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Spectrophotometric study of the interaction between a novel benzothiazolethioglycoside as antimicrobial agent with bovine serum albumin

Ayman B Farag, Adel M Michael*

Pharmaceutical Chemistry department, Faculty of Pharmacy, Ahram Canadian University

Abstract This study is concerned with the interaction of a newly synthesized antimicrobial agent (BTT) with bovine serum albumin (BSA). BTT is a novel benzothiazolethioglycosides that was prepared via reaction of the benzothiazole and 2-thioxoimidazolidin-4-one thiolate salts with tri- acetylated cyclic xylopyranosyl bromide. It was tested for antimicrobial activity against different bacteria and fungi and it exhibited interesting high antibacterial activities against Gram +ve bacteria while it showed no antibacterial activities against Gram -ve bacteria nor antifungal activity.

Spectrophotometry was the tool to elucidate the reaction product at pH 4 using Britton-Robinson buffer where the method was linear over the range 10-40 µg/mL. The develop method was validated using ICH guidelines and it was proved to be accurate and precise. BTT and BSA complex provides a model for revealing drug-protein interactions that helps the study of drug metabolism and transportation and can be a useful director for further drug design.

Keywords BTT; bovine serum albumin and spectrophotometry

1. Introduction

Bovine serum albumin (BSA) is one of the most significant bioactive molecules that can be used for the study of drug-protein interaction [1]. Albumin acts as the transporter and disposition agent of many drugs, has been frequently used as a model protein for investigating protein folding and ligand binding mechanism [2-3].

BTT is a new antibacterial that was prepared by reaction of the benzothiazole and 2-thioxoimidazolidin-4-one thiolate salts in the presence of tri- acetylated cyclic xylopyranosyl bromide [4]. Structure elucidation was done to confirm the prepared compound structure. It was proved to have antibacterial activity against Gram +ve bacteria. The aim of the present investigation was to study the affinity of benzothiazolethioglycosides derivative for BSA using UV–visible spectroscopy to understand the carrier role of serum albumin for such compound in the blood. This complex formation between biomacromolecules, especially between plasma proteins and drugs, represents a useful guideline for drug therapy and modeling [5-8]. The proposed method was simple, accurate and precise. The work does not require any sophisticated apparatus or computer programs.

2. Experimental:

2.1. Chemicals and reagents

- BSA (lyophilized powder): Sigma-Aldrich, Germany. It was prepared as 1 mg/mL solution in distilled water.

- Phenyl isothiocyanate: Sigma-Aldrich, Germany.
- Methanol and Ethanol: Sigma-Aldrich, Germany.
- Acetic acid, phosphoric acid and boric acid: El-Nasr company for chemicals, Egypt.
- Potassium Hydroxide: Sigma-Aldrich, Germany.
- Britton – Robinson (BR) buffer: prepared by mixing the acid mixture containing phosphoric acid (0.04 M), acetic acid (0.04 M) and boric acid (0.04 M). Buffer solutions of different pH values were adjusted by the necessary amount of 0.2 M NaOH [9].

2.2. Instruments and software

- All melting points were uncorrected on a Gallenkamp melting point apparatus. The IR spectra were recorded (KBr disk) on a Perkin Elmer 1650 FT-IR instrument. The NMR spectra were recorded on a Varian 500 MHz spectrometer in (CD3)2SO using $Si(CH_3)_4$ as an internal standard in Dusseldorf University, Germany. Elemental analyses were obtained from the Microanalytical Data Center at Cairo University, Egypt. Progress of the reactions was monitored by TLC using aluminum sheets coated with silica gel F254 (Merck). Viewing under a short-wavelength UV lamp effected detection. All evaporations were carried out under reduced pressure at 40 °C.
- Spectrophotometric measurements were carried out using a double - beam UV/Visible spectrophotometer model J-760 (Jasco, Japan) connected to ACER compatible computer, using 1.00 cm quartz cells. Scans were carried out in the range from 200-450 nm at room temperature.

