Drug Design, Development and Therapy

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ORIGINAL RESEARCH

Enzyme Inhibitory, Antioxidant And Antibacterial Potentials Of Synthetic Symmetrical And Unsymmetrical Thioureas

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Sumaira Naz[1]
Muhammad Zahoor[1]
Muhammad Naveed Umar[1]
Barkat Ali[1,2]
Riaz Ullah[3]
Abdelaaty A Shahat[3,4]
Hafiz Majid Mahmood[5]
Muhammad Umar
Khayam Sahibzada[6]

[1]Department of Chemistry, University of Malakand Chakdara, Dir Lower, Kpk 18800, Pakistan; [2]Department of Chemistry, GC University Faisalabad, Faisalabad, Punjab, Pakistan; [3]Medicinal, Aromatic and Poisonous Plants Research Center (MAPRC), College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia; [4]Phytochemistry Department, National Research Centre, Giza, Egypt; [5]Department of Pharmacology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia; [6]Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Kpk 25000, Pakistan

Correspondence: Muhammad Umar Khayam Sahibzada
Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, KPK 25000, Pakistan
Email umar.sahibzada@gmail.com

Muhammad Zahoor
Department of Chemistry, University of Malakand Chakdara, Dir Lower, KPK 18800, Pakistan
Email mohammadzahoorus@yahoo.com

Background: In this study, 2 symmetrical and 3 unsymmetrical thioureas were synthesized to evaluate their antioxidant, antibacterial, antidiabetic, and anticholinesterase potentials.

Methods: The symmetrical thioureas were synthesized in aqueous media in the presence of sunlight, using amines and CS_2 as starting material. The unsymmetrical thioureas were synthesized using amines as a nucleophile to attack the phenyl isothiocyanate (electrophile). The structures of synthesized compounds were confirmed through H^1 NMR. The antioxidant potential was determined using DPPH and ABTS assays. The inhibition of glucose-6-phosphatase, alpha amylase, and alpha glucosidase by synthesized compounds was used as an indication of antidiabetic potential. Anticholinesterase potential was determined from the inhibition of acetylcholinesterase and butyrylcholinesterase by the synthesized compounds.

Results: The highest inhibition of glucose-6-phosphatase was shown by compound **V** (03.12 mg of phosphate released). Alpha amylase was most potently inhibited by compound **IV** with IC_{50} value of 62 µg/mL while alpha glucosidase by compound **III** with IC_{50} value of 75 µg/mL. The enzymes, acetylcholinesterase, and butyrylcholinesterase were potently inhibited by compound **III** with IC_{50} of 63 µg/mL and 80 µg/mL respectively. Against DPPH free radical, compound **IV** was more potent (IC_{50} = 64 µg/mL) while ABTS was more potently scavenged by compound **I** with IC_{50} of 66 µg/mL. The antibacterial spectrum of synthesized compounds was determined against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Agrobacterium tumefaction* and *Proteus vulgaris*). Compound **I** and compound **II** showed maximum activity against *A. tumefaction* with MIC values of 4.02 and 4.04 µg/mL respectively. Against *P. vulgaris*, compound **V** was more active (MIC = 8.94 µg/mL) while against *S. aureus*, compound **IV** was more potent with MIC of 4.03 µg/mL.

Conclusion: From the results, it was concluded that these compounds could be used as antibacterial, antioxidant, and antidiabetic agents. However, further in vivo studies are needed to determine the toxicological effect of these compounds in living bodies. The compounds also have potential to treat neurodegenerative diseases.

Keywords: picolylamine, symmetrical thioureas, enzyme inhibition, anti-diabetic, antioxidant, Alzheimer's disease, antibacterial

Introduction

Thioureas are an important class of compounds that are used as intermediates/precursors in synthesis of many synthetic drugs.[1,2] A number of substituted derivatives of thioureas can be synthesized by simple condensation of different primary and secondary amines (aliphatic and/or aromatic) with iso-thiocyanate or its derivatives.[3] They can also be prepared by reaction of carbon disulphide and

amines (with or without catalyst).[4] N,N′-disubstituted thioureas, whether aliphatic, aromatic or heterocyclic can be used as building blocks in the synthesis of heterocyclic compounds (having a variety of applications in different fields). In organic synthesis, they played a very important role as catalysts in important organic reactions (Mannich and Biginelli reactions) and as chelating agents.[5] The diverse applications of these compounds in industry, agriculture, and medicine made them unique. Due to the presence of substituted sites in their structures, they have been used in drug design and synthesis.[6] Literature on thiourea derivatives shows that they possess biological activities like anti-bacterial,[7] anti-fungal, anti-cancer, analgesic, and antimalarial.[8–13] They have also been used as inhibitors of certain enzymes like acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase etc.[14–17]

