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Synthesis, Spectroscopic Characterization and Biological Evaluation of Some 6-Nitro-Benzothiazole-2-yl-Hydrazone Derivatives

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ARTICLE INFORMATION

Received May 24, 2018

Revised July 01, 2018

Accepted July 03, 2018

Published September 22, 2018

ABSTRACT

A series of 2-[(6-Nitro-benzothiazol-2-yl)-hydrazonomethyl]-substituted-phenol derivatives (3a-f) were synthesized and characterized by using elemental and spectroscopic analyses (FT-IR, UV-Vis, ¹H-NMR, ¹³C-NMR and Mass spectra). The synthesized compounds were screened for antimicrobial activities against two Gram-positive bacteria (*Bacillus subtilis* and *Streptomyces griseus*) two Gram-negative bacteria (*Salmonella typhi* and *Pseudomonas aeruginosa*) and three fungi (*Candida tropicalis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*). The antioxidant activities of these compounds (3a-f) were determined by hydrogen peroxide (H₂O₂) scavenging activity. The substitution iodo-group compound 3d is more potentially active than other synthesized compounds in antibacterial and antifungal activities and the most promising antioxidant activity shown by hydroxy and methoxy substituted compounds 3a and 3e.

KEYWORDS: 6-Nitro-benzothiazole-2-yl-hydrazone; spectroscopic analyses; antimicrobial and antioxidant activity.

INTRODUCTION

The numerous lives threaten by microorganism which is increasing their empire worldwide. These microorganisms effect on human life as well as the whole ecosystem. To confronting the effect of microorganism the scientist keeps trying to synthesis biological active organic molecules as well as its metal complexes. Benzothiazole moiety has wide spectrum of biological activities and important ring system of drugs, possessing several pharmacological functions with multiple therapeutic applications [1]. Biological and pharmaceutical activity increases when Benzothiazole ring coincident with hydrazine form 2-benzothiazol-2-yl-

hydrazine and finally coupled with appropriate ketones or aldehydes to form (E)-2-benzothiazole hydrazones [2].

Hydrazone derivatives are significant because of their versatile biological actions. The coincident hydrazones are azomethines containing triatomic grouping >C=N-N< which is differentiated from other members of this class due to presence of two interlinked nitrogen atoms in it. Many of physiologically active hydrazones find applications in biological, clinical, analytical and various other fields. Many researchers synthesized these compounds as target structures and evaluated their biological activities. Furthermore, the

hydrazones improving existing biological activity such as antibacterial, antifungal, antitumoral, antimalarial, anticonvulsant, anticancer, antiinflammatory, antioxidant and antitoxoplasma activity [3-8]. Hydrazone derivatives also widely applied in the field of herbicides, insecticides, acaricides, rodenticides and analytical reagents for their excellent bioactivity properties.

Biological activities of various hydrazones compounds are well reported in literature as drugs in order to combat diseases with less toxicity and large effects. The -OH group attached on the phenyl ring strongly influenced the antioxidative activity [9]. The presence of -NO₂, -Br and -OCH₃ groups to the substituted (E)-2-benzothiazole hydrazones enhanced the antimicrobial activities [10]. Keeping the view in mind, in this present study we synthesis a series of 6-nitro -2- benzothiazole hydrazones derivatives condensing 6-nitro-benzothiazol-2-yl-hydrazine with appropriate salicylaldehyde (**3a-e**), 2-hydroxy-1-naphthaldehyde (**3f**) and screened for their antibacterial, antifungal and antioxidant activity.

MATERIAL AND METHODS

Material and Physical measurement

All the chemicals used were of analytical reagent grade (AR) or chemically pure grade and purchased from SD-Fine Ltd and Sigma-Aldrich Chemicals Ltd. Melting points determined on electrical melting point apparatus using one end seal capillary. R_f value measured using solvent system chloroform: Ethyl acetate (7:3). Fourier transform infrared (FT-IR) spectra were recorded on Agilent Cary 630 FT-IR Spectrometer in the range 0-4000 cm⁻¹. UV-Vis spectra were performed on a Shimadzu UV-1800 spectrophotometer in DMF. The elemental analysis (C, H, N and S) was carried out with Thermo finnigan FLASH EA 1112 CHNS analyser. ¹H and ¹³C NMR spectra were run on Bruker Advance II, 400MHz, NMR spectrometer in DMSO. The mass spectrum was obtaining on JEOL GCMATE II GC-MS.