2.3. Solutions

- Standard stock solution of BTT: was prepared by dissolving 25 mg of BTT in 100 mL methanol (250 µg/mL).
- Standard working solution of BTT: was prepared by diluting 40 mL of the stock solution with methanol to 100-mL mark in a volumetric flask (100 µg/mL).

2.4. Procedures

2.4.1. Chemistry

Compounds **3** and **5** were prepared following reported procedures [4]. The general procedure is described as follow:

A mixture of **3** (0.01 mol) and phenyl isothiocyanate (0.01 mol) was stirred for 60 minutes in ethanol (25 ml) containing potassium hydroxide (0.01 mol) gave the in situ liberated intermediate potassium benzothiazole-2-thiolates salt **4**, which was treated with tri- acetylated cyclic xylopyranosyl bromide bromides (0.01mol) in 10 ml acetone and stirred at room temperature for 6 hour. The solution was evaporated and the formed residue was washed with distilled water to remove the formed potassium bromide salt. The resulting product was recrystallized from ethanol.

(*E*)-2-(benzo[d]thiazol-2-yl)-3-(2′,3′,4′-tri-O-acetyl-β-D-xylopyranosylthio)-3-(phenylamino) acrylonitrile 6.

Yellow solid, yield 74%, mp 193˚C (EtOH), yield 75%, IR (KBr) 3591 (NH), 2197 (CN), 1725 (CO); ^{1}H NMR (DMSO-d^6) δ 1.93-1.96 (3s, 9H, 3COCH3), 3.35 (q, 1H, H-5′); 3.63-3.72 (q, 1H, H-5′′); 3.98-4.03 (m, 1H, H-4′); 4.84-4.90 (t, 1H, H-2′); 5.19-5.24 (t, 1H, H-3′); 5.27-5.30 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1′)7.24- 8.37 (m, 9H, aromatic protons); ^{13}C NMR (DMSO-d6) δ 20.69-20.95 (3 $COCH_3$); 61.81 (C-5′); 67.84 (C-4′), 68.50 (C-2′), 73.14 (C-3`), 75.08 (C-1`), 124.51- 148.25 (C-aromatic); 169.55-170.36 (3 C=O), Anal.Calcd For $C_{27}H_{25}N_3O_7S_2$ (567.11); (Found: C, 57.20; H, 4.48; N, 7.33; O, 19.78; S, 11.25, calculated C, 57.13; H, 4.44; N, 7.40; O, 19.73; S, 11.30%).

2.4.2. Antimicrobial Activity

The newly synthesized compound was screened for their antimicrobial activity *in vitro* against Escherichia coli NRRL B-210 (Gram -ve bacteria), Bacillus subtilis NRRL B-543 (Gram +ve bacteria), Staphylococcus aureus NRRL B-313 using nutrient agar medium. The antifungal activity of the compound was tested against Candida albicans NRRL Y-477 using Sabouraud dextrose agar medium using agar diffusion method [12]. The area of zone of inhibition was measured using Ciprofloxacin (50µg/mL) as standard antibiotic and Ketaconazole (50µg/mL) was used as a reference antifungal. The

tested compound was dissolved in dimethyl sulfoxide (DMSO) to give a solution of (50µg/mL). The inhibition zone was measured in millimeters at the end of incubation period of 24 hours at 37°C.

2.4.3. Study of the reaction conditions

2.4.3.1. The optimum pH

Different pH values (3.3- 7) were investigated using Britton – Robinson buffer to obtain a complex of maximum stability.

2.4.3.2. The optimum buffer volume

Different buffer volumes (0.5-2 mL) were tried to obtain a complex of maximum stability.

2.4.3.3. The optimum volume of BSA

Different BSA solution volumes (0.3-1 mL) were tried to obtain a complex of maximum stability.