Oxidative stress, diabetes mellitus, neurodegenerative diseases, and antibacterial resistance are the burning issues of this era. Free radicals are continuously produced during normal metabolism and nearly 1/4th of the inhaled oxygen is converted into free radicals, which have deteriorating effects on biologically important molecules like protein and DNA. Although human bodies are equipped with efficient systems to cope with these free radicals, for the last two decades, humans' dependence on synthetically processed food has complicated the issue and the amount of free radicals now produced is high as compared to the body's free radical scavenging capacities. Therefore, there is a need for certain potent compounds to be taken additionally, to help the body in scavenging the free radicals. As mentioned earlier, thioureas have two substation sites that could be useful in such type of research.[18–21]

In neurodegenerative diseases, there is maximum hydrolysis of acetylcholine, the substance responsible for the transmission of nerve impulses at synoptic gaps between the two nerves. A practical approach applied to relieve the symptoms of neurodegenerative diseases is the use of inhibitors of acetylcholinesterase and butyrylcholinesterase (the enzymes responsible for hydrolyses of neurotransmitter acetylcholine).[22] An example of neurodegenerative disease is Alzheimer's disease (AD) where an uncontrolled hydrolysis of the mentioned neurotransmitter occurs, rendering the neurotransmission difficult or almost impossible. If acetylcholinesterase is inhibited, the hydrolysis of acetylcholine can be controlled which can be helpful in the symptomatic relief of dementia or AD.[18] Butyrylcholinesterase normally has a less significant role in hydrolysis of acetylcholine, but in the diseased condition, when acetylcholinesterase is inhibited by medicines, its therapeutic importance increases as it could hydrolyze the available acetylcholine, minimizing the effect of the medicines, thus exaggerates the disease and its inhibition is then desired.[19,23]

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, impairment of carbohydrate, lipid and protein metabolism, which is either due to insufficient secretion of insulin or insulin resistance. It is considered to be one of the chronic diseases after cancer and cardiovascular diseases. Among the different strategies used to control hyperglycemia, inhibition of the key enzymes of glucose metabolism like alpha amylase, alpha glucosidase and glucose-6-phosphatase is considered to be the most effective strategy.[20] α-Amylase cleaves polysaccharides at α-1,4 glycosidic linkages and the products (oligo and disaccharides) are further hydrolyzed to free glucose by intestinal α-glucosidase. Controlling/lowering activity of these two enzymes slows down the digestion of carbohydrates, absorption of glucose hence lowering the overall blood glucose level.[24,25] Glucose-6-phosphatase catalyses the last step of both glycogenolysis (breakdown of glycogen) and gluconeogenesis (synthesis of glucose from non-carbohydrate sources) pathways, as a result glucose level is maintained during fasting condition. Inhibition of this enzyme is also considered to be a good therapeutic target in disorders of carbohydrate metabolism and would be helpful in minimizing the severity of fasting hyperglycemia occurring in diabetic patients.[26]

Keeping in mind the therapeutic uses of thioureas, an attempt was made to synthesize novel symmetric and unsymmetric derivatives of thiourea and use them as remedy for oxidative stress, diabetes mellitus, and neurodegenerative disorders. The synthesized derivatives were also evaluated for their antibacterial potential against Gram positive and Gram negative bacterial strains.

Experimental

Materials

All the chemicals, reagents, and solvents used were of analytical grade and were used without any further purification. They were purchased from Sigma Aldrich Co, St Louis, MO, USA. The reaction progress was monitored using thin layer chromatography (TLC). The structures of synthesized compounds were confirmed by ^{1}H-NMR in $CDCl_3$ using NMR-Bruker apparatus.

Methods

Cinchona alkaloids are a group of naturally occurring compounds with promising biological activities due to the presence of primary and tertiary nitrogens in their structures. Keeping in mind the importance of these alkaloids, the simplest commercially available analog 2-picolyl amine was taken and a simpler symmetrical thiourea presented as compound **II** was synthesized using CS_2 (1 equiv) in water and in the presence of sunlight. After confirmation that 2-picolyl amine has the capability of forming thiourea, the starting material 2-picolyl amine was derivatized to dibenzyl picolyl amine (Precursor **P**). Compound **I** and **III** were then synthesized using dibenzyl picolyl amine as starting material by two different methods described in the following section. Compound **IV** was prepared from 2,4-dimethyl aniline and phenyl isothiocyanate. Phenyl isothiocyanate is a commercially available compound, while 2,4-dimethyl aniline was prepared from 2, 4-dimethyl nitrobenzene by reduction of nitro group into amines by heterogenous catalyst under high pressure in the presence of hydrogen. Compound **V** was prepared from phenyl isothiocyanate and 2-fluoro aniline. The 2-fluoro aniline was prepared by reduction of the nitro group as described previously.

