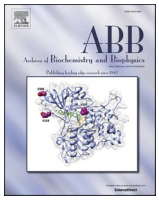
Archives of Biochemistry and Biophysics 761 (2024) 110159

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi



Synthesis of *N*-substituted 4-phenyl-2-aminothiazole derivatives and investigation of their inhibition properties against *h*CA I, II, and AChE enzymes

Abdullah Biçer [a,b,*], Cüneyt Çağlayan [c], Yeliz Demir [d], Cüneyt Türkeş [e], Ramazan Altundaş [f], Hasan Akyıldız [f], Şükrü Beydemir [g]

[a] Department of Chemistry, Faculty of Science, Bilecik Şeyh Edebali University, Bilecik, 11230, Türkiye
[b] Scientific Research Projects Coordinatorship, Bilecik Şeyh Edebali University, Bilecik, 11230, Türkiye
[c] Department of Medical Biochemistry, Faculty of Medicine, Bilecik Şeyh Edebali University, Bilecik, 11230, Türkiye
[d] Department of Pharmacy Services, Nihat Delibalta Göle Vocational High School, Ardahan University, Ardahan, 75700, Türkiye
[e] Department of Biochemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Erzincan, 24002, Türkiye
[f] Department of Chemistry, Faculty of Science, Gebze Technical University, Kocaeli, 41400, Türkiye
[g] Department of Biochemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, 26470, Türkiye

ARTICLE INFO

Keywords:
Bromination
Chlorination
Iodination
Hantzsch
*h*CA
AChE

ABSTRACT

In this study, thiazole derivatives containing sulphonamide, amide, and phenyl amino groups were synthesized to protect the free amino groups of 5-methyl-4-phenyl-2-aminothiazole and 4-phenyl-2-aminothiazole. Halogenated reactions of N-protected thiazole derivatives have been investigated. LCMS, FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy techniques were used to elucidate the structures of the synthesized compounds. Inhibition effects of the N-protected thiazole derivatives against human carbonic anhydrase I, II (*h*CA I, *h*CA II), and acetylcholinesterase (AChE) were investigated. The best results among the synthesized N-protected thiazole derivatives showed K_i values in the range of 46.85–587.53 nM against *h*CA I, 35.01–578.06 nM against *h*CA II, and in the range of 19.58–226.18 nM against AChE. Furthermore, in silico studies with the target enzyme of the thiazole derivatives **(9** and **11)**, which showed the best results experimentally, have examined the binding interactions of the related compounds at the enzyme active site.

1. Introduction

Synthesis of thiazole ring-containing molecules is becoming increasingly important, as thiazole derivatives are widely used in the pharmaceutical, and agricultural industries due to their biological activities. Thiazoles are important role in medicinal chemistry because they are found in the structure of natural compounds (vitamin B1, penicillin, and carbonic anhydrase enzyme) and biologically active molecules [1]. Thiazoles show properties such as antimicrobial, anti-inflammatory, antibacterial, antifungal, anticancer, anthelmintic, antiparasitic, anthelminitic, antipyretic, antiviral, anti-HIV-1, anti--Alzheimer's, adenosine Thiazoles are the important place in medicinal chemistry because they are found in the structure of natural compounds receptor antagonist, osteoporosis inhibitor and aldose reductase enzyme inhibitor [2–13]. Since 2-amino-1,3-thiazoles are found in many drug molecules and structures of bioactive molecules, they constitute one of the important research areas of organic chemistry [14,15] (Fig. 1).

Carbonic anhydrases (CAs, EC 4.2.1.1) are a group of metalloenzymes found in many living organisms. They play a crucial role in a fundamental physiological process, which is the reversible conversion of CO_2 into bicarbonate ions and protons, as well as facilitating other important physiological reactions [16,17]. So far, scientists have discovered 16 distinct α-CA isoforms in mammals. The catalytically active α-CAs all have an active site composed of a Zn (II) ion coordinated with three histidine residues, as well as either a hydroxide ion or a water molecule [18]. Humans (*h*) have fifteen distinct isoforms, with the catalytically active isoforms playing a crucial role in essential physiological processes related to biosynthetic reactions, electrolyte secretion in various organs and tissues, respiration, pH regulation, calcification, tumor formation, CO_2 transport between metabolizing lungs and tissues,

* Corresponding author. Department of Chemistry, Faculty of Science, Bilecik Şeyh Edebali University, Bilecik, 11230, Türkiye.
E-mail address: abdullah.bicer@bilecik.edu.tr (A. Biçer).

https://doi.org/10.1016/j.abb.2024.110159
Received 13 August 2024; Received in revised form 20 September 2024; Accepted 20 September 2024
Available online 24 September 2024

bone resorption, and numerous other pathological and physiological processes [19–21].

Due to the growing older population in most countries, Alzheimer's disease (AD) and other neurological illnesses are projected to become the second most common cause of mortality globally [22]. AD causes progressive cognitive impairment, which manifests as challenges in decision-making, language difficulties, mood fluctuations, learning difficulties, disorientation, and various behavioral disorders [23]. The cognitive decline caused by AD is linked to the fast breakdown of acetylcholine (ACh) by cholinesterases, such as acetylcholinesterase (AChE). Therefore, the act of inhibiting AChE has been suggested to have a neuroprotective effect. Undoubtedly, AChE inhibitors are the primary choice for pharmacological management of mild-to-moderate AD symptoms. AChE inhibitors were first used to treat myasthenia gravis characterized by a decrease in ACh receptors at the neuromuscular junction, resulting in muscle weakness [24,25]. AChE is a therapeutic target for managing ataxia, myasthenia gravis, glaucoma, senile dementia, and Parkinson's disease [26].

This study aimed to functionalize 5-methyl-4-phenyl-2-aminothiazole and 4-phenyl-2-aminothiazole compounds containing thiazole ring by halogenation reaction and to investigate the inhibition properties of the obtained products against *h*CAI, *h*CAII and AChE enzymes.

2. Results and discussion

2.1. Chemistry

Thiazole derivatives **(3, 4)** were synthesized from the Hantzsch reaction of acetophenone and propyphenone compounds with thiourea, respectively. Likewise, thiazole derivatives **(15, 16)** were synthesized from the Hantzsch reaction with N-phenyl thiourea. Then, from the reaction of the amino group of **(3, 4)** with methane sulfonyl chloride, benzene sulfonyl chloride, and tosyl chloride, thiazole derivatives **(5, 6, 7, 10, 11, 12)** containing sulfone amide group were synthesized. Thiazole derivatives containing amide group **(8, 9, 13, 14)** were synthesized from the reaction of amino groups of compounds **(1, 2)** with acetyl chloride benzoyl [27–40] (Scheme 1).

Interestingly, in the synthesis of N-protected thiazole derivatives, benzoic acid **(17)** was obtained as a by-product in the reaction of **(3)** with benzoyl chloride. In this reaction carried out under NEt_3 catalysis in THF at room temperature, compound **(9)** was obtained in 60 % yield, and by-product **(17)** was obtained in 20 % yield (Scheme 2). The structure of **(17)** was confirmed by melting point, ^{1}H NMR, ^{13}C NMR, and X-ray analysis. The data for the molecule numbered **(17)** are available in SI.

Halogenation reactions of *N*-protecting-5-methyl-4-phenyl-2-aminothiazole derivatives from the methyl group were performed with NCS, NBS, KBr-Oxone, HBr, Br_2, I_2–NaI reagents under different radical initiators, and reaction conditions [40–48] (Scheme 3). The experimental reaction conditions for the halogenation reactions of the 5-methyl-4--phenyl-2-aminothiazole derivatives **(10, 14,** and **16)** are tabulated in SI.

Thiazole compounds containing sulfonamide, benzamide, and phenyl-amino groups **(10, 14**, **16)** were subjected to halogenation reactions within the scope of functionalization studies of the methyl group. In this context, in compound **(14)** reaction many bromination agents (Br_2, NBS, HBr, NBS-DBP, NBS-Al_2O_3, KBr-Oxane, HBr–H_2O_2, Br_2-Pyridine), solvent system (chloroform, chloroform-H_2O, DMSO, AcOH, benzene, H_2O, EtOAc, ACN, acetone) and reaction condition ((room temperature, reflux, ultrasonic, 0 °C, room temperature-in dark) has been applied. Tiral reaction conditions are given in the SI. In the trial reactions, better results were obtained with chloroform solvent. Trial reactions were performed under different conditions using chloroform solvent. In these reactions, it was observed by LCMS follow-up that when the reaction time was extended for the complete reaction of the starting compound, the product formed was degraded in the reaction medium. In addition, in the next study for the isolation of the target product, the reaction was terminated after product formation was observed in the reaction by LCMS. When the reaction mixture was separated by column chromatography, it was observed that the target product was degraded by interaction with the column filler (SiO_2)

A similar situation was observed in bromination reactions with 5-methyl-4-phenyl-2-aminothiazole derivatives **(10**, **16)**. The formation of the product in the reaction medium was followed by LCMS and it was determined that the product deteriorated as the reaction progressed. Test reactions of these compounds are given in the SI.