2.4.4. Construction of calibration graph

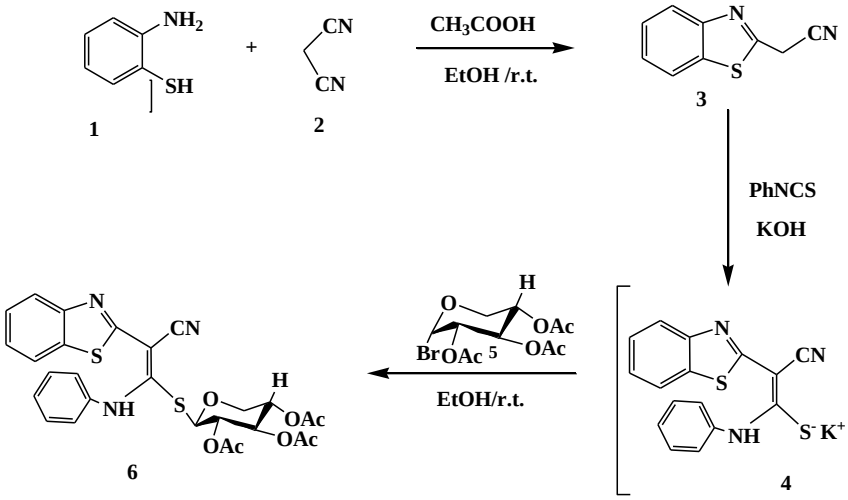
Different aliquots of BTT standard working solution (1-4 mL) were taken into 10-mL volumetric flasks. To each flask, 0.5 mL BSA solution and 1 ml BR buffer pH 4 were added. The volume was completed to the mark with distilled water. The absorbance was measured at 376 nm against blank. The calibration curve was constructed by plotting the absorbance of the formed complex versus the corresponding concentration and the regression equation was computed.

2.4.5. Method validation

Accuracy and precision were investigated using different concentrations of the complex. Intra- and inter-day precision should conform to the regulatory ICH guidelines [10].

3. Results and Discussion

BTT is a novel benzothiazolethioglycoside that was synthesized via one-pot reaction of the benzothiazole and 2-thioxoimidazolidin-4-one thiolate salts with tri- acetylated cyclic xylopyranosyl bromide. **Scheme 1** summarizes the steps followed in the preparation. Benzothiazole-2-acetonitrile **3**, chosen as the starting substance, was obtained from the reaction of 2-amino thiophenol**1**, acetic acid and malononitrile**2** in ethanol. Treatment of the benzothiazole**3** with phenylisothiocyanate in alcohol and in the presence of potassium hydroxide at room temperature for 1 hour, gave the in situ liberated intermediate potassium benzothiazole-2-thiolates salt **4,** which upon treatment with tri- acetylated cyclic xylopyranosyl bromide **5** at room temperature afforded the requisite S-glucoside **6**, in high yield [4, 11].



Scheme 1

The structure of BTT was elucidated with correct elemental analyses, NMR and IR spectra as shown in figures 1-3. The ^{1}H NMR spectrum data showed the anomeric proton as a doublet at δ 5.27-5.30 ppm with high coupling constant of $J_{1',2'}$ = 8.7 Hz, corresponding to a trans diaxial orientation of H-1′and H-

2′ protons indicated the presence of only β configuration of S-glycosidic bond. The other six glucose protons resonated at δ 3.35-5.30 ppm region, and the three acetoxy groups appeared as four singlets at δ 1.93-1.96 ppm. In addition, the aromatic protons of the aglycone part resonated at δ 7.24-8.37 ppm.

The antibacterial activity of BTT exhibited interesting high action against Gram +ve bacteria while it showed no antibacterial activities against Gram -ve bacteria nor antifungal activity which is showed in table 1.

