Research Article

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Synthesis, characterization, and pharmacological evaluation of thiourea derivatives

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Abstract: Thioureas and their derivatives are organosulfur compounds having applications in numerous fields such as organic synthesis and pharmaceutical industries. Symmetric thiourea derivatives were synthesized by the reaction of various anilines with CS_2 . The synthesized compounds were characterized using the UV-visible and nuclear magnetic resonance (NMR) spectroscopic techniques. The compounds were screened for *in vitro* inhibition of α -amylase, α -glucosidase, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE) enzymes and for their antibacterial and antioxidant potentials. These compounds were fed to Swiss male albino mice to evaluate their toxicological effects and potential to inhibit glucose-6-phosphatase (G6Pase) inhibition. The antibacterial studies revealed that compound **4** was more active against the selected bacterial strains. Compound **1** was more active against 2,2-diphenyl-1-picrylhydrazyl and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radicals, AChE, BuChE, and α -glucosidase. Compound **2** was more potent against α -amylase and G6Pase. Toxicity studies showed that compound **4** is safe as it exerted no toxic effect on any of the hematological and biochemical parameters or on liver histology of the

experimental animals at any studied dose rate. The synthesized compounds showed promising antibacterial and antioxidant potential and were very active (both *in vitro* and *in vivo*) against G6Pase and moderately active against the other selected enzymes used in this study.

Keywords: thiourea derivatives, pharmacological evaluation, acetylcholinesterase, glucose-6-phosphatase, biochemical and histopathological evaluation, *in silico* modeling

1 Introduction

Thiourea or thiocarbamide is an organosulfur compound with the formula $\text{SC}(\text{NH}_2)_2$. The term “thioureas” is used for the class of compounds with the general formula $(\text{R}_1\text{R}_2\text{N})(\text{R}_3\text{R}_4\text{N})\text{C}=\text{S}$ [1]. None of any other class of organic compounds has such a wide diversity and multiple applications as thioureas have. Indeed, in almost every branch of chemistry, thioureas have played their exceptional role [2]. Commercially, industrially, and academically, they are of huge importance. On commercial scale, thioureas are frequently used in photographic film, plastics, dyes, elastomers, and textiles [3,4]. Several derivatives of thioureas are used as pharmaceuticals, preservatives, rodenticides, and insecticides [5–8]. Thioureas have valuable uses in organic synthesis and are used as intermediates in several organic synthetic reactions [9].

Another very important, diverse, and effective area of thiourea applications is their biological activities. Again, none other class of organic compounds has as much biological activities as thioureas. These have been reported to have antifungal, antiviral, antibacterial, catalytic, anti-tubercular, analgesic, insecticidal, anti-inflammatory, herbicidal, anticonvulsant, anti-cancer, anti-thyroid, anthelmintic, and anti-phenoloxidase activities [9–16]. They also act as rodenticide and have anti-HIV, antiviral, high density lipoprotein (HDL)-elevating, antibacterial, analgesic,

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antidiabetic, anti-hypertensive, anti-epileptic, anticancer, DNA-binding, hypnotic, and anesthetic properties [9–16].

Free radicals with unpaired electrons are very unstable and reactive molecules. The most reactive molecules are different atomic and molecular forms of oxygen, which are called reactive oxygen species (ROS). Being unstable, they attack nearby molecules, snatching their electrons and thus making them reactive and unstable free radicals, initiating a free radical chain reaction. Free radicals are normally and continually produced in the body because of biochemical reactions, and they are normally scavenged by the body scavenging mechanism. If the body's self-defense system becomes incapable of eliminating them, then ROS can damage DNA, unsaturated lipids, and proteins and can lead to death of the cell. ROS are the cause of a variety of pathologic processes such as cancer, aging, inflammatory disease, reperfusion injury, diabetes, stroke, and dementia and several other lethal diseases [17–20].

Alzheimer's disease (AD) is a type of dementia that causes memory loss, problems with cognitive abilities, thinking, and behavior. Usually, its symptoms develop slowly and get worse with the passage of time, becoming serious enough to interfere with routine life and usually accounts for about 60–80% of dementia cases [19]. The two main types of cholinesterases present in the mammalian brain are acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). In patients with AD, a remarkable decrease in the concentration of acetylcholine (ACh) was observed. One possible way to stop and maintain proper ACh hydrolysis is inhibiting AChE, which is the enzyme responsible for its hydrolysis [17–20].

BuChE has a minor role in the normal, healthy brain, but its activity gradually increases in patients with AD. In the advanced stages of AD, when AChE is inhibited, BuChE usually can compensate for AChE and hydrolyze the remaining ACh present, thus making the situation worse. Recently, experimental evidence from the dual inhibition of both AChE and BuChE show enhanced therapeutic benefits in AD and other related dementias [17–20].

Diabetes mellitus is a disorder of carbohydrate metabolism, where the body is unable to either produce insulin or respond to it, which results in impairment of maintaining normal blood glucose levels. Insulin is a hormone secreted by β cells of islets of Langerhans in the pancreas. It triggers the uptake of glucose by the cells, thereby ensuring a proper blood glucose level and making it available to the cells to be used as an energy source. People with diabetes either have inactive β cells that cannot secrete insulin (type 1 diabetes mellitus) or have unresponsive muscles and adipose cells that are resistant to the actions of insulin (type 2 diabetes mellitus). In both cases, the concentration of glucose in blood increases, resulting in hyperglycemia. In the treatment

of diabetes mellitus, the main aim is to reduce blood glucose concentrations to normal range. One of the several strategies is to use such drugs that can control blood glucose level. One class of such drugs having the ability to inhibit two important enzymes involved in carbohydrate metabolism, namely, α -amylase and α -glucosidase, has been proved to be the most effective treatment [17–20].

In polysaccharides, the α -1,4-glycosidic bond is hydrolyzed by the α -amylase enzyme to yield oligosaccharides and disaccharides, which are further hydrolyzed by α -glucosidase (EC 3.2.1.20), an intestinal enzyme that splits their α -1,4-glycosidic linkages, yielding α -D-glucose molecules, which are then absorbed into the blood. Inhibition of these enzymes delays the digestion of carbohydrates and thus their consequent absorption, ultimately lowers the postprandial blood glucose concentration [17–20].

