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Benzothiazol-2-yl-hydrazone derivatives as potential antioxidant agents

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A series of benzothiazol-2-yl-hydrazone derivatives (**4a-i**) have been synthesized and characterized using elemental analysis, FT-IR, ^{1}H-NMR and Mass spectroscopy techniques. The synthesized compounds have been screened for antioxidant activity by DPPH radical scavenging activity method. The compounds bearing methoxy group 4c, 4e, 4f, 4g, 4h and 4i have shown promising antioxidant activity, better than the standard drug ascorbic acid.

Keywords: Benzothiazole, Hydrazone, Benzothiazol-2-yl hydrazone, Antioxidant

The bicyclic heterocycle benzothiazole is formed by the fusion of benzene ring with thiazole ring at 4, 5 position[1]. Benzothiazole is an important pharmacophore present in several bioactive compounds[2]. In recent years, 2-amino benzothiazole derivatives have acquired significance due to their multiple pharmacological functions such as antimicrobial, anti-inflammatory, antioxidant, anticonvulsant, antifungal, anthelmintics, analgesic, anticancer, antiviral, antimalarial, and anti-diabetic[3-10].

Hydrazones constitute the class of organic compounds containing azomethine segment that influences its properties[11]. Hydrazone derivatives possess diverse biological activities, for example, anti-inflammatory, analgesic, antiplatelet, antimicrobial, anticancer, antifungal, anti-tubercular, antiviral, anticonvulsant and antioxidant[12-18].

Reactive oxygen species (ROS) such as superoxide, hydroxyl, peroxyl and alkoxyl radical are generated in body through several metabolic processes. These free radicals are easily removed by natural antioxidant defensive mechanism of our body[19]. Accumulated evidences suggested that the production of ROS is responsible for the damage of essential biological macromolecules *viz* DNA, proteins, and phospholipids leading to the development of several pathological condition, including neurodegenerative, cardiovascular and autoimmune diseases[20-21]. Exogenous antioxidants are required to balance the redox system, when the endogenous defense mechanism fails to overcome the production of free radicals[22]. Antioxidants are very essential for cell survival. They act as inhibitor and scavengers of free radicals, thus protects our body from diseases[23]. In recent years, the development of new antioxidants has gained importance due to their beneficiary effect in delaying and preventing several conditions associated with oxidative stress.

Apart from wide spectrum of biological activities, benzothiazole-hydrazone derivatives have also been studied extensively as antioxidant agent[24]. In the present work, we have synthesized and characterized some new benzothiazole-hydrazone derivatives with the aim of investigating the effect of different substituents on antioxidant property.

Experimental Details

All the reagents and solvents were purchased from Sigma Aldrich. The melting points were obtained using a Thermoink precision melting point cum boiling point apparatus and are uncorrected. The progress of reaction and purity of compounds was controlled by thin layer chromatography (TLC) on silica gel plates using chloroform: ethylacetate (7:3) as mobile phase. The methanolic solution of the compounds were applied with capillary tubes and visualized by exposure to iodine vapours in the UV chamber at 254 nm. The FTIR spectra were recorded using KBr pellets on Shimadzu FTIR-8400s. The ^{1}H-NMR spectra were recorded on Bruker Advance III,

400 MHz spectrophotometer in DMSO using TMS as internal standard. The chemical shifts values were reported in δ ppm and coupling constants (*J*) in hertz. The splitting patterns were denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The mass spectra were recorded on Shimadzu LC-MS 8040. The elemental analysis was determined using Heraus Carlo Erba 1108 CHN analyzer. For *in vitro* antioxidant activity, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and methanol were purchased from Merck Millipore and Molychem, respectively, and absorbance was measured on Jasco V 600 spectrophotometer.

Synthesis procedure

6-substituted phenylthiourea (1a-i)[5]

In a round bottom flask, a warm saturated solution of ammonium thiocyanate (20 g in 30 mL) was added slowly to a mixture of substituted aniline (0.2 mol) and concentrated HCl (20-25 mL). The solution was boiled until turbidity appeared and then poured into cold water. The precipitate of substituted phenylthiourea was filtered off, washed with water, dried and recrystallized using dilute ethanol (70 % v/v).