As a chlorinating agent in the chlorination experiments of the methyl group of the thiazole molecules **(10**, **14)**; Although NCS, NCS-DBP, chloroform as solvent, and different reaction conditions (room temperature, reflux, ultrasonic, room temperature-in dark) were tried, the synthesis of the target products could not be realized (Scheme 4). Iodination reactions of thiazole molecules **(14)** were also tested. In this context, ethyl acetate and reaction conditions (reflux, ultrasonic) were applied as an iodinating agent (I_2-DBP, I_2–H_2O_2), but product formation was not observed. Trial reactions related to chlorination and iodine are given in the SI.

In the bromination reactions of compound **(4)**, NBS-DBP as bromine agent: chloroform, EtOAc, and benzene as solvents and different reaction conditions (room temperature, ultrasonic) was tried, but the synthesis of the target product **(24)** could not be realized (Scheme 4).

The desired product could not be isolated in the halogenation reactions of compound **(10, 14, 16)** obtained from propyphenonone. Compounds **(3, 8**, **15)** were synthesized starting from acetophenone. Compounds **(25–27)** were synthesized with bromination of compounds **(3, 8, 15)** with NBS at room temperature (Scheme 5).

The target product **(28)** could not be synthesized in the bromination reaction of the compound **(9).** Benzoic acid **(17)** was also formed in this reaction (Scheme 6). This reaction was performed at different times with the reaction indicated in Scheme 2. NMR and melting point data for benzoic acid obtained from both experiments are given in the SI section.

2.2. Enzyme inhibition studies

Evaluation of the effects of N-substituted 4-phenyl-2-aminothiazole derivatives on AChE, and both *h*CA isoenzymes (*h*CAI and *h*CAII) was the main objective of this study. The inhibition results are summarized in Table 1. Recently, *h*CAIs have mostly been utilized in clinical settings as anti-epileptic, diuretic, and anti-glaucoma medications. However, freshly developed compounds are currently being assessed in clinical trials as anti-obesity drugs/diagnostics or anti-tumor agents [49].

Erythrocytes contain cytosolic *h*CA I and *h*CA II, which play a crucial role in maintaining normal blood pH by producing HCO_3^- (Biçer et al., 2020). Pathological disorders such as oedema, epilepsy, and glaucoma

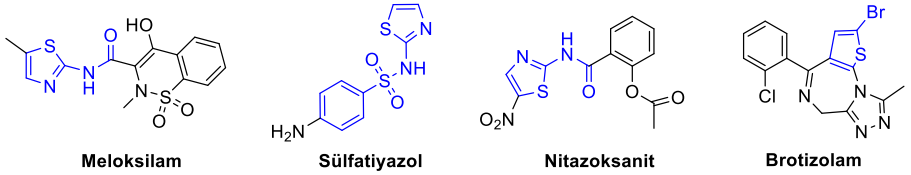
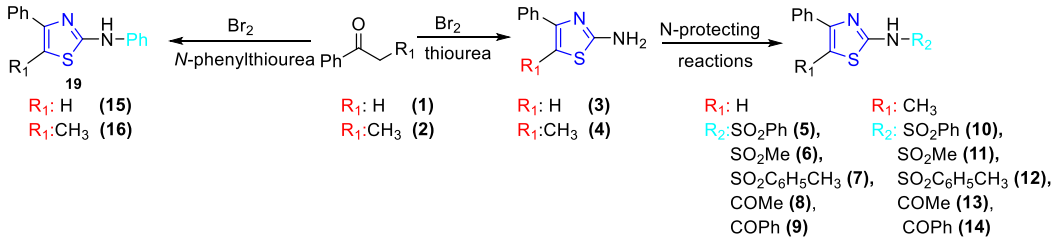
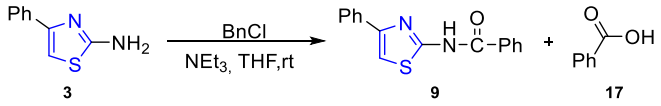


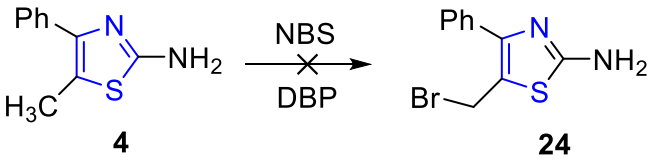
Fig. 1. Some bioactive thiazole compounds.



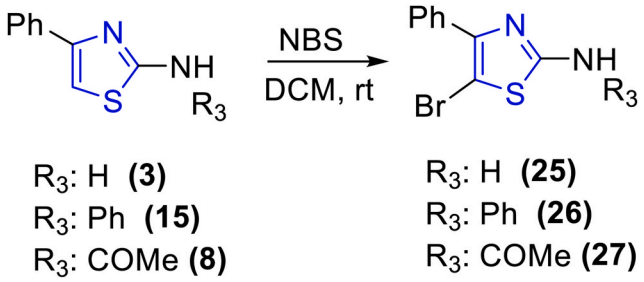
Scheme 1. General synthesis scheme of thiazole derivatives.



Scheme 2. Protection reaction of compound **(3)** with benzoyl chloride.



Scheme 4. Bromination test of compound **(4)** with NBS.

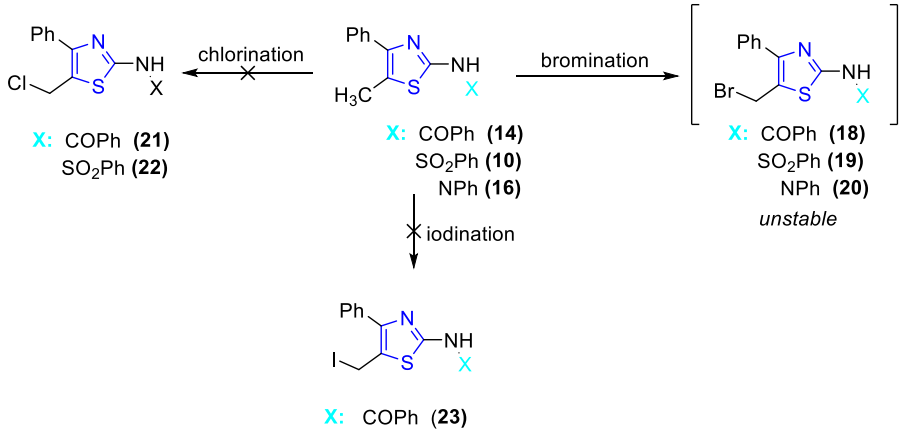


Scheme 5. Synthesis of bromo-containing thiazole derivatives.

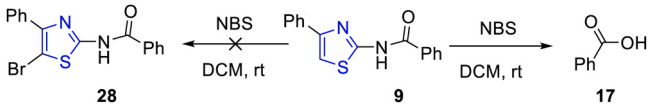
are induced by the dysregulation of *h*CA I and II isoenzymes in tissue cells [50]. The cytosolic enzyme *h*CA I was inhibited by the *N*-protecting-thiazole derivatives with K_i values ranging between 46.85 ± 7.18 and 587.53 ± 40.44 nM. In this study, the Ki for the positive control *h*CAI acetazolamide (AAZ), a recorded *h*CA I inhibitor, was 295.78 ± 17.88 nM against *h*CA I isoenzyme (Table 1). Compound **9**, containing a carbonyl group (COPh) and a phenyl ring**,** recorded the most powerful *h*CA I isoform with a K_i value of 46.85 ± 7.18 nM. The carbonyl group forms hydrogen bonds with residues in the active site, while the phenyl ring engages in hydrophobic interactions, leading to high binding affinity. Compound **7**, features a sulfonamide group ($SO_2C_6H_5CH_3$) and a phenyl ring, it turned out to be the second most effective inhibitor of the *h*CA I enzyme (K_i: 62.51 ± 8.80 nM). The sulfonamide group interacts with the zinc ion, and the phenyl ring stabilizes the complex through hydrophobic interactions. Compound **6**, wich includes a sulfonamide group (SO_2Me) and a smaller alkyl group, showed a less inhibition effect on *h*CA I enzyme (K_i: 587.53 ± 40.44 nM). The sulfonamide group interacts with the zinc ion, whereas the smaller alkyl group may have a lesser impact on hydrophobic stabilization, leading to a decrease in binding affinity. In general, *N*-protecting-thiazole derivatives **(5–14)** showed effective inhibition effects against *h*CA I enzyme.