Glucose-6-phosphatase (G6Pase) is an important enzyme of carbohydrate metabolism, which catalyzes the last step of gluconeogenesis and glycogenolysis, where it converts glucose-6-phosphate to glucose and inorganic phosphate [20,21]. Both glycogenolysis and gluconeogenesis result in the formation of glucose-6-phosphate from gluconeogenic precursors in liver and kidney and from glycogen in liver. It not only catalyzes the hydrolysis of glucose-6-phosphate but also catalyzes its resynthesis from glucose and a phosphoryl donor. This enzyme, therefore, plays an important role in the regulation of glucose homeostasis. Consequently, its insufficiency prompts glycogen storage disorder, which is an acquired metabolic condition, described by poor resistance to fasting, development impediment, and hepatomegaly, which results in amassing of glycogen and fat in the liver. G6Pase also has an important role in diabetes mellitus, where its activity is increased in liver, leading to high blood glucose level. Therefore, its inhibition can decrease hepatic glucose output. Recently, this enzyme has been identified as an important antidiabetic target [20,21].

Many compounds such as 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid, which is a well-known inhibitor of anion transporter, 2-hydroxy-5-nitrobenzaldehyde, and chlorogenic acid derivatives inhibit the translocases of G6Pase system, but only a few compounds have inhibitory activities on the G6Pase enzyme complex [22–24]. Recently, a series of 4-, 5-, 6-, 7-tetrahydrothienopyridines have been reported as potent competitive inhibitors of G6Pase [20]. There is a need to find out a compound that competitively inhibit the G6Pase; hence, it will be an alternative to the drugs that inhibit T1 translocase [20,21].

An organism's hematological and biochemical parameters of blood are indicative of its overall physiological status. A normal healthy organism without any toxic

metabolites in blood should maintain its blood indices in relatively normal or constant state. A disorder, disease, or toxicity may be expected if the blood indices are found to be significantly altered or a tissue damage is detected [25,26]. *In vivo* studies of certain synthetic compounds (metribuzin, etc.) in albino mice have shown marked alterations in hematological and biochemical parameters [27]. It is necessary that a chemical compound that is a drug or intended to be used for therapeutic purpose should be assessed for its possible toxicity or adverse effects through examining the changes it may or may not bring in blood parameters and in function or structure of tissues [25,26].

The aim of this study was to synthesize novel thiourea derivatives that have antidiabetic, antibacterial, antioxidant, and anticholinesterase properties. The synthesized compounds were also tested in *in vivo* using albino mice whether they can inhibit G6Pase or not. Furthermore, various hematological and biochemical parameters were studied and histopathological examinations were also carried out to find out the effect of the synthesized compounds on the overall metabolic status and on liver tissue to tell that whether they are safe enough to be used therapeutically or not.

2 Materials and methods

Chemicals such as 3,4-dichloroaniline, 2,6-dimethylaniline, ethyl 4-aminobenzoate, 4-nitroaniline, 4-methylaniline, and CS₂ were acquired from Sigma-Aldrich, Merck, and Alpha Acer and were used without further purification. Solvents including *n*-hexane, methanol, dichloromethane, ethanol, ethyl acetate, and chloroform were purchased from Sigma-Aldrich. The solvents were distilled before use. Thin layer chromatography (TLC) plates were used to monitor the reaction at regular intervals under ultraviolet irradiation.

2.1 Synthesis

2.1.1 Compound 1

Carbon disulfide (5 mmol) and 3,4-dichloroaniline (10 mmol) were added to 5 mL of water in a 50-mL round-bottom flask without using magnetic bar stirring, and the flask was corked and placed in the sunlight. After 7 h, the white solid was obtained in water. Thin-layer chromatography (10:90% ethyl acetate:*n*-hexane used as a mobile phase) was used to check the progress of the reaction. The product obtained was

recrystallized with ethanol, purified by column chromatography, and characterized using UV-visible and NMR techniques (Figure 1S).

Compound 1

2.1.2 Compound 2

Carbon disulfide (5 mmol) and 2,6-dimethylaniline (10 mmol) were added to 5 mL of water in a 50-mL round-bottom flask without using magnetic bar or stirring. The flask was corked and placed in the sunlight. After 5 h, the white solid was obtained in water. Thin-layer chromatography (10:90% ethyl acetate:*n*-hexane) was used to check the progress of the reaction. The product obtained was recrystallized with ethanol and further purified by column chromatography and characterized by UV-visible and NMR techniques (Figure 2S).

Compound 2

2.1.3 Compound 3

Carbon disulfide (5 mmol) and ethyl 4-aminobenzoate (10 mmol) were added to 5 mL water in a 50-mL round-bottom flask without using magnetic bar or stirring. The flask was corked and placed in the sunlight. After 5 h, the white solid was obtained in water. Thin-layer chromatography (10:90% ethyl acetate:*n*-hexane) was used to check the progress of the reaction. The product obtained was recrystallized with ethanol, purified by column chromatography, and characterized by UV-visible and NMR techniques (Figure 3S).

Compound 3

2.1.4 Compound 4

Carbon disulfide (5 mmol) and 4-nitroaniline (10 mmol) were added to 5 mL of water in a 50 mL round-bottom flask without using magnetic bar or stirring. The flask was corked

and placed in the sunlight. After 7 h, a white solid was obtained. Thin-layer chromatography (10:90% methanol:chloroform) was used to check the progress of the reaction. The product obtained was recrystallized with ethanol, purified by column chromatography, and characterized by UV-visible and NMR techniques (Figure 4S).

2.1.5 Compound 5

Carbon disulfide (5 mmol) and ethyl-4-methylaniline (10 mmol) were added to 5 mL of water in a 50 mL round-bottom flask without using magnetic bar or stirring. The flask was corked and placed in the sunlight. After 5 h, the white solid was obtained in water. Thin-layer chromatography (10:90% ethyl acetate:*n*-hexane) was used to check the progress of the reaction. The product obtained was washed with a mixture of ethyl acetate:*n*-hexane, purified by column chromatography, and characterized by UV-visible and NMR techniques (Figure 5S).

2.2 Instrumentation

Electrothermal melting point instrument (Model BioCote Stuart-SMP-10, Japan) was used to determine the melting point of the compounds in a sealed capillary tube. The ^1H -NMR spectra were recorded using an NMR Bruker apparatus at 300 MHz in CDCl_3 .

2.3 *In vitro* biological activities of the synthesized compounds

2.3.1 Antibacterial assay

Minimum inhibitory concentration (MIC) and agar well diffusion method were used to evaluate the antibacterial potential of the synthesized compounds. Four different bacterial strains *Pseudomonas aeruginosa* ATCC 10145,

Klebsiella pneumoniae ATCC 13883, *Enterococcus faecalis* ATCC 29212, and *Salmonella typhi* ATCC 6539 were used in this study. Nutrient agar and the Petri dishes were sterilized at 121°C for 15 min using autoclave (Jisico: J-NAS 45, Korea). Nutrient agar was poured into the sterilized Petri dishes under aseptic environment just before solidification. In this study, DMSO and ceftriaxone were used as negative and positive controls, respectively. Sample solutions of the synthesized thiourea compounds were prepared in DMSO (30 µg/mL). At the end of the incubation period, the average zones of inhibition were acquired and were compared with the positive control. Triplicate analysis was performed for the antibacterial assay.