6-substituted-2-benzothiazolamine (2a-i)[25]

In a beaker, the solution of substituted phenylthiourea (0.1 mol) in carbon tetrachloride (150 mL) was brominated using solution of bromine (5% v/v) in carbon tetrachloride untill orange-yellow colour persists. The slurry was kept overnight; the precipitated dibromide was filtered off, washed with carbon tetrachloride until the yellow colour disappeared. Later, the precipitates were dissolved in rectified spirit (200 mL) and subsequently basified with concentrated ammonia solution. The precipitate of substituted 2-amino benzothiazole was filtered, washed with water, dried and recrystallized from dilute ethanol (70 % v/v).

6-substituted benzothiazol-2yl-hydrazine (3a-i)[26]

In a round bottom flask, concentrated HCl (6 mL) was added dropwise with stirring to hydrazine hydrate (80%, 6 mL) by maintaining temperature at 5–10°C followed by addition of ethylene glycol (24 mL). Further 6-substituted-2-amino benzothiazoles (0.03 mol) was added in portion and the resulting mixture was refluxed for 2 h at 150-160°C. The mixture was cooled and poured onto crushed ice to afford the product which was then filtered and recrystallized from ethanol.

1-(4-substituted phenyl)-1-(6-substituted benzo thiazol-2-yl)hydrazone (4a-i)[27]

In a round bottom flask, the ethanolic solution of of 6-substituted benzothiazol-2yl-hydrazine (1.5 mmol) and appropriate aromatic ketones/aldehyde (2.2 mmol) was refluxed for 5 h in the presence of glacial acetic acid (5 mL). The reaction mixture was cooled and poured onto crushed ice to obtain the solid product. The product was filtered off, washed with methanol, dried and recrystallized from ethanol.

1-(4-bromophenyl)-ethanone-1-(6-nitro-benzothiazol-2-yl)-hydrazone (4a)

Yield 73.96%, Brown solid, m. p. 246-248°C. Rf: 0.68. UV spectrum (CH_3OH), λ_{max}, nm: 370. IR spectrum, ν, cm^{-1}: 3363 (N-H), 1653 (C=N), 534 (C-S), 1506 (N-O), 669 (Br), 2600 (S-H), 1394 (-), 1653 (C=C). ^1H NMR spectrum (DMSO), δ, ppm: 6.596-7.958 (m, 7H, *J*= 2.14, 2.00 Hz, Ar-H), 2.509 (t, 3H, *J*= 3.08 Hz, CH_3), 3.825 (s, 1H, *J*= 1.50 Hz, N-H). Mass spectrum, *m/z*: 392.69 $[M+1]^+$. Found, %: C 46.10; H 2.89; N 14.37. $C_{15}H_{11}BrN_4O_2S$. Calculated, %: C 46.05; H 2.83; N 14.32.

1-(4-bromophenyl)-methanone-1-(6-nitro-benzo thizol-2-yl) -hydrazone (4b)

Yield 70.90%, Brown solid, m. p. 236-238°C. Rf: 0.57. UV spectrum (CH_3OH), λ_{max}, nm: 370. IR spectrum, ν, cm^{-1}:3363 (N-H), 1653 (C=N), 534 (C-S), 1506 (N-O), 669 (Br), 2600 (S-H), 1394 (C-H), 1653 (C=C). ^1H NMR spectrum (DMSO), δ, ppm: 6.591-7.957 (m, 7H, *J*= 1.07, 1.00 Hz, Ar-H), 3.738 (s, 1H, *J*= 3.16 Hz, NH). Mass spectrum, *m/z*: 378.85 $[M+1]^+$.Found, %: C 44.61; H 2.49; N 14.89.$C_{14}H_9BrN_4O_2S$. Calculated, %: C 44.58; H 2.40; N 14.85.