Synthesis of 6-nitro-2-benzothiazolamine (1)

4-Nitro aniline (0.4 mol) was treated with HCl (50 %) to get aniline hydrochloride salt. A saturated solution of ammonium thiocyanate (0.8 mol) in water was added with constant stirring in above hydrochloride salt solution and

refluxed still solution become turbid. Cool the reaction mixture and poured into ice-water to obtain nitro-phenylthiourea precipitate was filter and washed with water and recrystallized from ethanol.

A solution of molecular bromine, Br₂ (0.1 mol) in glacial acetic acid was add slowly to the solution of nitro- phenylthiourea (0.1 mol) in glacial acetic acid and continuously stirred for 3hr at 5-10°C and 5 hr at room temperature formed yellow precipitate, dissolved in hot water and neutralized with KOH (saturated) solution and filtered with water washing. Finally, recrystallized from aqueous ethanol and dried, yield: 78%, colour: yellow powder, m.p.: 245°C, R_f : 0.50, IR (v cm⁻¹): 3444 (NH *str*), 3024 (CH-Ar), 1647 (C=N), 1448 (C=C), 1278 (C-NO₂), 697 (C-S-C). λ_{max} (nm): 230 and 275 π-π*, 313 n-π*. Anal. for C₇H₅N₃O₂S (Calcd.): C, 43.07; H, 2.58; N, 21.53; S, 16.43 %, (Found): C, 42.71; H, 2.30; N, 21.11; S, 16.5 %.

Synthesis of (6-nitro-benzothiazol-2-yl)-hydrazine (2)

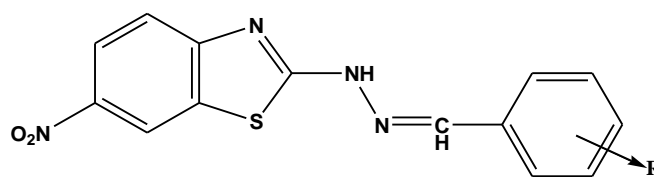
Conc. HCl (3 ml) was added slowly in hydrazine hydrate 99 %, (3 ml) with continuously stirring at 5-8 °C. Add to it glycerol (30 ml) and compound **1** (0.01 mol) were added at room temperature after complete addition mixture solution refluxed with stirring for 2 hr at 150-153 °C. The reaction solution become homogeneous and yellow green color appeared. At the end of reaction yellow precipitated form. Cool at room temp and poured into ice cooled water. The product was filtered and washes with water and recrystallized from absolute ethanol. Yield: 65%, yellow crystalline powder, m.p.: 274°C, R_f : 0.30, FTIR(v cm⁻¹): 3356 (NH *str*), 3062 (CH-Ar), 1659 (C=N), 1451(C=C),1296 (C-NO₂), 1047(N-N) 736 (C-S-C). λ_{max} (nm): 225 and 260 π-π*, 355 n-π*. Anal. for C₇H₆N₄O₂S (Calcd.): C, 40.00; H, 2.88; N, 26.65; S, 15.25 %, (Found): C, 40.31; H, 2.43; N, 27.09; S, 15.60 %.

Synthesis of 2-[(6 nitro-benzothiazol-2-yl)-hydrazonomethyl]-substituted-phenol

The ethanolic solution of compound **2** (0.01 mmol) was added drop wise into the ethanolic solution of substituted salicylaldehyde (**3a-e**) and 2-hydroxy-1-naphthaldehyde (**3f**) (0.01 mmol).add glacial acetic acid (2-3 drop) and resulting mixture was stirring under the refluxed for 2 hr. The reaction mixture was cool

at room temperature. Solid compound was separated. It was filtered, washed, recrystallized from ethanol and dried in vacuum over

anhydrous calcium chloride to get the pure final compound. Synthetic route shown in Fig.1.



