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Synthesis and evaluation of enzyme inhibitory potential of some derivatives of scopolamine

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This study was designed to synthesize and evaluate derivatives of scopolamine (**1**) as acetylcholine esterase and protease inhibitors. Scopolamine (**1**) was extracted from the aerial parts of *Datura innoxia* through bioassay guided fractionation. Five different derivatives of scopolamine (**1**) were synthesized, and identified through spectroscopic studies. Their acetylcholine esterase (AChE) and trypsin inhibitory potentials were determined through standard protocols and evaluated from the perspective of structure-activity relationship. The synthesized scopolamine derivatives (**2-6**) showed remarkable AChE inhibitory activity, except for scopoline (**6**). The results showed higher enzyme inhibition potential of the synthesized compounds (**2-5**) as compared to scopolamine (**1**). Maximum inhibition was exhibited by scopolamine *N*-oxide (89.9 ± 1.2%, IC_{50} = 37.4 ± 1.1 μM)), followed by scopolamine sulfonic acid (70.3 ± 0.8%, IC_{50} = 46.9 ± 1.0 μM) and *O*-methyl scopolamine (66.1 ± 1.2%, IC_{50} = 94.7 ± 0.8 μM). All derivatives showed moderate activity against trypsin; maximum activity was exhibited by **6** (54.0 ± 1.4%) with IC_{50} = 621.2 ± 3.7 μM.

Key Words: Enzyme inhibition, scopolamine, *Datura innoxia*

Introduction

Tropane alkaloids amount to a group of 200 compounds, which are predominantly produced by the members of family *Solanaceae*, comprising 100 genera and 3000 plant species.[1] Tropane alkaloids have also been found in various other plant families as well: *Convolvulaceae, Brasicaceae (Cruciferae), Erythroxylaceae, Euphorbiaceae,*

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Olaceae, *Proteaceae*, and *Rhizophoraceae*, but they are best known for their occurrence in the family *Solanaceae*.[2] Presence of a pyrrolidine and a piperidine ring sharing a common nitrogen atom and 2 carbon atoms is a characteristic of all tropane alkaloids. Various pharmaceutical industries are manufacturing over 20 active pharmaceutical substances containing tropane moiety in their structures; they are applied as mydriatics, antiemetics, antispasmodics, anesthetics, and bronchodilators[3]. Hyoscine, commonly known as scopolamine (**1**), was firstly isolated from *Scopola carniolica* in 1881, and later found in many other plants including many *Datura* spp. In many pharmaceutical products scopolamine (**1**) is used for a specific range of indications. Due to antispasmodic properties, n-butyl scopolamine bromide is used for many intestinal disorders and motion sickness.[4]

Enzymes are naturally occurring catalysts that regulate the metabolic activities in living organisms. Overactivity of enzymes inside living bodies causes many serious conditions like Alzheimer's disease.[5] After extensive and attentive research all over the world, it has been discovered that natural products have the potential to control the overactivity of many enzymes. These natural products, owing to enzyme inhibitory potential, are being used as natural therapeutics against several diseases. Currently it is preferable to use herbal medicines instead of synthetic ones due to their contra-indicative effects. Enzyme inhibitors block the active site of enzymes and in this way the actual metabolism of the substrate is inhibited and the body functions remain normal. Tacrine, galanthamine, rivastigmine, donopezil, and huperzine are some cholinesterase inhibitors currently used as promising drugs for the treatment of Alzheimer's disease,[6] but their clinical use is strictly limited because of several adverse effects such as hepatotoxicity and some pharmacokinetic disadvantages, and so the study of new compounds as cholinesterase inhibitors is required to discover more effective and targeted drugs. Many pathological disorders are caused by the abnormal regulation of proteolytic enzymes, resulting in tissue destruction or irregular processing of other proteins. Trypsin is a serine protease enzyme that catalyzes the hydrolysis of the peptide bond of protein in the small intestine.

In the course of our studies in the purification of acetylcholine esterase inhibitors from medicinal plants, we have already reported the AChE inhibitory potential of scopolamine (**1**).[7] The present research work was designed to produce many derivatives of scopolamine, keeping in view its greater enzyme inhibitory potential.