1-(4-methoxyphenyl)-ethanone-1-(6-nitro-benzo thizol-2-yl)-hydrazone (4c)

Yield 79.24%, Yellow solid, m.p. 224-226 °C. Rf: 0.71. UV spectrum (CH_3OH), λ_{max}, nm:370. IR spectrum, ν, cm^{-1}:3356 (N-H), 1631 (C=N), 536 (C-S), 1541 (N-O), 669 (C-Br), 2600 (S-H), 1394 (C-H), 1631 (C=C). ^1H NMR spectrum (DMSO), δ, ppm: 6.591-7.956 (m, 7H, *J*= 1.00 Hz, Ar-H), 4.092 (t, 3H, *J*= 2.05 Hz, Ar-OCH_3), 3.3781 (s, 1H, *J*= 3.00 Hz, N-H), 2.508 (t, 3H, *J*= 3.08 Hz, CH_3). Mass spectrum, *m/z*: 343.80 $[M+1]^+$.Found, %: C 56.19; H 4.18; N 16.39.$C_{16}H_{14}N_4O_3S$. Calculated, %: C 56.13; H 4.12; N 16.36.

Diphenylmethanone-1-(6-nitro-benzothizol-2-yl)-hydrazone (4d)

Yield 75.40%, Yellow solid, m.p. 214-216°C. Rf: 0.63. UV spectrum (CH_3OH), λ_{max}, nm: 370. IR spectrum, ν, cm^{-1}: 3365 (N-H), 1631 (C=N), 540 (C-S), 1506 (N-O), 3365 (C=O), 2600 (S-H), 1394 (C-H), 1631 (C=C). ^{1}H NMR spectrum (DMSO), δ, ppm: 6.890-9.468 (m, 13H, *J*= 2.94, 2.06, 1.01, 1.02, 0.63 Hz, Ar-H), 3.737 (s, 1H, *J*= 3.00 Hz, N-H). Mass spectrum, *m/z*: 375.85 $[M+1]^+$. Found, %: C 64.21; H 3.79; N 14.98.$C_{20}H_{14}N_4O_2S$. Calculated, %: C 64.16; H 3.77; N 14.96.

1-(4-methoxyphenyl)-methanone-1-(6-nitro-benzothizol-2-yl)-hydrazone (4e)

Yield 81.40%, Yellow solid, mp 232-234°C. Rf: 0.58. UV spectrum (CH_3OH), λ_{max}, nm: 336. IR spectrum, ν, cm^{-1}: 3363 (N-H), 1653 (C=N), 534 (C-S), 1506 (N-O), 2603 (S-H), 1394 (C-H), 1602 (C=C). ^{1}H NMR spectrum (DMSO), δ, ppm: 6.591-7.957 (m, 7H, *J*=1.07, 1.00 Hz, Ar-H), 4.092 (t, 3H, *J*= 2.05 Hz, Ar-OCH_3), 3.738 (s, 1H, *J*= 3.16 Hz, NH). Mass spectrum, *m/z*: 328.55 $[M+1]^+$. Found, %: C 54.91; H 3.72; N 17.10.$C_{15}H_{12}N_4O_3S$. Calculated,%: C 54.87; H3.68; N17.06.

Diphenylmethanone-1-(6-methoxy-benzothizol-2-yl)-hydrazone (4f)

Yield 79.21%, Brown solid, m. p. 228-230°C. Rf: 0.64. UV spectrum (CH_3OH), λ_{max}, nm: 260, 340. IR spectrum, ν, cm^{-1}:3402 (N-H), 1681 (C=N), 690 (C-S), 1589 (N-O), 1338 (C-N), 1394 (C-H), 1655 (C=C).^{1}H NMR spectrum (DMSO), δ, ppm: 6.890- 9.468 (m, 13H, *J*= 2.94, 2.06, 1.01, 1.02, 0.63 Hz, Ar-H), 4.092 (t, 3H, *J*= 2.05 Hz, Ar-OCH_3), 3.737 (s, 1H, *J*= 3.00 Hz, N-H). Mass spectrum, *m/z*: 360.75 $[M+1]^+$.Found, %: C 70.19; H 4.80; N11.73.$C_{21}H_{17}N_3OS$. Calculated, %: C70.17; H 4.77; N 11.69.