Preparation Of Dibenzyl Picolyl Amine (P)

The precursor dibenzyl picolyl amine was synthesized starting from acetylpyridine as more readily available starting material (Figure 1). The sodium hydride (NaH) was used to abstract proton from acetyl pyridine to make enolate which then attack on benzyl bromide (added dropwise). As a result, first substitution takes place. With reaction progress under same condition, a second substitution takes place. The reaction product was then treated with hydroxyl amine hydrochloride. As a result, oxime was obtained which was then reduced into amine. The amine was purified further by column chromatography using ethyl acetate and hexane as solvent system.[27] The product was obtained as yellowish viscous oil with 68% yield.

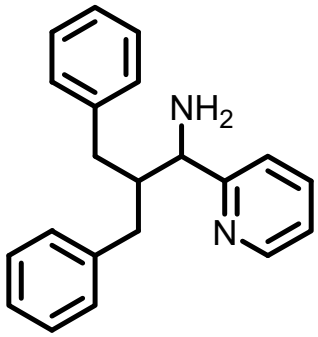

Figure 1 Chemical structure of 2-Benzyl-3-phenyl-1-(pyridin-2-yl)propan-1-amine (Precursor **P**).

Synthesis Of Symmetrical Thioureas (Compounds I And II)

Picolylamine/Picolylamine derivatives (1.0 mmol, 2.0 equiv) were mixed with carbon disulphide (0.5 mmol, 1.0 equiv) in 10 mL EtOH/H_2O (1:1) and placed in sunlight for 6–8 hrs. After 8 hrs, white precipitates were formed which were confirmed by NMR as symmetrical thioureas. The reaction mixture was kept at room temperature for 12 hrs until crystal formation.

Characterization Of Compound I

Brown crystals (yield 79%).

1HNMR (300 MHz, CDCl3-d) δ ppm: 4.5–4.7 (m, 8H), 5.2 (m, 2H), 5.4 (m, 2H), 6.8 (m, 4H), 6.9–7.1 (m, 8H), 7.2 (m, 8H), 7.5 (m, 2H), 7.7 (m, 2H), 8.1 (m, 2H), 8.5 (m, 2H), 8.9 (s, 2H). The chemical structure of compound **I** is given in Figure 2.

Characterization Of Compound II

Needle-like crystals (81% yield).

1HNMR (300 MHz, CDCl3-d) δ ppm 3.2 (d, 4H), 6.4 (d, 4H), 6.5 (d, 2H), 7.3 (m, 4H), 8.2 (s, 2H). The structural formula of compound **II** has been presented in Figure 3.

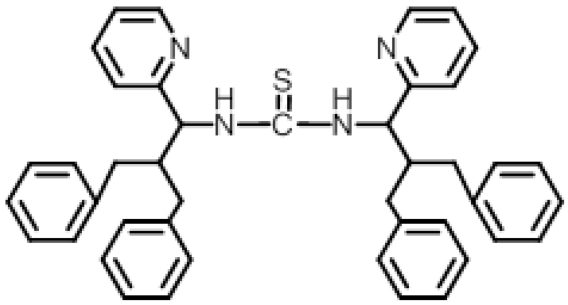

Figure 2 1,3-*bis*(2-benzyl-3-phenyl-1-(pyridine-2-yl)propyl)thiourea (Compound **I**).

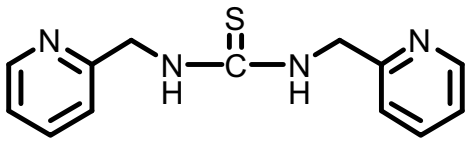

Figure 3 1,3-*bis*(1-(pyridin-2-ylmethyl)thiourea (Compound **II**).

Synthesis Of Unsymmetrical Thioureas (Compound III-V)

The required amines were mixed with phenyl iso-thiocyanate (both at the ratio of 1.0 equiv at a concentration of 1.0 mmol, 1.0) in 6 mL anhydrous acetone. The reaction mixture was then refluxed for 8–10 hrs at 52–56°C. Progress of the reaction was checked through TLC with EtOAc/n-hexane solution (3:7). The reaction mixture was then cooled using crushed ice. Precipitates formed were then filtered. Filtered precipitates were washed with water, dried and recrystallized using ethanol.

Characterization Of Compound III

Yellow crystals (yield 87%).

^{1}HNMR (300 MHz, $CDCl_3$-d) δ ppm: 2.32 (m, 1H), 2.59 (m, 2H), 2.77 (m, 2H), 3.08 (s, 1H), 7.04–7.28 (m, 15H), 7.37 (m, 2H), 7.65 (m, 2H), 7.82 (s, H, –NH), 8.47 (s, H, –NH). Compound **III** is presented in Figure 4.

Characterization Of Compound IV

Yield: 93%.

