Anticholinesterase Activity Screening of some Novel Dithiocarbamate Derivatives Including Piperidine and Piperazine Moieties

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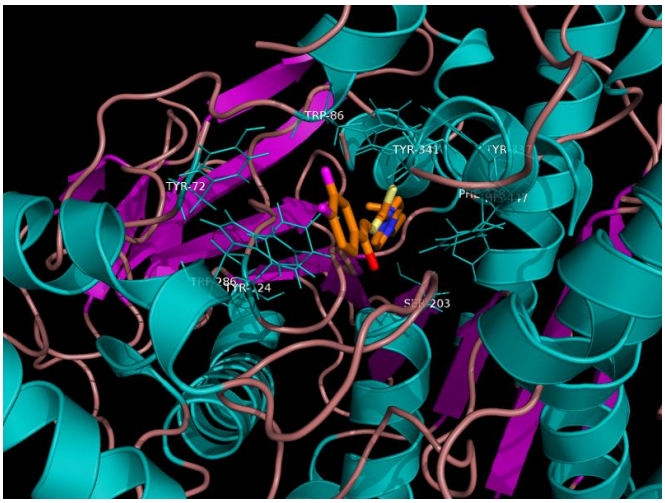
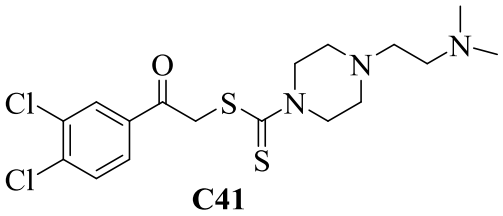
Abstract

The present study was undertaken to synthesize some novel lipophilic piperazine and piperidine dithiocarbamates and investigate their inhibitory potencies against cholinesterase enzymes. In the synthetic studies, 44 new compounds were isolated. The structures of the synthesized compounds were confirmed by spectroscopic analyses. Enzymatic studies were carried out using modified Ellman's assay against Acetylcholinesterase (AChE) and Butrylcholinesterase (BChE) enzymes, and it was observed that some of the compounds selectively inhibit AChE. Theoretical ADME predictions were calculated for selected compounds in the series. Enzyme kinetics and molecular

docking studies were performed for the most active compound C41 and nature of inhibition and interactions between enzyme and ligand were explained.





C41

Keywords

Alzheimer’s disease; Dithiocarbamate; Piperazine; Piperidine; AChE; BChE

Introduction

Alzheimer's disease (AD) is a chronic, irreversible, neurodegenerative disorder characterized by a progressive deterioration of intellectual functions, including memory, language, visuospatial skills, problem-solving ability, basic activities of daily living and ultimately causing death [1-3]. It is connected with a selective loss of cholinergic neurons, which occurs due to various neuropathological conditions such as amyloid plaques, neurofibrillary tangles and reduced levels of acetylcholine neurotransmitter [4]. One rational way to treat the AD's symptoms, is raising the ACh through the inhibition of acetylcholinesterase (AChE) that is responsible for hydrolysis of ACh in pre-synaptic areas [5,6]. Many efforts have been spent in the search for potent AChE inhibitors and four AChE inhibitors belonging to different chemical groups have been developed for the symptomatic treatment of mild to moderate stages of AD. These are tacrine [7], donepezil [8], rivastigmine [9] and galantamine [10], which have been approved by FDA [11, 12].

In the development of new anticholinesterase agents, carbamate is one of the most used moieties[13, 14]. The major reason for this approach is rivastigmine, a carbamate based cholinesterase inhibitor used widely for the treatment of AD. Dithiocarbamate, an isoster of carbamate, is also an important pharmacophore for anticholinesterase agents [15,16]. Replacement of carbamate with dithiocarbamate increases the lipophilicity, which is crucial for the delivery of central nervous system drugs to their site of action through the blood-brain barrier [17-19].

Functionalized piperidine scaffolds are found to form a very crucial core in many natural products, synthetic pharmaceuticals, and a wide variety of biologically active compounds [20,21]. Many researchers have synthesized piperidine-carrying compounds and investigated them for their anti-Alzheimer potential [22-28]. The chemical structure of donepezil, an important and widely

used drug for the treatment of AD patients in neurology clinics, is also based on a 1,4-disubstituted piperidine ring. Like piperidine, its bioisoster, piperazine has as well as been subjected to the development of novel anticholinesterase agents [29-38].