- R = 2-HO-3-MeO- (3a)
 = 2-HO-5-Br- (3b)
 = 2-HO-4-N(CH₂CH₃)₂- (3c)
 = 2-HO-3,5-I₂- (3d)
 = 2-HO-4-MeO- (3e)
 = 2-HO-5,6-benzo- (3f)

Fig. 1: Synthetic route for preparing Compounds. 3a-f.

2-[(6-Nitro-benzothiazol-2-yl)-

hydrazonomethyl]-6-methoxy-phenol (3a)

Yield: 65%; yellow solid, m.p. 262°C. R_f : 0.58. FTIR (ν cm⁻¹): 3455(NH *str*), 1621 (C=N), 707(C-S-C). λ_{max} (nm): 229 and 261 π - π^* , 372 n- π^* . ¹H NMR (400 MHz, DMSO-d₆, δ , ppm): 12.79 (brs, 1H, NH), 9.67 (brs, 1H, OH), 8.81-8.80 (d, J=2.12 Hz, 1H, Ar H), 8.53 (s, 1H, N=C-H), 8.19-8.16 (m, J =11.36 Hz, 1H, Ar H), 7.54-7.52 (s, 1H, Ar H), 7.31-7.29 (d, J=7.08 Hz, 1H, Ar H), 7.04-7.01 (m, J=9.25, 1H, Ar H), 6.89-6.86 (m, J =12.4 Hz, 1H, Ar-H), 3.85(s, 3H, -OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ , ppm): 165, 158.7, 158.2, 148, 146.1, 141.1, 123, 122, 120.4, 120.1, 119.3, 118.3, 117.1, 113.2, 55.8. ESI-MS: m/z [M⁺]: 345.5. Anal. for C₁₅H₁₂N₄O₄S (Calcd.): C, 52.32; H, 3.51; N, 16.27; S, 9.31 %, Found: C, 52.40; H, 4.03; N, 16.67; S, 9.33%.

2-[(6-Nitro-benzothiazol-2-yl)-

hydrazonomethyl]-4-bromo-phenol (3b)

Yield: 60 %; yellowish green, m.p. 247°C. R_f : 0.68. FTIR (ν cm⁻¹): 3696 (NH *str*), 1613 (C=N), 643 (C-S-C). λ_{max} (nm): 232 and 264 π - π^* , 393 n- π^* . ¹H NMR (400 MHz, DMSO-d₆, δ , ppm): 12.84 (brs, 1H, NH), 10.49 (brs, 1H, OH), 8.77 (s, 1H, Ar H), 8.41 (s, 1H, N=C-H), 8.18-8.15 (m, 1H, Ar H), 7.80-7.79 (s, 1H, Ar H), 7.53-7.51 (d, 1H, J=7.78 Hz, Ar H), 7.38-7.36 (m, 1H, Ar H), 6.90-6.87 (d, J=8.72 Hz, 1H, Ar H). ¹³C NMR (100 MHz, DMSO-d₆, δ , ppm): 155.6, 147, 141.3, 133.5, 130.3, 124.4, 122.3, 122, 118.5, 118.3, 115.6, 113.2, 110.8; ESI-MS: m/z [M⁺]: 395.2. Anal. Calcd. For C₁₄H₉BrN₄O₃S: C, 42.76; H,

2.31; N, 14.25; S, 8.15 %, Found: C, 42.51; H, 2.40; N, 13.67; S, 8.21 %.