The cytosolic enzyme *h*CA II was significantly inhibited by the *N*-protecting-thiazole derivatives with K_i values ranging between 35.01 ± 8.33 and 578.06 ± 72.98 nM. Among the synthesized derivatives, compound **(12)** exhibited the most potent *h*CA I isoform with a Ki value of 35.01 ± 8.33 nM. The compounds **(12)** are, the best inhibitor among these molecules (K_i:35.01 ± 8.33 nM) compared to the AAZ (K_i: 266.77 ± 33.87 nM), which is known as a specific inhibitor of *h*CA isoenzymes as a standard. Sulfonamide groups, especially when combined with phenyl rings, have robust affinities for the zinc ion in carbonic anhydrases and establish enduring complexes via hydrogen bonding and hydrophobic interactions. Carbonyl groups also enhance binding stability by forming hydrogen bonds. These findings indicate that by tailoring the positioning and characteristics of these functional groups, it is possible to develop enzyme inhibitors with greater potency.

The cholinergic enzyme AChE was significantly inhibited by the *N*-protecting-thiazole derivatives with K_i values ranging between 19.58 ± 3.28 and 226.18 ± 47.77 nM. The compound **(12)** has the highest



Scheme 3. Halogenation reactions of 5-methyl-4-phenyl-2-aminothiazole derivatives.



Scheme 6. Bromination reaction of thiazole derivative **(9)**.

Table 1
Enzyme inhibition results of N-protecting thiazole derivatives.

Compounds	K_i (nM)		
	*h*CA I	*h*CA II	AChE
5	142.87 ± 38.55	78.78 ± 13.21	66.19 ± 13.45
6	587.53 ± 40.44	578.06 ± 72.98	185.44 ± 41.13
7	62.51 ± 8.80	50.98 ± 14.89	33.64 ± 6.66
8	374.00 ± 33.57	214.58 ± 28.14	149.29 ± 24.21
9	46.85 ± 7.18	110.05 ± 22.18	75.78 ± 11.97
11	207.66 ± 38.65	169.34 ± 35.88	97.34 ± 15.04
12	79.75 ± 12.67	35.01 ± 8.33	19.58 ± 3.28
13	382.72 ± 29.87	370.81 ± 14.87	136.40 ± 19.21
14	73.89 ± 4.55	92.78 ± 16.37	44.93 ± 9.21
15	152.88 ± 43.78	118.00 ± 36.32	95.21 ± 16.42
25	463.07 ± 30.49	527.50 ± 58.86	226.18 ± 47.77
26	101.22 ± 23.31	111.97 ± 37.30	52.73 ± 2.33
27	286.02 ± 41.34	324.14 ± 41.11	150.65 ± 35.19
AAZ	295.78 ± 17.88	266.77 ± 33.87	–
THA	–	–	175.44 ± 13.87

inhibitory activity among these molecules, with a K_i value of 35.01 ± 8.33 nM. In comparison, tacrine (THA), a well-known selective inhibitor of AChE, has a standard K_i value of 175.44 ± 13.87 nM. This study clearly demonstrated that compound **(12)** had potent inhibitory effects against cholinergic enzymes. Compound **(12)** showed superior inhibition properties against both *h*CA II and AChE enzymes.

When *h*CA I and *h*CAII enzymes were compared with the standard compound AAZ, it was observed that the synthesized derivatives generally gave better inhibition results than the standard compound. N-protected thiazole derivatives showed a better inhibitory profile against AChE enzyme. Inhibition results at lower nM levels were obtained in studies with AChE enzyme.

2.3. Computational study

Molecular docking-assisted virtual screening has been employed in numerous drug discovery campaigns. Though there are significant advancements in computer-aided molecular docking, estimating the reliable ligand binding mode has remained challenging since ligand binding can cause a wide range of induced conformational changes to the protein. Generally, ligand-induced conformational changes (LICCs) are more pronounced for side chains than leading chains [51]. LICCs should often be addressed in docking studies to properly score the compounds to enhance the selection of hits and provide valuable insight into the binding determinants of the hits [52]. The binding affinities of the synthesized N-protecting thiazole derivatives were scrutinized utilizing X-ray crystallographic structures of *h*CA I (PDB ID 1AZM) [53], *h*CA II (PDB ID 3HS4) [54], and AChE (PDB ID 7XN1) [55]. Validation of the docking protocol was achieved by re-docking the co-crystallized native ligands AAZ and THA into the enzymes' active sites, ensuring methodological reliability as indicated by low RMSD values (<1.0 Å) and accurate reproduction of key interactions by the docking poses of the co-crystallized ligands. The investigation revealed that compounds **9** (targeting *h*CA I with K_I of 46.85 ± 7.18 nM) and **12** (targeting *h*CA II and AChE with K_Is of 35.01 ± 8.33 nM and 19.58 ± 3.28 nM, respectively) demonstrated notable binding affinity with predicted (Figs. 2–4).

In all CAs enzyme classes, a metal hydroxide species of the enzyme is the catalytically active species, acting as a strong nucleophile (at neutral pH) on the CO_2 molecule bound in a hydrophobic pocket nearby. This metal hydroxide species is generated from H_2O coordinated to the metal ion, which is found at the bottom of the active site cavity. The active center usually comprises Zn(II) ions in tetrahedral geometry, with three protein ligands in addition to the H_2O molecule/hydroxide ion. In many enzymes, generation of the metal hydroxide species from the metal-coordinated H_2O is the rate-determining step of the catalytic turnover, which for some α-CAs achieve kcat/KM values $>10^8$ $M^{-1}s^{-1}$, making CAs among the most effective catalysts known in nature. The metal ion ligands are three His residues (His94, His96, and His119) in α-CAs. The inhibition and activation of CAs are also well-understood processes, with most inhibitors binding to the metal center and activators binding at the entrance of the active site cavity and participating in the proton shuttling between the metal ion-bound H_2O molecule and the environment. This leads to the enhanced formation of the metal hydroxide, catalytically active enzyme species. In this research, compounds 9 and 12 entered a π-π stacking interaction with the His94 amino acid residue, respectively, *h*CA I and II isoforms (Figs. 2 and 3).

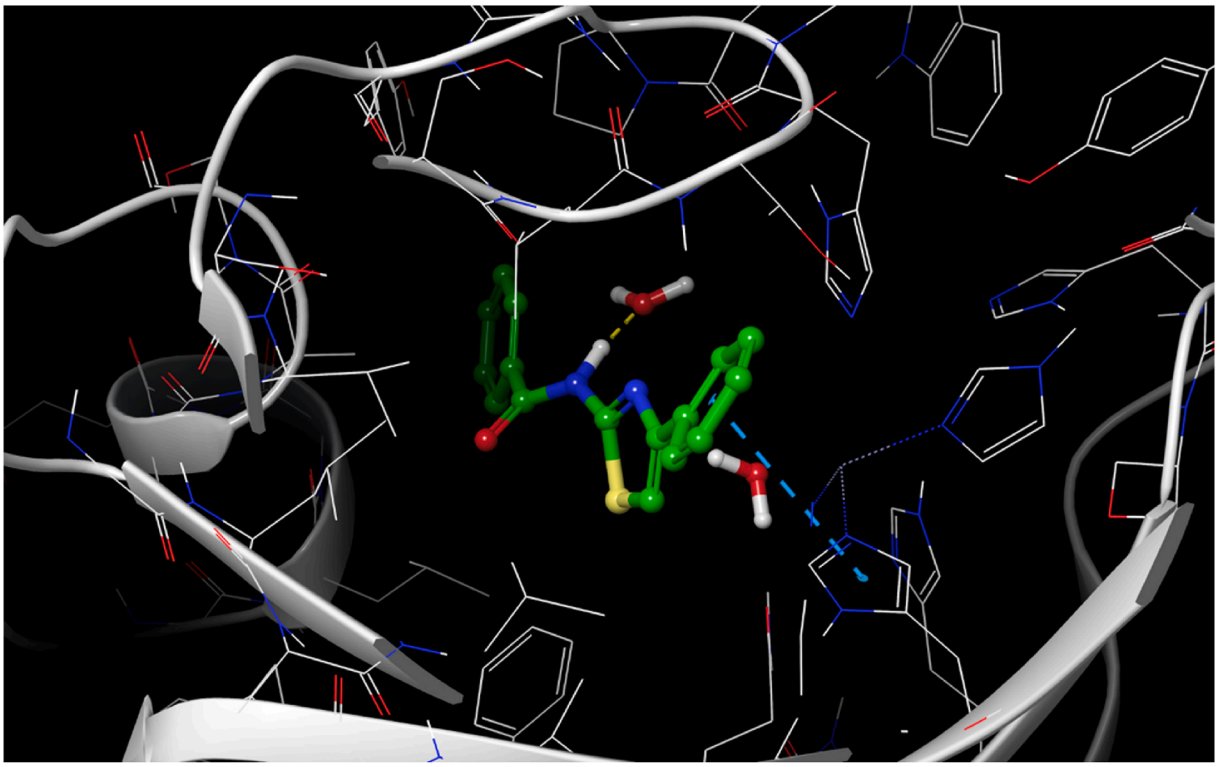
Being a drug target for AD, AChE has received wide attention over the years. The active AChE site is a narrow gorge of ~20 Å deep, with the catalytic site buried at the bottom of the gorge [56]. The active site comprises two subsites called 'esteratic' and 'anionic or aromatic' subsites. The esteratic subsite contains a catalytic triad of three amino acids: Ser203, His447, and Glu324. Residues Trp86 and Tyr337 constitute the 'anionic or aromatic' subsite of the catalytic site (CAS), making π-cation interactions with the quaternary group of the substrate [57]. The Tyr337, termed as a 'swinging gate', has a critical role in the binding of substrates and various inhibitors. Residues Trp286 and Asp72 contribute to the peripheral anionic site or peripheral aromatic site (PAS) located at the lip of the gorge, which has a vital role in routing the substrate ideally to the active site. The Phe295 and Phe297 contribute to the acyl pocket, which determines the specificity for ACh [57]. Herein, compound **12** interacted by π-π stacking with CAS amino acids Trp86 and Tyr337 (Fig. 4).