Materials and methods

Chemicals and instruments

IR spectra were recorded as KBr disks using a Perkin-Elmer 735B infrared spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 400 MHz and 100 MHz, respectively, using a Bruker Avance spectrometer. NMR samples were prepared in CD_3OD containing tetramethylsilane as an internal standard. MS spectra were measured with a MAT 312 instrument. Silica gel 60 was used for column chromatography. Nα-benzoyl-DL-arginine-paranitroanilide-HCL (BApNA), trypsin from bovine serum, and DMSO were purchased from Fluka. Acetylthiocholine iodide and 5,5'-dithiobis [2-nitrobenzoic acid] (DTNB) were purchased from Sigma (St. Louis, MO, USA), while erythrocytes (acetylcholine esterase) were obtained from the Biochemistry Lab, Mayo Hospital, Lahore. Solvents of analytical grade were purchased from Panerac (Spain). All other chemicals and reagents of analytical grade were from Merck (Germany).

Collection of plant material

Datura innoxia was collected from the Botanical Garden of Government College University, Lahore. The plant material was identified at the Department of Botany, GC University, Lahore, where a voucher specimen was submitted (GCU-BOT-395).

Extraction and isolation of scopolamine (1)

Aerial parts of *D. innoxia* were extracted in ethanol at room temperature. The crude extract was filtered and concentrated at reduced pressure using a rotary evaporator. The ethanolic extract was fractionated with n-hexane, chloroform (pH 9.0), and n-butanol successively.

The chloroform fraction at pH 9.0 of *D. innoxia* was subjected to column chromatography and eluted successively with n-hexane, chloroform, ethyl acetate, and methanol with increasing gradient polarity and 29 fractions were collected. The fractions obtained in chloroform-ethyl acetate (10:90) were combined, which, on evaporation, resulted in scopolamine (**1**) as a white solid (1.5 g).

Preparation of derivatives

O-acetylscopolamine (2)

Scopolamine (2.0 mmol) was taken in a round bottom flask (50 mL) and 4.0 mL of acetyl chloride was added to it. The reaction mixture was heated on a steam bath for 1.5 h,[8] then cooled to room temperature, neutralized with Na_2CO_3 (10%) solution, fractionated with ethyl acetate, and concentrated using a rotary evaporator. White crystals of *O*-acetyl scopolamine (**2**) were obtained with m.p. 190 °C (86% yield).

O-Methylscopolamine (3)

Dimethyl sulfate (1.0 mmol) and scopolamine (1.0 mmol) were mixed together in a round bottom flask (50 mL). Sodium hydroxide (5%, 200 mL) solution was added to it and the mixture was subjected to vigorous shaking.[9] The stirring was continued for 1 h, which resulted in white precipitates. The reaction mixture was then filtered, washed with cold water, and dried to yield *O*-methyl scopolamine (**3**) as a white powder (m.p. = 151 °C, 16% yield).

Chlorosulfonyl scopolamine (4)

Compound **4** was synthesized according to Furnis's method along with some modification.[10] Scopolamine (1.0 mmol) was taken in a round bottom flask (50 mL) and ice cooled chlorosulfonic acid (5.0 mmol) was added dropwise to it, keeping the reaction mixture in an ice bath so that the temperature would not exceed 5 °C. After complete addition of chlorosulfonic acid the reaction mixture was left for 4 h with continuous stirring and then left overnight in a refrigerator. The reaction mixture was fractionated with ethyl acetate and the organic layer was separated and evaporated under vacuum. Compound **4** was obtained as an oil with b.p. =148 °C (30% yield).

Scopolamine-*N*-oxide (5)

Compound **1** (0.1 mol) was dissolved in 5 mL of ethanol (95%) and 20 mL of hydrogen peroxide (30%) added.[8] The reaction mixture was stirred for 4 days at room temperature and excess H_2O_2 was evaporated under vacuum, which resulted in scopolamine-*N*-oxide (**5**) as a white amorphous powder (m.p. = 134 °C, 37% yield).

Scopoline (6)

Scopolamine (1.0 mmol) was dissolved in 100 mL of potassium hydroxide solution (10%)[11] and then refluxed for 17 h. The reaction mixture was then neutralized with dil. HCl (10%) and extracted with diethyl ether. The diethyl ether layer was washed with water, dried with anhydrous sodium sulfate, and evaporated under reduced pressure to yield scopoline (**6**) as a clear viscous oil (b.p. = 72 °C, 81% yield).

