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Acetylcholinesterase Inhibition Activity and Molecular Docking Studies of 3-α-Carboxy Ethyl/3-Benzamidoacetic Acid Rhodanine Derivatives

Sulaiman Shameema [1], Senthilkumaran Dhanapal [1], Mohan Hariharan [1], Chinna Senrayan Nandha Kumar [1], Arjunan Siva Muthu [1], Senniyappan Venkatachalapathi [2], Kaveri Sundaram [1,*]

[1] Department of Chemistry, Karpagam Academy of Higher Education, Coimbatore-641 021, Tamil Nadu, India
[2] Department of Chemistry, MP Nachimuthu M Jaganathan Engineering College, Erode-638 112, Tamil Nadu, India
* Correspondence: sundarg2010@gmail.com;

Scopus Author ID 55736140700

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Abstract: Based on the broad spectrum of therapeutic activities of rhodanine, we propose a new acetylcholinesterase (AChE) inhibitor having 3-α-carboxy ethyl/3-benzamide acetic acid-5-benzylidene rhodanine structural scaffold. Most of the tested compounds exhibited better inhibition activity against AchE compared with rivastigmine. Among them, 3f exhibited a potential AChE inhibitory activity with an IC_{50} value of 27.29 μM compared to rivastigmine with an IC_{50} value of 54.37 μM. Molecular docking studies were carried out to explore the binding modes of all compounds into the active site of acetylcholinesterase in order to rationalize the inhibitory efficacy of these derivatives. The structural-activity relationship was made based on the compounds' IC_{50} value and molecular docking studies. Electron-withdrawing groups in the benzene ring fare well when compared with the electron-donating groups. The results of the present study suggested that this new type of rhodanine could serve as a basis for developing new AChE inhibitors.

Keywords: rhodanine, acetylcholinesterase; rivastigmine; molecular ocking.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative syndrome and the greatest dominant form of dementia found to be increasing among older adults. Cholinergic system dysfunction, loss of cognitive function, and enhanced aggregation of β-amyloid peptides (Aβ) are the hallmarks of AD [1,2]. The exact cause of AD remains unexplored, but the above elements offer a base for the cholinergic and amyloid hypotheses for AD pathology, respectively. Based on the cholinergic hypothesis, the memory and cognitive signs of Alzheimer's disease are due to the paucity of acetylcholine in the brain caused by decreased production or increased activity of acetylcholinesterase (AChE) [3,4]. The amyloid peptide hypothesis characteristics of the genesis of AD for the enhanced aggregation of the Aβ peptide in the brain results in the death of neuronal cells and, finally, dementia [5].

Furthermore, aggregation of Aβ has been associated with increased plague deposition [6]. For the past ten years, enormous interest has been shown in developing inhibitors of tau and other tauopathies [7]. The recent report of a phase II clinical trial with the tau aggregation inhibitor MTC could hold promise for validating the concept. Therefore, preventing elevated

AChE activity in the brain is a potential therapy for AD. The present treatment for AD emphasizes the increase of cholinergic neurotransmission by diminishing AChE using inhibitors, for example, donepezil, rivastigmine, and galantamine, which have been shown to improve symptoms [8]. However, these drugs showed many side effects. Therefore, it is essential to identify new drugs for the suppression of AchE [9-11]. Since the introduction of epalrestat into clinical use for treating diabetic complications, rhodanine has become a well-recognized therapeutic class of compounds. A long-term clinical study with epalrestat shows that the structure can be bioavailable and well-tolerated [12]. In a more positive perspective, rhodanine derivatives can be considered desired compounds with tunable target affinity and selectivity in the optimization steps. Bulic et al. have described a series of 5-arylidene-rhodanine-3-acetic acids as tau aggregation inhibitors potentially useful in managing Alzheimer's disease and related dementias [13]. Martin Kratky et al. reported that compounds containing the rhodanine moiety recorded potent AChE inhibitory activity [14]. Recently, a series of 5-benzylidenerhodanine-3-acetamides having morpholino-, 4-benzylpiperidinyl-, or 4-arylpiperazinyl- moieties were synthesized, and their inhibitory activities against acetylcholinesterase (AChE) were evaluated [15]. This led us to concentrate on the synthesis and inhibition of AChE of the 3-α-carboxy ethyl/3-benzamide acetic acid rhodanine derivatives.