Moreover, the QikProp module of Schrödinger Suite 2024-2 for Mac was employed to evaluate the drug-likeness of the synthesized N-protecting thiazole derivatives, assessing ADME/T (absorption, distribution, metabolism, elimination, and toxicity) parameters, as detailed in Table 8 (SI). Generally, the ability to design drugs capable of penetrating the blood-brain barrier (QPlogBB; brain/blood partition coefficient) and affecting the desired biological response is a formidable challenge. On the other hand, peripherally-acting drugs need to possess specific physical-chemical properties that prevent them from crossing the QPlogBB. Fundamental physicochemical features of central nervous system (CNS) drugs are related to their ability to penetrate the BBB affinity and exhibit CNS activity [58]. CNS drugs show molecular weight, lipophilicity, and hydrogen bond donor and acceptor values that generally have a smaller range than general therapeutics. The results indicate that these oxime ethers possess drug-like properties [59] and comply with Lipinski's Rule of Five [60] and Jorgensen's Rule of Three [61,62].

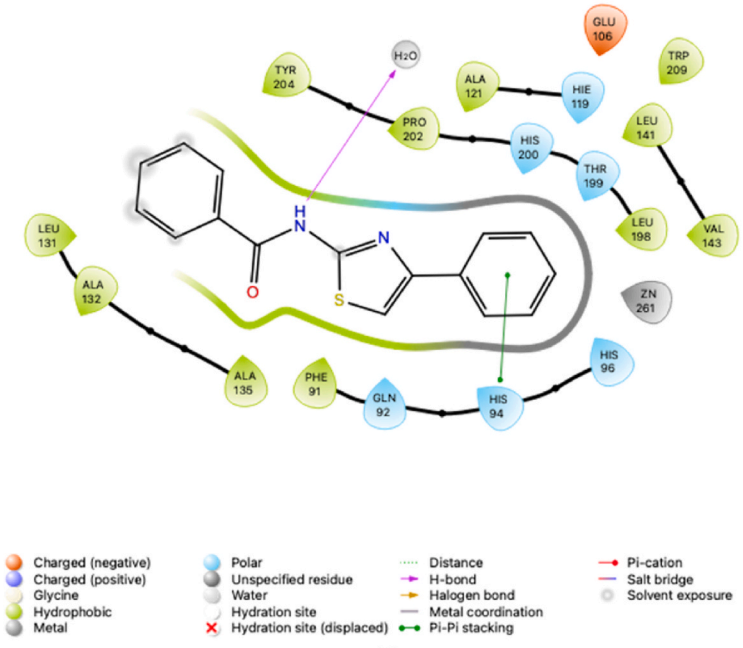
3. Experimental section

3.1. General

All chemicals were commercially available and used without further purification. Melting points were determined in a Gallenkamp melting point apparatus. All reactions were monitored by TLC (MERCK 105715 Silica Gel 60 F254 25 TLC Plates) with detection by UV light (254 nm). The IR spectra were recorded using a Shimadzu Infinity model FTIR spectrometer equipped with three reflections ATR attachment. NMR measurements were performed with a 500 MHz Bruker or 500 MHz Varian Mercury spectrometer. LCMS measurements were recorded with Shimadzu LabSolutions. X-ray diffraction studies were performed in Bruker Smart Apex II Quazar.

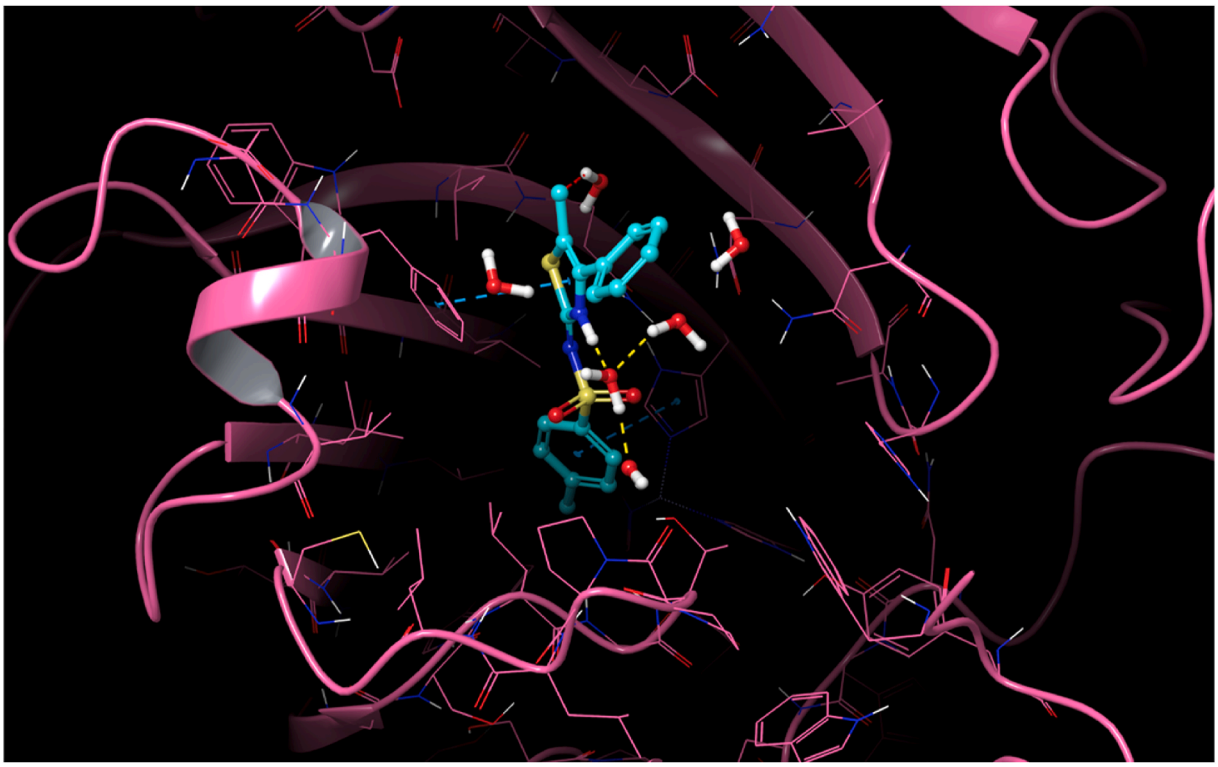


A

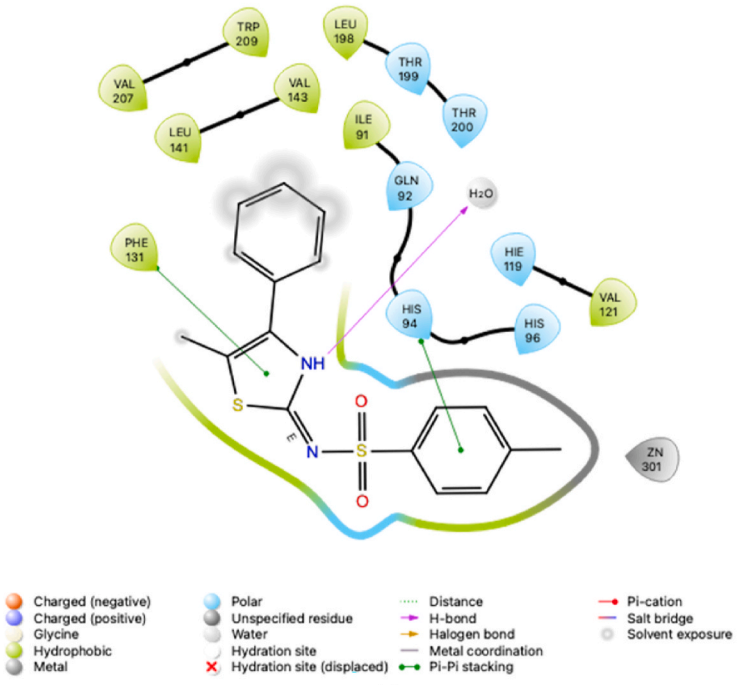


B

Fig. 2. The molecular docking analysis of the *h*CA I isoform (PDB ID 1AZM) with *N*-(4-phenylthiazol-2-yl)benzamide (**9**) revealed the three-dimensional docking pose of the compound (**9**) within the active site of 1AZM, as illustrated in Figure **A**. Subsequently, a two-dimensional interaction diagram (illustrated in Figure **B**) was constructed to detail the specific interactions between 1AZM and compound (**9**).

