the referent. The substances **4c**, **4g**, **4h**, **4j**, **4k**, **4m**, **4n** and **4p** showed slightly lower activity, the effect of which did not differ statistically from that of donepezil.

3.4. Structure-activity relationship of the studied compounds

In general, the results of molecular docking of the predicted structures are in a good agreement with the results of the primary pharmacological screening of anticholinester-

ase activity *in vitro* of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one. The most active compounds inhibiting AChE are thienopyrimidines, containing in the second position of the heterocycle pyrimidine-4(3*H*)-one fragment with hydroxy and methoxyphenyl substituents (**4d**, **4e** and **4f**).

Table 2 The effect of the studied compounds and GV-971 on the aggregation of amyloid particles.

Compounds	% of inhibition		
	3th day	6th day	9th day
4a	10.2±1.2*	34.5±1.6*	67.5±2.3*
4b	10.7±1.0*	42.5±2.5*	55.1±2.0*
4c	24.5±3.9*	32.2±3.8*	66.4±1.2*
4d	14.2±2.1*	38.6±2.4*	57.9±1.2*
4e	22.2±1.5*	39.1±2.2*	67.2±3.6*
4f	16.8±3.8*	38.1±1.5*	60.8±3.7*
4g	13.9±3.7*	33.7±2*	60.4±2.9*
4h	13.4±3.9*	42.9±3.6*	57.3±3.6*
4i	15.5±1.6*	36.5±3.7*	62.7±1.3*
4j	12.3±1.5*	45±2.9*	50±1.8*
4k	16.4±1.6*	55.3±2.3*	69.4±2.5*
4l	18.7±2.6*	38±2.9*	50±1.2*
4m	21.1±2.7*	49±3.5*	72.8±1.9*
4n	16.9±1.6*	31.2±3.3*	60.4±2.4*
4o	16.2±2.8*	34.9±3.1*	55.9±3.9*
4p	22.2±3.2*	43±1*	54.7±3.4*
4q	18.1±1.6*	32.2±2.8*	58±4*
4r	23.5±2.6*	41.3±3.4*	52.3±1.2*
4s	15.1±2.1*	41.2±3.4*	52.9±1.4*
GV-971	33.5±2.4	65.2±3.9	86.3±2.5

* – statistically significant relative GV-971 (ANOVA with the Tukey post-test, p<0,05)

Table 3 The effect of the studied compounds and donepezil on the acetylcholinesterase activity.

Compounds	IC_{50}, mg/ml
4a	6.31±0.091*
4b	5.36±0.087*
4c	3.10±0.031
4d	1.17±0.064*
4e	1.24±0.027*
4f	1.11±0.044*
4g	3.08±0.084
4h	3.75±0.058
4i	5.99±0.021*
4j	4.52±0.034
4k	3.19±0.044
4l	5.42±0.012*
4m	3.22±0.021
4n	3.68±0.092
4o	5.23±0.061*
4p	3.75±0.071
4q	5.82±0.025*
4r	4.92±0.074*
4s	4.57±0.096*
donepezil	2.40±0.06

* – statistically significant relative donepezil (ANOVA with the Tukey post-test, p<0,05)

These compounds are superior in the effectiveness to the drug Donezepil. It should be noted that for the acyclic precursors of azomethine derivatives of 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxamide, substances with similar substituents showed better activity. This fact indicates the significance of these pharmacophores. To a lesser extent, the **4c** and **4g** substances containing only hydroxyphenyl groups as a pharmacophore fragments exhibit the anticholinesterase activity. Among the compounds having a furan heterocycle, the compound **4p** has the greatest ability to inhibit AChE. The analysis of thecompounds **4j** and **4k** containing a tert-butyl radical in a hydroxyphenyl fragment allows us to judge its effect on the pharmacological properties of these structures. Particularly interesting is the remainder of the sterically hindered phenol contained in the **4k** compound. Among the halogen-derived target products, **4o** containing two bromine atoms in the 3,5 positions of the phenyl substituent showed the least activity. Compounds that do not contain hydroxy, methoxy and bromophenyl substituents have weak inhibitory properties of AChE, which is in a good agreement with the results of molecular docking and confirms the revealed tendency of the influence of electron-donating substituents in the 2-substituted phenyl fragment of the condensed thiophenpyrimidine system. Figure 3 shows the location of donepezil and **4d** in the active site of AChE.

