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The green synthesis and molecular docking of novel *N*-substituted rhodanines as effective inhibitors for carbonic anhydrase and acetylcholinesterase enzymes

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ABSTRACT

Recently, inhibition effects of enzymes such as acetylcholinesterase (AChE) and carbonic anhydrase (CA) has appeared as a promising approach for pharmacological intervention in a variety of disorders such as epilepsy, Alzheimer's disease and obesity. For this purpose, novel *N*-substituted rhodanine derivatives (***RhAs***) were synthesized by a green synthetic approach over one-pot reaction. Following synthesis the novel compounds, ***RhAs*** derivatives were tested against AChE and cytosolic carbonic anhydrase I, and II (hCAs I, and II) isoforms. As a result of this study, inhibition constant (Ki) were found in the range of 66.35 ± 8.35 to 141.92 ± 12.63 nM for AChE, 43.55 ± 14.20 to 89.44 ± 24.77 nM for hCA I, and 16.97 ± 1.42 to 64.57 ± 13.27 nM for hCA II, respectively. Binding energies were calculated with docking studies as −5.969, −5.981, and −9.121 kcal/mol for hCA I, hCA II, and AChE, respectively.

1. Introduction

In recent years, rhodanine (**1**, ***Rh***) has become a very important group of heterocyclic compounds in drug discovery [1–3]. The number of patents and scientific publications describing an abundance of the different biological activities of rhodanine-based molecules has been on the rise. Many natural and synthetic biological active compounds containing rhodanine core are being used as therapeutic agents like DNA helicase and HIV-1 inhibitors [4–6], antiviral agents [7] were shown to exhibit antibacterial and anticancer activities (Fig. 1) [8].

Therefore, there has been an increasing interest in developing innovative synthetic strategies for the decoration of rhodanine-cores [9,10]. Moreover, the rich chemistry of rhodanine (**1**) and the importance of the heterocyclic and metal complexes being steadily derived from this, encourage the further development of the green synthetic methods in this field [11,12]. Furthermore, the studies over 3-amino-2-thioxothiazolidin-4-one (**2**) are still not enough. While rhodanine derivatives with the exocyclic double bond at position five are usually synthesized by base-catalyzed Knoevenagel condensation between the rhodanine and aldehyde or ketone, it can be obtained azaylide (**A**) derivatives as well as Knoevenagel reaction derivatives via reaction of amino-rhodanine (**2**) with aldehyde or ketone. The direct synthesis of *N*-substituted rhodanine derivatives from 3-amino-2-thioxothiazolidin-4-one (**2**) is limited. One of these studies is carried out by Al-Romaizan and co-workers [13]. In this approach, the to *N*-substituted rhodanines were synthesized through ring closure reactions of dithioic formic acid hydrazones with functional reagents in the different medium (Fig. 3A). On the other hand, a plurality of synthesized organic molecules including functional groups such as alcohol, acid, ester and aldehyde groups have been reported as important enzyme inhibitors [14].

Enzymes are biocatalyst and biomolecules that are targeted for drug discovery and development due to their vital roles in many diseases [15]. Nearly half of the therapeutic agents currently having a market share perform their therapeutic effects by activating or inhibiting enzymes. Therefore, chemists and especially biochemists have focused on identify and optimize drug candidates that act as enzyme activators or inhibitors that molecules that bind to enzymes and decreases theirs activities [16,17]. Carbonic anhydrases (CAs, E.C.4.2.1.1) catalyze the rapid conversion of water (H_2O) and carbon dioxide (CO_2) to a proton (H^+) and bicarbonate anion (HCO_3^-) [18,19]. Seven different, genetically distinct CA families are known to date: the *α*-, *β*-, γ-, *δ*-, *ζ*-, n-

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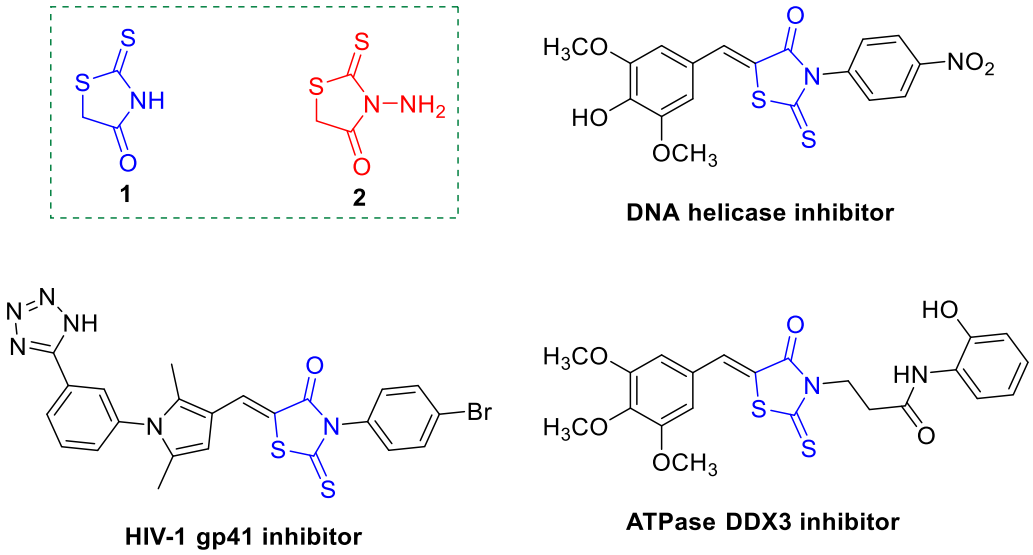


Fig. 1. Structures of rhodanine (**1**), 3-amino-2-thioxothiazolidin-4-one (**2**) and some rhodanine-based medicines.

and θ-CAs [20–22]. There are sixteen distinct CA isoenzymes commonly recorded in mammals belongs to α-CA family [23,24].

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

CA isoenzymes play an important role in multiple biochemical and physiological processes such as pH homeostasis, acid-base regulation, calcification, gluconeogenesis, ureagenesis, bone resorption, and tumorigenicity [25–27]. CA inhibitors (CAIs) are a class of pharmaceuticals or chemicals that inhibit the CA isoenzymes activities. Thus, CAIs have clinically usage as antiepileptics, diuretics and anti-glaucoma agents. They are also frequently used in the treatment of duodenal and gastric ulcers, idiopathic intracranial hypertension, osteoporosis and neurological disorders [28–30].

On the other hand, it was reported that Alzheimer's disease (AD) is the leading cause of dementia and other neurodegenerative diseases [31]. Acetylcholinesterase (AChE, E.C.3.1.1.7) is a specific cholinesterase and also is a hydrolytic enzyme of the serine class (Fig. 2). This enzyme is important for hydrolysis choline esters in the cholinergic synapses of the somatic system and central nervous system [32–34]. The specific and potent inhibitors of AChE cause an increase in ACh level in the brain, thereby increasing the transmission of electrical impulses [35,36]. Therefore, diverse AChE inhibitors are used for treating AD symptoms [37]. Recently, cholinesterase inhibitors compounds such as tacrine, donepezil, rivastigmine and galantamine have been used for the clinical treatment of AD. Moreover the use of these drugs is limited due to adverse effects such as gastrointestinal disturbance and hepatotoxicity [38–40]. Therefore, the investigation of novel non-toxic inhibitor compounds still remains important for the treatment of AD.

The synthesis of rhodanine derivatives and investigating their potential inhibitory actions are very popular. Furthermore there is very little study over the synthesis of amino-rhodanine derivatives, and also there is no any study investigating the effect of rhodanine derivatives on hCA isoenzymes and AChE enzyme activity. For this purpose in the present study, we synthesized of novel *N*-substituted rhodanine derivatives, which have differed nature, by a green synthetic approach (Fig. 3B) and investigated their inhibition effects on hCA enzymes and AChE enzyme activity.