2. Materials and Methods

2.1. Synthesis.

All the reactions were performed in oven-dried flasks. The values of the melting point of the synthesized compounds are uncorrected and obtained using an XT-5 digital melting point instrument. The Infrared spectroscopy data was obtained from a Shimadzu spectrometer (360 FT-IR). The ^{1}H and ^{13}C NMR spectral information were estimated at 400 and 125 MHz, individually on a Bruker-400 spectrometer utilizing TMS as internal standard and DMSO as dissolvable. Elemental analysis were determined by a PerkinElmer 240C elemental analyzer.

2.2. General procedure for the synthesis of compounds 3a–l.

The compound 3-α-carboxy ethyl rhodanine (I) was synthesized using the previously published procedure [16]. The compound 3-a-carboxy ethyl rhodanine (I) (0.003 mol) and substituted benzaldehydes (2a–l) (0.003 mol) were refluxed with anhydrous sodium acetate (0.003 mol) in glacial acidic for 4–6 h. After the completion of the reaction, which TLC checks, it was cooled, and the resulting mass was filtered, washed with water, dried, and recrystallized (ethanol) to afford the corresponding product (3a–l).

2.3. General procedure for the synthesis of compounds 3o-q.

The compound 3o-q was synthesized using the previously published procedure [17]. The compound rhodanine-3-hippuric acid (3n) (0.003 mol) and substituted benzaldehydes (2o-q) (0.003 mol) were refluxed with anhydrous sodium acetate (0.003 mol) in glacial acidic for 4–6 h. After the completion of the reaction, which TLC checks, it was cooled, and the resulting mass was filtered, washed with water, dried, and recrystallized (ethanol) to afford the corresponding product (3o-q).

2.4. Acetylcholine esterase inhibition assay.

In the AChE inhibition assays, ethanol (Merck) was used as the solvent. Acetylcholinesterase, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholine iodide, phosphate buffer, and galantamine hydrobromide (Sigma-Aldrich) were used in the AChE inhibition assay. The inhibitory effect of AchE on pure compounds was determined. The test solution contained 192 µL of acetylthiocholine iodide (AChI) as substrate, 240 µL (1.25 mM) of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), 1200 µL of phosphate buffer pH 8 in a test tube and 120 µL of pure test solution in ethanol. The blank solution was made by preferring 120 µl of buffer solution instead of the galantamine hydrobromide to act as a positive control. The reaction was initiated by adding 0.0325 µ/ml of AChE to the reaction mixture. The reaction was recorded at the wavelength of 412 nm using a UV-Vis spectrophotometer. The AChE activity was calculated as the percentage of inhibition following the equation.

$$Percentage\ of\ inhibition\ = \frac{Absorbance\ (control) - Absorbance\ (test)}{Absorbance\ (control)} \times 100$$

The results were given as mean ± standard deviation of three parallel experiments.

2.5. Protein preparation.

The 3D structure of the objective protein was recovered from the protein data bank (PDB ID: 1EVE). The main form of the Torpedo California AChE is a homodimer, covalently bound through the phosphatidylinositol group with the plasma membrane. AChE belongs to the α/β class protein. It also contains two active, esthetic, and anionic subsites. The protein structure obtained was prepared by removing water molecules for molecular coupling from their three-dimensional structure. The energy of the target protein was minimized before performing the docking simulations to describe the interaction *in vivo*.

2.6. Ligand preparation.

Two-dimensional structures (2D) of ligands were drawn using the ChemDraw Ultra 8.0 (Chem Office 2002). The 2D structures of the ligands were converted into 3D structures using Discovery Studio 4.5, and the energy minimization was carried out by the semi-empirical AM1 method.