To determine the MIC of the synthesized compounds, they (10–100 µg/mL) were added to the test tubes containing nutrient broth and inoculated with 5×10^5 cfu/mL of the selected bacterial strains and incubated at 37°C for 20 h. After 20 h incubation time, the bacterial growth in each tube was noted.

2.3.2 Antioxidant activity by DPPH method

Antioxidant activity of the synthesized compounds was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS assays, described by Naz et al. [28]. The stock solution of DPPH having concentration of 20 mg/100 mL of methanol was prepared, while stock solution of ABTS was prepared by dissolving 7 mM of ABTS and 2.45 mM of potassium persulfate separately in 100 mL of methanol. About 3 mL from each stock solutions was taken and diluted with methanol to adjust its absorbance of 0.7 at 515 nm. The stock solutions were covered with aluminum foil and placed in a cold and dark place for 24 h to ensure the formation of free radical. Sample solutions were prepared by dissolving 5 mg of each compound in 5 mL methanol (sample stock solution). A dilution series having concentrations of 62.5, 125, 250, 500, and 1,000 µg/mL were also prepared from stock compound solutions. Specified amount from working standards was mixed with specified amount of DPPH/ABTS solutions and incubated for specified interval of time, as described by Naz et al. [28]. Percentage of free radical inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100, \quad (1)$$

where A is the absorbance of pure DPPH in oxidized form and B is the absorbance of the sample.

2.3.2 Anticholinesterase activities of the synthesized compounds

Anticholinesterase activities of the synthesized compounds were evaluated using AChE and BuChE enzymes using Ellman's assay [14,29], which is based on acetylthiocholine iodide and butyrylthiocholine iodide hydrolysis by respective enzymes. The resultant product of hydrolysis is then combined with 5-thio-2-nitrobenzoate anion to form a yellow colored compound, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The DTNB is then detected by the spectrophotometer (T60 PG Instruments, UK) after 15 min of the reaction.

Phosphate buffer, DTNB, AChE and BuChE, and acetylcholine iodide and butyrylcholine iodide solutions were prepared as described in Naz *et al.* [32]. From working dilution, 1 mL was added to a series of test tubes, and 100 μ L of enzyme and DTNB solutions were added following 15 min incubation at 25°C. At the end of incubation, 100 μ L of the substrate AChEI/BChEI solution was added to the test tube and was left for 15 min to get the reaction completed. The absorbance of the mixture was monitored at 412 nm. Galantamine was used as a positive control.

Percent activities of AChE and BChE and their percent inhibition were calculated using the following equations:

$$V = \frac{\Delta \text{Abs}}{\Delta T}, \quad (2)$$

$$\% \text{ enzyme activity} = \frac{V}{V_{\max}} \times 100, \quad (3)$$

where V is the rate of reaction in the presence of inhibitor, ΔAbs is the change in absorbance, ΔT is the change in time, and $V_{\max}$ is the rate of reaction without inhibitor.

2.3.3 Antidiabetic property of the synthesized compounds

2.3.3.1 Alpha-amylase inhibition

Alpha-amylase inhibition assay was performed according to the procedure of Naz *et al.* [28]. Enzyme solution in phosphate buffer (25 μ L) after 10 min of incubation at 25°C was mixed with starch dissolved in phosphate buffer (25 μ L) and again incubated at the same interval of time and temperature. Then, 50 μ L DNS (3,5-dinitrosalicylic acid) was added to the mixture to stop the reaction, and absorbance was measured at 540 nm. The percent inhibition was determined from the

percentage (w/v) of maltose generated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{maltose}) \text{ test}}{(\text{maltose}) \text{ control}} \times 100, \quad (4)$$

where (maltose) test is the percentage (w/v) of maltose generated by the sample solution and (maltose) control is the percentage (w/v) of maltose generated by the control solution. Acarbose was used as the standard.

2.3.3.2 Alpha-glucosidase inhibition

The potential alpha-glucosidase inhibition was determined according to the procedure of Naz *et al.* [32]. One hundred microliters of the enzyme solution in phosphate buffer (0.50 unit/mL) was mixed with compound working dilutions and incubated for 15 min at room temperature. About 100 μ L of *p*-nitrophenyl- α -D-glucopyranoside was added to start the reaction, while 80 μ L Na_2CO_3 (0.2 M) was added to stop the reaction. The absorbance of the resultant mixture of the reaction was recorded at 405 nm. The percent inhibition was calculated using the following equation:

$$\begin{aligned} \text{Percent inhibition} \\ = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}}, \end{aligned} \quad (5)$$

where control absorbance is the absorbance of the control solution and sample absorbance is the absorbance of the sample solution.

2.3.3.3 G-6Pase inhibition

The G-6Pase activity was calculated on the basis of the rate of phosphate release under experimental conditions described by Fiske and Subbarow [21]. For calculating the enzyme activity, the same protocol was adopted as followed by Naz *et al.* [28]. The concentration of inorganic phosphate in each sample was determined from comparison with the standard phosphate calibration curve.

2.4 *In vivo* experiments

2.4.1 Experimental animals and their treatment

Swiss male albino mice, 24 weeks old, weighing 30–35 g, were obtained from the National Institute of Health,

Islamabad, and were housed in plastic cages having good space and sawdust containing bedding. The animals were given free access to water and food. The room temperature was maintained at 25°C. All experiments and overall handling of the animals were performed in accordance with the guidelines, “Ethics Committee for Animal Care & Use, Pakistan” and ARRIVE guidelines. Approval was obtained from the University Ethical Committee vide letter NO: UOM-EC/PHARM & BIOCHEM/2018/1.

2.4.1.1 Groups

The animals were divided into six groups. The group numbers were actually the arbitrary names of compounds **1**, **2**, **3**, **4**, and **5** fed to each group. Based on the quantity of dose the mice received, each of the five groups were subdivided in three groups (each having five mice): A (received 0.5 mg of dose/kg body weight of mice), B (1 mg of dose/kg body weight of mice), and C (received 1.5 mg of dose/kg body weight of mice). Following are the groups and subgroups:

Group CL (having five mice): The group receiving only the normal food and water.

Group 1 (fed with compound **1**): Subgroups: **1A**, **1B**, **1C**

Group 2 (fed with compound **2**): Subgroups: **2A**, **2B**, **2C**

Group 3 (fed with compound **3**): Subgroups: **3A**, **3B**, **3C**

Group 4 (fed with compound **4**): Subgroups: **4A**, **4B**, **4C**

Group 5 (fed with compound **5**): Subgroups: **5A**, **5B**, **5C**

2.4.1.2 Collection of blood and tissue samples

Blood samples were taken at the start of the experiment and on days 7, 14, and 21 for evaluation of the hematological and biochemical parameters. On the 22nd day, the mice were sacrificed by first euthanizing them with isoflurane and then killed through cervical dislocation, and the livers were collected for histopathology studies.