1HNMR (300 MHz, CDCl3-d) δ ppm 2.30 (s, 3H), 2.34 (s, 3H), 7.05 (d, 1H), 7.11 (d, 1H), 7.20 (s, 1H), 7.25 (m, 1H), 7.35–7.42 (m, 4H), 7.97 (s, 2H). Compound **IV** is presented in Figure 5.

Characterization Of Compound V

Yield: 96%.

1HNMR (300 MHz, CDCl3-d) δ ppm 7.0–7.49 (m, 9H), 7.8 (s, 1H, –NH), 7.9 (s, 1H, –NH). The chemical structure of compound **V** is given in Figure 6.

Anticholinesterase Assay

The acetylcholinesterase inhibitory potential of the synthesized compounds was examined using Ellman's assay.[28]

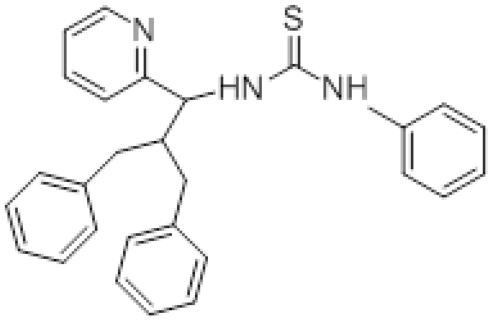

Figure 4 1-(2-benzyl-3-phenyl-1-(pyridine-2-yl) propyl)-3-phenylthiourea (Compound **III**).

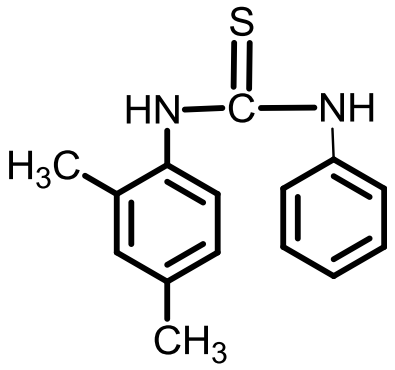

Figure 5 1-(2,4-dimethylphenyl)-3-phenylthiourea (Compound **IV**).

Acetylcholinesterase and butyrl choliesterase hydrolyses their respective substrates acetylthiocholine iodide and butyrylthiocholine iodide. The resulting product reacts with 5-thio-2-nitrobenzoate anion formed from DTNB resulting in the formation of yellow color product. The color changes were measured spectrophotometrically which were then converted into enzyme activity and percent inhibition.

Briefly, 205 µL compound dilutions (1000–125 µL/mL) were mixed with 5 µL of AChE (0.03 U/mL)/BChE (0.01 U/mL) and 5 µL DTNB and incubated in water bath at 30°C for 15 min. Then, 5 µL acetyl choline iodide/butyryl choline iodide (substrate). As a result, formation of yellow color anion (5-Thio-2-nitro benzoate) took place. The color change was measured after 4 mins at 412 NM using a double beam spectrophotometer (Thermo electron-corporation; USA). A blank solution was considered as a control. Galanthamine was used as positive control. Activity and inhibition of the selective enzymes were calculated by following relations:

$$V = \Delta Abs/\Delta t \quad (1)$$

$$\%\ enzyme\ activity = \frac{V}{Vmax} \times 100 \quad (2)$$

$$\%\ enzyme\ inhibition = 100 - \%\ enzyme\ activity \quad (3)$$

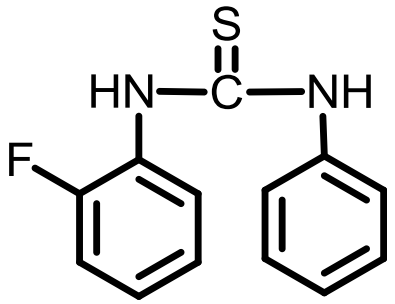

Figure 6 1-(2-fluorophenyl)-3-phenylthiourea (Compound **V**).

Where, V is the inhibitor dependent rate of reaction while, Vmax is the inhibitor independent rate of reaction.

Antidiabetic Potential Of The Synthesized Compounds

The antidiabetic potential of synthesized compounds was checked by monitoring their inhibitory effects on three important enzymes of carbohydrate metabolism viz alpha amylase, alpha glucosidase, and glucose-6-phosphatase.