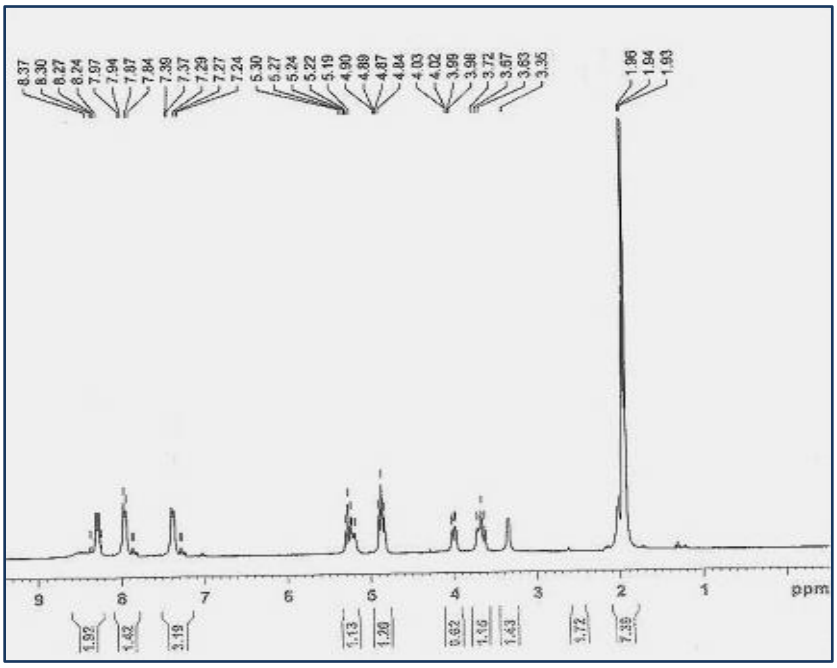


Figure 1:[1]H NMR spectrum of BTT

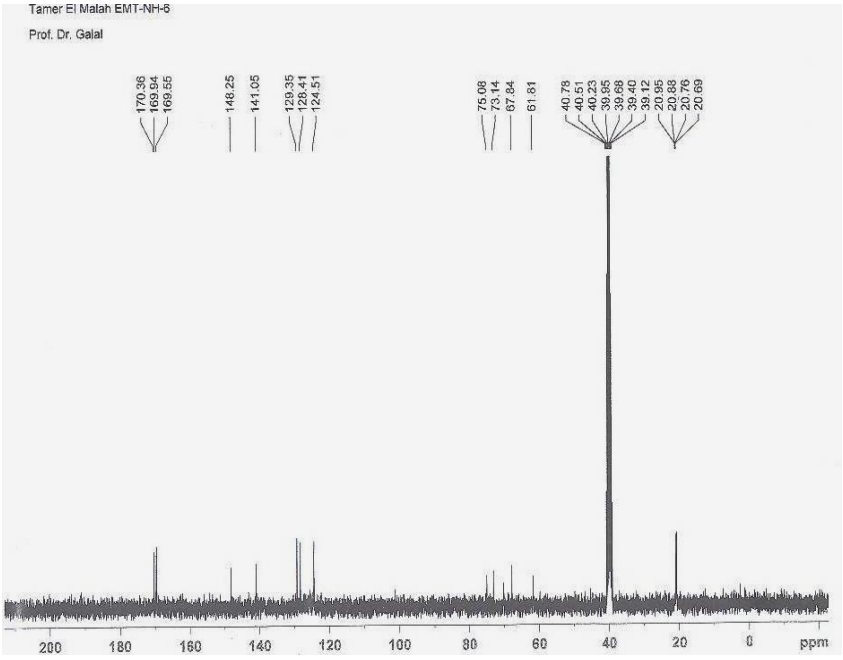


Figure 2: C-13 NMR spectrum of BTT

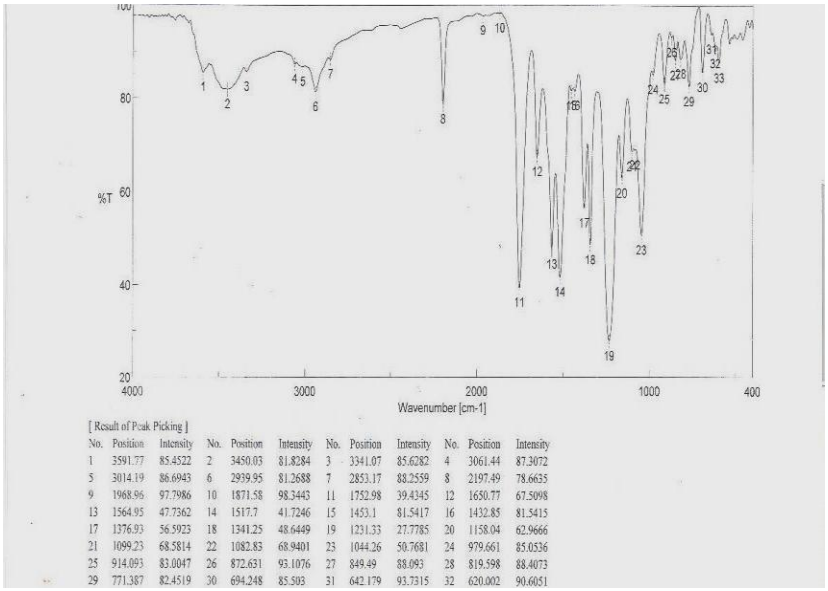


Figure 3: IR spectrum of BTT

Table 1: Inhibition zone in mm as a criterion of antibacterial and antifungal activities

BTT	**Inhibition zone in mm ***			
	Gram positive bacteria		**Gram negative bacteria**	**Fungi**
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Candida albicans***
	17.5	22	-ve	-ve
Reference Drugs:				
Ciprofloxacin	23	23	25	-ve
Ketaconazole	-ve	-ve	-ve	23

Highly active (inhibition zone > 20 mm), moderately active (inhibition zone 16-19.2 mm), slightly active (inhibition zone 11-15 mm), (-ve) no inhibition zone.