1-(4-nitrophenyl)-methanone-(6-methoxy-benzothizol-2-yl)-hydrazone (4g)

Yield 78.49%, Brown solid, m.p. 222-224 °C. Rf: 0.56. UV spectrum (CH_3OH), λ_{max}, nm: 260, 308. IR spectrum, ν, cm^{-1}:3363 (N-H), 1653 (C=N), 534 (C-S), 1539 (N-O), 518 (Br), 2835 (C-H), 1655 (C=C), 1244 (C=O). ^{1}H NMR spectrum (DMSO), δ, ppm: 6.596-7.958 (m, 7H, *J*= 2.14, 2.00 Hz, Ar-H), 4.092 (t, 3H, *J*= 2.05 Hz, Ar-OCH_3), 3.825 (s, 1H, *J*= 1.50 Hz, N-H). Mass spectrum, *m/z*: 329.40 $[M+1]^+$. Found, %: C54.91; H 3.72; N 17.11.$C_{15}H_{12}N_4O_3S$. Calculated, %: C54.87; H3.68; N17.06.

1-(4-bromophenyl)-methanone-(6-methoxy-benzothizol-2-yl)-hydrazone (4h)

Yield 75.54%, Brown solid, m.p. 254-256°C. Rf: 0.58. UV spectrum (CH_3OH), $\lambda_{max,}$ nm: 258. IR spectrum, ν, cm^{-1}:3566 (N-H), 1635 (C=N), 534 (C-S), 1506 (N-O), 669 (C-Br), 2835 (C-H), 1635 (C=C). ^{1}H NMR spectrum (DMSO), δ, ppm: 6.890-7.897 (m, 7H, *J*= 2.94, 2.06, 1.01, 1.02 Hz, Ar-H), 4.092 (t, 3H, *J*= 2.05 Hz, Ar-OCH_3), 3.737 (s, 1H, *J*= 3.00 Hz, NH). Mass spectrum, *m/z*: 363.30 $[M+1]^+$. Found, %: C 49.76; H 3.38; N 11.64.$C_{15}H_{12}BrN_3OS$. Calculated, %: C 49.73; H 3.34; N 11.60.

1-(diphenyl) methanone (6-methoxy-1,3-benzothizol-2-yl) hydrazone (4i)

Yield 69.86%, Black solid, m.p. 218-220°C. Rf: 0.54. UV spectrum (CH_3OH), λ_{max}, nm: 262, 306. IR spectrum, ν, cm^{-1}:3402 (N-H), 1697 (C=N), 520 (C-S), 1244 (C=O), 2985 (C-H), 1655 (C=C), 1338 (C-N). ^{1}H NMR spectrum (DMSO), δ, ppm: 6.986-8.638 (m, 12H, *J*= 0.97, 1.00, 1.07, 1.02, 1.07, 1.15 Hz, Ar-H), 4.092 (t, 3H, *J*= 2.05 Hz, Ar-OCH_3), 3.825 (s, 1H, *J*= 1.50 Hz, N-H). Mass spectrum, *m/z*: 284.10 $[M+1]^+$. Found, %:C 70.19; H 4.81; N 11.72. $C_{21}H_{17}N_3OS$. Calculated, %: C 70.17; H 4.77; N 11.69.

Biological screening

Antioxidant activity

The *in-vitro* antioxidant activity of all the synthesized compounds was evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity[28]. The degree of discoloration indicates the free radical scavenging efficiency of the compound. Stock solution of DPPH (100 µg/mL) in methanol was prepared. To 3.0 mL of methanol, 0.1 mL of DPPH stock solution was added and used as control. The absorbance was recorded at 516 nm. Various concentrations of the synthesized compounds (20-100 µg/mL) were prepared. 1.0 mL of each compound was diluted with 3.0 mL of methanol and 0.1 mL of DPPH stock solution. The test tubes were incubated for 30 min to complete the reaction. After 30 min, absorbance was recorded at 516 nm on UV-visible spectrophotometer against methanol as a blank and ascorbic acid as standard. The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ Scavenging} = (A_{control} - A_{sample} / A_{control}) * 100$$

Where, $A_{control}$ = Absorbance of control and A_{sample} = Absorbance of synthesized compounds.