The stock solutions of the compounds were prepared in 10% DMSO and phosphate buffer (pH 6.9) having a concentration of 20 mm. Different dilutions (1000–125 mg/mL) were prepared for each compound. About 200 µL of these dilutions was mixed with 200 µL of porcine α-amylase (2 U/mL) and pre-incubated for 10 mins at 30°C. Added to the mixture, was 200 µL of starch solution (1% w/v, made in 20 mM phosphate buffer, pH 6.9) and it was further incubated for 3 mins at 30°C. The reaction was stopped by adding 1 mL of 96 mM 3,5dinitrosalicylic acid (DNS). Again, the reaction mixture was incubated for 10 mins in boiling water bath (85–90°C) and then cooled at room temperature. Acarbose was used as positive control. Absorbance was monitored at 540 nm and percent inhibition was calculated using the following equation:

$$\%\ inhibition = \frac{\text{Control sample absorbance} - \text{Test sample absorbance}}{\text{Control absorbance}} * 100 \quad (4)$$

The α-glucosidase inhibition was carried out by mixing 100 µL of enzyme stock solution (0.5 unit/mL) with 600 µL stock phosphate buffer (pH 6.9) and 50 µL of each dilution prepared in the previous step and incubated at 37°C for 15 mins. To start the enzymatic reaction, 100 µL *p*-nitro-phenyl-α-D-glucopyranoside (5 mM) was added to the mixture which reached completion after 15 mins incubation at 37°C. To stop the reaction, 400 µL sodium carbonate (0.2 M) solution was added to the mixture. The absorbance of resulting mixture was measured at 405 nm. Percent inhibition of glucosidase was calculated using Formula 4. Acarbose was used as positive control and the same dilution was prepared by serial dilution.

The activity of the glucose-6-phosphatase was measured, which is based on the release of inorganic phosphate when the substrate glucose-6-phosphate is hydrolyzed by the enzyme. About 1 mL of 1 mM of the enzyme was taken, to which 1.5 mL tris (hydroxy methyl) amino methane buffer of pH 6.7 and 2.5 mL of 0.01 mM glucose-6-phosphate were added. The reaction mixture was incubated at 33°C for 1 hr. After the incubation, 1 mL 10% trichloroacetic acid was added to stop the reaction, and inorganic phosphate released during the reaction was measured by method of Fiske and Subbarow.[29]

Free Radical Scavenging Activities Of Synthesized Compounds

The free radical scavenging activities of synthesized compounds were determined following standard DPPH and ABTS protocols.[10] DPPH stock solution was prepared by dissolving 20 mg of DPPH in 100 mL methanol. Blank (control) solution was prepared by taking 3 mL from the DPPH solution and its absorbance was adjusted to 0.75 at 515 nm. For the development of free radicals in stock solution, it was covered and kept in the dark for about 24 hrs. Stock solution (in 5 mL of methanol) of each compound was prepared and a series of dilutions (1000, 500, 250, 125, 62.5 µg/mL) were then prepared from it, using dilution formula. About 2 mL from each dilution was mixed with 2 mL DPPH and allowed to react for 15 mins in the dark. The percent inhibition by DPPH was calculated with the following equation:

$$\%\ inhibition = \frac{A - B}{A} X\ 100 \quad (5)$$

Where; A = absorbance of pure DPPH in oxidized form.

B = absorbance of sample taken after 15 mins of reaction with DPPH.

Ascorbic acid (5 mg/5 mL) was taken as a standard and its different solutions (1000, 500, 250, 125 µg/mL) were prepared.

The ABTS free radical scavenging was carried out using standard protocol of Re et al.[30] About 7 mM of ABTS and 2.45 mM of potassium per sulphate were dissolved, each in 100 mL of methanol and were then mixed together. To develop free radicals of ABTS, the reaction mixture was kept in the dark for 12 hrs. Absorbance of blank control (3 mL) was adjusted to 0.75 at 745 nm by diluting it with 50% methanol. About 300 µL of each sample was taken, mixed with 3 mL of ABTS solution, and incubated for 15 mins at 25°C. Absorbance of the incubated mixture was measured at 745nm. The same procedure was done to prepare various dilutions of ascorbic acid (positive control). Percent free radical inhibition was calculated using Equation (5).

Anti-Bacterial Activities

The anti-bacterial activities of the synthesized compounds were determined against *Staphylococcus aureus*

(Gram-positive bacteria), *Agrobacterium tumefaction,* and *Proteus vulgaris* (Gram-negative bacteria) bacterial strain. Agar well diffusion method was used following the standard protocol.[31]

Statistical Analysis

All the experiments were performed in three replicates and the results have been presented as mean ± SEM.

Results And Discussion

Effect Of Synthesized Compounds On Acetylcholinesterase And Butyrylcholinesterase

The effectiveness of the synthesized compounds as inhibitors of the selected cholinesterases was tested. The activities of both the enzymes were suppressed to a lesser or greater extent. The results are presented in Tables 1 and 2 respectively, for acetylcholinesterase and butyrylcholinesterase. As is clear from Table 1, compound **III** was more potent against acetylcholinesterase with IC_{50} value of 63 µg/mL, followed by compound **II** (IC_{50} = 82 µg/mL). Compound **V** was moderately active with IC_{50} value of 182 µg/mL while compound **VI** and **I** were merely active (IC_{50} = 503 and 1002 µg/mL respectively).