2.4.1.3 Acute toxicity

Mice ($n = 90$) were taken, fasted overnight, and divided into five major groups (for the five compounds); each had further three subgroups and each sub-group had six mice. The subgroups were based on the three different doses they received (0.8, 1.6, and 2.4 mg/kg body weight). It was recorded that two of the six mice in the subgroup 3 (fed with the highest dose) of the major

group 2 died after 22 h. In the light of the acute toxicity, dose range for the subchronic toxicity was set as 0.5–1.5 mg/kg body weight.

2.4.2 G6Pase inhibition

Mice were sacrificed on the 22nd day of the experiment, their livers were collected, and fast frozen, each in a separate flask (Labconco Corporation, Kansas City, MO, USA). Liver homogenates were prepared in iced water, and enzyme extract was prepared by placing the homogenate in a flask at a shaker (Stuart SF1, Cole Parmer, UK) (maintained at 0°C by continuous addition of ice) for an hour, and the supernatant obtained is filtered and used as a source of G6Pase. Then, 1 mL of the obtained liver homogenate (enzyme source), 2.5 mL of 0.01 M glucose-6-phosphate (substrate), and 1.5 mL Tris(hydroxymethyl)aminomethane buffer (pH 6.7) were taken in a flask and incubated for an hour at 33°C. The reaction was stopped by the addition of 1 mL of 10% trichloroacetic acid. Inorganic phosphate released (one of the products) during the reaction was measured by the method of Fiske and Subbarow [21].

2.4.3 Hematological studies

About 0.2 mL of blood sample was drawn in 10% EDTA (anticoagulant) tube. Hematological parameters such as hematocrit, RBCs, hemoglobin, and leukocyte and platelet concentration were determined by using an automated hematology analyzer (Abbot CELL-DYN 3200, USA).

2.4.4 Biochemical studies

Another 0.2 mL of blood sample was collected without any EDTA to obtain serum after centrifugation. Serum was studied for biochemical parameters such as blood sugar, total cholesterol, triacylglycerol, alanine transaminase (ALT), and HDL using the HUMAN kit (HUMAN Diagnostic, Germany).

2.4.5 Histopathology studies

Specimens from the collected liver dissected, fixed in 10% formalin, and dehydrated using ethanol. Thin sections (about 8–10 μm) were cut from the specimens and studied under a light microscope (Olympus BH-2, Shinjuku, Tokyo, Japan) after staining [30].

2.5 Statistical analysis

The IC_{50} for % DPPH, ABTS, α -amylase, α -glucosidase, AChE, and BuChE was measured by linear regression analysis against the different concentrations of the tested compounds using Excel 2013 version. All results were presented as mean $\pm$ SEM (standard error of mean). Student's *t*-test and one-way ANOVA followed by Dunnett's post hoc multiple comparison test were used to determine *P* values. *P* values less than 0.05 were considered as significant.

3 Results and discussion

3.1 General mechanism for the synthesis of thiourea

General Mechanism:

In the first step, amine or amine derivative attacks as a nucleophile on the electrophilic carbon of CS_2 to form thiocarbamate intermediate. In the second step, H^+ transfer to form thiocarbamate. In the third step, another amine molecule attacks on the electrophilic center of carbamate to form thiourea. The H_2S is evolved during this mechanism.

3.2 Spectral analysis of the synthesized compounds

The spectral details of the synthesized compounds are described below. 1H -NMR spectra of compounds **1–5** are shown in Figures 1S–5S. As an internal reference, tetramethyl silane was used. Chemical shifts are given in a delta

scale (ppm). Abbreviations s, d, t, and m represent singlet, doublet, triplet, and multiplet, respectively.

3.2.1 Compound 1

1,3-Bis(3,4-dichlorophenyl)thiourea: IUPAC name, 1,3-bis(3,4-dichlorophenyl)thiourea, yield = 84%, m.p. = 174–180°C, 1H -NMR (300 MHz, $CDCl_3$): δ ppm 7.20 (d, 2H), 6.70 (d, 2H), 6.40 (d, 2H). Since this is a known compound, its structure elucidation data were already reported [31].

3.2.2 Compound 2

1,3-Bis(2,6-dimethylphenyl)thiourea: IUPAC name, 1,3-bis(2,6-dimethylphenyl)thiourea, yield = 82%, m.p. = 180–184°C, 1H -NMR (300 MHz, $CDCl_3$): δ ppm 6.90 (d, 4H), 6.70 (d, 4H), 2.10 (d, 4H). Since this is a known compound, its structure elucidation data were already reported [31].

3.2.3 Compound 3

Diethyl 4,4-thiocarbonylbis(azanediy)l)benzoate: yield = 78%, m.p. = 177–183°C, 1H -NMR (300 MHz, $CDCl_3$): δ ppm 7.60 (d, 4H), 6.50 (d, 4H), 4.30 (d, 2H) 1.30 (d, 2H). Since this is a known compound, its structure elucidation data were already reported [31].

3.2.4 Compound 4

1,3-Bis(4-nitrophenyl)thiourea: IUPAC name, 1,3-bis(4-nitrophenyl)thiourea, yield = 80%, m.p. = 188–193°C, 1H -NMR (300 MHz, $CDCl_3$): δ ppm 8.0 (d, 4H), 6.60 (d, 4H). Since this is a known compound, its structure elucidation data were already reported [31].

3.2.5 Compound 5

1,3-Di-*p*-tolylthiourea: IUPAC name, 1,3-bis(4-methylphenyl) thiourea, yield = 86%, m.p. = 181–185°C, $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm 6.90 (d, 4H), 6.30 (d, 4H), 2.30 (d, 2H) 1.30 (d, 2H). Since this is a known compound, its structure elucidation data were already reported [31].

3.3 Antibacterial spectrum of the synthesized compounds

The synthesized compounds were evaluated for their antibacterial potential against *K. pneumoniae*, *Pseudomonas aeruginosa*, *S. typhi*, and *Enterococcus faecalis*. Compounds **1** and **5** showed moderate antibacterial activities. The antibacterial studies are shown as zone of inhibition in millimeters in Table 1. Compound **2** showed substantial activities against the selected bacterial strains, whereas compounds **3** and **4** showed significant antibacterial activities; compound **4** was the highly potent one. Against *E. faecalis*, *P. aeruginosa*, *S. typhi*, and *K. pneumoniae*, compound **4** showed 29 mm (standard 30 mm), 24 mm (standard 25 mm), 30 mm (standard 31 mm), and 19 mm (standard 20 mm) zone of inhibitions, respectively.

