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Synthesis, Characterization and Antioxidant Action of Benzothiazole Based Compounds

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ABSTRACT
The objective of the present work was to synthesize novel benzothiazole derivatives and evaluate their antioxidant action in vitro. The multistep reaction pathway comprised of synthesizing three intermediate compounds and final compound was obtained by reaction with various aromatic aldehydes. The synthesized compounds (7a-e) were subjected to in vitro determination of antioxidant potential in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH and hydroxy radical scavenging using Iron-EDTA. It was observed from the result of DPPH scavenging that the presence of electron withdrawing substitution decreased the ability of the compounds to inhibit DPPH. The order of electronegativity affected the DPPH scavenging (Br >Cl> NO_2). On the other hand electron donating substituent (OH) in the compound improved the DPPH scavenging activity. The unsubsituted compound (**7a**) was found to be having a better antioxidant action in comparison to 7c, 7d and 7e. To conclude, the study was able to design novel benzothiazole derivatives with significant antioxidant action.
Keywords:-Benzothiazole, DPPH, radical scavenging, antioxidant, electron withdrawing

I. INTRODUCTION

Oxidative stress plays an essential role in the pathogenesis of chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer [1]. Long term exposure to increased levels of pro-oxidant factors can cause structural defects at a mitochondrial DNA level, as well as functional alteration of several enzymes and cellular structures leading to aberrations in gene expression. Free radicals play an essential role in several biological processes. Many of these are necessary for life, such as the intracellular destruction of bacteria by phagocytes, especially by granulocytes and macrophages. Researchers believe that free radicals are also involved in some cellular signaling processes, known as redox signaling [2]. At low-to-moderate amounts, ROS are beneficial both in regulating processes involving the maintenance of homeostasis as well as a wide variety of cellular functions [3].

Reactive oxygen species (ROS) are produced in normal cellular oxygen metabolism, effective in some biological systems [4]. The increase in ROS on the intracellular antioxidant capacity may result in a situation characterized by oxidative stress [5]. ROS are free radicals such as hydroxyl radicals, superoxide anion radicals, and nonradical species such as singlet oxygen and hydrogen peroxide. ROS attack hepatic and extrahepatic organs and induce oxidative stress [6]. Thus leading directly or indirectly causes degenerative diseases such as cancer, dementia, and aging.

Benzothiazoles (Figure 1) are fused membered rings, which contain the heterocycles bearing thiazole. Sulphur and nitrogen atoms constitute the core structure of thiazole and many pharmacologically and biologically active compounds [7].

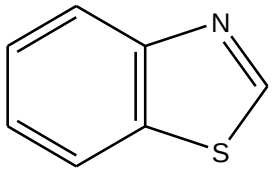

Figure 1.Benzothiazole

Recent studies exhibited that benzothiazoles and their derivatives are used for clinical, critical pharmaceutical and synthetic chemistry applications as chemical inhibitors of enzyme activities. Previous works reported that benzothiazoles derivatives possessed antimicrobial, antioxidant, antihyperglycemic, and anti-carcinogenic and anti-HIV activities [8-11]. In the present investigation we have synthesize new benzothiazole derivatives and evaluated the antioxidant activity of the synthesized compounds.

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II. MATERIAL AND METHODS

5-amino salicylic acid, 2-aminobenzenthiol was purchased from Loba, ethanol was obtained from Sigma Aldrich, chloracetylchloride, sulfuric acid, potassium hydroxide, hydrochloric acid, benzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde, chlorobenzaldehyde, hydroxybenzaldehyde, and all other chemicals used were of analytical or synthetic grade and purchased from CDH or Loba. Any chemical used in the study was used as received without any further purification. The melting point of each of the synthesized compounds was determined by open capillary method using melting point apparatus and is uncorrected.

The experimental scheme was adapted from the previous reports [12-15] by modification in conditions and optimized for obtaining highest yield. The typical steps involved in the reaction protocol are presented in Figure 2.