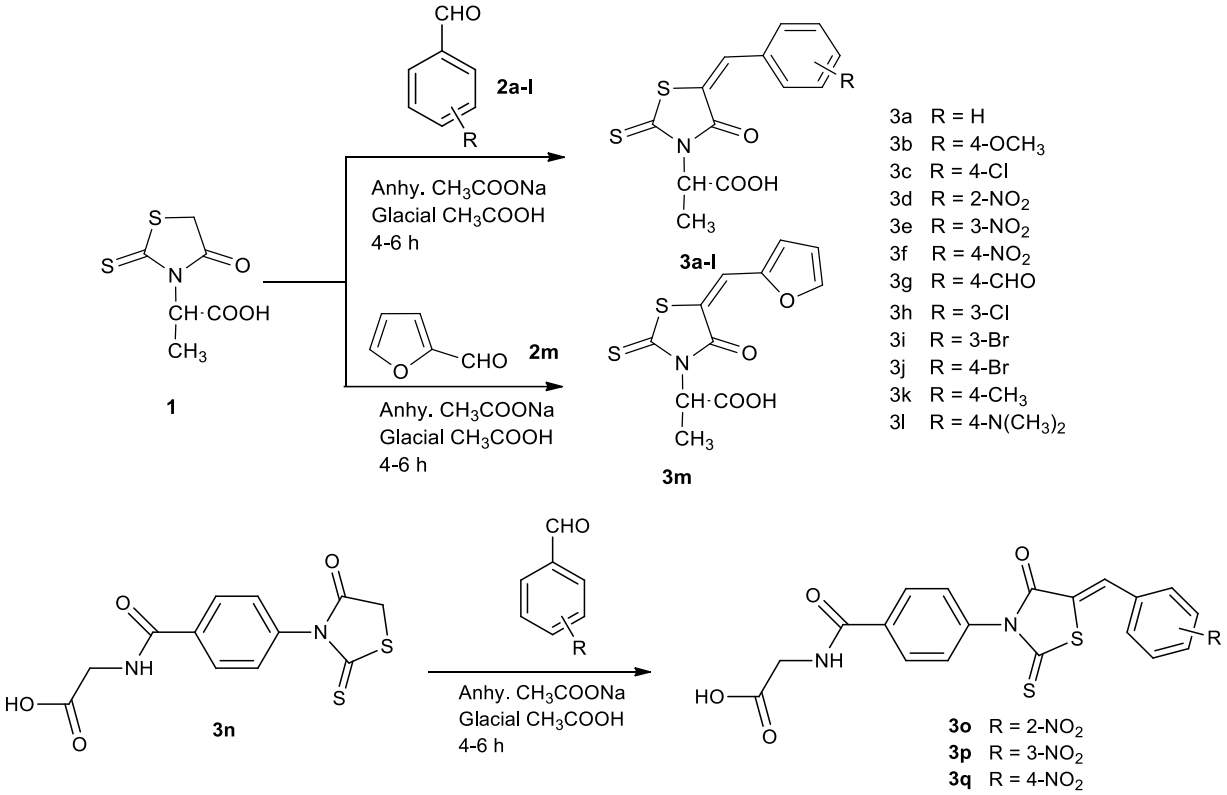
2.7. Docking protocol.

To examine the molecular-based interaction and affinity of the selected compounds to the target AChE protein, all the ligands were docked with the active site of the AChE. A bibliography survey identified the active sites of the target protein. The molecular docking studies were performed using AutoDock version 4.2. The best-docked structure was selected using docking score and hydrogen bond. The docking score has a negative regression coefficient towards the binding affinity. The outcomes were exported to Chimera 1.10, and a visual investigation of the binding interactions and modes of the compound with amino acid residues in the active sites was carried out using Discovery Studio 4.5.

3. Results and Discussion

3.1. Chemistry.

The 3-α-carboxy ethyl rhodanine was synthesized by dl-alanine and carbon disulfide reaction, as shown in Scheme 1. Knoevenagel condensation (Scheme 1) of 3-α-carboxy ethyl rhodanine with different substituted aromatic aldehydes yields compounds 3a-h. The structure of the synthesized compounds was elucidated by comparing the UV, IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analyses with literature [18]. The compounds 3a-g and 3o-q have a benzylidene-rhodanine moiety, and the compound 3m has a furan moiety.



Scheme 1. Synthesis of compounds 3a-q.