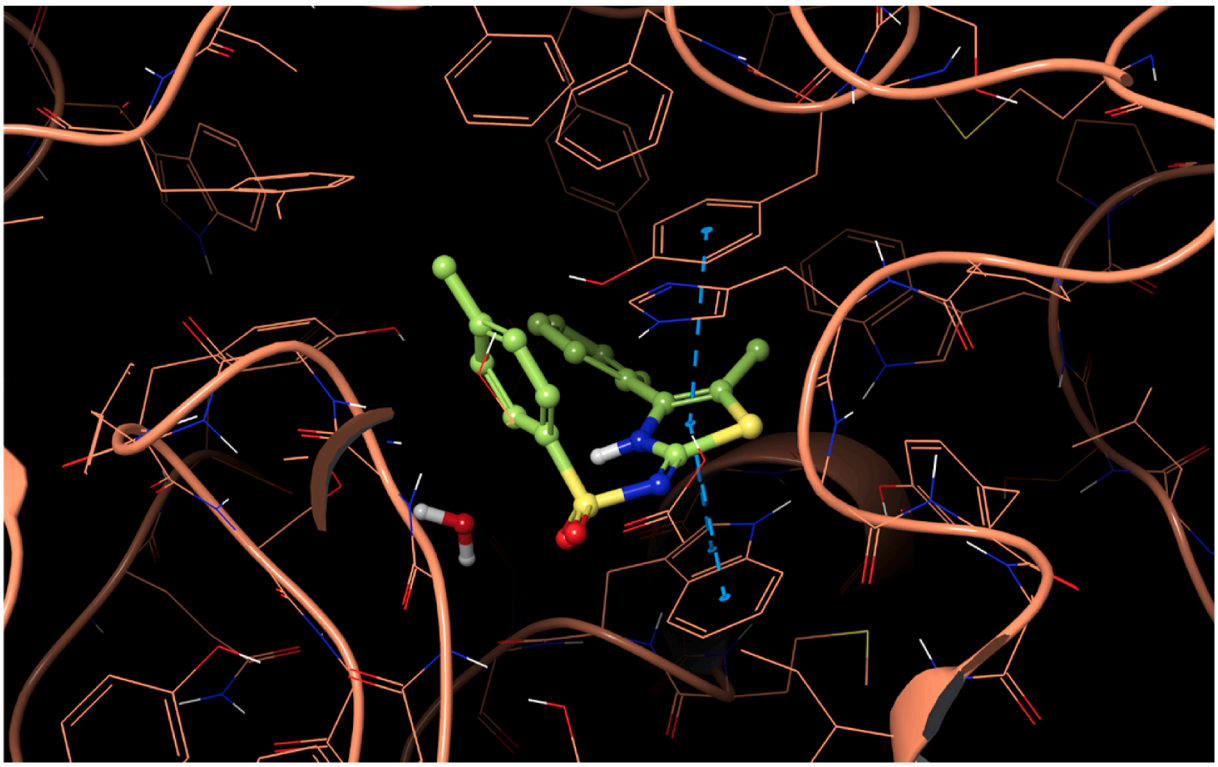


A

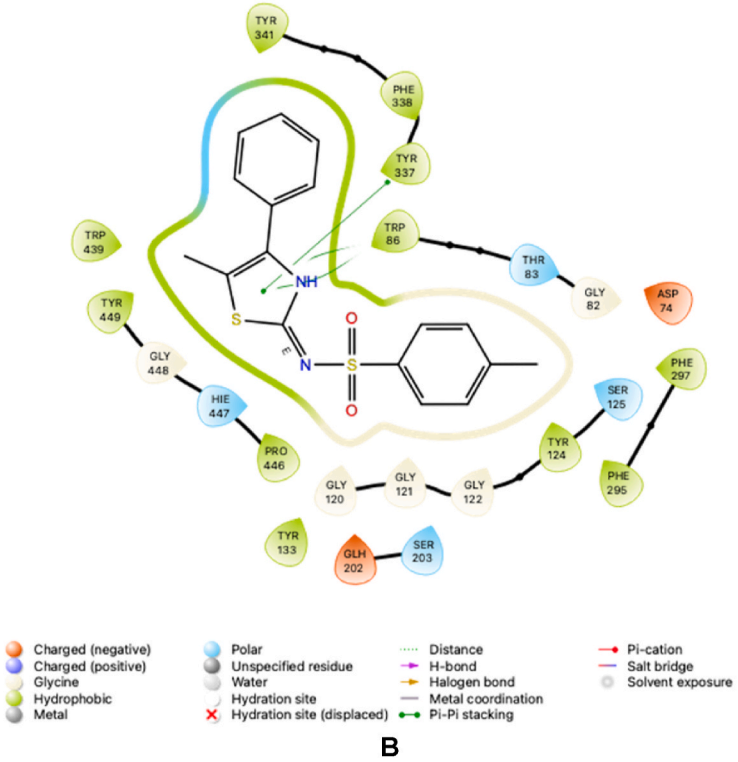


B

Fig. 3. The molecular docking analysis of the *h*CA II isoform (PDB ID 3HS4) with 4-methyl-*N*-(5-methyl-4-phenylthiazol-2-yl)benzenesulfonamide (**12**) revealed the three-dimensional docking pose of the compound (**12**) within the active site of 3HS4, as illustrated in Figure **A**. Subsequently, a two-dimensional interaction diagram (illustrated in Figure **B**) was constructed to detail the specific interactions between 3HS4 and compound (**12**).



A



B

Fig. 4. The molecular docking analysis of the AChE (PDB ID 7XN1) with 4-methyl-*N*-(5-methyl-4-phenylthiazol-2-yl)benzenesulfonamide (**12**) revealed the three-dimensional docking pose of the compound (**12**) within the active site of 7XN1, as illustrated in Figure **A**. Subsequently, a two-dimensional interaction diagram (illustrated in Figure **B**) was constructed to detail the specific interactions between 7XN1 and compound (**12**).

3.2. Chemistry

Thiazole derivatives **(3,4,15,16)** were synthesized by Hantzsch reaction with thiourea/N-phenylthiourea following bromination of the alpha position of acetophenone and propyphenone compounds. *N*-protecting-2-amino thiazole derivatives **(5–14)** were synthesized according to the literature by protection reactions of the free amino group of the thiazole compound with benzenesulfonyl chloride, mesityl chloride, tosyl chloride, acetyl chloride, and benzyl chloride. The spectroscopic data of the synthesized molecules are given in the SI section.

3.2.1. Synthesis of N-(4-phenylthiazol-2-yl)benzamide (9)

Compound **(3)** (1 equivalent, 0.100 g) was dissolved in 0.6 mL NEt_3 and 2 mL THF, benzoyl chloride (1 equivalent, 0.015 g) was added. After stirring the reaction at room temperature. The reaction was checked by TLC and it was observed that the starting compound was consumed after 5 h. The reaction mixture was neutralized with 10 % HCl solution and a yellow solid product precipitated. The precipitate was washed with plenty of water and dried. The crude product was purified by column chromatography with 30 % EtOAc/Hexane solvent system. Compound **(9)** was obtained in 60 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.10 (brs, NH), 7.86 (d, $J = 5$ Hz, 2H), 7.74 (d, $J = 5$ Hz, 2H), 7.49–7,45 (m, 1H), 7.38–7.31 (m, 4H), 7.28–7.24 (m, 1H), 7.16 (s, 1H). As a by-product, compound **(17)** was obtained in 20 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ:11.78 (brs, COOH, 1H), 8.13 (d, $J = 5$ Hz, 2H), 7.62 (t, $J = 5$ Hz, 1H), 7.49 (t, $J = 10$ Hz, 2H).

3.2.2. Synthesis of benzoic acid (17)

Compound **(9)** (0.1 g, 1 equiv) was dissolved in 4 mL DCM. At room temperature, a solution of NBS (0.1 g, 1 equivalent) in 3 mL DCM was added dropwise to the reaction medium. The color of the reaction mixture was initially yellow and became black after the addition of 3/2 of the NBS solution. The reaction was monitored by TLC. Product **(28)** could not be obtained in the reaction. Product **(17)** was obtained from the reaction medium in 30 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.30 (brs NH), 8.12 (d, $J = 5$ Hz, 2H), 7.62 (t, $J = 5$ Hz, 1H), 7.48 (t, $J =$ 7.5Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 172.28, 133.77, 130.23, 129.50, 128.50.