Acetylcholine esterase assay

Spectrophotometry was used to determine the inhibitory potential of the compounds against acetylcholine esterase enzyme isolated from red blood cells.[12,13] Acetyl thiocholine iodide was used as a substrate. Two milliliters of Tris buffer of pH 7.8 was taken in a test tube and 0.2 mL of compound (2 mg/mL) and 30 μL of enzyme were added to it. The reaction mixture was allowed to stand for 15 min. The coloring agent (50 μL) was added and then substrate (30 μL), followed by incubation for 20 min. The absorbance was measured at 412 nm and % inhibition was calculated using the following formula:

$$\% \text{ inhibition} = (\text{A} - \text{B})/\text{A} \times 100,$$

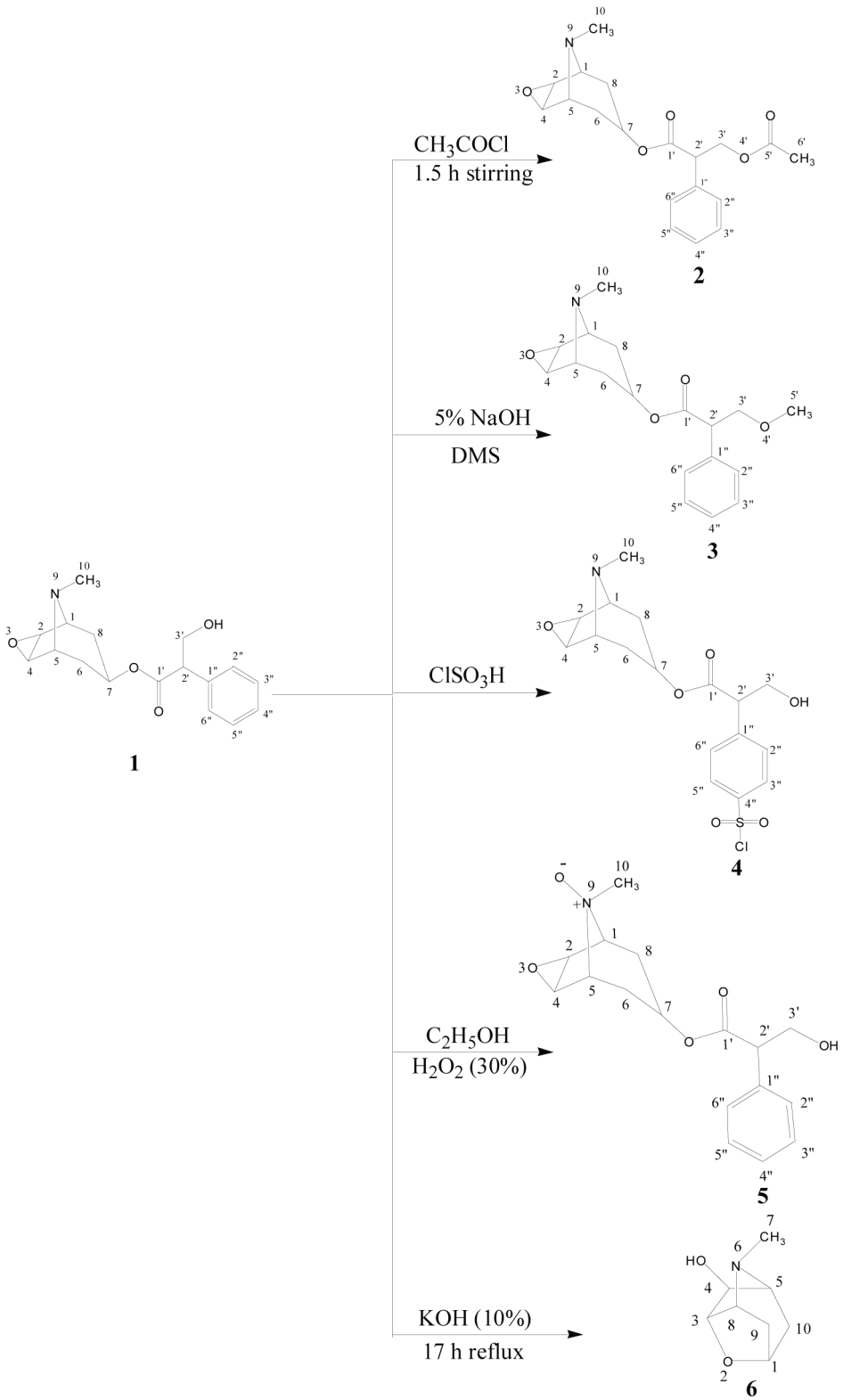
where A is the absorbance of blank and B is the absorbance of sample.