3.2. In vitro inhibition of acetylcholinesterases.

All the synthesized rhodanines were tested against AChE using the Ellman method [19]. The inhibition of the compounds was expressed as IC_{50} values, expressing the concentration of the substance required for 50% inhibition of the AChE enzyme. The obtained results were compared with the results of the standard drug (rivastigmine). The experiment was repeated three times. The diversity of the compounds 3a-l and 3o-q was made by introducing various substituents (-OCH_3, Cl, Br, $N(CH_3)_2$, CH_3, 2-NO_2, and CHO) in various positions of the benzene ring. Substituents at various locations on the cyclic or heterocyclic rings of biologically active compounds vary in electron thickness and interaction between the ligand and receptor, thereby altering the potential of the activity of the drug [18]. The IC_{50} values of the synthesized compounds with AChE enzyme were within the range between 27.29 – 112.58 µM (Figure 1).

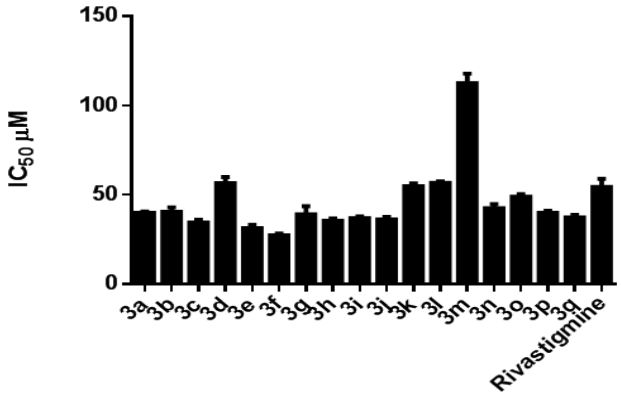


Figure 1. AChE inhibition of the synthesized compounds is expressed as the mean±SD (n=3 experiments).

Most of the compounds showed fairly good activity compared to that of the standard drug rivastigmine, as shown in Table 1. Compound 3f has exhibited the most effective AChE inhibition with an IC_{50} value of 27.39 µM. On the other hand, compound 3 m is identified to be the least effective inhibition with an IC_{50} value of 112.58 µM. This variation is attributed to greater lipophilicity, lack of hydrogen, and a slightly lower molar refractory or combination.

Table 1. AChE inhibition of the synthesized compounds is expressed as the mean ± SD (n=3 experiments).

Compound	R	IC_{50} value (µM)
3a	H	39.76±0.78
3b	4-OCH_3	40.44±2.45
3c	4-Cl	34.44±1.45
3d	2-NO_2	56.44±3.45
3e	3-NO_2	31.33±1.89
3f	4-NO_2	27.29±1.08
3g	4-CHO	38.97±4.61
3h	3-Cl	35.46±1.21
3i	3-Br	36.82±0.97
3j	4-Br	34.23±1.36
3k	4-CH_3	54.87±1.48
3l	4-$N(CH_3)_2$	56.74±0.87
3m	Furan	112.58±5.33
3n	-	42.47±2.12
3o	2-NO_2	48.92±1.43
3p	3-NO_2	39.86±1.17
3q	4-NO_2	37.34±1.31
Rivastigmine	-	54.37±4.52

Concerning the benzene ring substitution, in general, introducing chlorine, nitro, methoxy, or aldehyde moiety in the phenyl ring significantly influences the activity on the positive side compared with the original unsubstituted compound. The compound 3c with the 4-NO_2 group possesses good inhibition when compared with other substituents OCH_3, Cl, Br, $N(CH_3)_2$, CH_3, CHO, and furan). With respect to the structure-activity relationship between the synthesized compounds, the following observations were made. Notably, the compounds with the 3-α-carboxy ethyl group in the nitrogen atom of the rhodanine moiety fare well when compared with the compounds with hippuric acid moiety. This is attributed to the bulkier size of the hippuric acid moiety when compared with the 3-α-carboxy ethyl group. Also, irrespective of the substituent attached to the nitrogen atom of the rhodanine moiety, whether it is the 3-α-carboxy ethyl group as in compounds 3d-f or hippuric acid group as in compounds 3o-q, the nitro group in the para position 3f and 3q fares well when compared to the substitution of nitro group in the meta position 3e, 3p or ortho position 3d, 3o. Similarly, between the

compounds with meta-substituted –Cl and -Br groups and para-substituted –Cl and -Br groups, the compounds with para substitution 3c and 3j showed higher activity than the compounds with meta substitution 3h and 3i. Further compounds with electron-withdrawing substituents have inhibited the enzyme well compared to those with electron-donating substituents 3b, 3k, and 3l.

