



Synthesis of Benzothiazole Schiff's Bases and screening for the Anti-Oxidant Activity

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ABSTRACT

In this present study, schiff's bases were synthesized from *o*-Vanilidine refluxing with 2-amino-6-chloro benzothiazole, 2-amino-6-bromo benzothiazole and 2-amino-6-methyl benzothiazole. The synthesized compounds were purified by recrystallisation and characterized by IR, mass and ¹H NMR spectral data. The synthesized compounds were tested for DPPH radical assay and ABTS radical scavenging ant-oxidant activities. All the compounds were showed good activity compare to the standard. Among the synthesized compounds *o*-Vanilidine-2-amino-6-bromo benzothiazole shows IC₅₀ value 248.82 µg/mL in DPPH assay and *o*-Vanilidine-2-amino-6-chloro benzothiazole shows IC₅₀ value 52.36 µg/mL in ABTS assay.

Keywords: Benzothiazole; Schiff's base; DPPH assay; ABTS assay; IC₅₀ value

INTRODUCTION

Benzothiazole is one of the biologically active heterocyclic compound formed by the benzene ring fused with 1, 4 positions of thiazole rings. Benzothiazole Schiff bases has a wide variety of applications in analytical, biological, inorganic, medicinal and pharmaceutical fields due to its biological activities like anti-inflammatory, analgesic, antimicrobial, anticonvulsant, antitubercular, anticancer, antioxidant, anthelmintic [1-14]. Its potent and significant biological activities is due to the presence of azomethine (–C=N–) functional group. Because of above facts and wide variety of the applications, synthesis of this compound is of considerable interest in this present scenario to enhance the biological activity. In the present study, we have synthesized three derivatives of benzothiazole schiff's bases by condensing it with *o*-Vanilline. The synthesized compounds were tested for DPPH radical assay and ABTS radical scavenging ant-oxidant activities.

EXPERIMENTAL SECTION

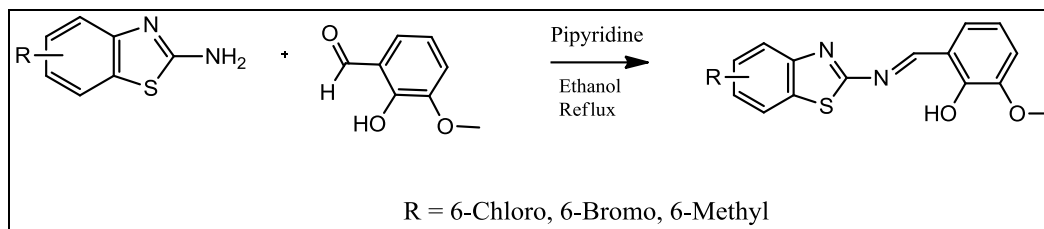
Materials and methods

All the reagents were of analytical grade and were prepared with doubly distilled water. 0.05 % (w/v) Schiff's base solutions were prepared by dissolving appropriate amounts of the substance in methanol. *o*-vanillin, DPPH(1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) were purchased from Sigma Aldrich (Germany).

Reactions were carried out in 50 mL three necked round bottom flask and previously washed with ethanol, oven dried. Melting point was recorded by open capillary method and it was uncorrected. Infrared Spectra were recorded in KBr disk on a Perkin Elmer FT-IR. ¹H NMR spectra on a Bruker advanced 600, MHz spectrometer in CDCl₃

and thermo DFS double focusing magnetic sector mass spectrophotometer for mass spectral measurements. Shimadzu UV double beam spectrophotometer was used for spectrophotometric measurements.

Reaction Scheme



General method for the synthesis of Schiff's bases

10 mmol of Benzothiazole derivative (6-Chloro,6-bromo,6-methyl) was dissolved in absolute ethanol (15 mL) and then *o*-vanillin (1.10 g) was added to this solution along with a drop of piperidine. The mixture was refluxed for 3 hours and kept for eight hours. The yellowish orange solid obtained was filtered and washed several times with cold ethanol, dried and recrystallized from carbon tetrachloride to yield yellow crystalline solid.

Analysis of *o*-Vanilidine-2-amino-6-chloro benzothiazole (VCBT)

C₁₅H₁₁ClN₂O₂S requires 56.52% C, 3.48% H and 8.79% N; found 56.50% C, 3.49% H and 8.79% N; yellow crystalline solid, m.p 224°C. IR (KBr, cm⁻¹): 3440(O-H), 1596(C=N), 1247 (C-O). ¹H NMR (CDCl₃, TMS, %) ppm: 3.9 (s,-OCH₃), 6.9 to 8.0 (m,aromatic protons), 9.3 (s,-CH=N), 12.38 (s,-OH). Mass spectrum M⁺ ion peak 318.

Analysis of *o*-Vanilidine-2-amino-6-bromo benzothiazole (VBBT)

C₁₅H₁₁BrN₂O₂S requires 49.60% C, 3.05% H and 7.71% N; found 49.58% C, 3.02% H and 7.74% N; yellow crystalline solid, m.p 234°C. IR (KBr, cm⁻¹) 3432(O-H) 1592 (C=N), 1259 (C-O). ¹H NMR (CDCl₃, TMS, %) ppm): 3.15(s,-OCH₃), 6.8 to 8.0 (m,aromatic protons), 9.3(s,-CH=N), 12.38 (s,-OH). Mass spectrum M⁺ ion peak 364.