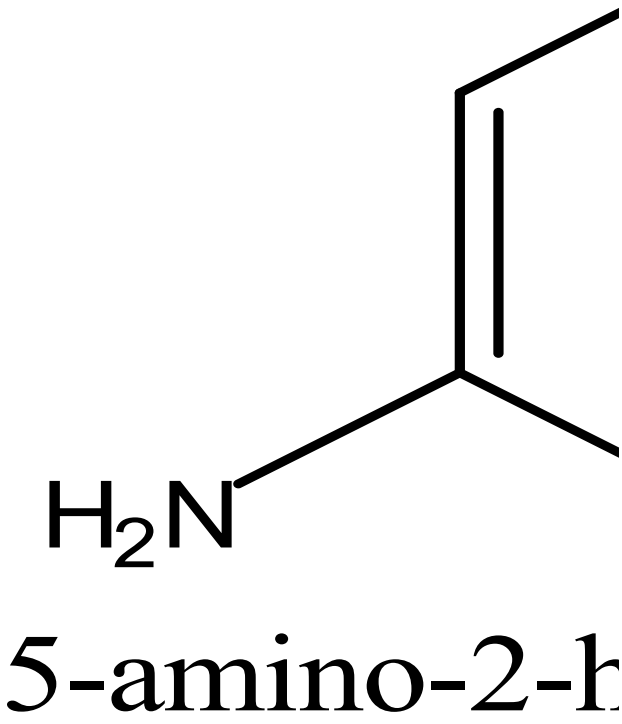

Figure 2.Reaction pathway for synthesis of benzothiazole derivatives

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The multistep reaction pathway comprised of synthesizing three intermediate compounds. The third intermediate was reacted with various aromatic aldehydes leading to the formation of the desired substituted product.

Synthesis of 4-amino-2-(benzo[d]thiazol-2-yl)phenol

A mixture of 5-amino-2-hydroxybenzoic acid (13 mmol) and 2-aminobenzenthiol (13 mmol) in polyphosphoric acid (10 ml) was heated at 200 °C for 4 h with stirring. The reaction mixture was poured into 400 ml of water and the precipitate obtained was filtered, neutralized with a solution of sodium carbonate (10% w/v), washed with water and dried [12].

Synthesis of N-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)-2-chloroacetamide

To a stirred solution of benzothiazole compound (0.04 mol) and triethylamine (Et_3N) (0.02 mol) in dioxane (50 ml), chloro acetyl chloride (0.02 mol) was added dropwise. The reaction mixture was refluxed for 13 h and the excess of solvent was evaporated using rotary vacuum evaporator. The solid obtained was washed with distilled water, filtered dried and crystallized from ethanol [13].

Synthesis of (E)-2-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenylimino)thiazolidin-4-one

A well stirred solution of the N-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)-2-chloroacetamide (0.01 mol) in methanol (50 mL) was added ammonium thiocyanate (0.01 mol) slowly over a period of 10 min. The mixture was refluxed for 7 h and the product obtained on cooling was filtered, washed with cold water and recrystallized from glacial acetic acid [14].

General method for synthesis of (2E,5E) – 2-(3-(benzo[d]thiazol-2 – yl) – 4 – hydroxyphenylimino) – 5 – benzylidenethiazolidin-4-one derivatives

A well-stirred solution of 2-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenylimino)thiazolidin-4-one (0.8 g, 4 mmol) in acetic acid (35 mL) was buffered with sodium acetate (8 mmol) and the appropriate arylaldehyde (6 mmol) was added. The solution was refluxed for 4 h and then poured into ice-cold water. The precipitate was filtered and washed with water and the resulting crude product was purified by recrystallisation from Dioxane [15].

(2E,5E) – 2-((3-(benzo[d]thiazol – 2 – yl) – 4-hydroxyphenyl)imino) – 5 – benzylidenethiazolidin – 4-one, 7a

Color: Reddish; **IR (KBr, cm^{-1}):**3164 (aromatic C–H str.), 1595, 1505, 1399 (C=C ring str.), 1651 (C=N), 1013 (N–N), 689 (C–S–C); **^{1}H NMR ($CDCl_3$, δ ppm):** 8.10 (C-H, Imine), 7.21–7.23 (Ar C-H), 4.21 (O-H); 3.53 (N-H), 2.6 (C-H, methylene)

(2E,5E) – 2-((3-(benzo[d]thiazol – 2-yl) – 4 – hydroxyl phenyl)imino) – 5-(4 – hydroxybenzylidene) thiazolidin – 4-one, 7b