Table 1 Effect Of The Compounds On The Activity Of Acetylcholinesterase Enzyme

Compound	Concentration (µg/mL)	%AChE Inhibition (mean ± SEM)	IC_{50} µg/mL
I	1000 500 250 125	68.11 ± 1.40 55.59 ± 1.23 46.10 ± 1.85 38.63± 1.33	1002
II	1000 500 250 125	78.67 ± 1.20 72.16 ± 2.01 59.21 ± 1.21 52.55 ± 1.73	82
III	1000 500 250 125	84.31 ± 1.12 75.66 ± 1.15 66.47 ± 2.08 62.22 ± 1.12	63
IV	1000 500 250 125	64.02 ± 1.70 50.04 ± 1.88 45.15 ± 1.83 41.99 ± 1.01	503
V	1000 500 250 125	70.25 ± 1.09 68.15 ± 1.25 56.34 ± 2.14 51.78 ± 1.72	182
Galantamine	1000 500 250 125	93.22 ± 0.81 88.14 ± 0.52 78.44 ± 0.61 64.53 ± 0.52	40

Table 2 Effect Of The Compounds On The Activity Of Butrylcholinesterase Enzyme

Compound	Concentration (µg/mL)	%BChE Inhibition (mean ± SEM)	IC_{50} µg/mL
I	1000 500 250 125	57.81 ± 0.54 56.26 ± 1.66 51.40 ± 2.05 48.13 ± 1.08	200
II	1000 500 250 125	70.13 ± 1.51 62.31 ± 2.11 59.15 ± 1.60 65.71 ± 1.28	82
III	1000 500 250 125	68.55 ± 1.07 63.83 ± 1.53 61.81 ± 1.54 58.26 ± 1.06	80
IV	1000 500 250 125	76.14 ± 1.90 60.50 ± 1.61 53.55 ± 1.07 49.83 ± 1.53	150
V	1000 500 250 125	62.67 ± 1.22 59.07 ± 1.76 55.09 ±1.04 53.07 ± 0.48	100
Galantamine	1000 500 250 125	92.30 ± 0.74 87.16 ± 1.23 78.42 ± 0.75 64.81 ± 0.73	55

The butyrylcholinesterase inhibitory activities of the synthesized compounds are presented in Table 2. Almost the same trend observed for acetylcholinesterase was also observed here with little variations. Compound **III** was more active with IC_{50} value of 80 µg/mL, followed by compound **II** with IC_{50} = 82 µg/mL. Comparatively improved activities were observed for compound **I, IV,** and **V** with IC_{50} values of 200, 150, and 100 µg/mL respectively.

Based on the results obtained it can be concluded that compound **III** and **II** have some therapeutic values in the treatment of neurodegenerative diseases. However, in vivo studies regarding their toxicology are needed.

Effect Of Synthesized Compounds On The Activity Of Alpha Amylase

Alpha amylase is a key enzyme of carbohydrate metabolism that makes glucose available for the absorption in the small intestine from starch sources. Inhibition of this enzyme would be helpful in lessening the burden of blood glucose in diabetic patients. Table 3 shows the inhibitory potential of the compounds on alpha amylase. Compound **IV** with IC_{50} value of 62 µg/mL was the most potent, followed by compound **I** (IC_{50} = 84 µg/mL). Compound **II, III,** and **V** were also quite potent, but less than **IV** and **I** with IC_{50} values of 135, 115, and 100 µg/mL respectively. All these compounds have inhibitory effects of alpha amylase which is clear from their IC_{50} values.

Effect Of Synthesized Compounds On The Activity Of Alpha Glucosidase

Glucosidase is another important enzyme for carbohydrate metabolism and it helps in glucose release from starch sources. In diabetic patients, its inhibition relieves the blood glucose burden. Compound **III** and **II** were more effective inhibitors of alpha glucosidase with IC_{50} values of 75 and 80 µg/mL respectively. Compound **IV** appeared moderately active with IC_{50} value of 180 µg/mL. Compound **I** and **V** were merely active with IC_{50} values of 500 and 1000 µg/mL respectively (Table 4).