Analysis - 2- of *o*-Vanilidine amino-6-methyl benzothiazole(VMBT)

C₁₆H₁₄N₂O₂S requires 64.41% C, 4.73% and 9.39% N; found 64.48% C, 4.7% H and 9.36% N; yellow crystalline solid, m.p 178°C. IR (KBr, cm⁻¹): 3434 (O-H), 1596 (C=N), 1258 (C-O). ¹H NMR (CDCl₃, TMS, δ): 2.76 (s,-CH₃), 3.96 (s,-OCH₃), 6.8-7.4 (m, aromatic protons), 9.3 (s,-CH=N), 12.53 (s,-OH). Mass spectrum M⁺ ion peak 298.

Antioxidant assays

Free radical scavenging ability by DPPH radical assay (1,1-diphenyl-2-picrylhydrazyl):

DPPH radical scavenging activity of isolated compound was determined as previously reported [15]. Various concentrations of test sample in an aliquot of 100 μl were mixed with 100 μl of 40 μM methanolic solution of DPPH (Himedia, Mumbai, India) in a 96-well microtiter plate. The decrease in absorbance at 517 nm was recorded after the incubation of 15 min at room temperature. The absorbance of the DPPH solution with only methanol and without sample was used as the control. The Butylated hydroxytoluene (BHT, Himedia, Mumbai) was used as a standard to compare the activity. Since the tested compound was yellow colored, appropriate blank readings at 517 nm were recorded for each tested dilution. The assay was carried out in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula.

$$\text{Percentage of inhibition} = [(A_C - A_S)/A_C] \times 100$$

Where A_C is the absorbance of the control and A_S is the absorbance of the sample/standard at 15 min. The IC₅₀ value was calculated graphically based on capacity of compound concentration to scavenge 50% of free radicals.

ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity :

ABTS assay was performed with modification of the previously reported method [16]. The ABTS stock solution was prepared by mixing equal volumes of 7.4 mM ABTS (Sigma-Aldrich, USA) solution and 2.6 mM potassium persulfate solution followed by incubation for 12 h at room temperature in the dark. A working solution was prepared freshly by adjusting the absorbance to 0.7±0.05. The reaction mixture consisted of 50 μl of the isolated compound at different concentrations (0.49-250 μg/ml in respective solvents) and 150 μl standardized ABTS

solution. The decrease in absorbance was measured at 734 nm after 15 min of incubation. Data for each assay was recorded in triplicate. Ascorbic acid (NICE Chemicals Pvt. Ltd., Cochin, India) was used as positive control. The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula:

$$\text{Percentage of scavenging} = [(A_C - A_S)/A_C] \times 100$$

Where A_C is absorption of control, A_S is absorption of tested extract solution. The IC_{50} value was calculated graphically based on capacity of compound concentration to scavenge 50% of free radicals.

RESULT AND DISCUSSION

Synthesis and characterization of Schiff's bases derived from 2-amino benzothiazole, 2-amino-6-chloro benzothiazole and 2-amino-6-bromo benzothiazole with o-Vanillin were done in the present work. The synthesized compounds were tested for anti-oxidant activities by DPPH and ABTS assay. All the compounds showed good activity both in DPPH Scavenging radical assay and ABTS assay, where as other compounds showed comparatively less activity. So, their IC_{50} values were calculated graphically based on capacity of compound concentration to scavenge 50 % of free radicals as shown in figure 1. Here VBBT compound shows very good activities and the IC_{50} value 248.82 and 54.4 $\mu\text{g/mL}$ by DPPH and VBTS assay respectively. The synthesized compounds have hydroxyl and methoxy groups, which show very good radical scavenging activities.

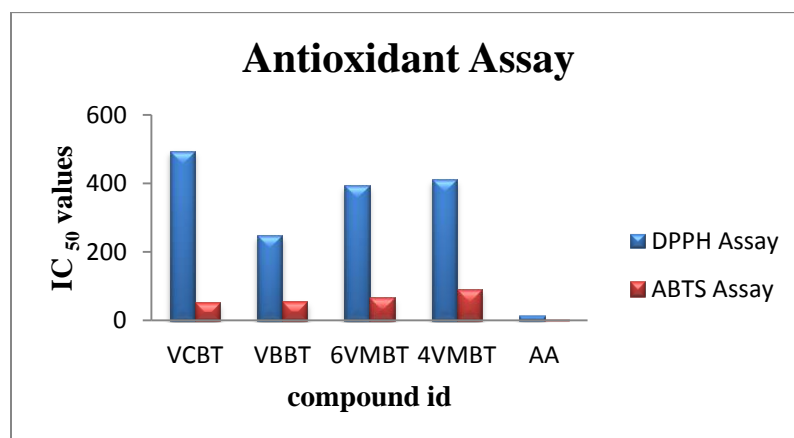


Figure 1: Anti-oxidant activity of the synthesized compounds

Some of the benzothiazole derivatives exhibited excellent anti-oxidant property as reported by Bhat *et al* [17], they synthesized benzothiazole guanidiny derivatives, in which compound containing hydroxyl and methoxy substituted compound shows the IC_{50} values at 27.50 $\mu\text{g/mL}$ concentration. Our results can be correlated with the benzothiazole azo ester derivatives as described by Bhat and Belagali 2014 [18], in which compound shows the activity at higher concentration.

CONCLUSION

Here we have synthesized the benzothiazole Schiff's bases in efficient way and characterized through IR, Mass and ^1H NMR spectroscopy. All the three compound tested for anti-oxidant activities both in DPPH and ABTS assay. Since the synthesized compounds has hydroxyl and methoxy group. So it shows very good radical scavenging activities.

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