The MIC values of each compound (in $\mu\text{g/mL}$) are shown in Table 1. The MIC values were in the range of 40–60 $\mu\text{g/mL}$, indicating the high antibacterial potency of the synthesized compounds. Here, again compounds **3** and **4** were more potent, and less amount was required to inhibit the bacterial growth.

3.4 Antioxidant activity of the synthesized compounds

The synthesized compounds were investigated for their antioxidant potential using the DPPH and ABTS assays. In the DPPH assay, compound **1** was found to have higher antioxidant activity, with the IC_{50} value of 45 $\mu\text{g/mL}$, followed by compound **3** (IC_{50} = 50 $\mu\text{g/mL}$). The IC_{50} values of compounds **2**, **4**, and **5** were 100, 105, and 112 $\mu\text{g/mL}$ (Table 2), respectively. As a positive control, ascorbic acid was used with the IC_{50} value of 20 $\mu\text{g/mL}$.

Comparable antioxidant activities of the synthesized compounds against ABTS free radical were observed. For compound **1**, the IC_{50} value was 52 $\mu\text{g/mL}$, almost similar to its IC_{50} value in the antioxidant activity using DPPH free

Table 1: Antibacterial spectrum of the synthesized compounds

Sample	Bacterial strain	Zone of inhibition of standard* (mm)	Zone of inhibition of sample* (mm)	MIC ($\mu\text{g/mL}$)
1	<i>Enterococcus faecalis</i>	30	27	40
	<i>Pseudomonas aeruginosa</i>	28	24	50
	<i>Salmonella typhi</i>	25	22	60
	<i>Klebsiella pneumoniae</i>	23	20	60
	<i>E. faecalis</i>	31	29	40
2	<i>P. aeruginosa</i>	27	24	40
	<i>S. typhi</i>	33	29	40
	<i>K. pneumoniae</i>	24	22	50
	<i>E. faecalis</i>	28	26	50
3	<i>P. aeruginosa</i>	25	24	40
	<i>S. typhi</i>	30	28	40
	<i>K. pneumoniae</i>	22	21	50
	<i>E. faecalis</i>	30	29	40
4	<i>P. aeruginosa</i>	25	24	40
	<i>S. typhi</i>	31	30	40
	<i>K. pneumoniae</i>	20	19	50
	<i>E. faecalis</i>	32	28	50
5	<i>P. aeruginosa</i>	28	26	50
	<i>S. typhi</i>	35	32	40
	<i>K. pneumoniae</i>	26	24	60

*Antibacterial activity expressed as zone of inhibitions expressed in millimeters.

Table 2: Antioxidant activities of the synthesized compounds

Compound	1	2	3	4	5	Ascorbic acid
DPPH IC_{50} ($\mu\text{g/mL}$)	45	100	50	105	112	20
ABTS IC_{50} ($\mu\text{g/mL}$)	52	103	56	109	120	30

Ascorbic acid was used as a positive control.

radical scavenging (Table 2). Compound **3** was also a potent antioxidant (IC_{50} = 56 $\mu\text{g/mL}$), followed by compounds **2**, **4**, and **5** (IC_{50} of 103, 109, and 120 $\mu\text{g/mL}$, respectively). Ascorbic acid was used as a positive control (IC_{50} = 30 $\mu\text{g/mL}$).

3.5 AChE inhibition by the thiourea derivatives

In AD, the inhibition of AChE is required to inhibit the ACh hydrolysis in the brain and hence to preserve the neurotransmitter acetyl choline. Many other thiourea derivatives

Table 3: Thiourea derivatives inhibitory potential against AChE and BuChE

Compound	1	2	3	4	5	Galantamine
AChE IC ₅₀ (μg/mL)	130	200	160	475	300	40
BChE IC ₅₀ (μg/mL)	125	300	123	490	495	55

Galantamine was used as a positive control.

that could inhibit the activity of AChE and BChE have also been investigated previously by several researchers [8–11]. The AChE inhibitory activity of the synthesized compounds as their IC₅₀ values is shown in Table 3. Among the different synthesized compounds, compound **1** showed maximum inhibition of AChE with IC₅₀ value of 130 μg/mL, followed by compound **3** with IC₅₀ value of 160 μg/mL. Compound **2** showed substantial inhibition with IC₅₀ of 200 μg/mL, followed by compounds **5** and **4** with the IC₅₀ of 300 and 475 μg/mL, respectively. The AChE percent inhibitions were compared with that of the standard galantamine (IC₅₀ = 45 μg/mL).

3.6 BuChE inhibition by thiourea derivatives

Currently, the treatment available for the treatment of AD is only symptomatic and 90% of the medications are cholinesterase inhibitors. The patients receiving only AChE inhibitors as medicines could not completely give response to the treatment as the activity of BChE becomes high in such patients to compensate for the loss of AChE activity; the ideal and required approach, therefore, is to develop such drug that could inhibit both the enzymes. The compounds were thus also tested for their potential BChE inhibition. BChE inhibitory activity of the synthesized compounds as their IC₅₀ values is shown in Table 3. Compound **3** showed maximum BChE inhibitory activity among the group with the IC₅₀ value of 123 μg/mL compared with the other four compounds. Compounds **2**, **4**, and **5** showed comparatively moderate inhibitory potential with the IC₅₀ values of 300, 490, and 495 μg/mL, respectively. In this study, galantamine (IC₅₀ = 60 μg/mL) was used as a positive control.

3.7 Alpha-amylase inhibition by thiourea derivatives

Alpha-amylase is an important enzyme of glucose metabolism. It hydrolyzes polysaccharides, starch, and glycogen to glucose and maltose. Acarbose, a non-

Table 4: Percent alpha-amylase and alpha-glucosidase inhibition by the synthesized compounds

Compound	1	2	3	4	5	Acarbose
Alpha-amylase IC ₅₀ (μg/mL)	122	115	250	200	130	50
Alpha-glucosidase IC ₅₀ (μg/mL)	126	200	250	450	275	60

Acarbose was used as a positive control.

insulin antidiabetic drug, works on the principle of alpha-amylase inhibition. The synthesized thiourea derivatives were investigated for their α-amylase inhibitory potentials, and the results are shown in Table 4. Compounds **1** and **2** were found to be highly potent against the selected enzyme, showing promising inhibitory potential with IC₅₀ of 122 and 115 μg/mL, respectively. Compound **5** was ranked the third with IC₅₀ of 130 μg/mL, followed by compounds **4** and **3** with IC₅₀ of 200 and 250 μg/mL, respectively (Table 5). The observed inhibitory potentials of the compounds were compared with that of the standard acarbose (IC₅₀ = 36 μg/mL).