Color: Dark Yellow; **IR (KBr, cm–1):** 3420 (Ar–OH), 3161 (aromatic C–H str.), 1657 (C=N), 1591, 1485, 1403 (C=C ring str.), 640 (C–S–C), 1063 (N–N); **^{1}H NMR ($CDCl_3$, δ ppm):** 8.09 (C-H, Imine), 6.63-7.67 (Ar C-H), 4.21 (O-H); 3.53 (N-H), 2.6 (C-H, methylene)

(2E,5E) – 2-((3-(benzo[d]thiazol – 2-yl) – 4 – hydroxyl phenyl)imino)-5-(4-chlorobenzylidene)thiazolidin – 4-one, 7c

Color: Brown; **IR (KBr, cm–1):** 3082 (aromatic C–H str.), 1597, 1520, 1412 (C=C ring str.), 1687 (C=N), 969 (Ar–Cl), 1054 (N–N), 673 (C–S–C); **^{1}H NMR ($CDCl_3$, δ ppm):** 8.14 (C-H, Imine), 7.29-7.63 (Ar C-H), 4.21 (O-H); 3.53 (N-H), 2.6 (C-H, methylene)

(2E,5E) – 2-((3-(benzo[d]thiazol – 2-yl) – 4 – hydroxyl phenyl)imino) – 5-(4-nitrobenzylidene)thiazolidin-4-one, 7d

Color: Dark Yellow; **IR (KBr, cm–1):** 3084 (aromatic C–H str.), 1683 (C=N), 1599, 1521, 1472 (C=C ring str.), 1341 (Ar–NO_2), 1049 (N–N), 663 (C–S–C); **^{1}H NMR ($CDCl_3$, δ ppm):**8.06 (C-H, Imine), 7.24-7.64, (Ar C-H), 4.21 (O-H); 3.53 (N-H), 2.6 (C-H, methylene),(2E,5E)-2-((3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)imino)-5-(4- bromobenzylidene)thiazolidin-4-one

Color: Dark Yellow; **IR (KBr, cm–1):** 3087 (aromatic C–H str.), 1688 (C=N), 1599, 1521, 1472 (C=C ring str.), 1344 (Ar–NO_2), 1053 (N–N), 665 (C–S); **^{1}H NMR ($CHCl_3$, δ ppm):**8.11 (C-H, Imine), 7.21-7.65 (Ar C-H), 4.21 (O-H); 3.53 (N-H), 2.6 (C-H, methylene),

Evaluation of antioxidant action

DPPH Scavenging Assay

The antioxidant action of the synthesized compounds was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

The free radical scavenging activity of the synthesized molecules was measured in terms of hydrogen donating or radical scavenging ability

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using the stable radical DPPH [16]. The test samples (10–100 μL) were prepared in DMSO and were mixed with 1.0 mL of DPPH solution and filled up with methanol to a final volume of 4 mL. Absorbance of the resulting solution was measured at 517 nm in a visible spectrophotometer. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Ao} - \text{At})}{\text{Ao}} \text{ X } 100$$

Where Ao is the absorbance of the control (blank, without sample) and At is the absorbance in the presence of the test samples. All tests were performed in triplicate and the results were expressed as mean values ± standard deviations.

Hydroxy radical scavenging activity

Various concentrations of test solutions (50, 100, 150, 200 and 250 µg/mL) were taken and 1 mL of iron EDTA solution, 0.5mL of EDTA solution, 1 mL of DMSO and 0.5mL of ascorbic acid were added to it. The mixture was incubated in a boiling water bath at 80 to 90°C for 15 min. After incubation, 1 mL of ice cold TCA and 3mL of Nash reagent were added and the reaction mixture was incubated at room temperature for 15 min. The absorbance was read at 412 nm [17]. The % hydroxyl radical scavenging activity is calculated by the following formula

$$\% HRSA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where, HRSA is the Hydroxyl Radical Scavenging Activity, Abs control is the absorbance of control and Abs sample is the absorbance of the test solution.

III. RESULTS AND DISCUSSION

Antioxidant Action

The synthesized compounds (7a-e) were subjected to in vitro determination of antioxidant potential in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH and hydroxy radical scavenging using Iron-EDTA.