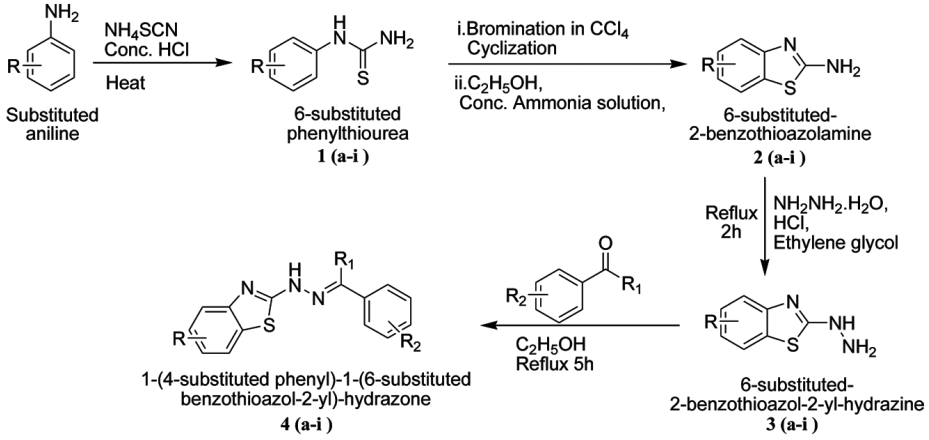
The IC50 value was calculated to evaluate the antioxidant activities. It is defined as the effective concentration, at which 50% of the radicals are scavenged, Lower the IC50 value greater is the antioxidant activity. The results are given in Table 1.

Results and Discussion

In the present study, the benzothiazol-2-yl hydrazone derivatives were synthesized according to the procedure depicted in Scheme 1. All the synthesized compounds were checked for their purity by TLC and melting point determination. The structures of the newly synthesized compounds were confirmed by spectral and elemental data. All the synthesized compounds were checked for their purity by the TLC and melting point determination. The UV absorption spectra showed characteristic peaks from 260-370 nm, indicating the presence of n- π* and π – π* transitions in the compounds due to carbonyl group and aromatic ring system, respectively. The synthesized compounds showed the IR absorption peaks at 3363 (NH), 1653 (C=N), 534 (C-S-C), 1506 (NO), 669 (C-Br), 2600 (SH), 1394 (CH) and 1653 (C=C) indicating that the values assigned to the functional group are in accordance with the structure of compounds. From the mass spectra of synthesized compounds, it was found that all the compounds showed almost expected molecular weight. The ^{1}H-NMR of the compounds showed multiplet at δ 6.596-9.468 due to aromatic protons, triplet at δ 4.092 due to aromatic methoxy and singlet at δ 3.737 due to -NH protons. All the synthesized compounds exhibited satisfactory data correlated well with the assigned structure. The synthesized compounds (4a-i) were screened for *in vitro* antioxidant activity by DPPH assay method. The data revealed that compound 4c, 4e, 4f, 4g, 4h and 4i displayed better antioxidant activity compared to the standard drug ascorbic acid as observed from the IC_{50} values. The promising antioxidant activity is exhibited by the compounds having electron releasing methoxy group. On the other hand, compound with electron withdrawing

Table 1 — In vitro antioxidant activity of compounds by the DPPH assay

Compound	Inhibition (%)					IC50 (μg/mL)
	20 μg/mL	40 μg/mL	60 μg/mL	80 μg/mL	100 μg/mL	
4a	31.81	35.27	44.09	51.49	57.79	76.02
4b	47.40	49.76	59.54	63.14	68.50	53.32
4c	52.35	64.09	68.34	70.23	80.15	41.06
4d	38.42	47.71	48.34	55.74	63.46	64.61
4e	59.11	66.61	73.70	78.89	80.00	35.42
4f	44.54	49.18	50.81	78.72	87.45	47.69
4g	59.84	66.77	73.22	78.11	82.51	35.11
4h	60.47	65.82	74.17	74.48	80.00	35.77
4i	36.06	73.07	76.69	80.94	81.41	39.69
Ascorbic acid (standard)	34.09	48.54	68.17	73.63	76.32	49.83



Scheme 1 — Synthesis of designed compounds (**4a-i**)

nitro group did not displayed satisfactory antioxidant activity. Hence, the data suggest that the different substituents can highly influence the biological activity and the present synthesized compounds can be used effectively as antioxidant agents.

Conclusion

We have synthesized a series of 1-(4-substituted phenyl)-1-(6-substituted benzothiazol-2-yl) hydrazone derivatives and confirmed their structures with spectral and elemental analysis. The *in vitro* antioxidant activity data revealed that most of the synthesized compounds were found to be potent than the standard drug. Based on the results, these hydrazones derivatives could be considered as useful scaffolds for development of antioxidant agents in future.

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