Table 5: Effect of the synthesized compounds on the activity of glucose-6-phosphatase

Compound	Concentration (μg/mL)	Total activity*	Control
1	1,000	25.60	91.0
	500	29.80	
	250	32.70	
	125	38.10	
2	1,000	04.20	91.0
	500	09.20	
	250	08.20	
	125	10.40	
3	1,000	18.50	91.0
	500	22.50	
	250	24.70	
	125	28.40	
4	1,000	15.10	91.0
	500	15.70	
	250	17.30	
	125	22.70	
5	1,000	12.20	91.0
	500	13.10	
	250	14.50	
	125	17.50	

*Activity expressed as mg of inorganic phosphate (P) released from the potassium salt of glucose-6-phosphate per hour at 33°C and pH 6.7.

3.8 Alpha-glucosidase inhibition by thiourea derivatives

Alpha-glucosidase is another important enzyme of glucose metabolism, which is found in the small intestine and hydrolyzes starch and maltose to glucose. The results are presented in Table 4, which shows that compound **1** was most potent against the enzyme with IC_{50} of 126 $\mu\text{g/mL}$, followed by compounds **2** and **3** with IC_{50} of 200 and 250 $\mu\text{g/mL}$, respectively. The least activity was recorded for compound **4** with IC_{50} of 450 $\mu\text{g/mL}$. The inhibitory potentials of the synthesized compounds were compared with that of acarbose used as standard ($IC_{50} = 34 \mu\text{g/mL}$).

3.9 G6Pase inhibition by synthesized thioureas

Inhibition of G6Pase by the synthesized compounds is shown in Table 5. Compound **2** was highly potent, followed by compound **4**, and compound **5** also showed very good inhibition. Compounds **1** and **3** exhibited moderate inhibition of the enzyme. The inhibitory pattern seemed to be competitive as the activity of the enzyme significantly decreased with increasing concentration of the compounds.

3.10 *In vivo* effect of the synthesized compounds on the activity of G6Pase

The synthesized compounds were tested for their G6Pase inhibitory potential *in vivo* using male Swiss Albino mice. Table 6 illustrates the effect of the compounds on the activity of G6Pase. It is clear from the table that the enzyme's activity is significantly decreased after oral administration of each of the five compounds when measured on the 22nd day of the experiment. Highest inhibitory activity was shown by compound **2** at all doses but the effect was maximum at 1.5 mg/kg body weight of mice. Likewise, compounds **3** and **4** also showed very good inhibition. Compounds **1** and **5** also inhibited the enzyme and decreased its activity significantly. The compounds were generally found to suppress the enzyme activity at one level or other. The mechanism of inhibition is not known, but the increase in inhibition with an increase in the amount of dose and time suggests that it may be competitive inhibition. Compounds **2** and **4** possessed the maximum potential, followed by compound **3**. A moderate inhibition was observed for compounds **1** and **5**.

Table 6: Effect of thiourea derivatives on the activity of glucose-6-phosphatase in Swiss albino mice

Description of groups		Total activity*
Group 1	1A	67.99 $\pm$ 1.735
	1B	58.3 $\pm$ 1.904
	1C	46.11 $\pm$ 0.6416
Group 2	2A	46.37 $\pm$ 1.412
	2B	38.38 $\pm$ 1.649
	2C	32.07 $\pm$ 1.113
Group 3	3A	56.04 $\pm$ 0.8536
	3B	47.42 $\pm$ 0.5892
	3C	37.59 $\pm$ 2.428
Group 4	4A	53.38 $\pm$ 1.896
	4B	44.55 $\pm$ 1.38
	4C	39.25 $\pm$ 1.616
Group 5	5A	63.95 $\pm$ 2.39
	5B	51.58 $\pm$ 1.633
	5C	41.22 $\pm$ 2.027
Control		84.55 $\pm$ 3.213

The values are expressed as mean $\pm$ SEM of $n = 5$ mice per subgroup.

*Activity here is expressed as mg of inorganic phosphate (P) released from the potassium salt of glucose-6-phosphate/hour at pH 6.7 and temperature 33°C.

The enzyme's activity is significantly decreased by oral administration of each of the five compounds when measured at the 22nd day of the experiment.

Therefore, all these can securely be presumed as potential inhibitors of G6Pase.

3.11 Effect on hematological parameters

Table 7 shows the effect of the compounds on various hematological parameters. Packed cell volume (PCV) was significantly decreased ($P < 0.001$) at all dose rates by compounds **1**, **2**, **3**, and **5**, and compound **4** had no significant effect on any dose. Erythrocyte concentration remains unaffected at all dose rates by all the test compounds. Hemoglobin was significantly decreased at dose rates of 0.5, 1.0 ($P < 0.01$), and 1.5 ($P < 0.01$) mg/kg body weight by compound **1**; a significant decrease ($P < 0.01$) by compound **2** at dose rates 0.5, 1.0, and 1.5 ($p < 0.001$)/kg body weight and a significant ($P < 0.05$ and 0.01) decrease by compound **3** at 0.5, 1.0, and 1.5 mg/kg body weight, respectively, were observed. Compound **4** had no significant effect on PCV. WBC count was significantly decreased ($P < 0.001$) at a dose rate of 1.5 mg/kg body weight by compound **1** and at all dose rates for compound **2**, whereas compound **5** significantly ($P < 0.05$) increased leukocyte count at higher doses. No significant effect was observed at any dose of compounds **3** and **4**. Monocyte

Table 7: Effect of thiourea derivatives on hematological parameters in mice after 30 days of exposure