Table 3 Effect Of The Compounds On The Activity Of Alpha Amylase Enzyme

Compound	Concentration (µg/mL)	%Inhibition (mean ± SEM)	IC_{50} µg/mL
I	1000 500 250 125	70.29± 0.31 68.08 ± 0.87 63.15 ± 1.41 60.23± 1.03	84
II	1000 500 250 125	59.13± 0.15 57.81 ± 2.25 56.04 ± 1.58 50.04 ± 0.10	135
III	1000 250 250 125	65.82 ± 1.13 62.49 ± 1.24 57.91 ± 0.43 50.07 ± 2.11	115
IV	1000 500 250 125	80.12 ± 2.02 79.18 ± 1.09 75.63 ± 0.63 69.04 ± 1.19	62
V	1000 500 250 125	62.82 ± 1.13 59.49 ± 1.24 55.91 ± 0.43 52.07 ± 2.11	100
Acarbose	1000 500 250 125	88.09 ± 1.13 72.98 ± 1.34 68.04 ± 1.88 58.13 ± 1.34	50

Table 4 Effect Of The Compounds On Activity Of Enzyme Alpha Glucosidase

Compound	Concentration (µg/mL)	%Inhibition (mean ± SEM)	IC_{50} µg/mL
I	1000 500 250 125	51.13± 0.15 47.81 ± 2.25 46.04 ± 1.58 40.04 ± 0.10	1000
II	1000 500 250 125	72.02 ± 1.70 71.04 ± 1.88 70.15 ± 1.83 69.99 ± 1.01	80
III	1000 500 250 125	84.67 ± 1.20 81.16 ± 2.01 79.21 ± 1.21 75.67 ± 1.20	75
IV	1000 500 250 125	65.92 ±0.23 60.46 ±0.083 55.88 ± 2.04 48.00 ± 1.34	180
V	1000 500 250 125	57.18 ± 1.46 52.14 ± 3.93 40.45 ± 1.59 32.49 ± 2.07	500
Acarbose	1000 500 250 125	94.02 ± 1.23 79.11 ± 2.12 68.14 ± 2.18 59.83 ± 2.04	60

Effect Of Synthesized Compounds On The Activity Of Glucose-6-Phosphatase

Glucose-6-phosphatase is another important enzyme of carbohydrate metabolism that plays a very important role in glycogenolytic and gluconeogenic pathways. Its inhibition is needed in case of diabetes mellitus and enhancement of its activity is desired in patients suffering from glycogen storage disease. The inhibition of this enzyme is interpreted from the mg of inorganic phosphate released during the reaction. The smallest amount of phosphate released in a given reaction indicates high inhibition.

Table 5 Effect Of The Compounds On Activity Of Enzyme Glucose-6-Phosphatase

Compound	Concentration µg/mL	Total Activity*	Control
I	1000 500 250 125	21.20 25.31 31.62 34.11	72.0
II	1000 500 250 125	08.78 10.07 13.10 15.60	72.0
III	1000 500 250 125	24.23 27.10 28.35 32.04	72.0
IV	1000 500 250 125	10.10 11.77 13.58 17.70	72.0
V	1000 500 250 125	03.12 04.16 05.21 05.66	72.0

Note: *The activity has been expressed as mg of P released from the potassium salt of glucose-6-phosphate per hour (at 33°C, pH 6.7).

Compound **V** and **II** were the most potent inhibitors of this enzyme as the amount of phosphate released was comparatively less than that of other compounds (Table 5). Compound **I, III,** and **V** were also quite potent when compared to control experiments. As stated earlier, enhancers of this enzyme are also required in case of glycogen storage disease patients, however, even though all of these compounds were inhibitors of this enzyme, none of them exhibited the enhanced role.

Free Radical Scavenging Activities Of The Compounds

Free radicals are the chemically reactive substances with singlet electron. Free radicals are continuously produced in the human body and are promptly scavenged by the body's defense system. However, when their production increases, supplementation of antioxidants are needed from outside, otherwise they damage the biologically important molecules (DNA and protein). All these synthesized compounds showed scavenging activities against the commercially available free radicals DPPH and ABTS, which is clear from Table 6. Compound **IV** was a more potent inhibitor of DPPH free radical (IC_{50} = 64 µg/mL) followed by compound **I** (IC_{50} = 67 µg/mL). Compound **V** and **III** were also quite effective in scavenging the DPPH radical and exhibited IC_{50} values of 70 and 79 µg/mL respectively. The least effective compound was compound **II**.

A totally different inhibitory pattern was observed in case of ABTS free radical. Compound **I** was more potent with IC_{50} value of 66 µg/mL, followed by compound **III** and **V** (IC_{50} = 80 and 85 µg/mL respectively). Compound **IV** was moderately effective while compound **II** was the least effective one.

Anti-Bacterial Activities Of The Compounds

The antibacterial spectrum of the synthesized compounds was determined against *A. tumefaction, P. vulgaris,* and *S. aureus*. The results have been presented in Table 7. Among the tested compounds, compound **I** was highly active against *A. tumefaction* with MIC value of 4.02 µg/mL followed by compound **II** (MIC = 4.02 µg/mL). Against the same bacteria, compound **III, IV** and **V** were also quite effective with MIC values of 11.62, 13.49, and 11.59 µg/mL respectively.

