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SYNTHESIS OF SOME NOVEL TETRAZOLE SUBSTITUTED BENZIMIDAZOLES AND THEIR EVALUATION AS ANTIOXIDANTS

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ABSTRACT

A series of tetrazole derivatives (3a-h) were synthesized and screened for antioxidant activity by nitric oxide free radical scavenging activity. All the synthesized compounds were structurally elucidated by spectral techniques such as IR, ^{1}H NMR spectroscopy and mass spectrometry. The compounds 3b, 3d 3f and 3h exhibited promising antioxidant activities which were found to be comparable with standard ascorbic acid.

KEY WORDS: **Nitric oxide scavenging assay, Tetrazole, Benzimidazole, Antioxidant.**

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INTRODUCTION

Oxidative stress plays a major role in the development of many chronic and degenerative ailments such as cancer, autoimmune disorders and is initiated by free radicals in the body.[1] Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital biomolecules are damaged. Also, heterocycles such as benzimidazole and tetrazole are known to possess various biological activities such as antiulcer[2-4], anti-inflammatory[5-7], antimicrobial[8-12], antifungal[13-14], anticancer[15-18]. Thus, literature survey suggests that benzimidazoles and tetrazoles help to reduce some of the dreadful effects of oxidative stress. In addition, the studies on predicted biological activities of synthesized derivatives by the application of PASS software[19] was also undertaken and it revealed that they possessed antioxidant activities. These observations have stimulated our interest to explore the synthesis of new potential compounds where mercaptobenzimidazole has been linked with an active nucleus tetrazole and evaluate them for antioxidant activity.

MATERIALS AND METHODS

All the reagents used in the synthesis are of analytical grade and hence used without further purification. The completion of reaction was monitored qualitatively by thin layer chromatography (TLC) performed on silica gel G coated plates of 0.3 mm thickness, using mobile phase benzene:methanol (9.5:0.5); respectively. The melting points of the organic compounds were determined by open capillary tube method, confirmed by digital melting point apparatus VEEGO model:VMP-DS and are quoted as uncorrected values. The spectral studies were conducted by standard methods. Infrared (IR) spectra were recorded in KBr using FTIR Prestige 21 (Schimadzu, Kyoto, Japan) spectrophotometer. The ^{1}H- NMR spectra were recorded in DMSO-d_6 on Variant 300 MHz spectrophotometer. Mass spectra were taken on a Bruker Daltonics Data Analyser by micro TOF method.

EXPERIMENTAL CHEMISTRY

Synthesis of mercaptobenzimidazole (1)

A mixture of o- phenylene diamine (0.1 mol), potassium hydroxide (0.1 mol), carbon disulfide (0.1 mol) and 95% ethanol (100 mL) with water (15 mL) was refluxed for 3 hrs. Later on, charcoal (1-1.5 g) was added and heated for 10 minutes. The reaction mixture was then filtered and to the filtrate, warm water (100 mL) was added and acidified with dilute acetic acid. The product separated as glistering white crystals, which were further recrystallized from ethanol.

Synthesis of 3-(1, 3-benzimidazol-2-yl-sulfanyl) propanenitrile (2)

Mercaptobenzimidazole (0.002 mmol) was refluxed with acrylonitrile (3 mmol), triethylamine (1-2 drops) and methanol (3-4 mL) in water bath for (11-12 hrs). The reaction mixture was then kept overnight and poured in ice cold water to get the crude which was filtered, washed with water and dried. It was recrystallized from methanol.

Synthesis of 2-{[2-(1H-tetrazole-5-yl)ethyl]sulfanyl}-1,3-benzimidazole (3)

3-(1,3-benzimidazol-2-yl-sulfanyl)propanenitrile (10 mmol), sodium azide (10 mmol), DMF (10 mL) and zinc chloride (10 mmol) were taken in a flask and the contents were heated in an oil bath for 6 hrs at 125°C. The residue was dissolved in distilled water (100 mL) and acidified with HCl upto pH 2. The solution was cooled to 5°C in ice bath. The product was removed by filtration, washed with water and dried. It was recrystallized from equimolar DMF-ethanol mixture to get compound. IR: 3124(N–H), 2872(C–H), 2957(C–H), 1552(C=N), 1296(–N–N=N–), 1382 and 1176(tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$): 3.5(t, 2H, S-CH_2-CH_2), 3.1(t, 2H, -CH_2-CH_2), 4.7(s, 2H, N-H of benzimidazole & tetrazole), 7.2–7.8(m, 4H, Ar-H); MS (ESI): 247.29 ($M+H^+$).