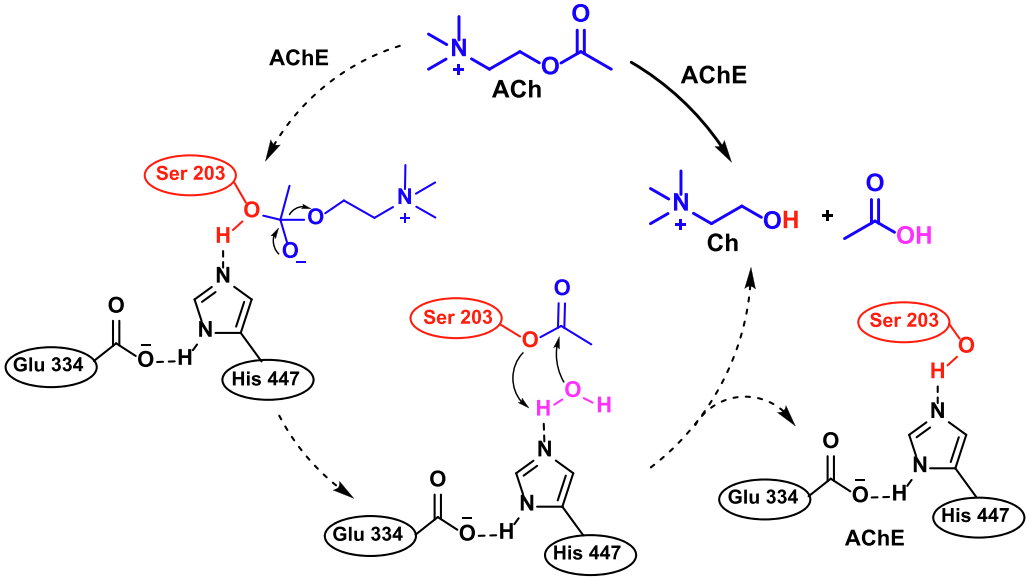


Fig. 2. Hydrolyzation reaction of acetylcholine in the presence of acetylcholinesterase (AChE) enzyme.

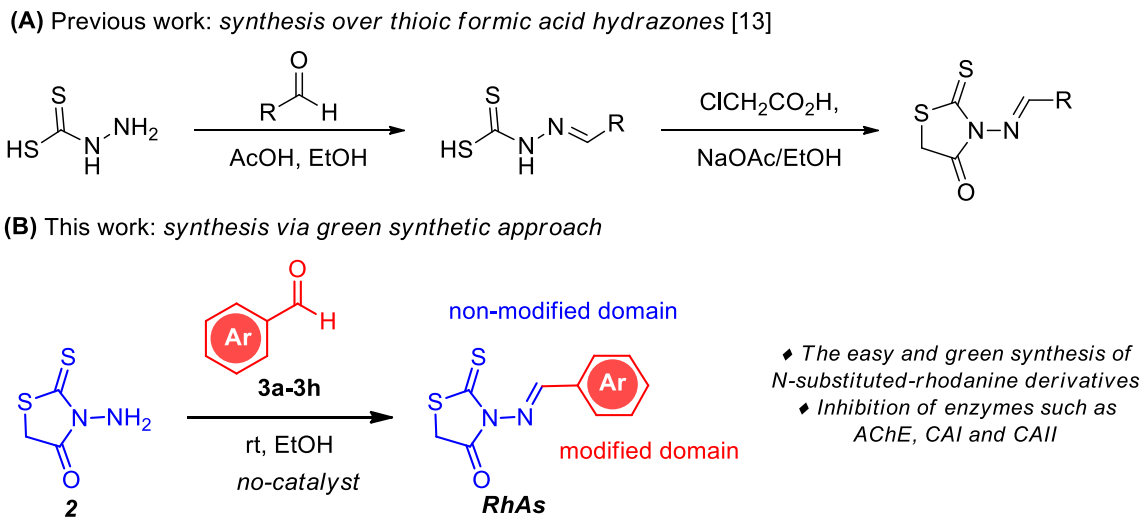


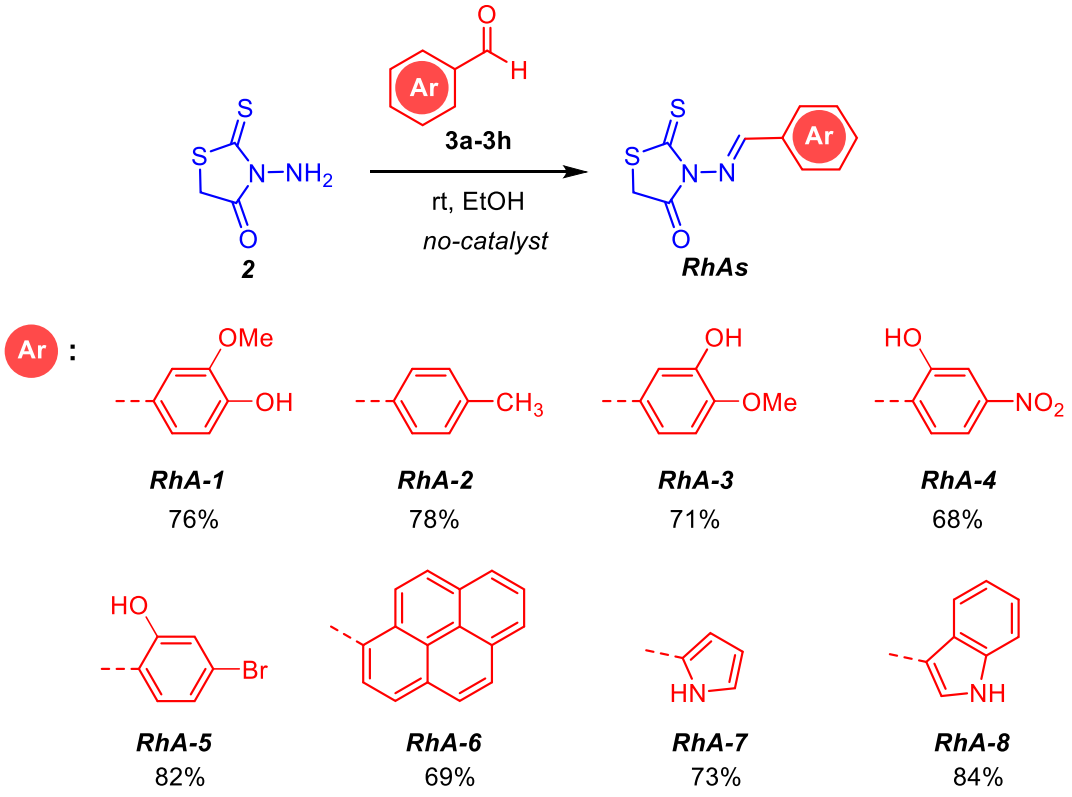
Fig. 3. Strategies for the synthesis of *N*-substituted rhodanines.

2. Results and discussion

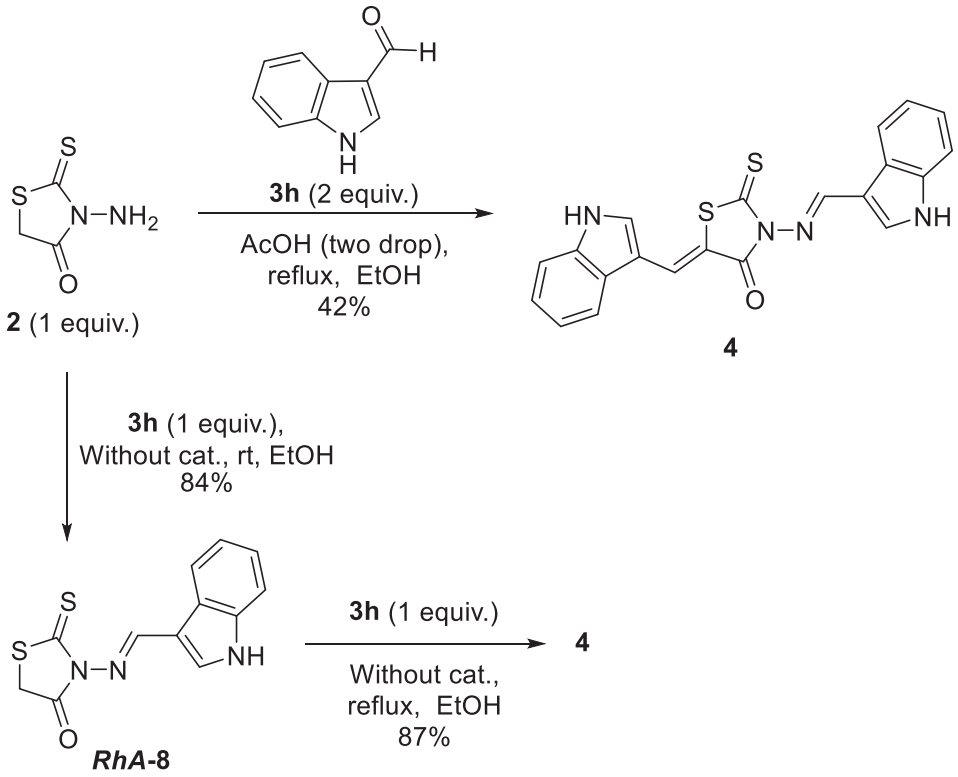
2.1. Chemistry

As mentioned above, in this work we interested in the synthesis and enzyme activity of *N*-substituted rhodanine (***RhAs***) derivatives starting from commercially available 3-amino-2-thioxothiazolidin-4-one (**2**) and aldehydes (**3a-3h**). For this purpose, initially, the reaction conditions were optimized by changing of parameters including temperature, time, catalysts and solvents for identification favorable reaction conditions. It was determined that the most favorable reaction conditions for the reactions were non-catalyzed reaction conditions in ethanol at room temperature. As a result of optimization studies, the synthesis of other ***RhAs*** derivatives was carried out under these conditions (Scheme 1). Moreover, since the synthesis of ***RhAs*** derivative ***RhA-7*** was synthesized at relatively a low yield (12%) at room temperature, the synthesis of ***RhA-7*** was carried out at the reflux temperature of ethanol.