Against *P. vulgaris,* compound **V** was more effective followed by compound **I** (MIC = 8.94 and 9.76 µg/mL respectively). The rest of the compounds were also quite effective against this bacterial strain.

The growth of *S. aureus* was more effectively inhibited by compound **IV** (MIC= 4.03 µg/mL). The rest of the compounds were moderately active. Comparatively, all these compounds exhibited good antibacterial actions against all the selected bacterial strains.

Conclusion

The wide applicability of thioureas in various fields, especially in the field of medicines, makes it quite practical to study biological activities of an already synthesized compound or to synthesize some more and determine their therapeutic significance, if any. In this study, five new thioureas were synthesized from chiral amines in the presence of sunlight (hence eco-friendly and economic procedure) which were then used as starting material in the synthesis of N-heterocyclic thiourea compounds. The compounds were tested for their antibacterial, antioxidant, anticholinesterase, and antidiabetic potentials. Glucose-6-phosphatase was potently inhibited by compound **V** and **II**, α-amylase by

Table 6 Estimation Of Free Radical Scavenging Potential Through DPPH And ABTS Assays

Compound	Concentration µg/mL	DPPH % I (mean ± S.E.M)	DPPH IC_{50} µg/mL	ABTS % I (mean ± S.E.M)	ABTS IC_{50} µg/mL
I	1000 500 250 125	86.67 ± 1.20 82.16 ± 1.01 80.21 ± 1.21 78.55 ± 1.73	67	81.67 ± 1.20 80.16 ± 2.01 78.21 ± 1.21 77.55 ± 1.93	66
II	1000 500 250 125	57.59 ± 1.23 54.10 ± 1.85 52.63± 1.33 50.02 ± 1.99	120	55.67 ± 1.12 52.07 ± 1.16 49.09 ±1.04 47.07 ± 0.46	400
III	1000 500 250 125	67.45 ± 0.095 66.07 ± 0.283 64.74 ± 0.267 61.14±0.177	79	69.11 ± 0.234 68.27 ± 0.532 66.25 ±0.123 64.77 ±0.087	80
IV	1000 500 250 125	79.15 ± 1.23 74.13 ± 1.51 69.31 ± 2.11 66.15 ± 1.70	64	78.15 ± 1.21 74.99 ± 1.51 70.13± 0.05 64.81 ± 1.05	100
V	1000 500 250 125	73.14 ± 1.10 71.50 ± 1.61 70.55 ± 1.27 68.83 ± 1.53	70	75.04 ± 0.10 73.18 ± 1.46 71.14 ± 1.90 69.45 ± 1.29	85
Ascorbic acid	1000 500 250 125	90.14 ± 0.76 85.11 ± 1.64 76.13±1.45 67.40±1.18	35	88.17 ± 0.43 85.45 ± 1.36 70.14 ± 1.16 55.30 ± 1.75	55

Table 7 Evaluation Of Antibacterial Potential Of The Synthesized Compounds

Samples	Bacterial Species					
	Conc. (µg/mL)	*Agrobacterium tumefaction*		*Proteus vulgaris*		*Staphylococcus aureus*	
		ZI (mm)	MIC µg/mL	ZI (mm)	MIC µg/mL	ZI (mm)	MIC µg/mL
Compound I	10 20 30	21 24 26	4.02	15 22 24	9.76	13 16 18	19.58
Compound II	10 20 30	21 23 26	4.04	12 22 26	12.22	12 19 23	14.83
Compound III	10 20 30	15 20 26	11.62	12 16 22	14.96	15 16 19	14.93
Compound IV	10 20 30	14 18 23	13.49	14 18 23	11.92	21 23 26	4.03
Compound V	10 20 30	15 20 25	11.59	16 21 24	8.94	12 14 16	24.11

Abbreviations: ZI, Zone of inhibition; MIC, Minimum inhibitory concentration.

compound **IV** and **I**, α-glucosidase by compound **III** and **II** while acetylcholinesterase and butyrylcholinesterase were inhibited by compound **III** and **II**. As free radical scavengers, compound **IV** was a more potent inhibitor of DPPH radical while compound **II** was the most effective against ABTS. Their antibacterial evaluation revealed that all these compounds were effective against *A. tumefaction*. Even though the in vitro experiments pointed out the good biological activities of these compounds, it is still not safe to assume that they are good therapeutic agents and further testing and in vivo experiments are needed to fully assess its pharmacological applicabilities.

Availability Of Data And Material

The data in the form of the thesis will be provided on demand.

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Author Contributions

S.N. and M.Z. conceived and designed the experiments; M. N.U., B.A., R.U. and M.U.K.S. performed the experiments; M.Z., A.A.S and H.M.M. analyzed the data; M.Z., and M. U.K.S contributed reagents and materials. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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