In the light of above knowledge, we herein introduce new compounds, which include a dithiocarbamate moiety along with piperidine/piperazine ring to assess their potential against cholinesterases.

Results and discussion

Chemistry

In the present study, some piperidine or piperazine-dithiocarbamate compounds were synthesized and evaluated for their inhibitory potency against cholinesterase enzymes [39,40]. The target compounds were gained in three reaction steps. Sodium dithiocarbamates (**A1-A11**) were prepared by the reaction of carbon disulfide and cyclic secondary amine in the presence of sodium hydroxide [41]. Secondly, halogenated derivatives (**B1-B4**) were synthesized via acetylation of anilines by chloroacetyl chloride to obtain the compounds **B1-B3** and bromination of 3,4-dichloroacetophenone gave the 2-bromo-3',4'-acetophenone (**B4**) [42,43]. Finally, the compounds synthesized in initial steps were reacted in acetone to achieve target compounds (**C1-C44**). The synthetic route of the compounds was outlined in Scheme 1.

[Insert Scheme 1]

The structures of the obtained compounds were elucidated from their spectral data. In the IR spectra, significant stretching bands belonging to N-H were observed at 3225-3437 cm^{-1}. The stretching bands for C=O and C=S were observed between 1625-1695 cm^{-1} and 1200-1246 cm^{-1}. In the ^{1}H-NMR spectra, methylene protons between carbonyl and dithioate group were recorded

as a singlet peak between 4.20-4.93 ppm. Trisubstituted benzene peaks were observed as two doublets and one doublets of the doublet with varying shifting values depending on the substituted group. Amide protons on the dihalogenated and dimethoxy substituted derivatives gave a singlet peak between 10.51-10.80 ppm and 10.18-10.21 ppm, respectively. In the ^{13}C-NMR spectra, all compounds have thiocarbonyl peaks between 192 and 198 ppm. While carbonyl peaks for compound **C1-C33** was recorded at 162 ppm -168 ppm, peaks of **C34-C44** observed between 190 ppm and 193 ppm. All other aromatic and aliphatic protons and carbons were recorded on near to the expected values. HRMS was also performed, and all measured mass and isotope scores were compatible with calculated values.

Cholinesterase Inhibitory Activity

The synthesized compounds **C1-C44** were assessed as AChE and BChE inhibitors by using in vitro modified Ellman's spectrophotometric method [44]. Donepezil was used as the reference drug.

Generally, it was understood that the synthesized compounds have more potent inhibitory activity against AChE enzyme as regards BChE enzyme. The compounds **C8**, **C9**, **C19**, **C30**, **C31**, **C41** and **C42** exhibited significant inhibition profiles against AChE enzyme at 10^{-3} M concentration (Table 1). On the other hand, none of the compounds displayed remarkable inhibitor activity on BChE enzyme. Compound **C41** was the most active compound against BChE with a 22.67 % inhibition potency at 10^{-3} M concentration (data not shown). These results show that the synthesized compounds are selective AChE inhibitors.

By looking at the inhibition potency (>50%) at 10^{-3} M, the compounds **C8, C9, C19, C30, C31, C41** and **C42** were investigated in further concentrations (10^{-5}-10^{-9} M) to calculate IC_{50} values

(Table S 1 Supplemental Materials). According to enzyme inhibition studies, compound **C41** is the most active derivative due to its inhibitory profile on AChE with an IC_{50} value of 11.82 µM. Enzyme inhibition results gave a chance to evaluate the structure activity relationships. Once the structures of synthesized compounds were examined, it was striking that dimethylaminoethyl and dimethylaminopropyl substituents on 4th position of piperazine ring have a great impact on enzyme inhibitory activity.

Kinetics Study

The kinetics of this series of AChE agents were studied in detail using the most effective compound **C41**. The nature of AChE inhibition, caused by this compound, was evaluated by the graphical analysis of steady-state inhibition data. Lineweaver - Burk plots determined the compound **C41** as a mix-typed inhibitor, because of the different intercepts on both y- and x-axes (Figure 1). The values of K_m and V_{max} were investigated by nonlinear regression were calculated as 6.6869 and 0.2853, respectively.