The bisindole alkaloids, which exhibit various interesting biological activities, are widely found in nature, and their analogs exhibit antitumor and antifungal activities [41,42]. In addition of mono-substituted rhodanine derivatives, we also synthesized bisindole-substituted rhodanine derivative **4** (Scheme 2). For this purpose, firstly, we tried to obtain of target compound bisindole alkaloid **4** from the reaction of two equivalents of 1*H*-indole-3-carbaldehyde (**3h**) with one equivalent of 3-amino-2-thioxothiazolidin-4-one (**2**). Unfortunately, the target bisindole-substituted rhodanine derivative **4** was obtained with a



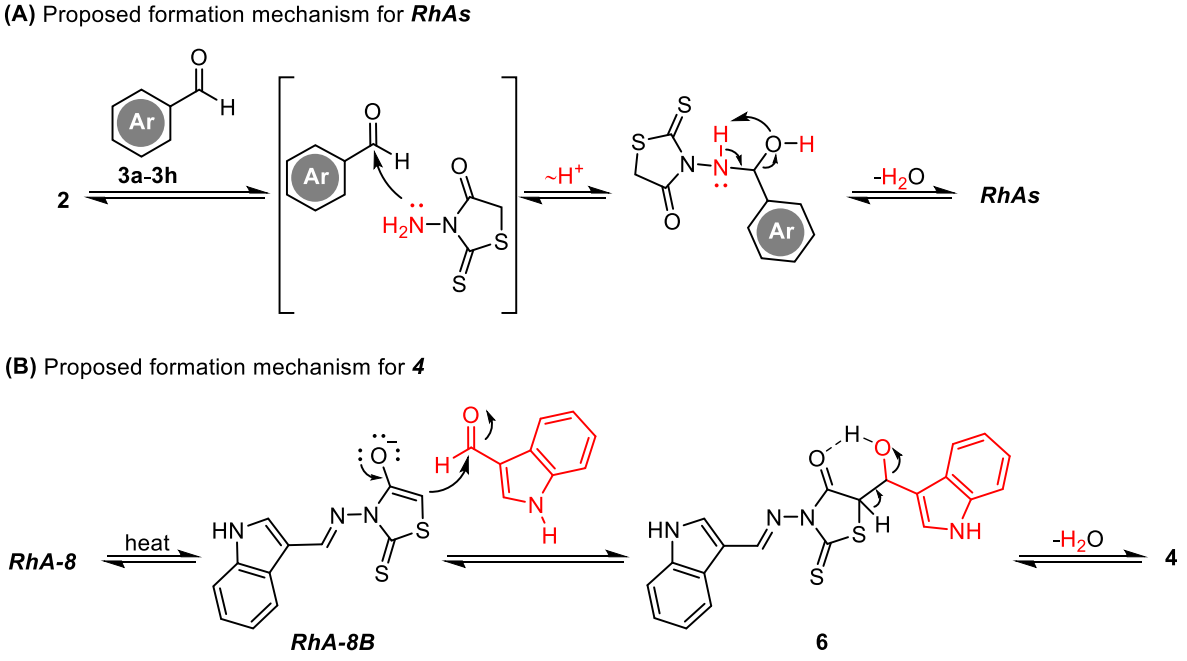
Scheme 1. Synthesis of *N*-substituted rhodanine derivatives.



Scheme 2. The synthesis of *N*, 5-bisindole-rhodanine derivative **4**.

low yield (42%) from the reaction that carried out in acid catalysis in ethanol. Upon this, the target bisindole-substituted rhodanine derivative **4** was obtained through a synthesis approach involving two easy and effective steps. The ***RhA*-8** was synthesized as per the reported procedure but without a catalyst in 84% yield. After synthesis of ***RhA*-8**, the target compound **4** was obtained from the reaction of ***RhA*-8** with 3-amino-2-thioxothiazolidin-4-one (**2**) without catalyst at the reflux temperature of ethanol in 87% yields.

A plausible mechanism for the reaction of one equivalent 3-amino-2-thioxothiazolidin-4-one (**2**) with one equivalent of aldehydes (**3a-3h**) is shown in Scheme 3. The mechanism for the formation of ***RhAs*** derivative involves classical condensation reaction of 3-amino-2-thioxothiazolidin-4-one (**2**) with aldehydes (**3a-3h**), which have different nature (Scheme 3A). It is supported in literature that ***RhA*-8** is in balance with the tautomerization product ***RhA*-8B** at high temperatures [43,44]. According to Scheme 3, we proposed a Knoevenagel condensation reaction mechanism based on for the formation of the other product **4** (Scheme 3B). The ^{1}H NMR spectra of ***RhA-8*** and **4**, which



Scheme 3. Proposed formation mechanism for ***RhAs*** and **4**.

have different nature, are shown in Figs. S3 and S4. When the ^{1}H NMR spectra of ***RhA-8*** and **4** are examined, it is seen that the NH (of indole core) proton peaks are resonance at 11.00 (s, NH, 1H) ppm and 12.41 (s, NH, 1H), 12.08 (s, NH, 1H) ppm, respectively. At the same time, it is seen that protons of -HC = N- (azaylide groups) in the structure of the target molecules ***RhA*-8** and **4** gave resonances signals at 8.60 (s, N = CH, 1H) and 8.93 (s, N = CH, 1H) ppm, respectively. Moreover, the most important evidence that bisindole-substituted rhodanine derivative **4** has been obtained is the disappearance of peak, which is resonance 4.16 (s, CH_2, 2H) ppm, in the structure of ***RhA*-8** and the presence of a new exogenous = CH peak in 8.15 (s, =CH, 1H) ppm. Detailed procedures and characterization can be found in the experimental section.

2.2. Biochemical results

In addition to synthesis of new *N*-substituted rhodanine derivatives, we also investigated the inhibition effects of ***RhAs*** on hCA I, and hCA II isoenzymes and AChE enzyme under *in vitro* conditions. The hCAs isoenzymes have been the subject of many kinds of research since the biological importance in many living organisms has been discovered [45]. Recently, these isoenzymes have become an attractive target for the design of novel activator and/or inhibitors compounds as previously described [46]. Thus, the inhibition act of the CA isoforms is noteworthy aims for several clinical applications such as antitumor, antiobesity, diuretics, antiglaucoma drugs and anticonvulsant factors/diagnostic tools [47,48].

The hCA I isoenzyme plays an important role in retinal and cerebral edema, and the inhibition of this isoenzyme can be a worth factor to reduce these adverse side effects [49]. The hCA I isoenzyme was strongly inhibited by the rhodanine core **2** and novel rhodanine derivatives (**4** and **RhAs**) with Ki values were found between 43.55 ± 14.20 and 89.44 ± 24.77 nM. Furthermore, ***RhA-8*** showed the most effective hCA I isoenzyme inhibition properties with K_i value of 43.55 ± 14.20 nM as competitive inhibition. The control and clinically used drug acetazolamide (AZA) demonstrated a K_i value of 110.11 ± 10.13 nM. Thus, the investigated novel rhodanine derivatives showed better inhibitory profiles compared to the AZA (Table 1 and Fig. 4). Therewithal, the physiologically dominant isoform hCA II is mostly associated with several diseases such as osteoporosis, epilepsy, glaucoma and renal tubular acidosis [50]. The hCA II was also efficiently inhibited by the rhodanine core **2** and novel rhodanine derivatives (**4** and ***RhAs***) investigated in this study. These compounds had powerful hCA II inhibition with K_i values ranging from 16.97 ± 1.42 to 64.57 ± 13.27 nM. These values are better than those of the clinically used drugs AZA (K_i of 116.97 ± 13.34 nM). The ***RhA-1*** is a competitively inhibits cytosolic dominant hCA II isoenzyme and has seven times more effective than AZA in inhibiting hCA II isoenzyme (Table 1 and Fig. 4).