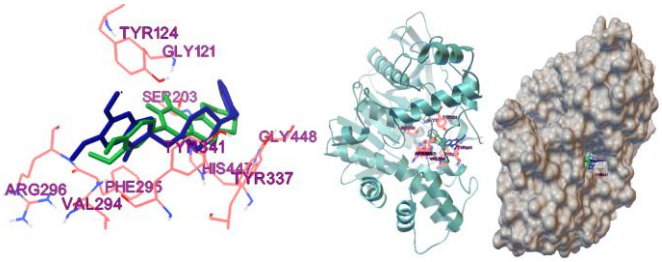


Figure 3 The location of donepezil determined by X-ray diffraction analysis (blue color) and the location of **4d** according to molecular docking (green color).

Figure 4 shows the location of the donezepil molecule corresponding to the data of X-ray diffraction analysis in the 4EY7 molecular complex and the positions of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one with hydroxy-methoxyphenyl fragments.

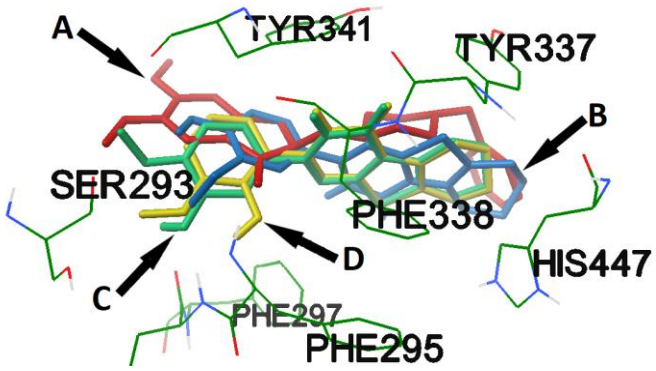


Figure 4 The location of donepezil determined by X-ray diffraction analysis (A – red color) and the location according to molecular docking: **4d** (B – blue color), **4e** (C – green color), **4f** (D – yellow color).

It can be seen that the aryl fragments **4d**, **4e** and **4f** with methoxy and hydroxy groups occupy a similar position with the same structural element of Donezepil. Thus, it is possible to assume similar molecular mechanisms of inhibition of AChE in the predicted compounds and their prototypes, as well as the importance of the hydroxy-methoxyphenyl fragment for the process of inhibition of the enzyme.

The study of the ability of synthesized compounds to aggregate amyloid particles allowed us to determine that the most active are tetrahydrothienopyrimidines with 5-bromo-2-hydroxy-3-methoxyphenyl (**4m**) and 3,5-di-tert-butyl-4-hydroxyphenyl (**4k**) substituents containing di-tert-butyl and bromine-substituted hydroxy-methoxyphenyl fragments in the second position of the pyrimidine-4(3*H*)-one heterocycle. Of the compounds with hydroxy-methoxyphenyl substituents, the substance **4e** containing an isovaniline residue in its structure showed the greatest activity. The compound **4a**, which has an unsubstituted phenyl substituent, also inhibits the aggregation of amyloid particles well.

The resulting combination of pharmacological properties of the studied objects, namely, the combination of the ability to suppress amyloidogenesis and anticholinestrease activity, opens up certain prospects in terms of the therapeutic use of these compounds. So, it is known that amyloidogenic processes underlie irreversible neurodegenerative diseases, in particular, Alzheimer's disease [32]. The development of drugs for the treatment of Alzheimer's disease is an extremely difficult task. Since 2003, extensive preclinical and clinical studies of promising molecules have been conducted, but none of them has been put into practice. As of 2021, not a single drug has been registered that directly affects the pathogenesis of the disease. But, at the same time, a purposeful search for substances that can prevent a neurodegeneration is ongoing [33]. According to Cummings et al., the most promising direction for the development of new therapeutic agents for the treatment of Alzheimer's disease is the suppression of the formation of β-amyloid. The most promising in this regard are purposefully obtained monoclonal antibodies, which are at different stages of clinical trials: Solanezumab; Gantenerumab; Crenezumab; Aducanumab [34]. But it is impossible to deny the possibility of using small molecules to suppress the formation of amyloid fragments. It should be emphasized that in addition to pathogenetic, symptomatic treatment is also important, which, as a rule, is aimed at eliminating cholinergic deficiency [35]. In this regard, the combination of pharmacological properties of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one may be a new vector of therapy for Alzheimer's disease, combining both the effect on the pathogenesis of the disease and the elimination of its leading symptoms.