3.3. hCAs and AChE inhibitory effect study

Verpoorte's approach [63] of measuring the change in absorbance at 348 nm was used to measure the activity of the *h*CA I and II and to ascertain the inhibitory effects of compounds [64]. The approach of Ellman group [65] was used to assess the novel synthesized compounds' effects on AChE activity *in vitro*. According to prior investigations, spectrophotometric measurements were made at 412 nm using AChI [66,67]. These analogues TAC and AZA were dissolved in DMSO at an initial concentration of 1 mg/mL. The resulting combination consisted of a minimal quantity of DMSO, around 1 %. Experiments were conducted to investigate the inhibitory processes of analogues by performing kinetic tests with different chemical concentrations and substrates in a controlled laboratory setting. The observed data was utilized to generate Lineweaver-Burk, determine K_i constants, construct Michaelis-Menten curves, and explore different forms of inhibition [68,69].

3.4. Computational study

In this study, the molecular docking analyses were conducted using the 2024-2 version of the Schrödinger Small-Molecule Drug Discovery Suite for Mac. Protein structures with PDB IDs 1AZM [53], 3HS4 [54], and 7XN1 [55], corresponding to *h*CA I, *h*CA II isoforms, and AChE, respectively, were retrieved from the RCSB Protein Data Bank and preprocessed for docking via the Protein Preparation Wizard module. The N-protecting thiazole derivatives were initially sketched using ChemDraw version 21 (PerkinElmer, Inc., Waltham, MA, USA) for Mac and subsequently optimized with the LigPrep module at pH 7.4 ± 0.5 under the OPLS4 force field utilizing Epik. The active site residues identified through the SiteMap tool were defined within the Receptor Grid Generation module to create the receptor grid in the Maestro interface. Docking of the ligands to *h*CAs and AChE was performed using the Glide application with default settings and the extra precision (XP) method. Moreover, the relative binding affinities were predicted using the Prime MM-GBSA method within the VSGB energy model and OPLS4 force field, applied to protein-ligand complexes 1AZM, 3HS4, and 7XN1. The QikProp tool was further employed to predict the ADME properties of all target compounds in this study.

4. Conclusions

In the halogenation reactions of 5-methyl-4-phenyl-2-aminothiazole derivatives, a bromination product was observed in the reaction medium, but no product could be isolated. Desired halogenation products were obtained in the bromination reactions of 4-phenyl-2-amino thiazole derivatives. Some of the thiazole derivatives obtained within the scope of derivatization studies were subjected to inhibition studies against *h*CA I, *h*CA II and AChE enzymes. All of the synthesized *N*-protecting-thiazole derivatives effectively inhibited the metabolic enzymes of *h*CA I and *h*CA II at the nanomolar levels. Among the synthesized molecules, compound **(9)** against *h*CA I enzyme and compound **(12)** against *h*CA II and AChE enzymes gave the best inhibition results. *In silico* studies for both compounds **(9)** and **(12),** it was analyzed that they interact with the active sites of enzymes.

CRediT authorship contribution statement

Abdullah Biçer: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Cüneyt Çağlayan:** Writing – original draft, Resources, Methodology, Investigation. **Yeliz Demir:** Writing – original draft, Resources, Methodology, Investigation. **Cüneyt Türkeş:** Writing – original draft, Visualization, Software, Resources. **Ramazan Altundaş:** Supervision, Methodology. **Hasan Akyıldız:** Investigation. **Şükrü Beydemir:** Software.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgement

The authors are grateful to TUBITAK (Project Number: 118C503) for financial support. We are thankful to Dr. Mustafa Güzel from Istanbul Medipol University for the use of laboratory facilities and support in obtaining LCMS spectra.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.abb.2024.110159.

References

[1] W. Zhou, S. Ni, H. Mei, J. Han, Y. Pan, Cyclization reaction of N-allylbenzothioamide for direct construction of thiazole and thiazoline, Tetrahedron Lett. 56 (27) (2015) 4128–4130, https://doi.org/10.1016/j.tetlet.2015.05.047.

[2] M.C. Bagley, K.E. Bashford, C.L. Hesketh, C.J. ve Moody, Total synthesis of the thiopeptide promothiocin A, J. Am. Chem. Soc. 122 (2000) 3301–3313, https://doi.org/10.1021/ja994247b.

[3] A.V. Stachulski, C. Pidathala, E.C. Row, R. Sharma, N.G. Berry, M. Iqbal, J. Bentley, S.A. Allman, G. Edwards, A. Helm, J. Hellier, B.E. Korba, J.E. Semple, J. Rossignol, Thiazolides as novel antiviral agents. 1. Inhibition of hepatitis B virus replication, J. Med. Chem. 54 (2011) 4119–4132, https://doi.org/10.1021/jm200153p.

[4] A. Ayati, S. Emami, A. Asadipour, A. Shafiee, A. Foroumadi, Recent applications of 1,3-thiazole core structure in the identification of new lead compounds and drug discovery, Eur. J. Med. Chem. 97 (2015) 699–718, https://doi.org/10.1016/j.ejmech.2015.04.015.

[5] J. Rossignol, Review Nitazoxanide: a first-in-class broad-spectrum antiviral agent, Antiviral Res 110 (2014) 94–103, https://doi.org/10.1016/j.antiviral.2014.07.014.

[6] G.V. Zaychenko, O.M. Lyapunov, O.P. Bezugla, The place of meloxicam in the series of modern non-steroidal anti-inflammatory drugs, News of Pharmacy 86 (2016) 69–76. http://nbuv.gov.ua/UJRN/VPhC_2016_2_19.

[7] F.L. Hu, J.C. Jia, Y.L. Li, G.B. Yang, Comparison of H2-receptor: antagonist and proton-pump inhibitor-based triple regimens for the eradication of Helicobacter pylori in Chinese patients with gastritis or peptic ulcer, J. Int. Med. Res. 31 (2003) 469–474, https://doi.org/10.1177/147323000303100601.

[8] T.B. Fogg, D. Semple, Combination Therapy with H2 and H1 Antihistamines in Acute, Non Compromising Allergic Reactions, BestBets. Manchester, Manchester Royal Infirmary, England, 2007.

[9] N. Siddiqui, M.F. Arshad, W. Ahsan, M.S. Alam, Review article thiazoles: a valuable insight into the recent advances and biological activities, Int. J. Appl. Pharm. Sci. 3 (2009) 136–143. ISSN 0975-248X.

[10] M. Gümüş, İ. Koca, Y. Sert, A. Dişli, E.N. Yenilmez Tunoğlu, L. Tutar, Y. Tutar, Triad pyrazole–thiazole–coumarin heterocyclic core effectively inhibit HSP and drive cancer cells to apoptosis, J. Biomol. Struct. Dyn. 41 (23) (2023) 14382–14397, https://doi.org/10.1080/07391102.2023.2181643.

[11] O.B. Dundar, M.C. Unlusoy, E.J. Verspohl, R. Ertan, Synthesis and antidiabetic activity of novel 2,4-thiazolidinedione derivatives containing a thiazole ring, Arzneimittel Forschung-Drug Research 56 (9) (2006) 621–6250, https://doi.org/10.1055/s-0031-1296762.

[12] O. Bozdag-Duendar, E.J. Verspohl, N. Das-Evcimen, R.M. Kaup, K. Bauer, M. Sarikaya, B. Evranos, R. Ertan, Synthesis and biological activity of some new flavonyl-2,4-thiazolidinediones, Bioorg. Med. Chem. 16 (14) (2008) 6747–6751, https://doi.org/10.1016/j.bmc.2008.05.059.

[13] O. Bozdag-Dundar, N. Das Evcimen, M. Ceylan-Unlusoy, R. Ertan, M. Sarikaya, Some new thiazolyl thiazolidinedione derivatives as aldose reductase inhibitors, Med. Chem. Res. 16 (1) (2007) 39–47, https://doi.org/10.1007/s00044-007-9008-9.

[14] A.A. Husain, K.S. Bisht, Thiocyanation and 2-amino-1,3-thiazole formation in water using recoverable and reusable glycosylated resorcin[4]arene cavitands, J. Org. Chem. 85 (15) (2020) 9928–9935, https://doi.org/10.1021/acs.joc.0c01150.

[15] K. Shibasaki, H. Togo, 2-Amino-4-arylthiazoles through one-pot transformation of alkylarenes with NBS and thioureas, Eur. J. Org. Chem. 14 (2019) 2520–2527, https://doi.org/10.1002/ejoc.201900100.