AChE inhibitors such as tacrine, galantamine, donepezil and rivastigmine have been approved by the Food and Drug Administration (FDA) for the treatment of AD [51,52]. Moreover, all of these drugs don't achieve significant improvement due to the side effects, such as nausea, diarrhea, vomiting and anorexia [53,54]. Therefore, it is urgent to find novel compounds, which have low toxicity and high AChE inhibitory activity. In this study, the AChE inhibition profiles of the rhodanine-based azaylide compounds evaluated here were quite interesting. Most of these compounds effectively inhibited AChE enzyme activity at the nanomolar level. The 3-amino-rhodanine (**2**) and novel rhodanine derivatives (**4** and ***RhAs***), effectively inhibited AChE enzyme with K_i values in the range of 66.35 ± 8.35 to 141.92 ± 12.63 nM. Also, tacrine (TAC), used as a standard AChE inhibitor in this study, demonstrated K_i value of 145.19 ± 17.53 nM toward AChE. Furthermore, it was found that the most potent AChE inhibition observed in compound of ***RhA-5*** as competitive inhibition. K_i value of ***RhA-5*** was found as 66.35 ± 8.35 nM. On the other hand, Ki value was calculated as 145.19 ± 17.53 nM for TAC as clinical used AChE inhibitor (Table 1).

In order to understand hCAs inhibition mechanism by novel rhodanine derivatives should be reminded that the active domain of CAIs contains an active Zn^{2+} ion site, which is a strong Lewis acid that binds to and activates a substrate water molecule to catalyze the reversible hydration reaction of carbon dioxide. According to this, the Zn^{2+} ion is situated at the bottom of the active site, being coordinated by three histidine residues (His 94, His 96 and His 119) and H_2O molecule [55]. On the other hand, the sulfonamide derivatives are the most important inhibitors of hCAs. According to researches, on the basis of X-ray crystallographic structures of sulfonamide inhibitors, it has been reported that the deprotonated sulfonamide was coordinated to the Zn^{2+} ion of the enzyme [56,57]. In this context, it was reported that the novel organic ligands, which are containing similar groups with sulfonate amide, have also shown similar inhibition effects. There are also a large number of heteroatoms in the structure of the new rhodanine derivatives synthesized in this study. It is observed that studies between new rhodanine derivatives and hCAs and AChE enzymes have a significant inhibitory effect against enzymes compared to literature data. In the light of all these data, the mechanism of the interaction between the ***RhA-1*** and hCA II enzyme is proposed as in Fig. 5. According to this, its NH moiety of ***RhA-1*** participates in an active Zn^{2+} ion site is engaged, and as a result, the enzyme is inhibited. Molecular docking studies were conducted to confirm this proposed mechanism, which is in the light of literature data and the obtained results confirm the

Table 1
The inhibition values of novel *N*-substituted rhodanines ***RhAs*** against human carbonic anhydrase isoenzymes I and II (hCA I and II), and acetylcholinesterase (AChE).

Compounds	IC_{50} (nM)						K_i (nM)		
	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
2	73.00	0.9603	57.75	0.9743	99.00	0.9748	47.78 ± 10.21	52.56 ± 14.88	80.30 ± 6.92
RhA-1	57.75	0.9851	38.50	0.9860	86.46	0.9828	50.06 ± 5.45	16.97 ± 1.42	98.70 ± 13.78
RhA-2	63.50	0.9770	63.00	0.9877	118.32	0.9861	83.35 ± 5.98	56.58 ± 2.86	141.92 ± 12.63
RhA-3	64.35	0.9755	49.50	0.9817	115.50	0.9773	50.43 ± 12.94	54.38 ± 5.48	94.98 ± 5.75
RhA-4	64.26	0.9610	49.60	0.9795	113.65	0.9686	76.71 ± 11.55	56.81 ± 7.14	90.20 ± 10.90
RhA-5	62.06	0.9706	36.47	0.9746	86.62	0.9796	51.96 ± 6.35	31.86 ± 7.02	66.35 ± 8.35
RhA-6	69.30	0.9859	55.77	0.9840	115.53	0.9038	78.06 ± 13.17	64.57 ± 13.27	96.15 ± 3.15
RhA-7	60.56	0.9793	69.45	0.9750	98.45	0.9912	89.44 ± 24.77	54.73 ± 7.75	86.26 ± 11.25
RhA-8	53.30	0.9417	46.20	0.9994	103.65	0.9901	43.55 ± 14.20	46.61 ± 10.28	79.55 ± 4.80
4	64.35	0.9742	69.30	0.9806	98.34	0.9822	44.73 ± 6.66	55.57 ± 8.26	80.04 ± 4.21
AZA*	118.32	0.9887	112.18	0.9763	–	–	110.11 ± 10.13	116.97 ± 13.34	–
TAC**	–	–	–	–	162.56	0.9664	–	–	145.19 ± 17.53

* AZA (acetazolamide) was used as a positive control for human carbonic anhydrase I and II isoforms (hCA I and II).
** TAC (tacrine) was used as a positive control for acetylcholinesterase (AChE).

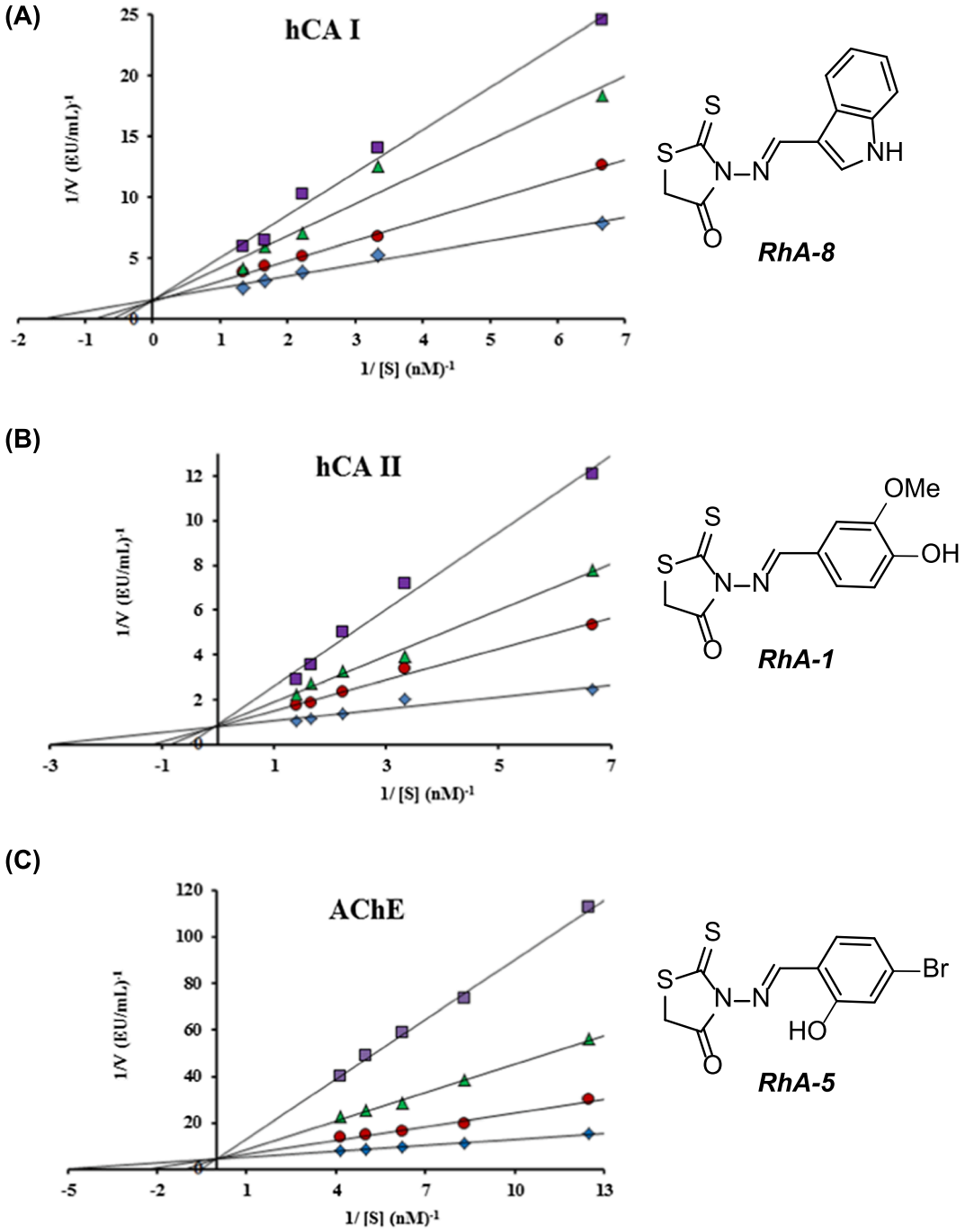


Fig. 4. Determination of Lineweaver-Burk graphs for excellent inhibitors of hCAs (hCA I and hCA II) and acetylcholinesterase (AChE).

proposed mechanism (Fig. 8).

In recent studies, it was reported that indole bearing thiadiazole analogs and indole base oxadiazole derivatives had β-glucuronidase inhibition effects. Also, oxindole-based chalcones demonstrated anti-glycation potentials against glycation of proteins. Additionally, Schiff bases of tryptamine act as potent inhibitors of nucleoside triphosphate diphosphohydrolases [58].