3.3. Docking studies.

In cholinergic synapses, AChE is the enzyme known for the quickened hydrolysis of the neurotransmitter acetylcholine (ACh). Medicinally, this has been directed in the treatment of Alzheimer's disease. Acetylcholinesterase inhibitors have been accepted for the suggestive treatment of Alzheimer's sickness by preventing the hydrolysis of acetylcholine for over 10 years. The X-ray crystallographic structure of AChE (PDB id: 1EVE) showed that the active site of the receptor is a catalytic triad and possesses the residues Ser200, Glu327, and His440, an active site of the peripheral anionic site having Trp279 and some aromatic residues such as Trp84 and Phe330 of the hydrophobic site [20, 21].

The binding affinity of the compounds examined experimentally was studied by molecular coupling studies using AutoDock 4.2 [22]. A molecular docking study was carried out using the crystal structure of X-ray AChE (code PDB 1EVE) [23]. The observed binding energy is depicted in Table 2.

Table 2. Docking score (kcal/mol) and hydrogen bonding of the synthesized compounds with AChE protein (1EVE).

Compound	Interaction with amino acids	Binding affinity
3a	Gly118, Ser200, His440	-9.0
3b	Gly118, Ser200, His440	-9.0
3c	Tyr121, Ser122	-9.4
3d	Gly118,Gly118, Ser200	-8.9
3e	Gly118,Gly118, Ser200, His440	-9.2
3f	Ser200, His440, Gly113, Gly113, Glu199	-9.3
3g	Gly118, Ser200, His440	-9.2
3h	Gly118, Ser200, His440	-8.9
3i	Gly118, Ser200, His440	-8.6
3j	Tyr121, Ser122	-9.1
3k	Gly118,Gly118, Ser200	-8.6
3l	Gly118,Gly118, Ser200, His440	-9.0
3m	Gly118, Ser200, His440	-8.7
3n	Gly118,Gly118, Ser200, His440	-8.4
3o	Gly118,Gly118, Ser200	-8.5
3p	Gly118,Gly118, Ser200, His440	-8.8
3q	Ser200, His440, Gly113, Gly113, Glu199	-9.1

Three-dimensional (3D) modeled molecular surfaces of compounds 3c and 3f into the binding site of 1EVE are shown in Figures 2 and 3.

Visual analysis of the lowest energy docked position of compound 3c (IC_{50} = 27.29 μM) showed the formation of two hydrogen bonds with Tyr121 and Ser122. Tyr334 showed three different interactions, namely, pi-pi stacked interaction with the rhodanine ring, pi-sulfur interaction with the sulfur atom of the thiocarbonyl group present in the rhodanine moiety and pi-sigma interaction with the methylene group attached to the rhodanine ring. In addition, the pi-pi stacked interaction with the hydrophobic site residue Phe330 and the interaction of halogen with Arg289 are also observed.