Protease inhibition activity

The protease inhibitory potential of isolated and its synthesized derivatives was evaluated using the colorimetric method of Jedinak et al. with some modification.[14] Tris buffer (100 mM) of pH 7.5 (1.0 mL), trypsin (0.3 mL), and the tested compound (0.1 mL) were incubated at room temperature for 10 min. BApNA (50 μL) was added to the reaction and the absorbance read at 410 nm after an incubation period of 30 min at 37 °C. Phenylmethylsulfonylfluoride (PMSF) was used as standard inhibitor. The % inhibition was calculated by using the following formula:

$$q\% = \frac{A - B}{A} \times 100,$$

where A is the absorbance of blank and B is the absorbance of the tested compound.



Scheme 1. Synthesis of scopolamine (**1**) derivatives.

Scopolamine (1)

9-methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]non-7-yl-3'-hydroxy-2'-phenyl propanoate

FTIR (KBr, cm^{-1}) V_{max}: 3323 (Ar-H, N-H), 2957 (C-H), 1727 (C=O), 1032 (C-N), 1490 (C=C), 1234 (C-O-C). EIMS m/z (Int. rel., %): 303.1 (M$^+$, 5.49), 154.1 (16.51), 138.1 (73.56), 108.1 (50.72), 94.1 (100.0), 57.0 (15.07). ^{1}H-NMR (400 MHz, CD_3OD): δ 1.82 (2H, m, H-6a, H-8a), δ 2.05 (2H, m, H-6b, H-8b), δ 2.83 (3H, s, H-10), δ 3.31 (4H, m, H-1, H-2, H-4, H-5), δ 3.82 (2H, m, H-2', H-3'a), δ 4.15 (1H, *dd*, $J_{2',3'a}$ = 8.5 Hz, $J_{3'a,3'b}$ = 10 Hz, H-3'b), δ 5.03 (1H, m, H-7), δ 7.30-7.37 (5H, m, H-2", H-3", H-4", H-5", H-6"). ^{13}C-NMR (100 MHz, CD_3OD): δ 29.1 (C-6), 29.1 (C-8), 41.1 (C-10), 53.8 (C-2'), 55.7 (C-1), 55.7 (C-5), 58.1 (C-2), 58.1 (C-4), 64.0 (C-3'), 64.5 (C-7), 127.2 (C-4"), 129.4 (C-3"), 129.4 (C-5"), 130.6 (C-2"), 130.6 (C-6"), 137.3 (C-1"), 172.5 (C-1').

***O*-Acetyl scopolamine (2)**

9-methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]non-7-yl-3'-(acetyloxy)-2'-phenyl propanoate

FTIR (KBr, cm^{-1}) V_{max}: 3327 (Ar-H, N-H), 2965 (C-H), 1750 (C=O), 1032 (C-N), 1490 (C=C), 1237 (C-O-C). EIMS m/z (Int. rel., %): 345.4 (M$^+$, 11.51), 154.2 (29.31), 138.1 (72.08), 113.2 (40.19), 94.1 (100.00). ^{1}H-NMR (400 MHz, CD_3OD): δ 1.91 (2H, m, H-6a, H-8a), δ 2.11 (2H, m, H-6b, H-8b), δ 2.21 (3H, s, H-6'), δ 2.51 (3H, s, H-10), δ 3.34 (4H, m, H-1, H-2, H-4, H-5), δ 3.64 (2H, m, H-2', H-3'a), δ 3.91 (1H, *dd*, $J_{2',3'a}$ = 8.2 Hz, $J_{3'a,3'b}$ = 10 Hz, H-3'b), δ 4.96 (1H, m, H-7), δ 7.04-7.37 (5H, m, H-2", H-3", H-4", H-5", H-6"). ^{13}C-NMR (100 MHz, CD_3OD): δ 20.7 (C-6'), 28.4 (C-6), 28.4 (C-8), 41.1 (C-10), 54.5 (C-2"), 55.1 (C-1), 55.1 (C-5), 56.7 (C-2), 56.7 (C-4), 65.1 (C-3'), 67.7 (C-7), 127.3 (C-4"), 128.1 (C-3"), 128.1 (C-5"), 130.7 (C-6"), 130.7 (C-2"), 137.0 (C-1"), 170.6 (C-5') 171.9 (C-1').