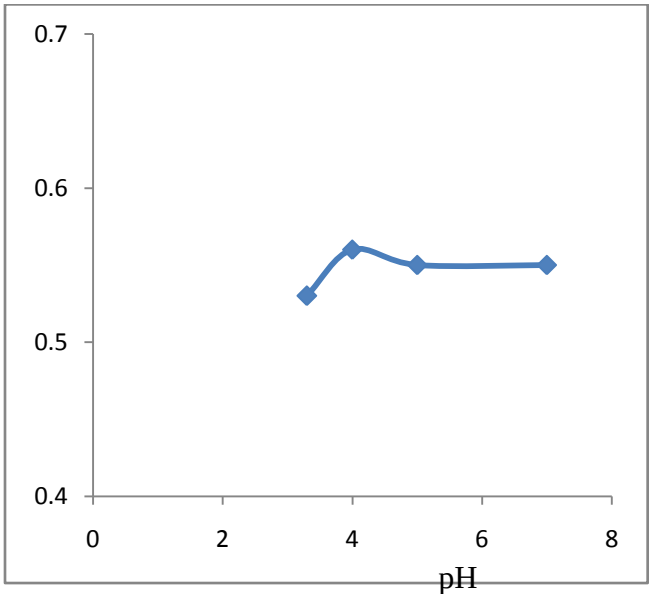


Figure 4: Effect of pH on the formed complex between BTT (20 µg/mL) and BSA using BR buffers

This work is concerned with the spectrophotometric study of the interaction between BTT and BSA. Detailed study

of the reaction conditions was carried out. Different pH values using BR buffer were tried ranging from 3.3 to 7 where the most suitable one was found to be pH 4 as shown in figure 4. The optimum buffer volume was proven to be 1 mL. Different volumes of BSA solution were tried and it was concluded that 0.5 mL gave the highest absorbance of the formed complex.