2-[(6 nitro-benzothiazol-2-yl)-

hydrazonomethyl]-5-diethylamino-phenol (3c)

Yield: 75 %; reddish brown, m.p. 268°C, R_f : 0.66. FTIR (ν cm⁻¹): 3391 (NH *str*), 1606 (C=N), 686(C-S-C). λ_{max} (nm): 227 and 258 π - π^* , 376 n- π^* . ¹H NMR (400 MHz, DMSO-d₆, δ , ppm): 12.56 (brs, 1H, NH), 10.19 (brs, 1H, OH), 8.75 (s, 1H, Ar H), 8.34 (s, 1H, N=C-H), 8.17-8.15 (d, J =8.08 Hz, 1H, Ar H), 7.39-7.37 (d, J =8.12 Hz, 2H, Ar H), 6.30-6.28 (d, J =7.84 Hz, 1H, Ar H), 6.14 (s, 1H, Ar H), 3.38 (S, 4H, -CH₂), 1.11 (s, 6H, -CH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ , ppm): 165.7, 158.9, 150.3, 140.7, 122.6, 122.6, 122, 121.9, 118.3, 117.7, 116.8, 106.6, 104.1, 97.1, 43.8, 12.5. ESI-MS: m/z [M⁺]: 385. Anal. for C₁₈H₁₉N₅O₃S (Calcd.): C, 56.09; H, 4.97; N, 18.17; S, 8.32 %, Found: C, 56.18; H, 4.71; N, 18.31; S, 8.64 %.

2-[(6 nitro-benzothiazol-2-yl)-

hydrazonomethyl]-4, 6 diiodo-phenol (3d)

Yield: 55 %; yellow solid, m.p. 251°C, R_f : 0.64. FTIR (ν cm⁻¹): 3263 (NH *str*), 1609 (C=N), 639(C-S-C). λ_{max} (nm): 247 and 261 π - π^* , 389 n- π^* . ¹H NMR (400 MHz, DMSO-d₆, δ , ppm): 12.94 (brs, 1H, NH), 11.70 (brs, 1H, OH), 8.77 (s, 1H, Ar H), 8.43 (s, 1H, N=C-H), 8.20-8.17 (dd, J=11.24 Hz, 1H, Ar H), 8.04 (s, 1H, Ar H), 7.91-7.90 (s, 1H, Ar H), 7.40-7.39 (m, 1H J=6.6 Hz, Ar H). ¹³C NMR (100 MHz, DMSO-d₆, δ , ppm): 170.6, 162.2, 152.2, 146.9, 141.5, 139.8, 135.5, 131, 130.5, 127.1, 124.8, 122.1, 118.7, 113.9. ESI-MS: m/z

[M⁺]: 566. Anal. for C₁₄H₈N₄I₂O₃S (Calcd.): C, 29.70; H, 1.42; N, 9.90; S, 5.66 %, Found: C, 29.66; H, 1.57; N, 9.55; S, 5.51 %.

2-[(6 nitro-benzothiazol-2-yl)-hydrazonomethyl]-5-methoxy-phenol (3e)

Yield: 72 %; yellow orange, m.p. 252°C, R_f: 0.52. FTIR (ν cm⁻¹): 3337 (NH *str*), 1619 (C=N), 600(C-S-C). λ_{max} (nm): 227 and 264 π-π*, 392 n-π*. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 12.83 (brs, 1H, NH), 9.82 (brs, 1H, OH), 8.80 (s, 1H, Ar H), 8.47 (s, 1H, N=C-H), 8.19-8.16 (dd, J = 11.2 Hz, 1H, Ar H), 7.54 (brs, 1H, Ar H), 7.22 (s, 1H, Ar H), 6.92-6.85 (m, 2H, Ar H), 3.74 (s, 3H, -OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): 162.1, 159.5, 158.3, 141, 139.9, 130.8, 122.5, 121.9, 118.2, 117.7, 116.8, 112.5, 106.6, 100.8, 55.26. ESI-MS: m/z [M⁺]: 344.

2-[(6 nitro-benzothiazol-2-yl)-hydrazonomethyl]-naphthol (3f)

Yield: 68 %; yellow brown, m.p. 242°C, R_f: 0.74. FTIR (ν cm⁻¹): 3671 (NH *str*), 1570 (C=N), 725(C-S-C). λ_{max} (nm): 236 and 264 π-π*, 391 n-π*. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 12.78 (brs, 1H, NH), 11.30 (brs, 1H, OH), 9.15 (s, 1H, Ar H), 8.81 (s, 2H, Ar H), 8.19-8.16 (d, J=11.28 Hz, 1H, Ar H), 7.92-7.86 (m, 2H, Ar H), 7.61-7.58 (m, 1H, Ar H), 7.50-7.48 (s, 1H, N=C-H), 7.42-7.39 (m, 1H, Ar H), 7.25-7.23 (d, J=8.92 Hz, 1H, Ar H). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): 157.4, 144.9,