2.3. Molecular docking studies

Following *in vitro* studies, we carried out molecular docking studies to understand the inhibition mechanism of most active ***RhAs*** on hCA I, hCA II, and AChE receptors. We firstly prepared ligands and receptors for docking studies. Then, binding site of prepared receptors was detected. SiteScores were calculated to determine whether binding sites are catalytic active site. SiteScores of the receptors were 1.063, 0.971, and 1.090 for hCA I, hCA II, and AChE receptors, respectively. The scores indicated that predicted binding sites exhibited catalytic active sites properties. Moreover, Dscores were calculated to understand whether catalytic active sites are druggable. Dscore of the receptors were 1.061, 0.945, and 1.113 for hCA I, hCA II, and AChE receptors, respectively. According to the Dscore, catalytic active sites of the receptors are druggable. We also used a catalytic active site to evaluate docking hits as seen in Fig. 6.

The ADME results of the selected ***RhAs*** were analyzed by comprising to properties of 95% of known drugs and demonstrated in Table 2. Molecular weight, partition coefficient, hydrogen bond acceptors (AHB) and hydrogen bond donors (DHB) of the ***RhAs*** are

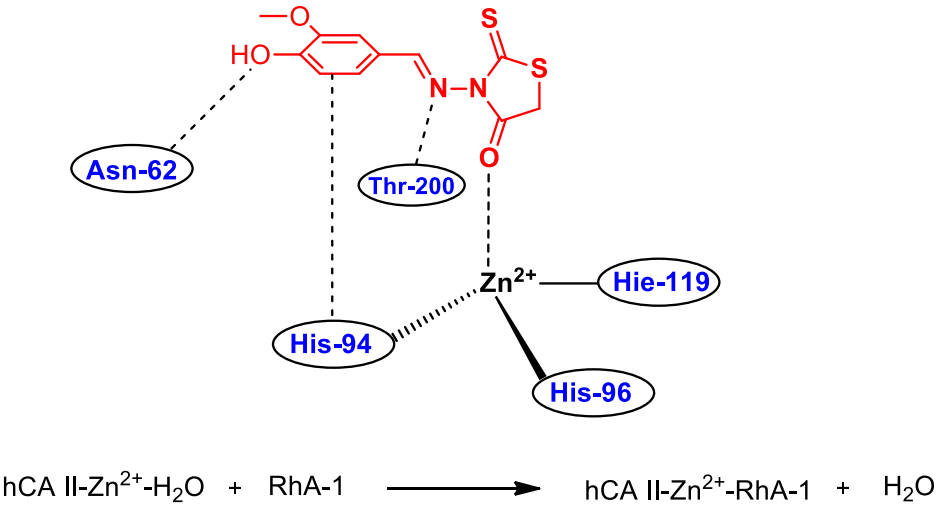


Fig. 5. Proposed hCA I inhibition mechanism by ***RhA-1***.

compatible with Lipinski's rule. Moreover, IC_{50} values for HERG K^+ channel blockage (logHERG) were below 25.0 for each selected ***RhAs***. Brain/blood partition coefficient of selected ***RhAs*** was noteworthy range. The selected ***RhAs*** can easily diffuse through the cell membrane because of their MDCK values. Also, human serum albumin binding value was slightly below an acceptable range. Lastly, the molecules can be easily absorbed by orally because of their high percentage of human oral absorption.

We carried out docking process of co-crystallized ligands and most active ***RhAs*** into the predicted catalytic active site of the receptors. We began by checking accuracy of the docking methodology before selected ***RhAs*** were docked into receptors. Docking validation results shown in Fig. 7. When the figure was examined, co-crystallized ligands and re-docked ligands very closely located into the catalytic active site of the receptors. The results revealed the accuracy of the docking process.

Moreover, we calculated the binding score of selected ***RhAs*** on the receptors. The binding scores are presented in Table 3. The scores have shown that selected ***RhAs***, especially ***RhA-5***, have good binding affinity against their receptors compared to standard inhibitors' binding affinity of the receptors. Best-scored ligand into catalytic active sites was approved as best pose of the ligand. 2D interaction diagrams of best-posed ligands were illustrated in Fig. 8. Indole ring of ***RhA-8*** was formed hydrogen bond with Pro 201 residue and interacted through π-π interaction with Hie64 and His 200 residues of hCA I receptor (Fig. 8a). Detailed binding mode of ***RhA-8*** has shown that indole ring formed aromatic hydrogen bound with Pro 202 residues (Fig. 9a). 4-Hydroxy-3-methoxybenzylidene moiety of ***RhA-1*** was formed hydrogen bond with Asn 62 and Thr 200 residues. Also, 2-thioxothiazolidin moieties were interacted with Zn 265 of hCA II (Fig. 8b). Moreover, benzyl ring formed aromatic hydrogen bond as seen Fig. 9b. 4-Bromo-2-hydroxybenzylidene moiety of ***RhA-5*** was formed hydrogen bond with Tyr124 and Hip 447 residues and interacted through π-π interaction with Tyr 337, Phe 338, and Tyr 341 residues of AChE receptor (Fig. 8c). In our previously studies, we detected similar interaction for each receptor [46,47,59]. We think that His 64 and His 200 residues of hCA I, His94 and Thr 200 residues of hCA II and Tyr 124, Phe 295, and Tyr 337 residues of AChE are key residues for inhibition for the enzymes. Also, in the case of AChE, residues Tyr124 and Tyr337 are located at the peripheral anionic site while Phe 295 belongs to the active site namely the acyl pocket [60].

3. Conclusion

The easy synthesis of new rhodanine derivatives containing important aromatic groups and investigating their potential inhibitory actions is very popular. In the present study, we synthesized of *N*-substituted rhodanine derivatives ***RhAs***, which have differed nature, by a green synthetic approach (Fig. 3b) and investigated their inhibition effects on hCAs (hCA I and hCA II) and AChE enzymes activity *in vitro*. Initially, novel ***RhAs*** derivatives**,** which are the skeleton of natural and important medicinal chemicals, were synthesized via a green approach without catalyst. Following synthesis, we investigated their hCA I, and hCA II isoenzymes and AChE enzyme inhibition properties. As a result

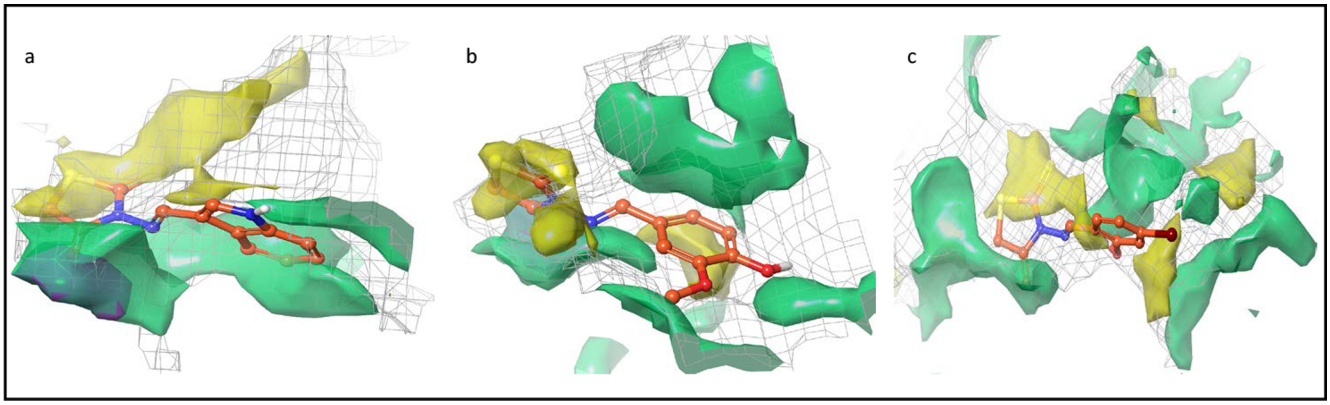


Fig. 6. Catalytic active sites; (a) ***RhA-8***-hCA I, (b) ***RhA-1***-hCA II, and (c) ***RhA-5***-AChE. Catalytic active sites are represented as grey mesh, hydrophilic site is represented as green surface, hydrophobic site is represented as yellow surface, and metal binding site is represented as purple surface.

Table 2
Pharmaceutically properties of the most active RhAs.