DPPH radicals scavenging activity

DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colourstoichometrically with the number of electrons consumed, which is measured spectrophotometricallty at 517 nm. The deep purple color of DPPH decreases if the compound exhibits antioxidant action (Table 1).

Table 1.% DPPH Scavenging by synthesized compounds

Test compound	DPPH Scavenging %				
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µf/mL
Ascorbic Acid (standard)	94.37 ± 0.702	-	-	-	-
7a	18.43 ± 0.135	22.74 ± 0.633	45.69 ± 1.021	61.81 ± 0.702	74.86 ± 0.634
7b	35.8 ± 0.361	52.9 ± 1.513	65.47 ± 1.464	79.45 ± 0.551	91.22 ± 0.781
7c	13.54 ± 1.033	16.93 ± 0.765	27.51 ± 1.234	42.88 ± 1.033	55.25 ± 0.535
7d	9.69 ± 0.535	11.42 ± 0.702	18.66 ± 1.021	29.74 ± 0.135	41.89 ± 0.633
7e	14.21 ± 1.464	18.38 ± 2.354	31.32 ± 0.666	46.95 ± 0.721	61.03 ± 1.066

It was observed from the result of DPPH scavenging that the presence of electron withdrawing substitution decreased the ability of the compounds to inhibit DPPH. The order of electronegativity affected the DPPH scavenging (Br >Cl> NO_2). On the other hand electron donating substituent (OH) in the compound improved the DPPH scavenging activity. The unsubsituted compound (**7a**) was found to be

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having a better antioxidant action in comparison to 7c, 7d and 7e.

The IC_{50} of the compounds for DPPH scavenging was calculated by plotting concentration vs. % DPPH inhibition.

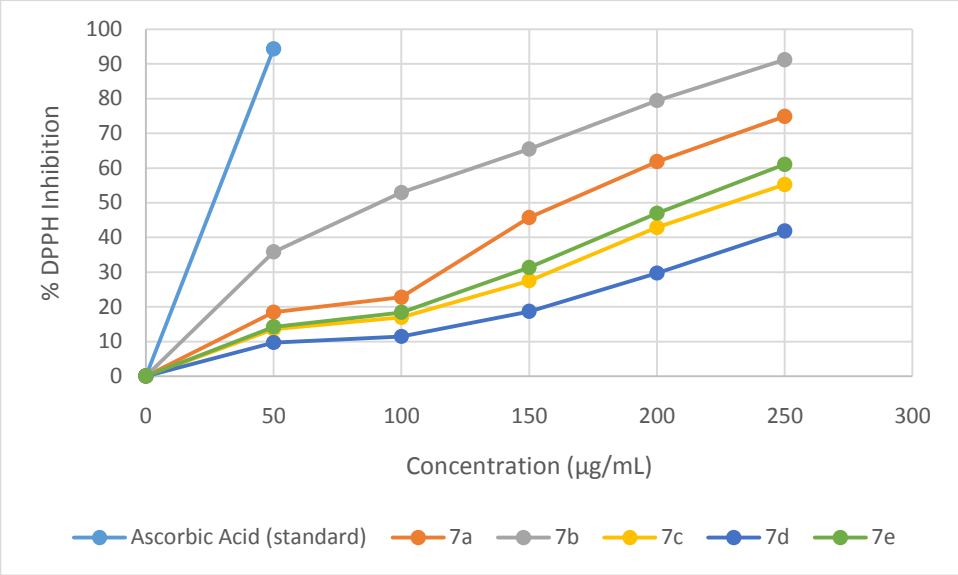

Figure 3.Plot of %DPPH scavenging vs. concentration

The IC_{50} values were calculated from Figure 3 and were found to be 26.49, 167.27, 112.92, 236.95, 323.68 and 214.74 µg/mL for ascorbic acid, 7a, 7b, 7c, 7d and 7e respectively.

Hydroxy radical scavenging assay

HRS assay is used to find the scavenging activity of free hydroxyl radicals like hydrogen peroxide (which damage the body cells) in the presence of different concentrations of plant sample. The model used is ascorbic acid-iron-EDTA model of hydroxyl radical generating system. This is a totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radicals. The results obtained are presented in Table 4.