144.7, 141.2, 136.3, 132.9, 131.1, 128.8, 128.1, 127.8, 123.5, 123.1, 122.6, 122, 118.5, 118.2, 109.8. ESI-MS: m/z [M⁺]: 364. Anal. for C₁₈H₁₂N₄O₃S (Calcd.): C, 59.33; H, 3.32; N, 15.38; S, 8.80 %, Found: C, 59.19; H, 3.46; N, 15.39; S, 8.16 %.

BIOLOGICAL EVALUATION

Antibacterial and Antifungal activities

All the synthesized compounds (**3a-f**) were screened for their antibacterial and antifungal activities by disc diffusion method [2, 11]. The antibacterial and antifungal activities were done at 100 μg/ml concentrations in DMF solvent. The antibacterial activity of these compounds tested against two Gram-positive (*Bacillus subtilis* and *Streptomyces griseus*) two Gram-negative (*Salmonella typhi* and *Pseudomonas aeruginosa*) and three fungus (*Candidi tropicalis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*). The bacterial stains were incubated at 37 °C for 24 hrs and fungal stains were incubated at 37° C for 72 hrs. To compare the antibacterial activities (Streptomycin) and antifungal activities (Nystatin) was used as standard drug under the similar conditions. Whatman filter paper disc diameter is 5 mm. The zone of inhibition was measured in mm after incubation of plates as shown in fig 2 and 3. All experiments performed as triplicate and take the mean values [11].