***General procedure for synthesis of derivatives*:**

1-{5-[2- (benzimidazol-2-yl-sulfanyl)ethyl]-2H-tetrazol-2yl}phenyl ethanone (3a)

Compound 3 (1g, 4 mmol) was refluxed in acetic anhydride (3g, 30 mmol) for 15 minutes. The reaction mixture was cooled and poured into cold water (20 mL). The contents were then boiled to decompose the excess acetic anhydride. The compound 3a was recrystallized from equimolar aqueous ethanol-DMF mixture. IR: 2949, 2877(C–H), 1483.26(C–H), 1247.94(–N–N=N–), 3024.35(aromatic ring), 1346.31 and 1176.88(tetrazole ring), cm^{-1}; ^{1}H-NMR ($CDCl_3$) d: 2.8(s, 3H, CH3), 3.5(t, 2H, S-CH_2-CH_2), 3.1(t, 2H, -CH_2-CH_2), 7.0–8.0(m, 4H, Ar-H); MS (ESI): 289.33 ($M+H^+$).

2-{[2-(2-benzyl-2H-tetrazol-5yl)ethyl]sulfanyl}benzimidazole (3b)

Compound 3 was treated with an equimolar amount of benzyl chloride in 10% w/v sodium bicarbonate solution (10 mL). The mixture was shaken vigorously in a stoppered test tube. The contents were acidified with 20% v/v hydrochloric acid and the product obtained was filtered. The dried compound was recrystallized from equimolar aqueous ethanol-DMF mixture. IR: 2873.0(C–H), 1643.35(C=O), 1415.75(C–H), 1176.58 and 1348.24(tetrazole ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$) d: 2.6(t, 2H, -CH_2-CH_2), 4.7(s, 1H, N-H (benzimidazole)), 7.0–8.5(m, 9H, Ar-H); MS (ESI): 365.42 ($M+H^+$).

1-{5-[2-(benzimidazol-2-yl-sulfanyl)ethyl]-2H-tetrazol-2yl}methanone (3c)

Compound 3 was treated with an equimolar amount of benzoyl chloride in 10% w/v sodium bicarbonate solution (10 mL). The mixture was shaken vigorously in a stoppered test tube. The contents were acidified with 20% v/v hydrochloric acid and the product obtained was filtered. The dried compound was recrystallized from equimolar aqueous ethanol-DMF mixture. IR: 2920(C–H), 2810(C–H), 1525.69(C=O), 1471, 1280(–N–N=N–), 3088(aromatic ring), 1130.29 and 1323.17(tetrazole ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$) d: 4.8(t, 2H, S-CH_2-CH_2), 3.6(t, 2H, -CH_2-CH_2), 4.6(s, 1H, N-H of benzimidazole)), 7.1–8.1 (m, 9H, Ar-H); MS (ESI): 351.4 ($M+H^+$).

4-({5-[2-(1,3-benzimidazole-2-ylsulfanyl)ethyl]-2H-tetrazol-2yl}sulfonyl)benzoic acid (3d)

Compound 3 was treated with an equimolar amount of benzene sulphonyl chloride in 10% w/v sodium bicarbonate solution (10 mL). The mixture was shaken vigorously in a stoppered test tube. The contents were acidified with 20% v/v hydrochloric acid and the product obtained was filtered. The dried compound was recrystallized from equimolar aqueous ethanol-DMF mixture. IR: 2920.23(C–H), 1470.69(C–H), 1311.59 and 1138(SO_2), 3088.03(aromatic ring), 1219.01 and 1082.07(tetrazole ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$) d: 4.8(t, 2H, S-CH_2-CH_2), 3.2(t, 2H, -CH_2-CH_2), 4.7(s, 1H, NH-benzimidazole), 7.1–8.0(m, 9H, Ar-H); MS (ESI): 387.5 ($M+H^+$).

1-{5-[5- methoxy- 2- (benzimidazol-2-yl-sulfanyl)ethyl]-2H-tetrazol-2yl}phenyl ethanone (3e)

Compound 3 (1g, 4 mmol) was refluxed in acetic anhydride (3g, 30 mmol) for 15 minutes. The reaction mixture was cooled and poured into cold water (20 mL). The contents were then boiled to decompose the excess acetic anhydride. The compound 3e was recrystallized from equimolar aqueous ethanol-DMF mixture. IR: 2867(C–H), 1161,1380 (–N–N=N–), 3016(aromatic CH str), 1768(C=O), 1041(C-O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) d: 2.9(s, 3H, CH3), 2.2(s, 3H, CH3), 3.3(t, 2H, -CH_2-CH_2), 6.9-8.2(m, 4H, Ar-H); MS (ESI): 319.7 ($M+H^+$).