4. Conclusions

In the course of the research, a method for the synthesis of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one was proposed, according to which new representatives of this class of organic compounds were obtained. Among the studied compounds there are substances with the high anticholinesterase activity. The most active are tetrahydrothienopyrimidine derivatives containing hydroxy-methoxyphenyl substituents in their structure. The compounds with fragments of 5-bromo-2-hydroxy-3-methoxyphenyl and 3,5-di-tert-butyl-4-hydroxyphenyl have the highest antiamyloid activity. As a result of the studies, the expediency of searching for new highly effective compounds for the treatment of neurodegenerative diseases in the series of tetrahydrobenzthienopyrimidine-4(3*H*)-one was confirmed.

Supplementary materials

No supplementary materials are available.

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Conflict of interest

The authors declare no conflict of interest.

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References

1. Weber SA, Patel RK, Lutsep HL. Cerebral amyloid angiopathy: diagnosis and potential therapies. Expert Rev Neurother. 2018;18(6):503–513. doi:10.1080/14737175.2018.1480938
2. Sağlık BN, Osmaniye D, Acar Çevik U, Levent S, Kaya Çavuşoğlu B, Özkay Y, Kaplancıklı ZA. Design, synthesis, and structure-activity relationships of thiazole analogs as anticholinesterase agents for alzheimer's disease. Mol. 2020;25(18):4312. doi:10.3390/molecules25184312
3. Bacalhau P, San Juan AA, Marques CS, Peixoto D, Goth A, Guarda C, Silva M, Arantes S, Caldeira AT, Martins R, Burke AJ. New cholinesterase inhibitors for Alzheimer's disease: Structure Activity Studies (SARs) and molecular docking of isoquinolone and azepanone derivatives. Bioorg Chem. 2016;67:1–8. doi:10.1016/j.bioorg.2016.05.004
4. Riswanto FDO, Rawa MSA, Vikneswaran M, Salin NH, Istyastono EP, Hariono M, Wahab HA. Anti-cholinesterase activity of chalcone derivatives: synthesis, *in vitro* assay and molecular docking study. Medicinal Chemistry 2021;17(5):442–452. doi:10.2174/1573406415666191206095032
5. Jang C, Yadav DK, Subedi L, Venkatesan R, Venkanna A, Afzal S, Lee E, Yoo J, Ji E, Kim SY, Kim m. Identification of novel acetylcholinesterase inhibitors designed by pharmacophore-based virtual screening, molecular docking and bioassay. Sci Rep. 2018;8:14921. doi:10.1038/s41598-018-33354-6
6. Hatice ED. Pyrimidines: Molecular docking and inhibition studies on carbonic anhydrase and cholinesterases. Biotechnol Appl Biochem. 2022. doi:10.1002/bab.2329
7. Bharate JB, Ådén J, Gharibyan A, Adolfsson DE, Jayaweera SW, Singh P, Vielfort K, Tyagi M, Bonde M, Bergström S, Olofsson A, Almqvist F. $K_2S_2O_8$-mediated coupling of 6-amino-7-aminomethyl-thiazole-pyridine with aldehydes to construct amyloid affecting pyrimidine-fused thiazolino-2-pyridones. Org Biomol Chem. 2021;19;9758–9772. doi:10.1039/D1OB01580J
8. Bortolami M, Pandolfi F, Tudino V, Messore A, Madia VN, De Vita D, Di Santo R, Costi R, Romeo I, Alcaro S, Colone M, Stringaro A, Espargaro A, Sabate R, Scipione l. New pyrimidine and pyridine derivatives as multitarget cholinesterase inhibitors: design, synthesis, and *in vitro* and *in cellulo* evaluation. ACS Chem Neurosci. 2021;12(21):4090–4112. doi:10.1021/acschemneuro.1c00485
9. Javed MA, Ashraf N, Saeed Jan M, Mahnashi MH, Alqahtani YS, Alyami BA, Alqarni AO, Asiri YI, Ikram M, Sadiq A, Rashid U. Structural modification, *In vitro, in vivo, ex vivo,* and *in silico* exploration of pyrimidine and pyrrolidine cores for targeting enzymes associated with neuroinflammation and cholinergic deficit in Alzheimer's Disease. ACS Chem Neurosci. 2021;12(21):4123–4143. doi:10.1021/acschemneuro.1c00507
10. Manzoor S, Prajapati SK, Majumdar S, Raza MK, Gabr MT, Kumar S, Pal K, Rashid H, Kumar S, Krishnamurthy S, Hoda N. Structural modification, *in vitro, in vivo, ex vivo,* and *in silico* exploration of pyrimidine and pyrrolidine cores for targeting enzymes associated with neuroinflammation and cholinergic deficit in Alzheimer's disease. Eur J Med Chem. 2021;215:113224. doi:10.1016/j.ejmech.2021.113224