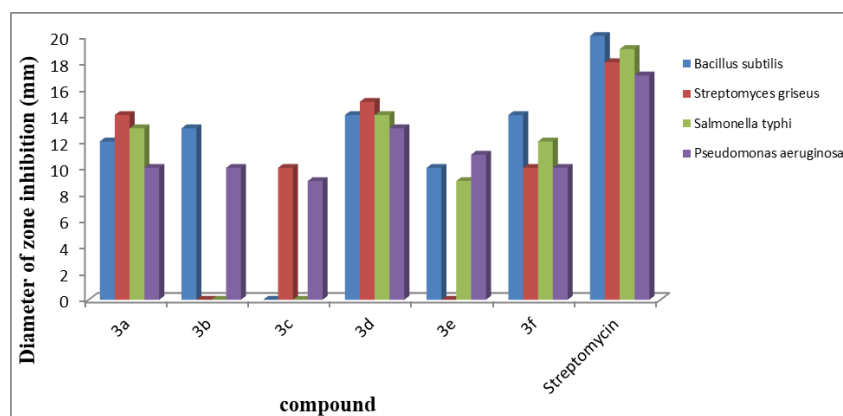


Fig 2: Antibacterial Activities of Compound **3a-f**

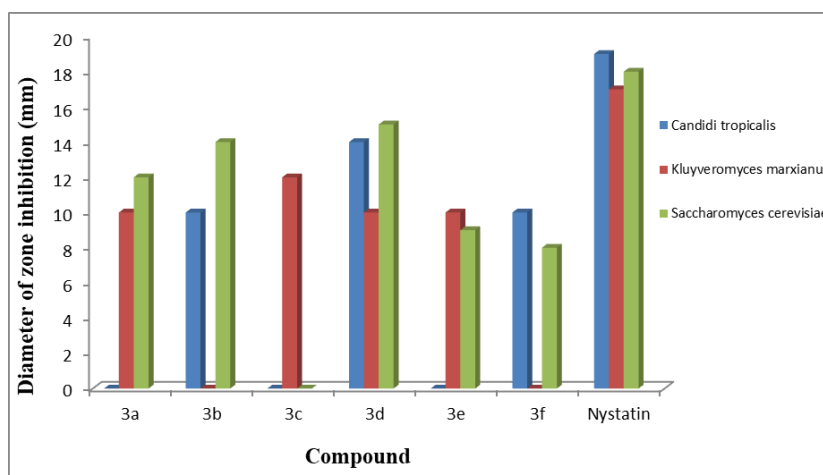


Fig 3: Antifungal Activities of Comp. 3a-f

Antioxidant activity

Hydrogen peroxide (H₂O₂) scavenging activity

The hydrogen peroxide scavenging activity of the synthesized hydrazones compounds (3a-f) was determined according to the method described by Y. Harinath, K. Seshaiyah. *et al*. For each of the above assay, tests compounds were run in triplicate and mean values taken as the result. In the assay the reaction mixtures consists 2.5 ml of phosphate buffer (0.15 M, pH= 7.4), 1 ml 945 μM EDTA-Fe(II), 0.5 ml 114 μM safranin, 1 ml 3% H₂O₂ and diluted to various concentrations (25, 50 and 100 μg/ml) were prepared in DMF. This reaction mixture was vigorously shaken to appear a clear

homogeneous solution. The reaction mixture without test compound was used as the control. The reaction mixtures were incubated at 37° C for 1 hr. in a water bath. The absorbance of the resulting mixture was read at 520 nm. The scavenging ratio (%) was calculated from the following formula:

$$\text{Scavenging ratio (\%)} = \frac{A_i - A_o}{A_c - A_o} \times 100$$

where A_i is the absorbance of the tested compound present in reaction mixture;

A_o is the absorbance of absence tested compound; and A_c is the absorbance of the absence the tested compound, EDTA-Fe (II) and H₂O₂ [14]. Experiments result shown in fig.4.

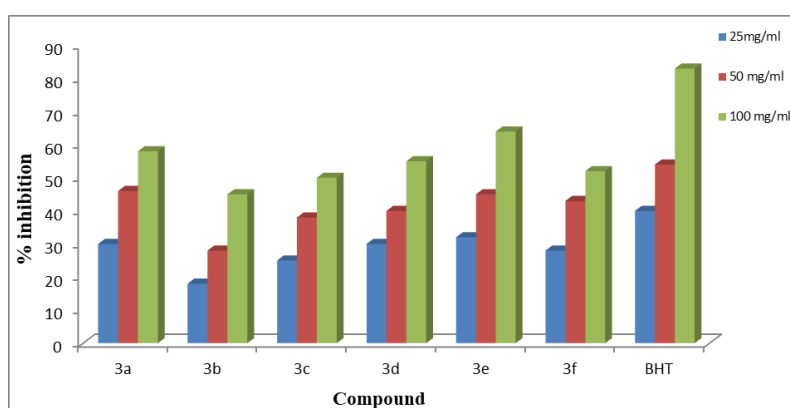


Fig 4: Antioxidant Activities of Comp. 3a-f.

RESULTS AND DISCUSSION

Synthesis of Compound (3a-f)

The synthesis of 6-Nitro-1,3-benzothiazol-2-yl hydrazone derivatives (3a-f) involves the

cyclization of 4-nitro aniline with ammonium thiocyanate by bromination in present of acetic acid formation of compound 1. then refluxed with hydrazine hydrate and Compound 1 in