***O*-Methyl scopolamine (3)**

9-methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]non-7-yl-3'-(methoxy)-2'-phenyl propanoate

FTIR (KBr, cm^{-1}) V_{max}: 3302 (Ar-H, N-H), 2920 (C-H), 1759 (C=O), 1132 (C-N), 1472 (C=C), 1258 (C-O-C). EIMS m/z (Int. rel., %): 317.2 (M$^+$, 15.27), 193.2 (6.42), 177.0 (5.45), 154.2 (38.70), 138.4 (68.05), 113.1 (40.17), 94.1 (100.00). ^{1}H-NMR (400 MHz, CD_3OD): δ 1.91 (2H, m, H-6a, H-8a), δ 2.14 (2H, m, H-6b, H-8b), δ 2.47 (3H, s, H-10), δ 3.06 (4H, m, H-1, H-2, H-4, H-5), δ 3.36 (3H, s, H-5'), δ 3.79 (2H, m, H-2', H-3'a), δ 4.03 (1H, *dd*, $J_{2',3'a}$ = 8.5 Hz, $J_{3'a,3'b}$ = 10 Hz, H-3'b), δ 4.96 (1H, m, H-7), δ 7.12-7.28 (5H, m, H-2", H-3", H-4", H-5", H-6"). ^{13}C-NMR (100 MHz, CD_3OD): δ 27.4 (C-6), 27.4 (C-8), 41.7 (C-10), 53.6 (C-2'), 57.0 (C-5), 57.0 (C-1), 58.1 (C-2), 58.1 (C-4), 59.2 (C-5'), 63.3 (C-7), 71.1 (C-3'), 127.3 (C-4"), 128.3 (C-3"), 128.3 (C-5"), 130.2 (C-2"), 130.2 (C-6"), 136.8 (C-1"), 173.3 (C-1').

Chlorosulfonyl scopolamine (4)

9-methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]non-7-yl-2[4"-(chlorosulfodithioyl)phenyl]-3'-hydroxy propanoate

FTIR (KBr, cm^{-1}) V_{max}: 3317 (Ar-H, N-H), 2955 (C-H), 1731 (C=O), 1043 (C-N), 1369 (SO_2Cl), 1306 (C=C), 1245 (C-O-C). EIMS m/z (Int. rel., %): 401.8 (M$^+$, 4.52), 207.0 (8.43), 177.2 (9.04), 154.2

(39.41), 138.1 (78.07), 121.2 (22.17), 108.2 (44.05), 94.1 (100.00), 81.2 (24.04), 57.5 (27.30), 43.2 (58.17). ^{1}H-NMR (400 MHz, CD_3OD): δ 1.91 (2H, m, H-6a, H-8a), δ 2.11 (2H, m, H-6b, H-8b), δ 2.52 (3H, s, H-10), δ 3.32 (4H, m, H-1, H-2, H-4, H-5), δ 3.86 (2H, m, H-2', H-3'a), δ 4.25 (1H, *dd*, $J_{2',3'a} = 8.5$ Hz, $J_{3'a,3'b} = 10$ Hz, H-3'b), δ 5.01 (1H, m, H-7), δ 7.42-7.78 (4H, dd, $J = 8.0, 2.2$ Hz, H-2", H-3", H-5", H-6"). ^{13}C-NMR (100 MHz, CD_3OD): δ 28.2 (C-6), 28.2 (C-8), 41.5 (C-10), 52.6 (C-2'), 54.7 (C-1), 54.7 (C-5), 56.4 (C-2), 56.4 (C-4), 59.9 (C-3'), 63.9 (C-7), 124.1 (C-3"), 124.1 (C-5"), 128.3 (C-2"), 128.3 (C-6"), 141.1 (C-1"), 143.4 (C-4"), 173.3 (C-1').