11. Shahid NM, Azam Khan J, Kazmi I, Rashid U. Design, synthesis, and bioevaluation of indole core containing 2-arylidene derivatives of thiazolopyrimidine as multitarget inhibitors of cholinesterases and monoamine oxidase A/B for the treatment of Alzheimer disease. ACS Omega. 2022;7(11):9369–9379. doi:10.1021/acsomega.1c06344
12. Mariki A, Anaeigoudari A, Zahedifar M, Pouramiri B. design, green synthesis, and biological evaluation of new substituted tetrahydropyrimidine derivatives as acetylcholinesterase inhibitors. Polycycl Aromat Compd. 2021. doi:10.1080/10406638.2021.1933102
13. Kumar V, Kumar B, Ranjan Dwivedi A, Mehta D, Kumar N, Bajaj B, Arora T, Prashar V, Parkash J, Kumar V. Design, synthesis and evaluation of O-Pentyne substituted diphenylpyrimidines as monoamine oxidase and acetylcholinesterase inhibitors. Chem Sel. 2020;5:8021. doi:10.1002/slct.202002425
14. Zribi L, Pachòn-Angona I, Bautista-Aguilera ÒM, Diez-Iriepa D, Marco-Contelles J, Ismaili L, Iriepa I, Chabchoub F. Triazolopyridopyrimidine: A new scaffold for dual-target small molecules for Alzheimer's disease therapy. Mol. 2020;25(14):3190. doi:10.3390/molecules25143190
15. Chiriapkin AS, Kodonidi IP, Pozdnyakov DI, Zolotych DS. Synthesis and QSAR of new azomethine derivatives as agents for the treatment of Alzheimer's disease. Pharmacologyonline. 2021;3:563–584. https://pharmacologyonline.silae.it/files/archives/2021/vol3/PhOL_2021_3_A062_Chiriapkin.pdf
16. Amawi H, Hussein N, Boddu SHS, Karthikeyan C, Williams FE, Ashby CRJ, Raman D, Trivedi P, Tiwari AK. Novel Thienopyrimidine Derivative, RP-010, Induces β-Catenin fragmentation and is efficacious against prostate Cancer Cells. Cancers. 2019;11:711. doi:10.3390/cancers11050711
17. Amawi H, Karthikeyan C, Pathak R, Hussein N, Christman R, Robey R, Ashby CR, Trivedi P, Malhotra A, Tiwari AK. Thienopyrimidine derivatives exert their anticancer efficacy via apoptosis induction, oxidative stress and mitotic catastrophe. Eur J Med Chem. 2017;138:1053–1065. doi:10.1016/j.ejmech.2017.07.028
18. Wang YD, Johnson S, Powell D, McGinnis JP, Miranda M, Rabindran SK. Inhibition of tumor cell proliferation by thieno[2,3-d]pyrimidin-4(1*H*)-one-based analogs. Bioorg Med Chem Lett. 2005;15(16):3763–3766. doi:10.1016/j.bmcl.2005.05.127
19. Zhang Y, Luo L, Han C, Lv H, Chen D, Shen G, Ye F. Design, Synthesis, and Biological Activity of Tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine Derivatives as Anti-Inflammatory Agents. Mol. 2017;22(11):1960. doi:10.3390/molecules22111960
20. Ouyang L, Zhang L, Liu J, Fu L, Yao D, Zhao Y, Zhang S, Wang G, He G, Liu B. Discovery of a small-molecule bromodomain-containing protein 4 (BRD4) inhibitor that induces AMP-activated protein kinase-modulated autophagy-associated cell death in breast cancer. J Med Chem. 2017;60(24):9990–10012. doi:10.1021/acs.jmedchem.7b00275
21. El-Mekabaty A, Fouda AE-AS, Shaaban IEI. Convenient synthesis of functionalized thieno[2,3-d]pyrimidine-4-ones and thieno[2,3-b]pyridine-4-ones bearing a pyridine moiety with anticipated antioxidant activity. J Heterocyclic Chem. 2020;57:2928–2935. doi:10.1002/jhet.4002
22. Kawade DP, Chaple DR, Khedekar PB. Synthesis, physicochemical characterization and analgesic evaluation of some new thieno[2,3-D]Pyrimidin-4(3*H*)-one derivatives. Int J Pharm Chem Anal. 2020;7(1):32–38. doi:10.18231/j.ijpca.2020.005
23. Pal K, Raza MK, Legac J, Ataur Rahman M, Manzoor S, Rosenthal PJ, Hoda N. Design, synthesis, crystal structure and antiplasmodial evaluation of tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine derivatives. RSC Med Chem. 2021;6:970–981. doi:10.1039/d1md00038a
24. Shi-Ben W, Xian-Qing D, Yan Z, Yan-Ping Y, Zhe-Shan Q, Li-Ping G. Synthesis and evaluation of anticonvulsant and antidepressant activities of 5-alkoxytetrazolo[1,5-c]thieno[2,3-