Compounds	[a]MW	[b]DHB	[c]AHB	[d]logPo/w	[e]logHERG	[f]logBB	[g]MDCK	[h]logKhsa	[i]% Hum. Oral Abs.
RhA-1	282.332	1	5.50	2.051	−4.314	−0.505	1510.730	−0.330	90.778
RhA-5	331.201	1	4.75	2.552	−4.505	−0.215	4840.722	−0.221	94.648
RhA-8	275.343	1	4.00	2.872	−4.889	−0.174	2781.760	−0.009	100.000

[a] Molecular weight (< 500 Da).
[b] Number of hydrogen bond donors (< 5).
[c] Number of hydrogen bond acceptors (< 10).
[d] Octanol/water partition coefficient (recommended range: −2.0 to 6.5).
[e] IC50 value for blockage of HERG K + channels (acceptable range: above 25.0).
[f] B/blood partition coefficient (recommended range: –3.0 to 1.2)
[g] Cell permeability in nm/*sec* (< 25 is poor and > 500 is great).
[h] Binding to human serum albumin (acceptable range: 21.5 to 1.5).
[i] Percentage of human oral absorption (< 25% is poor and > 80% is high).

of this study, new rhodanine derivatives showed inhibition at the nanomolar levels against these enzymes. The *N*-substituted rhodanine derivatives ***RhA*-8, *RhA*-1** and ***RhA*-5** were good inhibitors for hCA I, and hCA II isoenzymes and AChE enzyme, respectively. Moreover, we identified the binding modes of these inhibitors with molecular docking studies. In this context, synthesized by a green and easy synthetic approach candidates for anticholinergic and antiepileptic applications. Furthermore, it is thought that there will be a need for clinical studies before application is recommended.

4. Experimental section

4.1. General information

All chemicals and solvents were commercially available from Sigma-Aldrich or Merck. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 (1 0 0)-MHz Bruker spectrometer. Infrared spectra were recorded on a Mattson 1000 FT-IR spectrophotometer. High-resolution mass spectrometric analysis was carried out on an Agilent 1260 Infinity Series Q-TOF LC/MS (ESI/MS). The reaction progress was monitored by thin-layer chromatography (TLC, 0.25 mm-thick precoated silica plates).

4.2. Synthesis of organic compounds

*General procedure for synthesis of N-substituted-rhodanine derivatives **RhAs:*** To a solution of aldehydes (**3a-3h**, 1.0 equiv.) in ethanol (10 mL) was added slowly to the solution of 3-amino-2-thioxothiazolidin-4-one (**2**, 1.0 equiv.) in EtOH. The reaction mixture was stirred at room temperature without a catalyst for between 4 h and 12 h, and was monitored by TLC. After, the mixture product was recrystallized from EtOH. After recrystallization, *N*-substituted-rhodanine derivatives **(*RhAs*)** were obtained as follows.

(E)-3-((4-hydroxy-3-methoxybenzylidene)amino)-2-thioxothiazolidin-4-one [61]: The product ***RhA-1*** was obtained as yellow solid (76% yield). Mp: 160.5–161.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.58 (s, OH, 1H), 8.48 (s, N = CH, 1H), 7.41 (d, J = 1.6 Hz, =CH, 1H), 7.25 (dd, J = 7.7, 1.6 Hz, =CH, 1H), 7.06 (d, J = 7.7 Hz, =CH, 1H), 4.33 (s, CH_2, 2H), 3.86 (s, OCH_3, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.8, 170.5, 169.8, 152.3, 147.0, 123.6, 113.0, 111.8, 55.7, 34.7 (Fig. S1); ESI-MS (*m/z*) $[M + H]^+$ calcd. for $C_{11}H_{10}N_2O_3S_2$ 283.02, found: 283.12; IR (KBr, cm^{-1}): 3112 cm^{-1} (=C–H, aromatic H), 1717 cm^{-1} (C=O), 1638 cm^{-1} (O=C-*N*-C=S), 1544 cm^{-1} (C–C, stretch in ring), 1439, 1332 cm^{-1} (C=S).

(E)-3-((4-methylbenzylidene)amino)-2-thioxothiazolidin-4-one: The product ***RhA-2*** was obtained as pale yellow solid (78% yield). 139–141 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.66 (s, N = CH, 1H), 7.80 (d, J = 8.0 Hz, =CH, 2H), 7.37 (d, J = 8.0 Hz, =CH, 2H), 4.35 (s, CH_2, 2H), 2.39 (s, CH_3, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.8, 170.6, 169.7, 143.6, 129.8, 129.1, 128.9, 34.7, 21.3 (Fig. S2); ESI-MS (*m/z*) [M] calcd for $C_{11}H_{10}N_2OS_2$ 250.02, found: 250.11; IR (KBr, cm^{-1}): 3161 cm^{-1} (=C–H, aromatic H), 1729 cm^{-1} (C=O), 1615 cm^{-1} (O=C–*N*–C=S), 1414, 1311 cm^{-1} (C=S), 1129 cm^{-1} (C–N, stretch peak).

(E)-3-((3-hydroxy-4-methoxybenzylidene)amino)-2-thioxothiazolidin-4-one: The product ***RhA-3*** was obtained as yellow solid (71% yield). Mp: 152–153 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.57 (s, OH, 1H), 8.47 (s, N = CH, 1H), 7.40 (s, =CH, 2H), 7.26 (d, J = 7.5 Hz, =CH, 1H), 7.07 (d, J = 7.5 Hz, =CH, 1H), 4.34 (s, CH_2, 2H), 3.85 (s, OCH_3, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.2, 170.9, 170.2, 152.7,

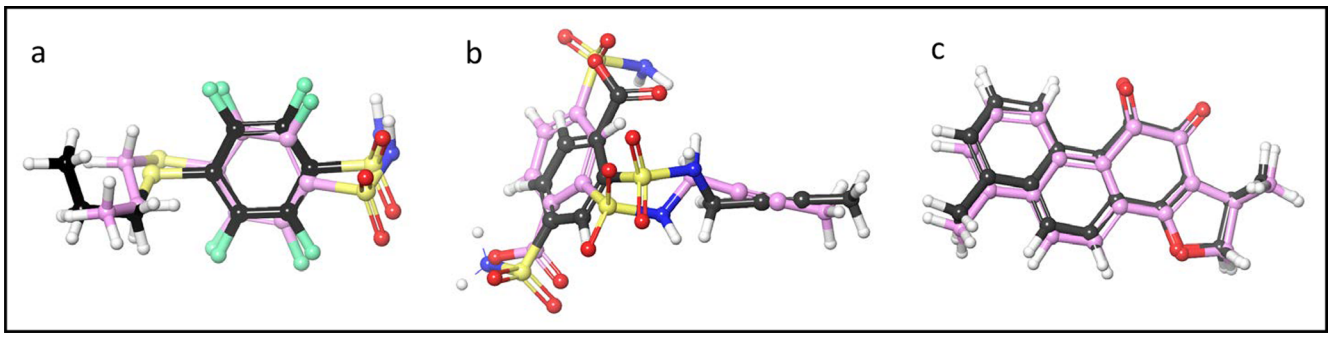


Fig. 7. Docking methodology test. (a) 3TV-hCA I, (b) 51 J-hCA II, and (c) 1YL-AChE. The poses of co-crystallized ligands are represented in black color ball and stick modelling while that of docked ligands is represented in pink color ball and stick mode.

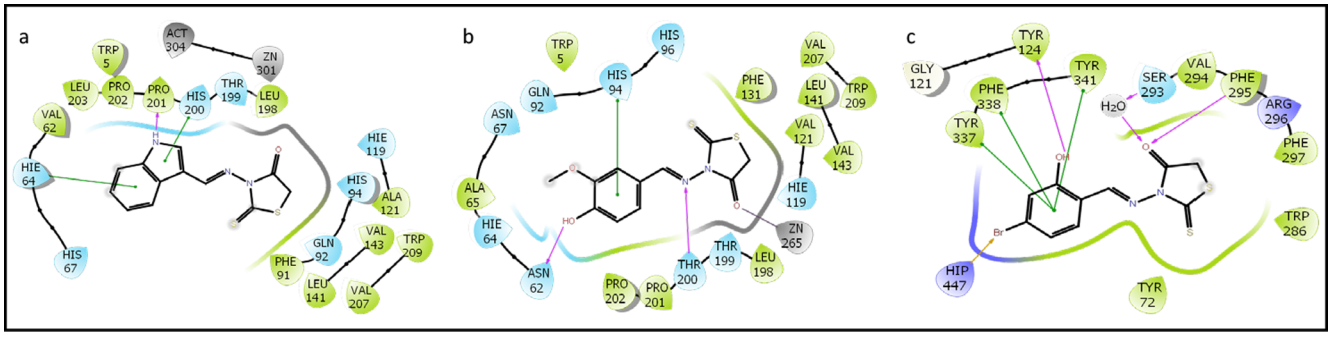


Fig. 8. 2D receptor-ligand interaction diagram; (a) ***RhA-8***-hCA I, (b) ***RhA-1***-hCA II, and (c) ***RhA-5***-AChE.