Scopolamine-*N*-Oxide (5)

9-methyl-9-oxy-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]non-7-yl-3'-hydroxy-2'-phenyl propanoate

FTIR (KBr, cm^{-1}) V_{max}: 3150 (Ar-H, N-H), 2891 (C-H), 1720 (C=O), 1056 (C-N), 1492 (C=C), 1239 (C-O-C), 967 (N-O). EIMS m/z (Int. rel., %): 319.1 (M^+, 21.40), 154.1 (31.21), 138.2 (56.07), 113.0 (48.32), 94.1 (100.00). ^{1}H-NMR (400 MHz, CD_3OD): δ 1.91 (2H, m, H-6a, H-8a), δ 2.34 (2H, m, H-6b, H-8b), δ 3.14 (2H, d, $J_{2,3} = 6$ Hz, H-2, H-4), δ 3.21 (3H, s, H-10), δ 3.85 (2H, m, H-2', H-3'a), δ 4.32 (1H, *dd*, $J_{2',3'a} = 8.5$ Hz, $J_{3'a,3'b} = 10$ Hz, H-3'b), δ 4.65 (2H, m, H-, H-5), δ 5.27 (1H, m, H-7), δ 7.05-7.42 (5H, m, H-2", H-3", H-4", H-5", H-6"). ^{13}C-NMR (100 MHz, CD_3OD): δ 28.4 (C-6), 28.4 (C-8), 45.86 (C-10), 54.7 (C-2'), 56.4 (C-2), 56.4 (C-4), 56.9 (C-3'), 63.9 (C-1), 63.9 (C-5), 65.7 (C-7), 127.3 (C-4"), 128.1 (C-3"), 128.1 (C-5"), 130.7 (C-2"), 130.7 (C-6"), 138.6 (C-1"), 172.1 (C-1').

Scopoline (6)

6-methyl-2-oxa-6-azatricyclo[3.3.1.0^{3,7}]nonan-4-ol

FTIR (KBr, cm^{-1}) V_{max}: 3367 (Ar-H, N-H), 2881 (C-H), 1053 (C-N), 1218 (C-O-C). EIMS m/z (Int. rel., %): 155.2 (M^+, 18), 136.2 (21.03), 111.2 (43.51), 93.1 (78.40), 71.3 (100.00), 43.4 (54.05). ^{1}H-NMR (400 MHz, CD_3OD): δ 1.68 (2H, m, H-9a, H-10a), δ 2.21 (2H, m, H-9b, H-10b), δ 2.41 (3H, s, H-7), δ 2.95 (1H, m, H-5), δ 3.96 (1H, m, H-4), δ 4.24 (3H, m, H-1, H-3, H-8). ^{13}C-NMR (100 MHz, CD_3OD): δ 30.1 (C-10), 33.2 (C-9), 40.6 (C-7), 55.8 (C-5), 57.3 (C-8), 70.2 (C-4), 72.6 (C-1), 81.2 (C-3).

Results and discussion

Enzymes play a vital role in the control of many physiological functions of cells. Overactivity of enzymes within the living system may induce several diseases. Keeping in view the enzyme inhibition potential of scopolamine (**1**),[7] its 5 different structural alterations were synthesized (Scheme 1) in order to evaluate their inhibition potential against acetylcholine esterase and protease. The most important aspect of our study was to find the structural modification of scopolamine (**1**), which can be more potent against these enzymes. Scopolamine (**1**) was extracted from the chloroform fraction (pH 9.0) of the ethanolic crude extract of *Datura innoxia* using column chromatography followed by thin layer chromatography.

The structures of scopolamine (**1**) and its derivatives (**2-6**) were confirmed with spectroscopic techniques. The ^{1}H-NMR spectrum of compound **2** exhibited a typical multiplet signal at δ 4.96 due to its low field position, attributed to H-7, characteristic of a tropane skeleton.[15] Five aromatic protons appeared in multiplet form at