e]pyrimidine derivatives. Eur J Med Chem. 2012;56:139–144. doi:10.1016/j.ejmech.2012.08.027
25. Ghayour F, Mohammad S, Mohammad R, Ghashang M. ZnO-CeO2 nanocomposite: efficient catalyst for the preparation of thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives. Main Group Metal Chem. 2018;41(1–2):21–26. doi:10.1515/mgmc-2017-0038
26. Dzhavakhishvili SG, Gorobets NYu, Musatov VI, Desenko SM, Paponov BV. Three possible products from the reactions of gewald's amide with aromatic aldehydes. J Heterocycl Chem. 2008;45(2):573–577. doi:10.1002/jhet.5570450243
27. Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, Franklin MC, Height J. Structures of human acetylcholinesterase in complex with pharmacologically important ligands. J Med Chem. 2012;55(22):10282–10286. doi:10.1021/jm300871x
28. Chiriapkin AS, Kodonidi IP, Larsky MV. Targeted Synthesis and Analysis of Biologically Active Azomethine Derivatives of 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxamide. Drug Dev Regist. 2021;10(2):25–31. doi:10.33380/2305-2066-2021-10-2-25-31
29. Chiriapkin A, Kodonidi I, Ivchenko A, Smirnova L. Synthesis and Prognosis of Anti-inflammatory Activity of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3*H*)-one. Bull Sci Pract. 2021;7(12):25–33. doi:10.33619/2414-2948/73/02
30. Wang W, Zhao C, Zhu D, Gong G, Du. W. Inhibition of amyloid peptide fibril formation by gold-sulfur complexes. J Inorg Biochem. 2017;171:1–9. doi:10.1016/j.jinorgbio.2017.02.021
31. Jończyk J, Godyń J, Stawarska E, Marak-Mlodawska B, Jelen M, Pluta K, Malawska B. Dual Action of Dipyridothiazine and Quinobenzothiazine Derivatives-Anticancer and Cholinesterase-Inhibiting Activity. Mol. 2020;25(11):2604. doi:10.3390/molecules25112604
32. Mahgoub N, Alexopoulos GS. Amyloid Hypothesis: Is There a Role for Antiamyloid Treatment in Late-Life Depression? Am J Geriatr Psychiatry. 2016;24(3):239–247. doi:10.1016/j.jagp.2015.12.003
33. Tolar M, Abushakra S, Sabbagh M. The path forward in Alzheimer's disease therapeutics: Reevaluating the amyloid cascade hypothesis. Alzheimers Dement. 2020;16(11):1553–1560. doi:10.1016/j.jalz.2019.09.075
34. Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline: 2019. Alzheimers Dement (NY). 2019;5:272–293. doi:10.1016/j.trci.2019.05.008
35. Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, Khachaturian AS, Vergallo A, Cavedo E, Snyder PJ, Khachaturian ZS. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain. 2018;141(7):1917–1933. doi:10.1093/brain/awy132