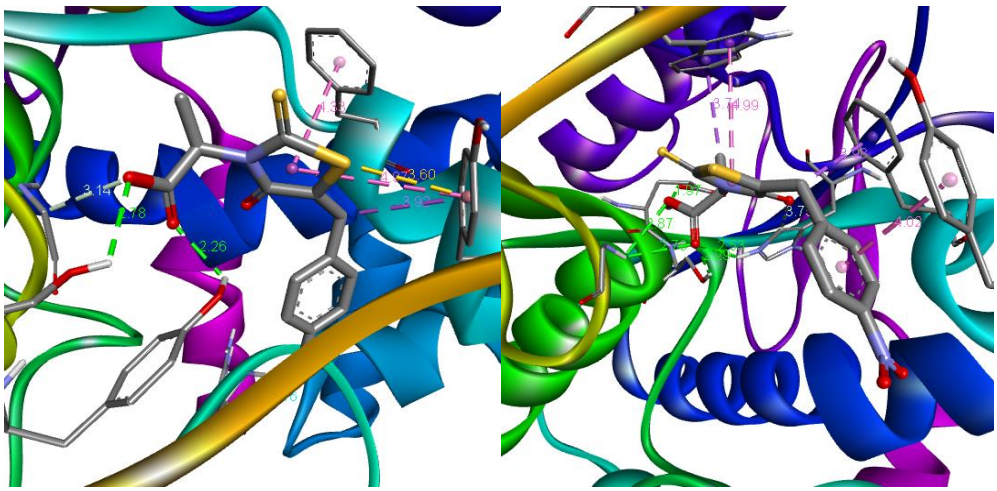


Figure 2. 3D binding interaction of the best scoring compound 3c and 3f with protein (1EVE).

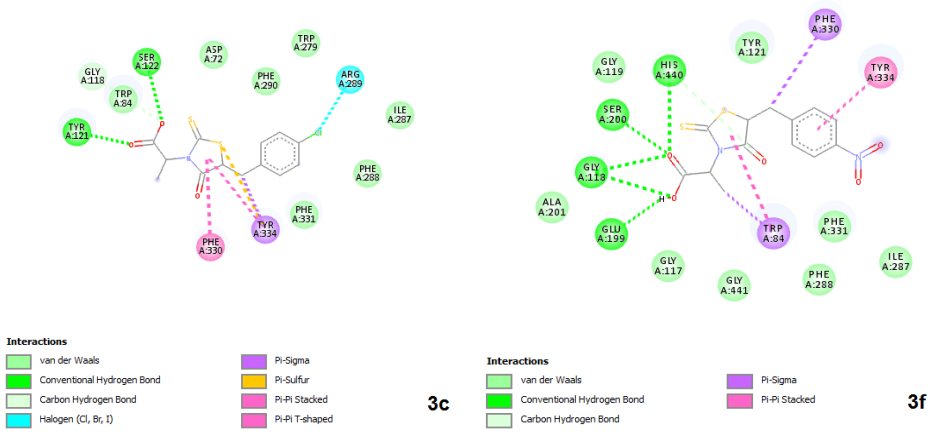


Figure 3. 2D binding interaction of the best scoring compound 3c and 3f with protein (1EVE).

The second lowest coupled energy of compound 3f (IC_{50} = 30.44 ± 1.45) showed that the carboxyl group of the rhodanine moiety interacts strongly by forming hydrogen bonds with the Ser200 and His440 residues present in the active site of the catalytic triad and also formed two more hydrogen bonds with Gly113 and one with Glu199. The benzylidine group establishes another important pi-sigma interaction with Phe330 of the hydrophobic active site. Another hydrophobic active site residue, Trp84, showed two types of interactions, namely pi-pi stacked interaction with methyl group in rhodanine residue and pi-sigma interaction with rhodanine ring.

Most of the substituents, like nitro CHO, are strongly electron-withdrawing in nature and alter the molecular structure by conjugation. However, the halogen compounds don‘t enter into conjugation and distribute the electron at different sites compared to other substituents. The 4-nitro substituent induced a uniformly more potent inhibition of AChE than all the other substituents. The distribution of electrons in compound 3f is more suitable for forming hydrogen bonds, so compound 3f had the lowest docking score with the 1EVE protein. In addition, this has been further confirmed by the in vitro studies against AChE, and the IC_{50} value is 27.29 µM.

4. Conclusion

Seventeen N-substituted-5-benzylidene rhodanines (3a-q) were synthesized with satisfactory yield and screened for their *in vitro* AChE inhibitory activity. Compound 3c showed more potent acetylcholinesterase inhibition activity. However, all the compounds exhibited comparable acetylcholinesterase inhibition activity. The structure-activity relationships were performed for the synthesized compounds. The 4-nitro substituent induced uniformly more potent inhibition of the AChE enzyme than the corresponding chloro and other substituents. This paper shows a significant scope for further developing AChE inhibitors with rhodanine moiety.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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