[Insert Figure 1]

Theoretical Determination of ADME Properties

The theoretical prediction of ADME properties (molecular weight, log P, TPSA, number of hydrogen donors and acceptors, volume) for the active compounds **C8**, **C9**, **C19**, **C30**, **C31**, **C41** and **C42** were determined and presented in Table S 2 (Supplemental Materials) along with violations of Lipinski's rule [45]. This rule suggests that, an orally active drug has no more than one violation. As seen in Table S 2, all calculated physicochemical parameters for the compounds are compatible with Lipinski's rule [45]. Furthermore, the most active compound **C41** has an ideal lipophilic character (logP=3.63) which is required to cross the central nervous

system (CNS) [46]. Besides, TPSA, described to be a predictive indicator of membrane penetration, is positive (26.79) and as AChE inhibitors have to pass different membranes and reach the CNS, this supports the potential of compound **C41**.

Molecular Docking

In order to designate the binding modes between the ligand and the receptor, docking studies were carried out using the X-ray crystal structure of *Homo sapiens* AChE (*h*AChE PDB ID: 4EY7), which is very similar to Electrophorus electricus AChE *(EeAChE)* include E2020 as ligand [47]. The compound **C41** was docked into the active site of *h*AChE. To identify the most likely interaction with the receptor, low energy docked coordinates were selected.

The best docking poses, showing interactions with the active site, are viewed in Figure S1. When the docking studies are analyzed, it is clearly understood that the compound **C41** is very compatible with the pocket of the active site. It interacts with the amino acids, Phe295, Tyr72, Tyr337, Trp86, His447, Ser203, Glu202. Compound **C41** settles down formation of H bond between the oxygen atom of the carbonyl group and the amino group of the Phe295 residue. The formation of π-π interactions between piperazine moiety and phenyl ring of Tyr337 amino acid is also sighted in the gorge. Due to the formation of H bond between aliphatic nitrogen atom and the carboxyl group of Glu202 residue, there are increased interactions with the active site. The ethyl group, among the rest of the piperazine moiety and aliphatic nitrogen atom sets up van der Waals interactions with the amino acids of the binding side. These interactions equipoise the ligand in the active site of the enzyme.

Conclusion

In the present study, cholinesterase inhibitory potency of some 44 novel piperazine and piperidine dithiocarbamate derivatives investigated. 2-(4-(2-Dimethylaminoethyl)piperazin-1-yl-dithiocarbamoyl)-3',4'-dichloroacetophenone (**C41**) was found as the most active compound AChE with a IC_{50} value of 11.82 µM. Enzyme kinetic studies revealed this compound as a mixed type inhibitor. Molecular modeling studies indicated the importance of 4-(2-dimethylaminoethyl) and ketonic carbonyl for interaction between AChE and ligand. Consequently, findings of this study may have an impact on chemists to synthesize similar compounds that may have higher potency against cholinesterase enzymes.

Experimental

Chemistry

The chemicals used in the syntheses were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points of the synthesized compounds were recorded on a MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were uncorrected. ^{1}H NMR and ^{13}C NMR spectrums were recorded on a Bruker 500 MHz digital FT-NMR spectrometer and Bruker Fourier 300 (Bruker Bioscience, Billerica, MA, USA) respectively, in DMSO-$d_{6.}$ Operating frequencies of the experiments were 500 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. The IR spectra was obtained on a Shimadzu, IR Prestige-21 (Shimadzu, Tokyo, Japan). LCMS studies were performed on Shimadzu LCMS-IT-TOF (Shimadzu, Tokyo, Japan). The purities of compounds were checked by TLC on silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany).

Materials and methods

*General procedure for the synthesis of sodium dithiocarbamate derivatives **A1-A11***

Substituted piperazine or piperidine derivative (15 mmol) was dissolved in EtOH (200 mL), and sodium hydroxide (15 mmol) was added. The mixture was cooled in an ice bath, and CS_2 (150 mmol) was added in portions. The reaction was allowed to stir for 1 h at room temperature. The solvent and excess of CS_2 were removed under reduced pressure [48]. The residue was washed with dry ether, and the raw product was recrystallized from ethanol.

General procedure for the synthesis of 2-chloro-N-(3,4-disubstitutedphenyl)acetamide derivatives ***B1-B3***

The appropriate 3,4-disubstituted aniline (50 mmol) and TEA (60 mmol, 8.45 mL) in THF (150 mL) were mixed on ice bath. Chloroacetyl chloride (60 mmol, 4,81 mL) in THF (20 mL) was added dropwise to this solution. After completion, the reaction mixture was stirred at room temperature for 1 h. The precipitated product was filtered washed with water, dried and then recrystallized from ethanol.