Table 3
Binding scores (kcal/mol) of the most active compounds in the catalytic sites of human carbonic anhydrase isoenzymes I and II (hCA I and II), and acetylcholinesterase (AChE).

Compounds	IFD Glide Score		
	hCA I	hCA II	AChE
RhA-1	–	−5.981	–
RhA-5	–	–	−9.121
RhA-8	−5.969	–	–
AZA*	−9.016	−9.560	–
TAC**	–	–	−9.579

* Acetazolamide (AZA) was used as a standard inhibitor for human carbonic anhydrase isoenzymes I, and II (hCA I, and II).

** Tacrine (TAC) was used as a standard inhibitor for acetylcholinesterase (AChE) enzyme.

147.4, 124.6, 124.0, 113.4, 112.2, 56.1, 35.1 (Fig. S3); ESI-MS (*m/z*) [M] calcd for $C_{11}H_{10}N_2O_3S_2$ 282.02, found: 282.03; IR (KBr, cm^{-1}): 3191 cm^{-1} (=C—H, aromatic H), 1726 cm^{-1} (C=O), 1617 cm^{-1} (O=C—*N*—C=S), 1452, 1321 cm^{-1} (C=S), 1125 cm^{-1} (C—N, stretch peak).

(E)-3-((2-hydroxy-4-nitrobenzylidene)amino)-2-thioxothiazolidin-4-one: The product ***RhA-4*** was obtained as yellow solid (68% yield). Mp: 169–171 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.01 (s, OH, 1H), 8.97 (s, N = CH, 1H), 7.98 (d, J = 1.6 Hz, =CH, 1H), 7.61 (d, J = 7.7 Hz, =CH, 1H), 6.98 (d, J = 7.7 Hz, =CH, 1H), 4.32 (s, CH_2, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.1, 169.7, 164.8, 157.8, 136.9, 130.4, 129.6, 119.8, 110.7, 34.7 (Fig. S4); ESI-MS (*m/z*) $[M + H]^+$ calcd for $C_{10}H_7N_3O_4S_2$ 298.31, found: 298.38; IR (KBr, cm^{-1}): 3151 cm^{-1} (=C—H, aromatic H), 1725 cm^{-1} (C=O), 1632 cm^{-1} (O=C—*N*—C=S), 1421, 1311 cm^{-1} (C=S).

(E)-3-((4-bromo-2-hydroxybenzylidene)amino)-2-thioxothiazolidin-4-

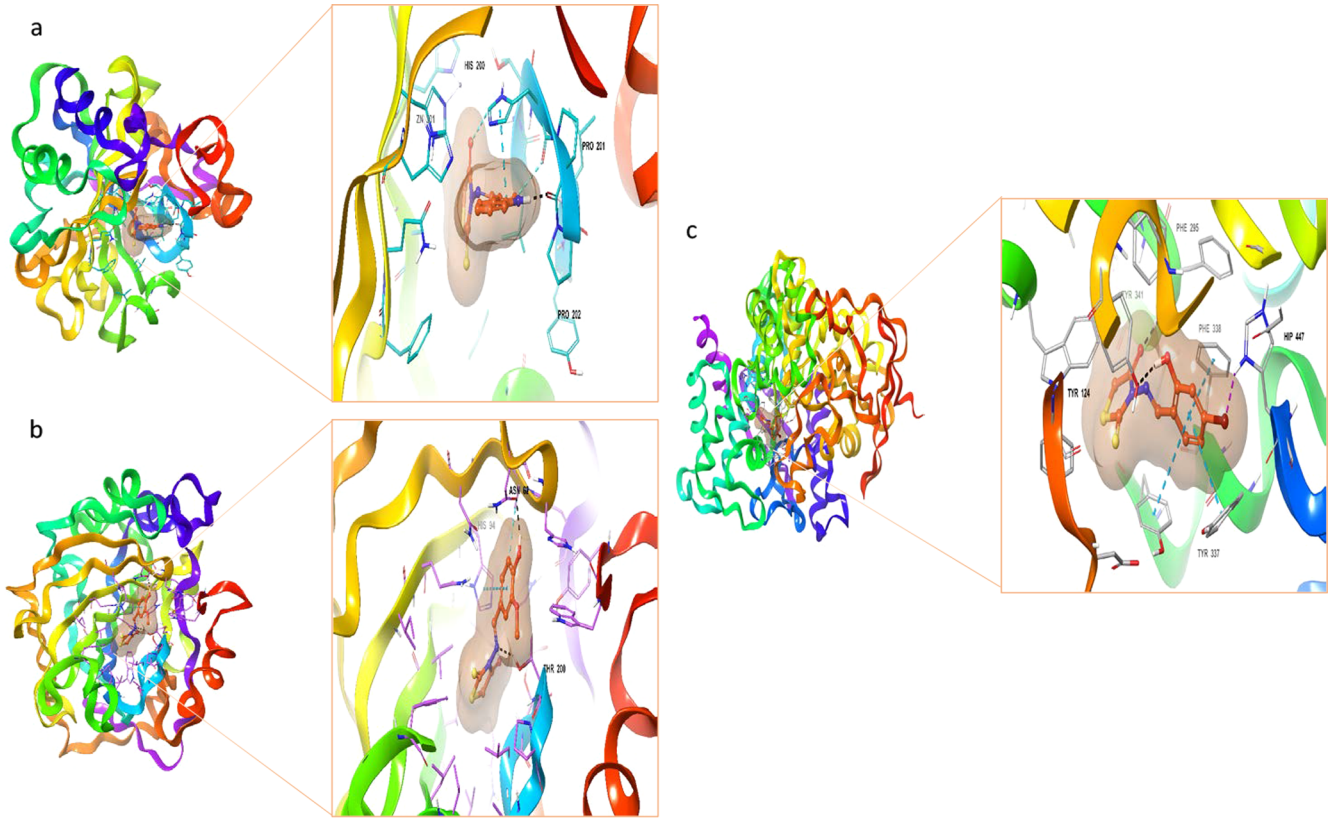


Fig. 9. 3D detailed binding mode of the most active ***RhAs*** into hCA I, hCA II, and AChE receptors. (a) Best-pose of ***RhA-8*** into catalytic active site of hCA I, (b) Best pose of ***RhA-1*** into catalytic active site of hCA II, and (c) Best-pose of ***RhA-5*** into catalytic active site of AChE. Receptors are presented in the ribbon model. ***RhAs*** are represented in ball and stick modelling which orange color carbon and residues are represented in thick tube modelling which hCA I with cyan color, hCA II with purple color, and AChE with grey color.

one: The product ***RhA-5*** was obtained as yellow solid (82% yield). Mp: 221–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.00 (s, OH, 1H), 8.96 (s, N = CH, 1H), 7.97 (d, J = 2.6 Hz, =CH, 1H), 7.62 (dd, J = 8.8, 2.6 Hz, =CH, 1H), 6.98 (d, J = 8.8 Hz, =CH, 1H), 4.32 (s, CH_2, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.1, 169.7, 164.8, 157.8, 136.9, 129.6, 119.8, 119.2, 110.7, 34.7 (Fig. S5); ESI-MS (m/z) [M] calcd for $C_{10}H_7BrN_2O_2S_2$ 330.92, found: 330.95; IR (KBr, cm^{-1}): 3191 cm^{-1} (=C−H, aromatic H), 1724 cm^{-1} (C=O), 1618 cm^{-1} (O=C−*N*−C=S), 1427, 1315 cm^{-1} (C=S), 1115 cm^{-1} (C−N, stretch peak).

(E)-3-((pyren-1-ylmethylene)amino)-2-thioxothiazolidin-4-one: The product ***RhA-6*** was obtained as orange solid (69% yield). Mp: > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.76 (s, N = CH, 1H), 8.87 (d, J = 8.5 Hz, =CH, 1H), 8.67 (d, J = 8.5 Hz, =CH, 1H), 8.43–8.34 (m, =CH, 5H), 8.25 (d, J = 8.5 Hz, =CH, 1H), 8.16 (t, J = 8.5 Hz, =CH, 1H), 4.47 (s, CH_2, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.0, 170.0, 169.8, 134.0, 130.6, 130.3, 129.9, 129.8, 129.7, 127.3, 126.8 (2C), 126.6 (2C), 125.1, 124.0, 123.8, 123.3, 122.5, 34.9 (Fig. S6); ESI-MS (m/z) [M + H]$^+$ calcd for $C_{20}H_{12}N_2OS_2$ 365.05, found: 365.07; IR (KBr, cm^{-1}): 3158 cm^{-1} (=C−H), 1712 cm^{-1} (C=O), 1627 cm^{-1} (O=C−*N*−C=S), 1424, 1317 cm^{-1} (C=S).