presence of glycerol and Finally refluxing Compound **2** with substituted salicylaldehyde (**3a-e**) and 2-hydroxy-1-naphthaldehyde (**3f**) to gives the (E) 6-Nitro-benzothiazole hydrazone derivative. Synthesized derivatives were characterized by elemental analysis, FTIR, UV-Vis, ^1H NMR, ^{13}C NMR and mass spectra. Spectral analysis results of all derivatives are in full agreement with the proposed structures. The IR spectra for these derivatives shows the NH, C=N and C-S-C peak observe at 3263-3696, 1570-1621 and 600-725 cm^{-1} respectively. In the UV-Vis spectrum $\pi-\pi^*$ transitions of the aromatic rings are observed in the 227-275 nm region whereas the absorption band at 313- 392 nm may be assigned to the $n-\pi^*$ transitions of the C=N bond. The ^1H NMR spectrum exhibits signals at 12.56-12.94 ppm for -NH protons, the -OH protons signals were observed at 9.46-10.49 ppm and signals observed at 8.34-8.53 ppm for N=C-H proton. The aromatic protons are observed as a multiplet in the range 6.14-8.80ppm. A peak at 3.85 and 3.74 ppm is attributed to proton of the $-\text{OCH}_3$ in compound **3a** and **3e** respectively. In compound **3c** signals observed at 3.38 ($-\text{CH}_2$) and 1.11 ($-\text{CH}_3$) ppm. The ^{13}C NMR spectral data support to the proposed structures which exhibits the N=C-S signal at 170-155 ppm and C=N signal at 122-128 ppm [6]. The signal for the $-\text{OCH}_3$ group observes at 55.8 and 55.26 ppm in compound **3a** and **3e**. While in compound **3c** signal seen at 43.3 and 12.5 ppm assigned to $-\text{CH}_2$ and $-\text{CH}_3$ respectively.

Antimicrobial activities

The synthesized compounds (**3a-f**) were evaluated for their anti-bacterial activity against *Bacillus subtilis*, *Streptomyces grieseus*, *Slamonella typhi* and *Pseudomonas aeruginosa* which showed that compound **3d** is more active than other synthesized compounds. The compound **3a**, **3d** and **3f** showed anti-bacterial activity against all the tested microorganisms. The anti-fungal activity against *Candida tropicalis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* showed that compound **3d** is more potentially active than other synthesized compounds while other has least antifungal activity. Compounds **3d** show Maximum anti-bacterial and anti-fungal activity due to substitution iodo group present in it.

Iodine exhibits microbial activity and highly effective against bacteria, fungi and yeasts [18].

Antioxidant activity

All the compounds (**3a-f**) were tested for their *in vitro* antioxidant activity by hydrogen peroxide free radicals scavenging activities. The investigation of antioxidant screening revealed that all the synthesized compounds showed potent to moderate in hydrogen peroxide scavenging activity when compared to the standard butylated hydroxyl toluene (BHT). Amongst the test compounds **3e** and **3a** have shown strong inhibitory effect. However compounds **3d** and **3f** showed mild inhibitory effect in the hydrogen peroxide radical scavenging activity. The $-\text{OH}$ and $-\text{OCH}_3$ group strongly influenced the antioxidative activity. The most promising antioxidative activity showed hydroxy and methoxy substituted compounds **3a** and **3e**. The results showed that the hydrogen peroxide scavenging activity is in the order of BHT > **3e** > **3a** > **3d** > **3f** > **3c** > **3b**.

CONCLUSION

In summary, the work reported involved the synthesis and spectroscopic characterization of 6-Nitro-benzothiazole-2-yl-hydrazone derivatives (**3a-f**) prepared by condensation process. The spectral data (FTIR, UV-Vis, ^1H NMR, ^{13}C NMR and mass spectra) are in fully confirmed the composition and structure of synthesized compounds. The nature of substituent and substitution attachment on the benzene ring may have a considerable effect on the biological activities. The presence of nitro and iodo group has enhanced the antimicrobial activities activity and the substituent hydroxy and methoxy group strongly influenced in the antioxidative activity.

ACKNOWLEDGEMENTS

Authors are thankful to the Prof. Dr. M.V. Waykole, Principal, P.O. Nahata College, Bhusawal. Principal, D. D. N. Bhole College, Bhusawal and Principal, Smt. G. G. Khadse College, Muktainagar. Also thankful to the Dr. Kishor J. Patil, Bio-Tech Department. M. J. College, Jalgoan for moral support and providing the laboratory facilities. We are sincerely thankful to the Director, SAIF-IIT Bombay. Punjab University, Chandigarh providing instrumental facility.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research article.

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Cite this article as:

S.S.Kolate, G.P.Waghulde, C.J. Patil, C.H Sarode. Synthesis, Spectroscopic Characterization and Biological Evaluation of Some 6-Nitro-Benzothiazole-2-yl-Hydrazone Derivatives. *J Pharm Chem Biol Sci* 2018; 6(3):143-150