Table 4.% HRSA by synthesized compounds

Test compound	% HRSA				
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µf/mL
Ascorbic Acid (standard)	89.20 ± 0.557	-	-	-	-
7a	21.29 ± 0.721	24.65 ± 1.033	49.54 ± 1.033	66.75 ± 0.702	84.06 ± 1.913
7b	36.37 ± 1.913	52.23 ± 0.666	68.63 ± 0.839	89.8 ± 1.9	93.67 ± 2.354
7c	14.91 ± 0.721	18.68 ± 0.702	30.55 ± 1.033	45.37 ± 1.633	61.09 ± 2.033
7d	10.58 ± 0.721	14.29 ± 0.361	21.91 ± 1.464	33.26 ± 0.633	45.52 ± 1.033
7e	17.37 ± 0.361	21.24 ± 1.513	35.83 ± 1.033	51.05 ± 0.702	65.28 ± 0.634

Similar to DPPH scavenging, it was observed from the result of hydroxy radical scavenging that the presence of electron withdrawing substitution decreased the ability of the compounds to inhibit hydroxy radical. The order of electronegativity affected the hydroxy

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radical scavenging (Br >Cl> NO_2). On the other hand electron donating substituent (OH) in the compound improved the hydroxy radical scavenging activity. The unsubsituted compound (**7a**) was found to be having a better antioxidant action in comparison to 7c, 7d and 7e.

The IC_{50} of the compounds for hydroxy radical scavenging was calculated by plotting concentration vs. % HRSA (Figure 4).

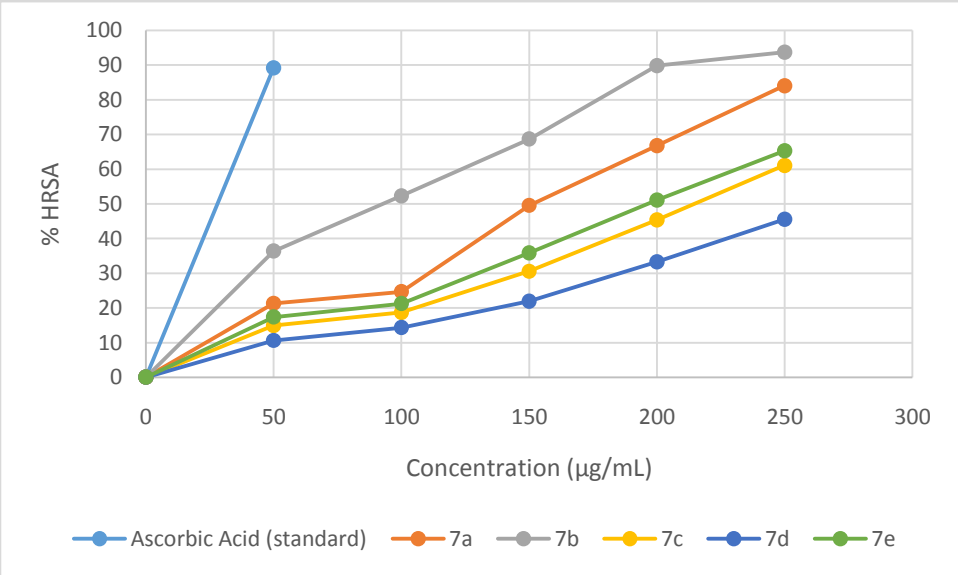

Figure 4.Plot of %HRSA vs. concentration

The IC_{50} values obtained were 28.02, 151.94, 106.59, 217.38, 292.75 and 197.06 µg/mL for ascorbic acid, 7a, 7b, 7c, 7d and 7e respectively.

The compounds exhibited better hydroxy radical scavenging in comparison to hydrogen radical scavenging (DPPH).

IV. CONCLUSION

Owing to the previous reports on benzothiazole derivatives antimicrobial, antioxidant, antihyperglycemic, and anti-carcinogenic and anti-HIV activities, the present work aimed to synthesis novel benzothiazole-thiazolidine-one derivatives and evaluates the antioxidant activity. The four step reaction pathway was able to produce the desired compounds in sufficient yield and purity. The antioxidant activity, as assessed using two in vitro models (DPPH and hydroxy radical scavenging assay) suggested the effect of substitution on the activity. The activity was dose dependent and also was less in compounds with electron withdrawing substituents. To conclude, the study was able to design novel benzothiazole derivatives with significant antioxidant action.

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