2-{[2-(2-benzyl-2H-tetrazol-5yl)ethyl]sulfanyl}benzimidazole (3f)

Compound 3 was treated with an equimolar amount of benzyl chloride in 10% w/v sodium bicarbonate solution (10 mL). The mixture was shaken vigorously in a stoppered test tube. The contents were acidified with 20% v/v hydrochloric acid and the product obtained was filtered. The dried compound was recrystallized from equimolar aqueous ethanol-DMF mixture. IR: 1469(C–H), 1693(C=O), 1220 and 1313(tetrazole ring),

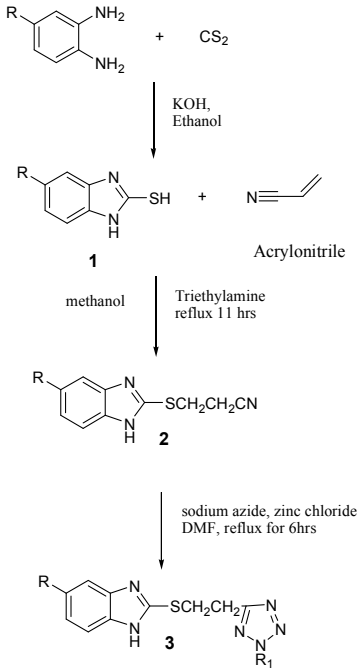
3084(aromatic CH str) 1024(C-O)cm^{-1}; ^{1}H-NMR ($CDCl_3$) d: 3.2(t, 2H, -CH_2-CH_2), 4.4(s, 1H, N-H of benzimidazole), 3.9(s, 3H, CH_3), 7.1- 8.6(m, 9H, Ar-H); MS (ESI): 395.01 ($M+H^+$).

1-{5-[5-methoxy-2-(benzimidazol-2-yl-sulfanyl)ethyl]-2H-tetrazol-2yl}methanone (3g)

Compound 3 was treated with an equimolar amount of benzoyl chloride in 10% w/v sodium bicarbonate solution (10 mL). The mixture was shaken vigorously in a stoppered test tube. The contents were acidified with 20% v/v hydrochloric acid and the product obtained was filtered. The dried compound was recrystallized from equimolar aqueous ethanol-DMF mixture. IR: 2920(C–H), 1670(C=O), 1481(–N–N=N–), 3084(aromatic CH str), 1062(C-O), 2120(C=N)cm^{-1}; ^{1}H-NMR ($CDCl_3$) d: 3.2(t, 2H, -CH_2-CH_2), 3.8(s, 3H, CH_3), 4.9(s, 1H, N-H of benzimidazole), 6.9–7.9 (m, 9H, Ar-H); MS (ESI): 381.20 ($M+H^+$).

4-({5-[5-methoxy-(1,3-benzimidazole-2-yl sulfanyl)ethyl]-2H-tetrazol-2yl}sulfonyl)benzoic acid (3h)

Compound 3 was treated with an equimolar amount of benzene sulphonyl chloride in 10% w/v sodium bicarbonate solution (10 mL). The mixture was shaken vigorously in a stoppered test tube. The contents were acidified with 20% v/v hydrochloric acid and the product obtained was filtered. The dried compound was recrystallized from equimolar aqueous ethanol-DMF mixture.IR: 2854.23(C–H), 1498.42(C–H), 1378 and 1127(SO_2), 3074(aromatic ring), 1020(C-O), 1376.09 and 1183.07(tetrazole ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$) d: 4.9(t, 2H, S-CH_2-CH_2),3.9(s, 3H, CH_3), 4.8(s, 1H, NH of benzimidazole), 7.1–8.5(m, 9H, Ar-H); MS (ESI): 387.07 ($M+H^+$).



3a : R=-H, R_1=-C_2H_3O
3b : R=-H, R_1=-C_8H_7O
3c : R=-H, R_1=-C_7H_5O
3d : R=-H, R_1=-$C_6H_5O_2S$
3e : R=-OCH_3, R_1=-C_2H_3O
3f : R=-OCH_3, R_1=-C_8H_7O
3g : R=-OCH_3, R_1=-C_7H_5O
3h : R=-OCH_3, R_1=-$C_6H_5O_2S$

Scheme 1
General route for the synthesis of 2-{[2-(1H-tetrazol-5-yl)ethyl]sulfanyl}-1,3-benzimidazole