[16] C.T. Supuran, A simple yet multifaceted 90 years old, evergreen enzyme: carbonic anhydrase, its inhibition and activation, Bioorg. Med. Chem. Lett. 93 (2023) 129411, https://doi.org/10.1016/j.bmcl.2023.129411.

[17] C.T. Supuran, Novel carbonic anhydrase inhibitors, Future Med. Chem. 13 (22) (2021) 1935–1937, https://doi.org/10.4155/fmc-2021-0222.

[18] A.S. El-Azab, A.A. Abdel-Aziz, H.A. Ghabbour, S. Bua, A. Nocentini, H. M. Alkahtani, N.A. Alsaif, M.H.M. Al-Agamy, C.T. Supuran, Carbonic anhydrase inhibition activities of schiff's bases based on quinazoline-linked benzenesulfonamide, Molecules 27 (22) (2022) 7703, https://doi.org/10.3390/molecules27227703.

[19] A. Angeli, V. Kartsev, A. Petrou, M. Pinteala, V. Brovarets, R. Vydzhak, S. Panchishin, A. Geronikaki, C.T. Supuran, Carbonic anhydrase inhibition with sulfonamides incorporating pyrazole- and pyridazinecarboxamide moieties provides examples of isoform-selective inhibitors, Molecules 26 (22) (2021) 7023, https://doi.org/10.3390/molecules26227023.

[20] P. Güller, U. Atmaca, U. Güller, U. Çalışır, F. Dursun, Antibacterial properties and carbonic anhydrase inhibition profiles of azido sulfonyl carbamate derivatives, Future Med. Chem. 13 (15) (2021) 1285–1299, https://doi.org/10.4155/fmc-2020-0387.

[21] T. Tunç, S. Abdurrahmanoğlu, A. Günel, Z. Alım, N. Demirel, Influence of chirality of benzimidazole amine hybrids on inhibition of human erythrocytes carbonic anhydrase I, II and acetylcholinesterase, Chemistry & biodiversity 20 (6) (2023) e202300207, https://doi.org/10.1002/cbdv.202300207.

[22] T. Umar, R. Meena, Mustehasan, P. Kumar, A.A. Khan, Recent updates in the development of small molecules as potential clinical candidates for Alzheimer's disease: a review, Chem. Biol. Drug Des. 2022 100 (2022) 674–681, https://doi.org/10.1111/cbdd.14133.

[23] S. Sepehri, M. Saeedi, B. Larijani, M. Mahdavi, Recent developments in the design and synthesis of benzylpyridinium salts: mimicking donepezil hydrochloride in the treatment of Alzheimer's disease, Front. Chem. 2022 10 (2022) 936240, https://doi.org/10.3389/fchem.2022.936240.

[24] M. Tonelli, M. Catto, B. Tasso, F. Novelli, C. Canu, G. Iusco, L. Pisani, A.D. Stradis, N. Denora, A. Sparatore, Multitarget therapeutic leads for alzheimer's disease: quinolizidinyl derivatives of Bi- and tricyclic systems as dual inhibitors of cholinesterases and β-amyloid (Aβ) Aggregation, ChemMedChem 10 (2015) 1040–1053, https://doi.org/10.1002/cmdc.201500104.

[25] H. Akıncıoğlu, İ. Gülçin, Potent acetylcholinesterase inhibitors: potential drugs for Alzheimer's disease, Mini Rev. Med. Chem. 20 (8) (2020) 703–715, https://doi.org/10.2174/1389557520666200103100521.

[26] C. Türkeş, S. Akocak, M. Işık, N. Lolak, P. Taslimi, M. Durgun, İ. Gülçin, Y. Budak, Ş. Beydemir, Novel inhibitors with sulfamethazine backbone: synthesis and biological study of multi-target cholinesterases and α-glucosidase inhibitors, J. Biomol. Struct. Dyn. 40 (19) (2022) 8752–8764, https://doi.org/10.1080/07391102.2021.1916599.

[27] H.R. Maradiya, Disperse dyes based on 2-aminothiazole derivatives for cellulose triacetate fabric, J. Saudi Chem. Soc. 16 (2012) 69–74, https://doi.org/10.1016/j.jscs.2010.10.021.

[28] Ş.D. Doğan, "N-Aril-N'-Heteroaril-Substitue Üre Türevlerinin sentezi", AKÜ FEmüBİD 16 (2016) 021202 https://doi.org/10.5578/fmbd.28135, 222-229.

[29] N.J. Thumar, M.P. Patel, Synthesis, characterization, and biological activity of substituted thiazole-5-carboxaldehydes and their ylidenenitriles derivatives, Phosphorus, Sulfur, and Silicon Relat. Elem. 184 (2009) 2720–2732, https://doi.org/10.1080/10426500802583520.

[30] S. Zheng, Q. Zhong, Y. Xi, M. Mottamal, Q. Zhang, R.L. Schroeder, J. Sridhar, L. He, H. McFerrin, G. ve Wang, Modification and biological evaluation of thiazole derivatiands as novel inhibitors of metastatic cancer cell migration and invasion, J. Med. Chem. 57 (2014) 6653–6667, https://doi.org/10.1021/jm500724x.

[31] D. Sikazwe, M.L. Bondarev, M. Dukat, J.B. Rangisetty, B.L. Roth, R.A. Glennon, "Binding of sulfonyl-containing arylalkylamines at human 5-HT6 serotonin receptors", J. Med. Chem. 49 (2006) 5217–5225, https://doi.org/10.1021/jm060469q.

[32] C.Y. Tsai, M. Kapoor, Y.P. Huang, H.H. Lin, Y.C. Liang, Y.l. Lin, S.C. Huang, W. N. Liao, J.K. Chen, J.S. Huang, M.H. Hsu, "Synthesis and evaluation of aminothiazole-paeonol derivatives as potential anticancer agents", Molecules 21 (2016) 145, https://doi.org/10.3390/molecules21020145.

[33] C. Galiana-Roselló, P. Bilbao-Ramos, M.A. Dea-Ayuela, M. Rolón, C. Vega, F. Bolás-Fernández, E. García-España, J. Alfonso, C. Coronel, M.E. González-Rosende, In vitro and in vivo antileishmanial and trypanocidal studies of new N-benzene- and N-naphthalenesulfonamide derivatives, J. Med. Chem. 56 (2013) 8984–8998, https://doi.org/10.1021/jm4006127.

[34] A.V. Stachulski, C. Pidathala, E.C. Row, R. Sharma, N.G. Berry, M. Iqbal, J. Bentley, S.A. Allman, G. Edwards, A. Helm, J. Hellier, B.E. Korba, J.E. Semple, J. Rossignol, Thiazolides as novel antiviral agents. 1. Inhibition of hepatitis B virus replication, J. Med. Chem. 54 (2011) 4119–4132, https://doi.org/10.1021/jm4006127.

[35] S. Mıwatashı, Y. Arıkawa, T. Matsumoto, K. Uga, N. Kanzaki, Y.N. Imaı, S. Ohkawa, Synthesis and biological activities of 4-Phenyl-5-pyridyl-1,3-thiazole derivatives as selective adenosine A3 antagonists, Chem. Pharm. Bull. 56 (8) (2008) 1126–1137, https://doi.org/10.1248/cpb.56.1126.

[36] M. Farouk Elsadek, B. Mohamed Ahmed, M. Fawzi Farahat, An overview on synthetic 2-aminothiazole-based compounds associated with four biological activities, Molecules 26 (2021) 1449, https://doi.org/10.3390/molecules26051449.

[37] J.R. Hitchin, J. Blagg, R. Burke, S. Burns, M.J. Cockerill, E.E. Fairweather, C. Hutton, A.M. Jordan, C. McAndrew, A. Mirza, D. Mould, G.J. Thomson, I. Waddell, D.J. Ogilvie, Development and evaluation of selective, reversible LSD1 inhibitors derived from fragments, Med. Chem. Commun. 4 (2013) 1513–1522, https://doi.org/10.1039/C3MD00226H.

[38] J.R.C. Prevost, A. Kozlova, B. Es Saadi, E. Yıldız, S. Modaffari, D.M. Lambert, L. Pochet, J. Wouters, E. Dolušić, R. Frédérick, "Convenient one-pot formation of highly functionalized 5-bromo-2 aminothiazoles, potential endocannabinoid hydrolase MAGL inhibitors", Tetrahedron Lett. 59 (2018) 4315–4319, https://doi.org/10.1016/j.tetlet.2018.10.055.

[39] J.P. Marina, R.F. Pradilla, "2-Arylimino-3-thiazolines–Formation of unusual tautomers of 2-arylamino thiazoles by a modifies hantzsch synthesis, Tetrahedron Lett. 26 (5) (1985) 1835–1838, https://doi.org/10.1016/S0040-4039(00)94750-6.