Compounds	Subgroups	PCV (%)	RBCs ($10^6 \mu\text{L}$)	Hb (g/dL)	WBCs ($10^3 \mu\text{L}$)	Differential count ($10^3 \mu\text{L}$)		Platelets ($10^3 \mu\text{L}$)
						L	M	
1	1A	40.79 $\pm$ 1.81***	7.05 $\pm$ 1.24	11.61 $\pm$ 3.22*	9.64 $\pm$ 2.31	5.13 $\pm$ 1.22	2.00 $\pm$ 0.00	179 $\pm$ 3.61***
	1B	37.05 $\pm$ 1.88***	7.36 $\pm$ 1.35	11.12 $\pm$ 4.59**	9.86 $\pm$ 1.27	5.25 $\pm$ 2.18	2.00 $\pm$ 0.00	185.6 $\pm$ 1.14***
	1C	33.03 $\pm$ 1.41***	6.31 $\pm$ 2.21	10.81 $\pm$ 2.56**	10.1 $\pm$ 3.28*	5.12 $\pm$ 2.14	2.00 $\pm$ 0.00	192.1 $\pm$ 1.96***
2	2A	37.82 $\pm$ 2.52***	4.76 $\pm$ 3.22	10.83 $\pm$ 0.78**	12.44 $\pm$ 2.29***	6.56 $\pm$ 2.36	2.00 $\pm$ 0.00	197.8 $\pm$ 1.15***
	2B	32.19 $\pm$ 1.42***	4.2 $\pm$ 3.28	10.1 $\pm$ 2.53***	12.61 $\pm$ 4.34***	7.12 $\pm$ 3.12	2.00 $\pm$ 0.00	201.8 $\pm$ 1.34***
	2C	30.25 $\pm$ 2.51***	4.1 $\pm$ 1.29	9.39 $\pm$ 3.34***	12.85 $\pm$ 2.42***	7.52 $\pm$ 1.24	2.00 $\pm$ 0.00	212 $\pm$ 4.36***
3	3A	39.64 $\pm$ 3.38***	4.72 $\pm$ 2.14	11.85 $\pm$ 2.22*	10.14 $\pm$ 1.24*	6.32 $\pm$ 2.32	2.00 $\pm$ 0.00	177.9 $\pm$ 3.32***
	3B	38.23 $\pm$ 1.43***	4.69 $\pm$ 2.35	11.64 $\pm$ 2.59*	10.36 $\pm$ 3.31*	6.49 $\pm$ 1.27	2.00 $\pm$ 0.00	184.4 $\pm$ 4.14***
	3C	35.18 $\pm$ 2.25***	4.48 $\pm$ 1.21	11.13 $\pm$ 1.49**	10.62 $\pm$ 1.12**	7.66 $\pm$ 4.44	2.00 $\pm$ 0.00	190 $\pm$ 2.57***
4	4A	44.01 $\pm$ 1.66	7.04 $\pm$ 2.36	14.74 $\pm$ 1.47	6.84 $\pm$ 2.66	5.09 $\pm$ 3.22	2.00 $\pm$ 0.00	137. $\pm$ 1.21
	4B	43.25 $\pm$ 1.89	7.66 $\pm$ 4.24	14.97 $\pm$ 1.15	6.41 $\pm$ 4.01	5.24 $\pm$ 2.16	2.00 $\pm$ 0.00	142. $\pm$ 1.48
	4C	44.73 $\pm$ 3.74	7.44 $\pm$ 3.39	14.2 $\pm$ 4.35	7.44 $\pm$ 3.59	5.28 $\pm$ 4.68	2.00 $\pm$ 0.00	135. $\pm$ 1.13
5	5A	39.51 $\pm$ 3.67***	5.87 $\pm$ 2.39	11.91 $\pm$ 3.64*	9.6 $\pm$ 1.35	5.67 $\pm$ 3.29	5.574 $\pm$ 0.27**	168.1 $\pm$ 1.47***
	5B	40.18 $\pm$ 1.53***	5.77 $\pm$ 4.11	11.57 $\pm$ 2.51*	9.82* $\pm$ 1.16	5.32 $\pm$ 2.18	5.81 $\pm$ 0.14**	172.2 $\pm$ 1.92***
	5C	39.31 $\pm$ 0.45***	5.43 $\pm$ 2.57	11.26 $\pm$ 2.57**	10.21 $\pm$ 2.69*	5.46 $\pm$ 0.89	6.2 $\pm$ 0.07**	177.1 $\pm$ 1.55***
Control		44.62 $\pm$ 1.58	7.76 $\pm$ 3.79	14.86 $\pm$ 2.75	7.16 $\pm$ 2.83	5.24 $\pm$ 1.78	2.00 $\pm$ 0.00	141 $\pm$ 4.69

Data are expressed as mean $\pm$ SD of $n = 6$. Numbers 1–5 represent compounds 1–5; A, 0.5 mg/kg body weight; B, 1 mg/kg body weight; and C, 1.5 mg/kg body weight. Significant difference was measured using one-way analysis of variance, followed by Bonferroni's *post hoc* test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control group, and *** $P > 0.001$ means significant increase compared to the control.

Table 8: Effect of thiourea derivatives on biochemical parameters in mice after 30 days of administration

Compounds	Subgroups	Glucose (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	ALT (μ /L)
1	1A	86.43 $\pm$ 1.69	115.7 $\pm$ 2.34	95.26 $\pm$ 1.86 ^{###}	40.23 $\pm$ 2.21 ^{***}	62 $\pm$ 1.81 ^{###}
	1B	85.6 $\pm$ 2.04	117 $\pm$ 1.61 ^{###}	99 $\pm$ 1.58 ^{###}	38.16 $\pm$ 3.61 ^{***}	71.14 $\pm$ 2.79 ^{###}
	1C	87.4 $\pm$ 2.15	119 $\pm$ 2.56 ^{###}	106.8 $\pm$ 1.43 ^{###}	38.79 $\pm$ 1.68 ^{***}	77.4 $\pm$ 4.87 ^{###}
2	2A	85.65 $\pm$ 1.12	118.1 $\pm$ 1.39 ^{###}	103.12 $\pm$ 1.69 ^{###}	37.53 $\pm$ 3.17 ^{***}	65.82 $\pm$ 2.22 ^{###}
	2B	87.43 $\pm$ 3.41	118.4 $\pm$ 4.36 ^{###}	108.04 $\pm$ 3.36 ^{###}	35.27 $\pm$ 1.77 ^{***}	71 $\pm$ 3.162 ^{###}
	2C	89 $\pm$ 2.91	121.4 $\pm$ 2.48 ^{###}	110.08 $\pm$ 2.76 ^{###}	35.05 $\pm$ 1.86 ^{***}	81.6 $\pm$ 2.702 ^{###}
3	3A	84.43 $\pm$ 2.23	115.9 $\pm$ 3.94	97 $\pm$ 1.81 ^{###}	39.1 $\pm$ 1.44 ^{***}	61.42 $\pm$ 1.26 ^{###}
	3B	86.75 $\pm$ 2.26	117.8 $\pm$ 1.37 ^{###}	101 $\pm$ 1.47 ^{###}	36.31 $\pm$ 1.81 ^{***}	59.73 $\pm$ 1.97 ^{###}
	3C	86.91 $\pm$ 1.53	119.7 $\pm$ 1.19 ^{###}	105.2 $\pm$ 1.94 ^{###}	35.6 $\pm$ 1.17 ^{***}	68.2 $\pm$ 2.58 ^{###}
4	4A	73.52 $\pm$ 2.96 ^{***}	106.1 $\pm$ 2.41 ^{***}	77.08 $\pm$ 2.41 ^{***}	51.10 $\pm$ 1.43 ^{###}	48.65 $\pm$ 1.49
	4B	70.2 $\pm$ 1.94 ^{***}	97.14 $\pm$ 2.29 ^{***}	74.49 $\pm$ 2.19 ^{***}	54.70 $\pm$ 1.51 ^{###}	50.14 $\pm$ 2.79
	4C	69.8 $\pm$ 3.78 ^{***}	92.2 $\pm$ 3.24 ^{***}	73.6 $\pm$ 2.48 ^{***}	57.20 $\pm$ 0.87 ^{###}	48 $\pm$ 2.91
5	5A	83.34 $\pm$ 1.95	115.3 $\pm$ 1.74	96.25 $\pm$ 1.86 ^{###}	37.78 $\pm$ 2.45 ^{***}	62.26 $\pm$ 1.83 ^{###}
	5B	84.6 $\pm$ 2.48	117.7 $\pm$ 4.19 ^{###}	98.15 $\pm$ 2.13 ^{###}	38.07 $\pm$ 2.17 ^{***}	63.4 $\pm$ 1.17 ^{###}
	5C	85.84 $\pm$ 3.07	119 $\pm$ 3.06 ^{###}	102.34 $\pm$ 1.68 ^{###}	38.86 $\pm$ 1.73 ^{***}	69.45 $\pm$ 1.88 ^{###}
Control		87.2 $\pm$ 2.49	110.4 $\pm$ 2.68	82.6 $\pm$ 3.78	45.4 $\pm$ 1.63	49.2 $\pm$ 2.87