Synthesis of 3',4'-dichloro-2-bromo acetophenone ***B4***

3,4-Dichloroacetophenone (50 mmol, 9.45 g) was dissolved in AcOH (250 mL) and catalytic amount of HBr was added. This solution was placed on an ice bath and bromine (60 mmol, 3,09 mL) was added dropwise. The reaction was routinely checked by TLC. After completion of reaction, the mixture was poured into the iced-water, precipitated products was filtered, washed with water, dried and then recrystallized from ethanol.

General synthesis procedure for target compounds

The corresponding sodium dithiocarbamate derivative (**A1-A11**) (5 mmol) and halogenated compound (**B1-B4**) (5 mmol) were dissolved in acetone and refluxed for 2 h. After TLC control, the solvent was evaporated. The residue was washed with water, dried and then recrystallized

from ethanol to afford final compounds (**C1-C44**). Complete characterization data for C2-C44 are presented in the Supplemental Materials and representative ^{1}H NMR spectra are given in Figures S 1 – S 2.

2-(*4-Methylpiperazin-1-yl-dithiocarbamoyl)-N-(3,4-dimethoxyphenyl)acetamide* ***C1***

Yield: 79 %, M.P. = 110.4 - 111.8 °C, FTIR (ATR, cm^{-1}): 3256 (N-H), 1663 (C=O), 1229 (C=S), 1051, 843, 729. ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 2.23 (3H, s, $–CH_3$), 3.08-3.14 (4H, m, piperazine $–CH_2$-), 3.72 (6H, s, $–OCH_3$), 4.08 (2H, s, piperazine $–CH_2$-), 4.28 (2H, s, $–SCH_2$-), 4.61 (2H, s, piperazine $–CH_2$-), 6.89 (1H, d, *J*=8.50 Hz, aromatic –CH-), 7.10 (1H, d, *J*=8.50 Hz, aromatic –CH-), 7.29 (1H, s, aromatic –CH-), 10.18 (1H, s,–NH-). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 45.2, 48.6, 50.1, 51.8, 52.4, 55.7, 56.3, 105.1, 111.5, 113.3, 132.4, 136.6, 144.3, 149.9, 166.2, 195.8. HRMS (m/z): $[M+H]^+$ calcd for $C_{16}H_{23}N_3O_3S_2$: 370.1254; found 370.1245.

Enzyme Inhibition Assay

Inhibition potency of the compounds against AChE and BuChE has been determined using Ellman's method [44]. Enzyme solutions were prepared in gelatin solution (1%), at a concentration of 2.5 units/mL. Synthesized compounds and donepezil were prepared at 10^{-3} M and 10^{-4} M concentrations using 2% DMSO. AChE or BuChE solution (20µL/well) and compound solution (20µL/well) were added to phosphate buffer (140 µL/well, pH 8± 0.1) and incubated at 25°C for 5 min. The reaction was started by adding the chromogenic reagent 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) (20 µL/well, 10 mM) and the substrates acetylthiocholine iodine (ATCI) or butrylthiocholine iodine (BTCI) (10 µL/well, 75 mM) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor was processed. Control and inhibitor readings were

corrected with blank-reading. All processes were assayed in four independent wells. The same procedure was followed for further concentrations (10^{-5}-10^{-9} M, 20 µL/well) of donepezil and selected compounds indicating ≥50% inhibition at the concentration of 10^{-3} M. The IC_{50} value was calculated from the plots of enzyme activity against concentrations by applying regression analyses on GraphPad Prism Version 5.

Enzyme Kinetics

The same materials were used in the cholinesterase inhibition assay. The compound **C41** was prepared at IC_{50} concentration that was calculated in enzyme assay and then added to the wells (20 µL/well). AChE was added to the plate (20 µL/well) and enzyme inhibitor mixture was incubated at 25°C for 5 min. The reaction was started by adding DTNB (20 µL/well) and the various concentrations (150, 75, 37.5, 18.75, 9.375, 4.6875, 2.3437, 1.1718, 0.5859 and 0.2929 µM) of substrate (ATCI) (10 µL/well). The production of the yellow anion was recorded for 10 min at 412 nm. A parallel control without inhibitor was used for comparison. All processes were assayed in four independent wells. The results were analyzed as Lineweaver-Burk plots using Microsoft Office Excel 2013.