[40] A. Biçer, R. Altundaş, Formylation reactions of N-protecting 2-Amino-4-phenyl thiazole compounds, J. Mol. Struct. 1289 (2023) 135840, https://doi.org/10.1016/j.molstruc.2023.135840.

[41] A.B. Reddy, R.V. Hymavathi, T. Chandrasekhar, M. Naveen, G. Narayana Swamy, Synthesis and antimicrobial activity of a new class of methyleneamine-linked bis-heterocycles, J. Heterocyclic Chem. 48 (2011) 1175–1180, https://doi.org/10.1002/jhet.638.

[42] C. Rajendiran, P. Nagarajan, M.T. Chary, Eco-friendly water as a solvent for the one-pot synthesis of 2-aminothiazoles, J. Chem. Pharm. Res. 8 (2) (2016) 813–820. http://jocpr.com/vol8-iss2-2016/JCPR-2016-8-2-813-820.pdf.

[43] B. Krieg, J. Mittner, N-Bromsuccinimid-Bromierung von substituierten Methylthiazolen, Liebigs Ann. Chem. 2 (4) (1981) 623–632, https://doi.org/10.1002/jlac.198119810410.

[44] E.J. Uzelac, S.C. Rasmussen, Synthesis of brominated thiazoles via sequential Bromination− debromination methods, J. Org. Chem. 82 (11) (2017) 5947–5951, https://doi.org/10.1021/acs.joc.7b00495.

[45] L. Chen, H. Zhu, J. Wang, H. Liu, "One-Pot NBS-promoted synthesis of imidazoles and thiazoles from ethylarenes in water", Molecules 24 (2019) 893, https://doi.org/10.3390/molecules24050893.

[46] R.B. Mohan, G.T. Reddy, N.C.G. Reddy, "Substrate directed regioselective monobromination of aralkyl ketones using N-bromosuccinimide catalysed by active aluminium oxide: α-bromination versus ring bromination", ISRN Organic Chemistry (11) (2014) https://doi.org/10.1155/2014/751298. ID 751298.

[47] V. Benin, A.T. Yeates, D. Dudis, "Preparation of halogenated derivatives of thiazolo [5,4-d]thiazole via direct electrophilic aromatic substitution", J. Heterocyclic Chem. 45 (2008) 811–819, https://doi.org/10.1002/jhet.5570450328.

[48] J.K. Kajorinne, J.C.M. Steers, M.E. Merchant, C.D. MacKinnon, "Green halogenation reactions for (hetero)aromatic ring systems in alcohol, water, or no solvent", Can. J. Chem. 96 (12) (2018) https://doi.org/10.1139/cjc-2018-0259.
[49] A. Biçer, R. Kaya, G. Yakalı, M.S. Gültekin, G.T. Cin, İ. Gülçin, Synthesis of novel β-amino carbonyl derivatives and their inhibition effects on some metabolic enzymes, J. Mol. Struct. 1204 (2020) 127453, https://doi.org/10.1016/j.molstruc.2019.127453.
[50] A. Biçer, P. Taslimi, G. Yakalı, I. Gülçin, M.S. Gültekin, G.T. Cin, Synthesis, characterization, crystal structure of novel bis-thiomethylcyclohexanone derivatives and their inhibitory properties against some metabolic enzymes, Bioorg. Chem. 82 (2019) 393–404, https://doi.org/10.1016/j.bioorg.2018.11.001.
[51] I. Kufareva, R. Abagyan, Methods of protein structure comparison, Homology modeling: Methods and protocols (2012) 231–257, https://doi.org/10.1007/978-1-61779-588-6_10.
[52] T.M. Frimurer, G.H. Peters, L.F. Iversen, H.S. Andersen, N.P.H. Møller, O.H. Olsen, Ligand-induced conformational changes: improved predictions of ligand binding conformations and affinities, Biophys. J. 84 (4) (2003) 2273–22810, https://doi.org/10.1016/S0006-3495(03)75033-4.
[53] S. Chakravarty, K.K. Kannan, Drug-protein interactions: refined structures of three sulfonamide drug complexes of human carbonic anhydrase I enzyme, J. Mol. Biol. 243 (2) (1994) 298–309, https://doi.org/10.1006/jmbi.1994.1655.
[54] K.H. Sippel, A.H. Robbins, J. Domsic, C. Genis, M. Agbandje-McKenna, R. McKenna, High-resolution structure of human carbonic anhydrase II complexed with acetazolamide reveals insights into inhibitor drug design, Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun. 65 (10) (2009) 992–995, https://doi.org/10.1107/S1744309109036665.
[55] K.V. Dileep, K. Ihara, C. Mishima-Tsumagari, M. Kukimoto-Niino, M. Yonemochi, K. Hanada, K.Y. Zhang, Crystal structure of human acetylcholinesterase in complex with tacrine: implications for drug discovery, Int. J. Biol. Macromol. 210 (2022) 172–181, https://doi.org/10.1016/j.ijbiomac.2022.05.009.
[56] C.T. Supuran, Carbonic anhydrase inhibitors, Bioorg. Med. Chem. Lett. 20 (12) (2010) 3467–3474, https://doi.org/10.1016/j.bmcl.2010.05.009.
[57] J.L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker, I. Silman, Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein, Science 253 (5022) (1991) 872–879, https://doi.org/10.1126/science.1678899.
[58] M. Harel, I. Schalk, L. Ehret-Sabatier, F. Bouet, M. Goeldner, C. Hirth, J.L. Sussman, Quaternary ligand binding to aromatic residues in the active-site gorge of acetylcholinesterase, Proc. Natl. Acad. Sci. 90 (19) (1993) 9031–9035, https://doi.org/10.1073/pnas.90.19.9031.
[59] M.S. Alavijeh, M. Chishty, M.Z. Qaiser, A.M. Palmer, Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery, Neurotherapeutics 2 (2005) 554–571, https://doi.org/10.1602/neurorx.2.4.554.
[60] A. Ratković, K. Pavlović, D. Barić, Ž. Marinić, I. Grgičević, I. Škorić, Modeling and synthesis of novel oxime derivatives as potential cholinesterase inhibitors, J. Mol. Struct. 1200 (2020) 127149, https://doi.org/10.1016/j.molstruc.2019.127149.
[61] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 64 (2012) 4–17, https://doi.org/10.1016/S0169-409X(96)00423-1.
[62] E.M. Duffy, W.L. Jorgensen, Prediction of properties from simulations: free energies of solvation in hexadecane, octanol, and water, JACS 122 (12) (2000) 2878–2888, https://doi.org/10.1021/ja993663t.
[63] J.A. Verpoorte, S. Mehta, J.T. Edsall, Esterase activities of human carbonic anhydrases B and C, J. Biol. Chem. 242 (18) (1967) 4221–4229, https://doi.org/10.1016/S0021-9258(18)95800-X.
[64] D. Osmaniye, C. Türkeş, Y. Demir, Y. Özkay, Ş. Beydemir, Z.A. Kaplancıklı, Design, synthesis, and biological activity of novel dithiocarbamate-methylsulfonyl hybrids as carbonic anhydrase inhibitors, Arch. Pharm. 355 (8) (2022) e2200132, https://doi.org/10.1002/ardp.202200132.
[65] G.L. Ellman, K.D. Courtney, V. Andres Jr, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (2) (1961) 88–95, https://doi.org/10.1016/0006-2952(61)90145-9.
[66] N. Lolak, S. Akocak, M. Durgun, H.E. Duran, A. Necip, C. Türkeş, M. Işık, Ş. Beydemir, Novel bis-ureido-substituted sulfaguanidines and sulfisoxazoles as carbonic anhydrase and acetylcholinesterase inhibitors, Mol. Divers. 27 (4) (2023) 1735–1749, https://doi.org/10.1007/s11030-022-10527-0.
[67] S. Akocak, P. Taslimi, N. Lolak, M. Işık, M. Durgun, Y. Budak, C. Türkeş, İ. Gülçin, Ş. Beydemir, Synthesis, characterization, and inhibition study of novel substituted phenylureido sulfaguanidine derivatives as α-glycosidase and cholinesterase inhibitors, Chem. Biodiversity 18 (4) (2021) e2000958, https://doi.org/10.1002/cbdv.202000958.
[68] C. Türkeş, Y. Demir, Ş. Beydemir, Calcium channel blockers: molecular docking and inhibition studies on carbonic anhydrase I and II isoenzymes, J. Biomol. Struct. Dyn. 39 (5) (2021) 1672–1680, https://doi.org/10.1080/07391102.2020.1736631.
[69] M. Erdoğan, M. Serdar Çavuş, H. Muğlu, H. Yakan, C. Türkeş, Y. Demir, Ş. Beydemir, Synthesis, theoretical, in silico and in vitro biological evaluation studies of new thiosemicarbazones as enzyme inhibitors, Chem. Biodiversity 20 (11) (2023) e202301063, https://doi.org/10.1002/cbdv.202301063.