Data are expressed as mean $\pm$ SD of $n = 6$. Numbers 1–5 represent compounds 1–5; A, 0.5 mg/kg body weight; B, 1 mg/kg body weight; and C, 1.5 mg/kg body weight. Significant difference was measured using one-way analysis of variance, followed by Bonferroni's *post hoc* test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control group, and ^{###} $P > 0.001$ means significant increase compared to the control.

count was significantly ($P < 0.01$) increased at higher doses by compound 5. Platelet count was significantly increased ($P < 0.01$) at all dose rates by all compounds except compound 4. It is essential that the safety of a compound to be used as drug must properly be assessed, as metabolites of synthetic drugs may cause cellular destruction and tissue damage, which may lead to several immunological, biochemical, and hematological responses. Hence, along with enzyme inhibitory studies, the effect of the compounds on various blood indices and histology of the liver was also examined, and as the alterations in blood indices or tissue damage can occur either rapidly or slowly, the assays were carried out for different duration of time (10, 20, and 30 days) and at different dose levels (0.5, 1.0, and 1.5 mg/kg body weight of mice). In this study, only the results of 30-day exposure are represented. The effects observed are suggestive of the inflammation/infection occurred during exposure of the animals to the aforementioned compounds.

3.12 Effect on biochemical parameters

Table 8 represents the effect of the five compounds on hematological parameters of mice after 30 days of administration. We can see that blood glucose level after 30 days of dose administration was significantly ($P < 0.001$) reduced by compound 4 at all doses; other compound did not have any significant effect. An increase in triglycerides (TG) levels was observed at higher doses by

compounds 1 and 3 and at all dose rates by compounds 2 and 5; a significant decrease ($P < 0.001$) in TG levels was caused by compound 4. Cholesterol level was increased ($P < 0.001$) at all doses by compounds 1, 2, 3, and 5, and compound 4 at all dose rates significantly ($P < 0.001$) decreased its level. HDL level was decreased ($P < 0.001$) by compounds 1, 2, 3, and 5, while it was increased by compound 4 at all doses. ALT level was significantly increased ($P < 0.001$) by all compounds except compound 4.

The increased levels of TG, cholesterol, and ALT in the presence of compounds 1, 2, 3, and 5 compared with that of the control propose an underlying infection/inflammation caused by the administration of the compounds.

3.13 Effect on liver histology

Figure 1 shows the effect of the compounds on histology of the mice liver after 30 days of oral administration. Compound 1 has caused a moderate degree of necrosis and inflammation (slide 1C) at 1.5 mg/kg body weight of mice. Compound 2 at 1.5 mg/kg body weight (slide 2C) shows inflammation, necrosis, swelling, and vacuolization of hepatocytes. Compound 3 has caused a higher degree of inflammation and necrosis (slide 3C). Almost normal hepatocyte and parenchyma can be seen for compound 4 at 1.5 mg/kg body weight of mice. Compound 5

Figure 1: Effect of thiourea derivatives on liver (1, 2, 3, 4, and 5 indicate the respective thiourea derivative. C indicates the amount of dose given which is 1.5 mg/kg body weight. Livers were collected at day 30 of the experiment).

shows necrosis, inflammation, and hepatocyte swelling at 1.5 mg/kg body weight of mice.

The liver histology was studied for all the three studied doses, but in this study, the results of the highest dose administration (1.5 mg/kg body weight) are presented. The histology findings indicate liver tissue damage in case of compounds 2 and 3 at all doses and compounds 1 and 5 at higher doses, and compound 4 showed no or less damage to the tissue. The findings of these three studies suggest that compound 2 is most toxic of all, the toxicity is highest at 1.5 mg/kg body weight of mice and 30 days, followed by compound 3, which is also highly toxic. Compound 5 showed moderate toxicity, and compound 1 had mildly toxic effects. Compound 4 can be generally assumed as safe, but it would be safer if used in lesser or moderate quantities.

4 Conclusions

The study was mainly devised to address three different yet tangentially interlinked biological conditions, viz., type 2 diabetes, oxidative stress, and AD. The synthesized compounds exhibited promising inhibitory activities against the studied free radicals and enzymes. Of all the five enzymes, highest *in vitro* inhibition against G6Pase was shown by all the compounds in general and by compounds 2 and 4 in

specific. The *in vitro* inhibition of G6Pase was further confirmed and supported in the *in vivo* inhibition study, which also showed that the activity of the enzyme was appreciably inhibited by the compounds, whereas the activity of both α -amylase and α -glucosidase were also effectively inhibited by compounds 1 and 2, respectively. Compounds 1 and 3 exhibited considerable antioxidant potentials and also inhibited the activities of AChE and BuChE in an effective way. This provides a clue that these compounds may be effective in symptomatic treatment of AD. Furthermore, a notable antibacterial activity was shown by compounds 2 and 4 pointing toward their possibility to be used in treating various diseases caused by bacteria. The compounds were also found to have antilipidemic and anti-hyperglycemic properties *in vivo*. To find out whether these compounds are safe for the systemic use or not, they were evaluated for their toxic effect on different blood indices and histology of liver. The *in vivo* studies showed that only compound 4 can be assumed to be safe (safer in moderate doses); compound 1 is mildly toxic and could be used only in lower doses; and compounds 2, 3, and 5 are toxic and should not be used at all. Further research and clinical trials are needed to fully determine their biological spectrum.

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