BSA reacts with BTT to form a product that can be measured spectrophotometricaly as shown in figure 5. Study of the albumin microspheres-drug interaction would be of great potential value for screening the pharmacokinetic and pharmacodynamic profiles [13-14].

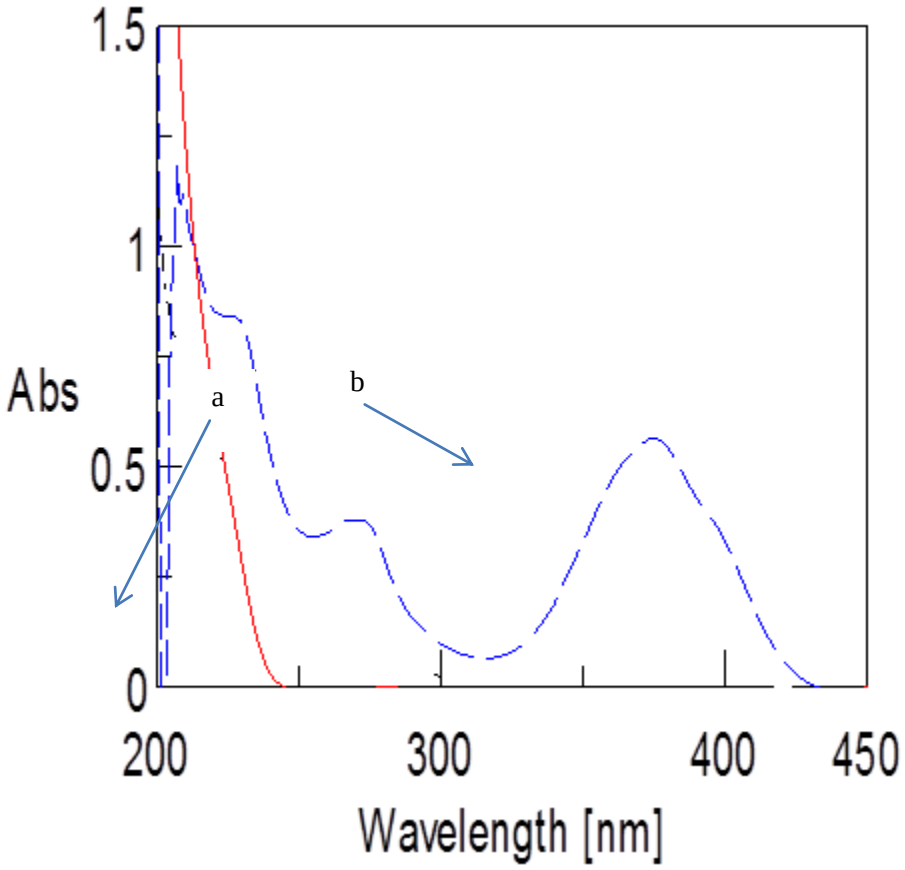


Figure 5: absorption spectra of: a- BSA solution (50 µg/mL), b- The reaction product of BTT solution (20 µg/mL) with BSA solution (50 µg/mL)

Table 2: Validation parameters of the proposed method

Parameter	Value
Linearity	
Regression equation:	
Slope	0.026
Intercept	0.006
Correlation coefficient (r)	0.9996
Range	10- 40 µg/mL
Accuracy	
Mean ± RSD	102.26 ± 1.678
Precision	
Intraday (Mean ± RSD)	102.935 ± 1.332
Interday (Mean ± RSD)	98.80 ± 2.619

4. Conclusion

In this work, novel and simple spectrophotometric method was applied for the study of interaction of the newly synthesized antimicrobial agent (BTT) with bovine serum albumin. The proposed method is time saving and environmentally-friendly since no harmful organic solvents were used. The developed method does not require sophisticated experimental setup. The method was shown to be accurate, simple, rapid and repeatable. The interaction can be used to enhance the efficacy of therapeutic agents and can be a useful director for drug modeling and design.

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