ANTIOXIDANT EVALUATION

Nitric oxide free radical scavenging activity

All the synthesized compounds were tested for their antioxidant properties by using nitric oxide free radical scavenging method. Test compounds with each of 50 mL concentrations were previously dissolved in DMSO and ascorbic acid as standard. These were taken in separate test tubes and the volume was uniformly made up to 150 mL with methanol. To each test tube, 2 mL of sodium nitroprusside (10 mmol) in phosphate buffer solution was added and the solutions were incubated at room temperature for 150 minutes. Similar procedure was repeated with methanol as blank which served as control. After the incubation, 5 mL of Griess reagent was added to each test tube including control and standard. The absorbance of chromophore formed was measured at 546 nm on UV-visible spectrometer. The IC_{50} values for each test compounds as well as standard were calculated[20-21]. % scavenging = [Absorbance of control - Absorbance of sample/Absorbance of control] X 100 The results of this study are summarised in table 1.

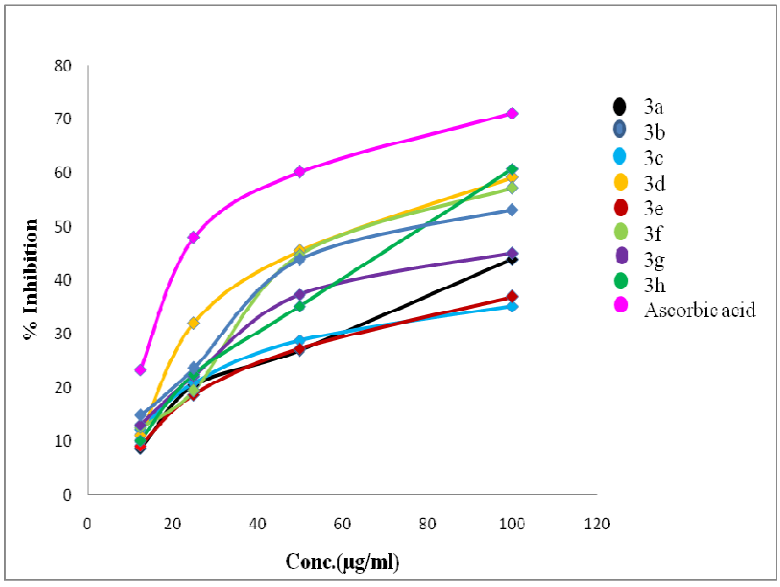
Table 1

Antioxidant activity by Nitric oxide free radical scavenging assay

CONCENTRATION	12.5 µg	25 µg	50 µg	100 µg
3a	8.75	20.45	27.08	43.97
3b	14.84	23.64	43.94	53.17
3c	12.18	20.91	28.75	35.22
3d	11.05	32.05	45.51	59.21
3e	9.18	18.73	27.27	36.94
3f	12.63	19.46	44.81	57.19
3g	13.15	22.19	37.33	45.11
3h	10.15	22.54	35.19	60.79
Ascorbic acid (standard)	27.31	48	60.16	71.73

Figure 1

% Radical scavenging activity of compounds 3a-3h



RESULTS AND DISCUSSION

Chemistry

Mercaptobenzimidazole undergoes cyanoethylation reaction with acrylonitrile in the presence of potassium hydroxide as a base. The yield of the compound 2 was found to be quantitative and which then

undergoes cyclization to give tetrazole 3 by treating them with sodium azide and zinc chloride in dimethylformamide (Scheme 1). The proton on the secondary amino group of tetrazole at first position is labile and is readily abstractable. This presents a suitable position for preparation of various derivatives through substitution reactions.

Biological evaluation

The antioxidant activity of all synthesized compound 3a-3h was carried out by using nitric oxide scavenging assay at various concentrations viz., 12.5, 25, 50, 100 µg/mL with ascorbic acid as standard. The results of this activity are depicted in table 1 and fig. 1. From the antioxidant studies, it was observed that as concentration increases, the % scavenging increases linearly for the test compounds along with standard. From the results, it was confirmed that the compounds 3b, 3d, 3f, 3h showed antioxidant activity comparable to the standard.

CONCLUSION

A series of some novel substituted 2-{[2-(1H-tetrazole-5-yl) ethyl] sulfanyl}-1,3-benzimidazoles were synthesized and were evaluated for antioxidant activity by employing nitric oxide scavenging assay method. The results of antioxidant activity revealed that the compounds 3b, 3d, 3f, 3h exhibited antioxidant activity comparable to that of the standard compound ascorbic acid. The compounds mentioned above are formed by substitution of electron donating substituents on the secondary nitrogen. Hence, it may be surmised that electron donation on this nitrogen might be responsible for increase in antioxidant activity which is not observed for the other derivatives where the substituents have electron withdrawing nature.

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