Theoretical Calculation of ADME Parameters

In order to evaluate pharmacokinetic profiles (ADME) of the active compounds (**C8**, **C9**, **C19**, **C30**, **C31**, **C41** and **C42**), some physicochemical parameters were predicted using the Molinspiration property calculation program.

Molecular Docking

Docking studies were performed using Autodock Vina [49]. The coordinates of *Homo sapiens* AChE (*h*AChE PDB ID: 4EY7) were obtained from the Protein Data Bank (PDB) [50]. For

docking studies premier protein was prepared by removing all non-protein molecules, including water, ions, any co-crystallized solvent and ligands. Autodock Tolls (ADT, version 1.5.6) [51], was used to prepare the ligand and the receptor, which were saved in pdbqt format and also to add hydrogens and fractional changes for protein and ligands. Autodock Vina was used to dock the ligand into the active site of hAChE. The parameters of the docking were formed as follows: center_x=3.102, center_y=-40.295, center_z=30.604, size_x=60, size_y=72, size_z=74. Docking of the compound **C41** was performed in a limited grid box described by Autodock tools (ADT, version 1.5.6). Docked ligand was analyzed, and the results were visualized by PyMOL 1.6.X [52] molecular graphics system, version 1.8.

Conflicts of Interest

The authors declare no conflict of interest

Acknowledgments

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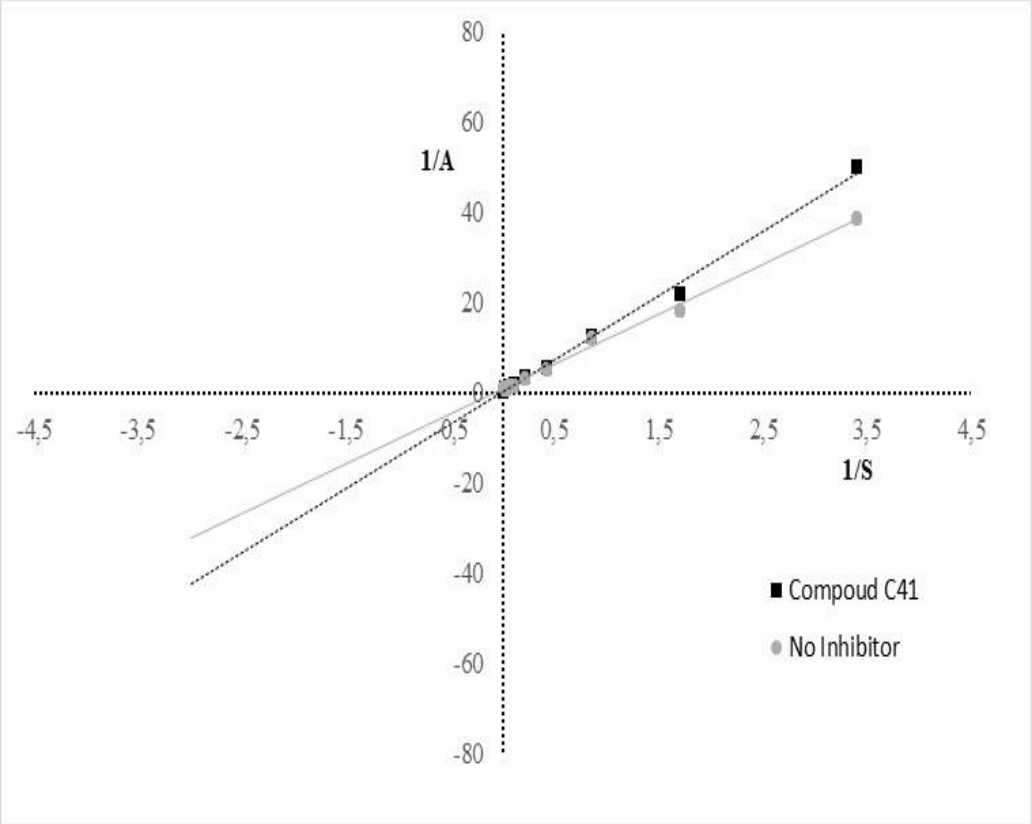
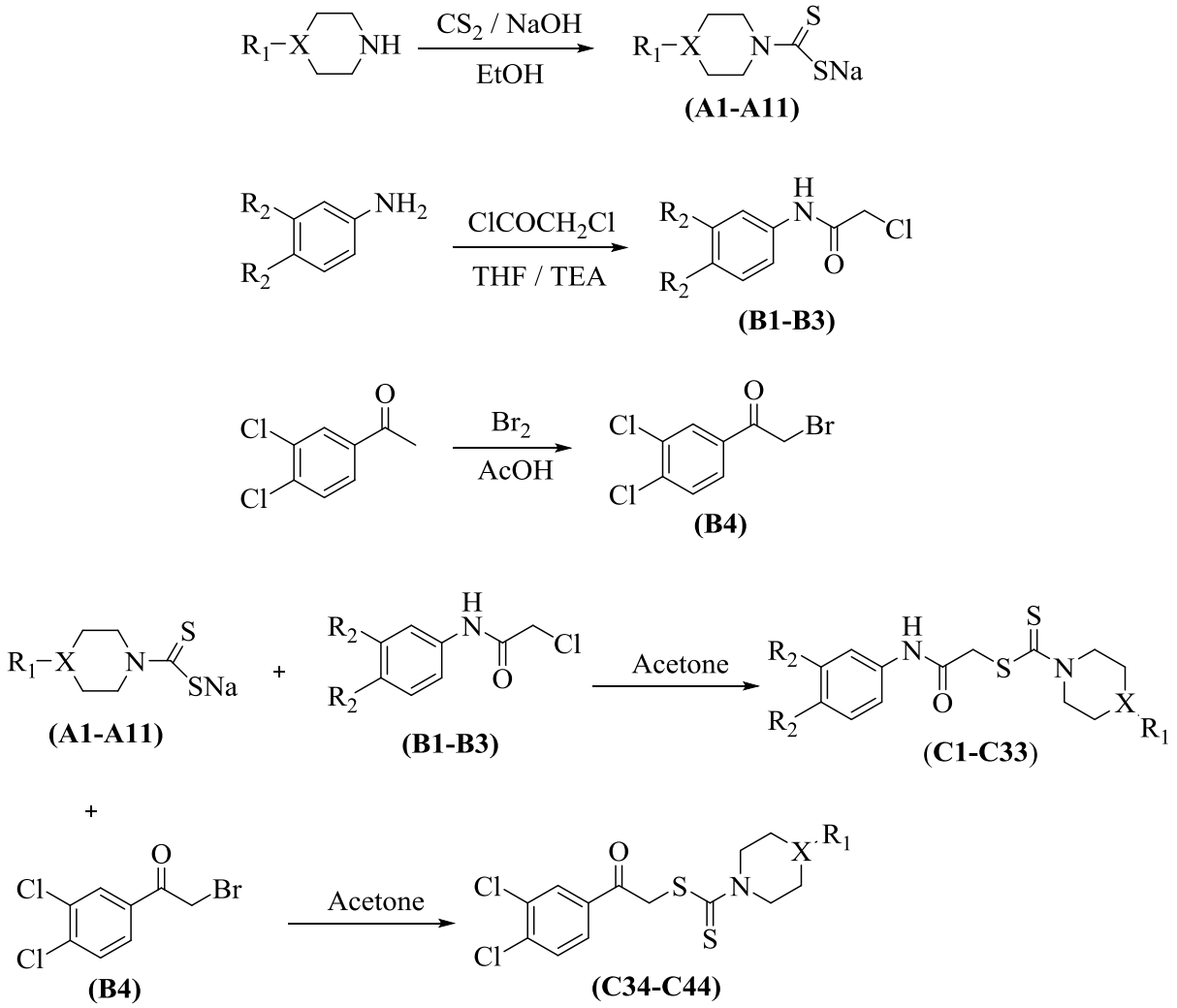


Figure 1: Lineweaver–Burk plots for compounds **C41** (IC_{50} = 11.82 μM). Substrate (ATCI-Acetylthiocholin iodine) concentrations used: 150, 75, 37.5, 18.75, 9.375, 4.6875, 2.3437, 1.1718, 0.5859 and 0.2929 mM. 1/V: 1/velocity of reaction [1/(absorbance/1 min)], 1/S: 1/substrate concentration (1/μM ATCI).



R_1:	CH_3,Ph,Bn,4-CH_3-Bn, 4-OCH_3Bn,4-F-Bn,4-CF_3-Bn,2-$(NCH_3)_2C_2H_4$, 3-$(NCH_3)_2C_3H_6$	CH_3, Bn
X:	N	CH
R_2:	F, Cl, OCH_3	

Scheme 1: Synthesis way for target compounds