δ 7.04-7.37, suggesting mono substituted phenyl. The appearance of 2 more carbon signal as compared to **1** indicated the addition of an acetyl group, which was further confirmed by one 3H singlet at δ 2.21 due to H-6'. The appearance of 1 more signal at δ 3.36 of 3 hydrogen integration suggested the addition of a methyl group in compound **3**. Four double doublet signals appeared in the range of δ 7.42-7.78 (J = 8.0, 2.2 Hz), which indicated the addition of a chlorosulfonic group at the para position of the aromatic ring of compound **4**. The signal of the aromatic protons at δ 7.42 was found correlated with the double doublet at δ 7.78 in the COSY spectrum of **4**. The analysis of COSY 45° spectrum of compound **2-4** showed the presence of a -$CHCH_2$- fragment in the structure. The methine protons of compound **2-4**, which appeared in the range of δ 3.64-3.86, showed geminal coupling of H-3b with H-3a at δ 3.91-4.25. Furthermore, methylene protons (H-6a and H-8a) of compounds **2-4** at δ 1.91 showed a strong interaction with the protons (H-6b and H-8b) at δ 2.11-2.14 in the COSY spectrum. The structure of N-oxide derivative (**5**) was confirmed by the downfield shifting of CH_3 (H-10) from δ 2.83 to δ 3.21 in compound **5**. The breakdown of ester linkage of scopolamine (**1**), which resulted in the formation of scopoline (**6**), was confirmed by the absence of signals at δ 7.30-7.37 due to the aromatic ring and the absence of carbonyl group stretching at 1727 cm^{-1} in the IR spectrum. Furthermore, the appearance of the M^+ peak at m/z 155.2 in the EI spectrum of compound **6**, which was 148 mass units (equivalent to $C_9H_8O_2$) less than the mass of scopolamine, was also in accordance with the loss of ester linkage during hydrolysis of compound **1**.

Scopolamine (**1**) belongs to the class of tropane alkaloids. There are many interesting sites in the structure of scopolamine, which can be modified with different substituents in order to check their effect on the activity of the molecule. In the literature the structure-activity relation (SAR) of many nitrogen-containing AChE inhibitors such as tacrine, physostigmine, benzylamines, benzyl piperidine, benzioxazoles, and huperzine A has been reported.[6] All of them gave an overall conclusion that these drugs bind to acetylcholine esterase through the nitrogen-containing heterocyclic part of the molecule.[16] It has reported in the literature that quaternary ammonium salts act as strong acetylcholine esterase inhibitors.[17] Therefore, in the present study, scopolamine was subjected to N-oxidation using H_2O_2, resulting in compound **5**, which was the most active analogue among all other derivatives with IC_{50} 37.4 $\pm$ 1.1 μM.

In previous reports regarding the SAR of AChE inhibitors, it was concluded that the substitution o then benzene ring enhances the activity of the molecule.[6] In the present study, substitution of the sulfonic acid group on the aromatic ring is found to markedly improve AChE activity. Scopolamine sulfonic acid (**4**) showed enhanced activity with IC_{50} = 46.9 $\pm$1.0 μM. This reaction is of unique significance as it can be further used to produce sulfonamides, which are an important class of drugs.[18] Given the comparatively small variation in AChE inhibition for the O-acetyl substituent (IC_{50} = 201.5 $\pm$ 1.8 μM), it is possible that this substituent does not participate in binding. However, O-methyl analogue of scopolamine (**3**) showed enhanced activity in comparison with unsubstituted compound. The only derivative that showed reduced activity was the hydrolyzed compound scopoline (**6**). It is interesting to note that compound **6**, which was found inactive in the acetylcholine esterase assay, showed enhanced activity against protease with IC_{50} = 621.2 $\pm$ 3.7 μM. None of the other synthesized compounds (**2-5**) showed significant activity against protease (Table).

Table. Acetylcholine esterase and proteases inhibition potential of scopolamine and its derivatives.

Compounds	AChE		Trypsin	
	% inhibition	IC_{50} (μM) %	inhibition	IC_{50} (μM)
Scopolamine	53.2 ± 1.1	196.2 ± 2.6	42.1 ± 1.0	-
O-acetyl scopolamine	55.5 ± 0.9	201.5 ± 1.8	29.3 ± 1.2	-
O-methyl scopolamine	66.1 ± 1.2	94.7 ± 0.8	37.6 ± 0.9	-
Scopolamine sulfonic acid	70.3 ± 0.8	46.9 ± 1.0	45.9 ± 0.7	-
Scopolamine-*N*-oxide	89.9 ± 1.2	37.4 ± 1.1	35.2 ± 0.6	-
Scopoline	37.3 ± 0.7	-	54.0 ± 1.4	621.2 ± 3.7
PMSF	-	-	85.2 ± 1.1	117.5 ± 1.3

-= not calculated

Conclusions

In this study 5 different derivatives of scopolamine were synthesized and their structures were identified by EIMS and ^{1}H-NMR spectroscopic techniques. The compounds were screened for their AChE and protease inhibitory potential. All synthesized compounds effectively inhibited the activity of AChE, except for scopoline (**6**). Among all the synthesized compounds only scopoline (**6**) showed positive results against proteases.

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