(E)-3-(((1H-pyrrol-2-yl)methylene)amino)-2-thioxothiazolidin-4-one: The product ***RhA-7*** was obtained as a black solid (73% yield) from reaction **2** with 1H-pyrrole-2-carbaldehyde (**3 g**) at reflux temperature of EtOH. Mp: 156–157 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.06 (bs, NH, 1H), 8.29 (s, N = CH, 1H), 7.16 (d, J = 1.4 Hz, =CH, 1H), 6.82 (d, J = 1.4 Hz, =CH, 1H), 6.28 (t, J = 1.4 Hz, =CH, 1H), 4.32 (s, CH_2, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.7, 170.3, 161.5, 126.2, 125.0, 119.3, 110.5, 34.5 (Fig. S7); ESI-MS (m/z) [M + H]$^+$ calcd for $C_8H_7N_3OS_2$ 326.29, found: 326.32; IR (KBr, cm^{-1}): 3142 cm^{-1} (=C−H), 1721 cm^{-1} (C=O), 1640 cm^{-1} (O=C−*N*−C=S), 1413, 1324 cm^{-1} (C=S).

(E)-3-(((1H-indol-3-yl)methylene)amino)-2-thioxothiazolidin-4-one [62]: The product ***RhA-8*** was obtained as red solid (84% yield). Mp: 204–205 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 11.09 (bs, NH, 1H), 8.73 (s, N = CH, 1H), 8.37 (d, J = 7.7 Hz, =CH, 1H), 8.04 (d, J = 3.0 Hz, =CH, 1H), 7.55 (d, J = 7.7 Hz, =CH, 1H), 7.30–7.22 (m, =CH, 2H), 4.30 (s, CH_2, 2H); ^{13}C NMR (100 MHz, acetone-d_6): δ 197.3, 170.8, 165.9, 141.3, 135.3, 130.8, 124.4, 123.8, 122.5, 120.2, 112.9, 34.6 (Fig. S8); ESI-MS (m/z) [M] calcd for $C_{12}H_9N_3OS_2$ 275.34, found: 275.38; IR (KBr, cm^{-1}): 3202 cm^{-1} (=C−H), 1718 cm^{-1} (C=O), 1626 cm^{-1} (O=C−*N*−C=S), 1428, 1317 cm^{-1} (C=S).

The synthesis of (Z)-5-((1H-indol-3-yl)methylene)-3-(((E)-(1H-indol-3-yl)methylene)amino)-2-thioxo thiazolidin-4-one (**4**):

Procedure A. To a solution of 1*H*-indole-3-carbaldehyde (**3h**, 489.7 mg, 3.37 mmol) in ethanol (10 mL) was added slowly to the solution of 3-amino-2-thioxothiazolidin-4-one (**2**, 250 mg, 1.69 mmol) in ethanol and was added to acetic acid (2 drops) as a catalyst. The reaction mixture was refluxed overnight, and the mixture was cooled to room temperature. The red product formed was recrystallized from ethanol, filtered, and dried in vacuo. Compound **4** (286 mg, 42%) was obtained as red solid after recrystallization.

Procedure B. To a solution of ***RhA-8*** (300 mg, 1.09 mmol) in ethanol (10 mL) was added slowly a solution of 3-amino-2-thioxothiazolidin-4-one (**2**, 158.2 mg, 1.09 mmol) in ethanol and was added to acetic acid (2 drops) as a catalyst. The reaction mixture was refluxed for 8 h, and was monitored by TLC. After the completion of the reaction, the mixture was cooled to room temperature. The red product formed was recrystallized from ethanol, filtered, and dried in vacuo. After recrystallization, **4** (382 mg, 87%) was obtained as red solid (Mp: > 300 °C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.41 (bs, NH, 1H), 12.08 (bs, NH, 1H), 8.82 (s, N = CH, 1H), 8.22 (d, J = 7.7 Hz, =CH, 1H), 8.14–8.13 (m, =CH, 2H), 8.00–7.97 (m, =CH, 2H), 7.54–7.52 (m, =CH, 2H), 7.31–7.22 (m, =CH, 3H), 5.96 (s, =CH, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 206.9, 171.1, 165.7, 157.0, 147.8, 144.8, 138.2, 137.3, 136.4, 135.6, 135.1, 130.7, 126.7, 124.2, 123.4, 122.3, 121.6, 113.3, 112.6, 111.2, 110.3 (Fig. S9); IR (KBr, cm^{-1}): 3242 cm^{-1} (=C−H), 1729 cm^{-1} (C=O), 1612 cm^{-1} (O=C-*N*-C=S), 1414, 1315 cm^{-1} (C=S); ESI-MS (m/z) [M] calcd for $C_{21}H_{14}N_4OS_2$ 402.49, found: 402.12.

4.3. Biochemical studies

4.3.1. hCA I and II isoenzymes purification and inhibition studies

In this study, hCA I and II isoenzymes were purified from fresh human blood erythrocytes using by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography [63] as described previously [64]. To control the purity of both isoenzymes, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied [65]. The measurement of hCA I and II isoenzymes was investigated by the method of Verpoorte et al. [66]. One enzyme unit of CA esterase activity is defined as hydrolysis of 1 micromol PNA to *p*-nitrophenol and acetate in 1 min [67]. This method is based on the hydrolysis of p-nitrophenylacetate (PNA) to *p*-nitrophenol by CA isoenzymes. The p-nitrophenol has a maximum absorbance at 348 nm using a spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan). Bradford method was used to measure the protein content at 595 nm [68]. Bovine serum albumin (BSA) was used as standard protein. Acetazolamide (AZA) was used as a positive control for hCA I, and II isoenzymes. The IC_{50} values were calculated from activity (%) versus compounds plots. To obtain Ki values, three different concentrations were used. The Lineweaver-Burk graphs were drawn and calculations were realized.

4.3.2. Determination of AChE activity and inhibition studies

AChE activities were measured according to the spectrophotometric method of Ellman et al. [69]. AChE enzyme obtained from *Electrophorus electricus* (C3389) was supplied from market (Sigma Aldrich chemical Company, Germany). 5,5′-dithio-bis(2-nitro-benzoic) acid (DTNB) was utilized for the measurement of the AChE activity. As the substrate of the enzyme AChE in the enzymatic reaction, acetylcholine iodide (AChI) was used. Briefly, 100 μL of buffer solution (1.0 M Tris/HCl, pH 8.0) and different concentration of sample solutions (5–100 μL) dissolved in pure water were added to 15 μL of AChE enzyme solution (5.32×10^{-3} EU). Then 50 μL of DTNB (0.5 mM) was added. Subsequently, the reaction was initiated by the addition of 50 μL of AChI (10 mM). Activity of this enzyme was performed spectrophotometrically at a wavelength of 412 nm. One AChE enzyme unit is the amount of enzyme that hydrolyzes 1.0 micromol of ACh to choline and acetate per minute at pH 8.0 at 37 °C [70].

4.4. Molecular docking studies

Receptor and ligand were prepared using the Small Drug Discovery Suit Package of Maestro 11.9. The x-ray crystal structures of CA I, CA II, and AChE receptors (PDB ID: 4WR7, 5AML, and 4M0E, respectively) were downloaded from RCSB Protein Data Bank. The crystal structures were chosen so that they have high resolution and best percentile rank. The crystal structures were repaired and prepared in three steps. (I) Bond order and charges were assigned and subsequently, all missing hydrogen atoms were attached to the crystal structure. (II) Missing side chains on the structure were filled. (III) amino acids were ionized at pH 7.0 ± 2.0, water molecules that were formed less than 3 contacts with the protein or ligand were removed and energy was minimized. 2D structures the most active compounds were sketched and following their 3D structures were created with Maestro 11.9. Their correct molecular geometries and protonation state at 7.0 ± 2.0 were prepared [47,59,71].

Potential binding site of the receptors was predicted and Sitescore and Dscore of the binding site were calculated using SiteMap module at Maestro 11.9. Catalytic active site properties of the binding site were designated using these score [46,59,72]. ADME analysis was carried out using QikProp module in Maestro11.9. The analysis provides information about pharmacokinetic properties including absorption,

distribution, metabolism, and excretion of the compounds. The drug-likeness of the compounds was evaluated on basis of Lipinski's rule of five (mol MW, logPo/w, donorHB, andaccptHB) [46,73,74].

Docking studies were carried out at three steps using Induced Fit Docking module at Maestro 11.9. The same module was used for the accuracy of the docking process. (I) The centroid box was generated around the selected co-crystallized ligand in the catalytic site. (II) The side chains were automatically trimmed based on B-factor. (III) The closest residues to the ligand were refined within 3.4 Å of ligand pose. The accuracy of the docking process was tested by re docking co-crystallized ligand into the active site of the receptors [46,59,75].

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103096.

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