

Synthesis And Antibacterial Activities Of Benzothiazole Derivatives Of Sulphonamides

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Abstract:

This study aims to synthesize hybrid compounds "via" the coupling of sulphonamide and benzothiazole into one structure that may have improved antibacterial property. The N-(biphenyl-4-yl) thiourea (1) used for the synthesis of the targeted sulphonamides was obtained by reacting diphenylamine and ammonium thiocyanate at room temperature. Cyclization of N-(biphenyl-4-yl)thiourea gave 2-amino-6-phenylbenzothiazole (2) which reacted with benzenesulphonyl chloride and para-toulene sulphonyl chloride to give the targeted sulphonamides (3a & 3b). The synthesized compounds were characterised using melting point, infra-red spectroscopy, nuclear magnetic resonance and elemental analysis. Anti-bacterial screening of the synthesised compounds indicated that all the compounds showed anti-bacterial properties, except 2-amino-6-phenylbenzothiazole that did not show any activity on *Escherichia coli*.

Keywords: Antibacterial, Benzothiazole Derivatives, Sulphonamide, Synthesis, Thiourea

1. Introduction:

Antibacterial resistance is a global public health problem that has hampered the effective prevention and treatment of wide range of bacterial diseases [1,2]. Antibacterial resistance is aggravated by the misuse or overuse of antibacterial agents in people and animal [3]. Some bacteria are multidrug resistant and the major examples are *Staphylococcus aureus* and *Escherichia coli* [3]. These multidrug resistant organisms have increased the cost of healthcare "via" prolonged stay in hospital and more intensive care [2]. World Health Organisation (WHO) reported that the death caused by methicillin resistant *S. aureus* infection is higher by 64% as against the non-methicillin resistant *S. aureus* infection [2].

One of the WHO strategies for the control of antimicrobial resistance is to encourage investment in new medicine research and development [4]. In accordance with this recommendation many research team are engrossed in the synthesis of new antimicrobial drug with better efficacy and lower toxicity [2,5]. The most exploited derivatives are sulphonamides, thiourea and benzothiazole [6]. The antimicrobial, antioxidant, antiviral and antitumor properties of thiourea derivatives have been reported [3,6]. Sulphonamide derivatives with antimicrobial, analgesic, anti-inflammatory, antitumor and antimalarial activities have been documented [6-9]. Benzothiazole derivatives are heterocyclic compounds with diverse biological activities such as antimicrobial, anticancer, antidiabetic, anti-inflammatory and analgesic activities [10]. Despite the vast number of antimicrobial agent available for the treatment of microbial infection, the problems of antimicrobial resistance is yet to be resolved. This study aims to synthesize hybrid compounds via the coupling of sulphonamide and benzothiazole into one structure that may have improved antibacterial property.

2. Materials and Methods

The chemicals used were of analytical grade and used without further purification. Melting points were determined with an open capillary melting point apparatus and are uncorrected. Reaction completion was monitored with thin layer chromatography (TLC) using silica gel-G (Marck 60). All the compounds were purified by column chromatography using silica gel mesh 120-160 (Marck). Infrared spectrophotometer was recorded on FT-IR (KBr) cm^{-1} at National Research Institute for Chemical Technology Zaria (NARICT) with Shimadzu FT-IR 8400s as described by Chinaka and Coworkers [11]. ^1H and ^{13}C of compounds 1 and 2 were recorded in CDCl_3 on Agilent f1 spectrometer at Central Laboratory of Kwame Nkrumah University of Science and Technology Ghana while ^1H and ^{13}C of compounds 3a & 3b were recorded in CDCl_3 on Bruker WM 300 f1 and 300 f2 spectrometer at Central Drug Research Institute Lucknow India as described by Chinaka and Coworkers [11].

2.1. Synthesis of N-(biphenyl-4-yl)thiourea (1)

A solution of 2 g of diphenylamine and 2 g of ammonium thiocyanate in 15 mL of methanol was stirred at room temperature until the salt dissolved and became light brown in colour. On completion of the reaction which was monitored with TLC, 2 M H_2SO_4 was added until the pH of the solution was 2. The orange colour precipitate obtained was filtered with suction pressure and crystallized with water. Yield of 56% (1.12 g); mp of 56-58°C; FT-IR (KBr) cm^{-1} gave absorption bands at 1527.67m ($-\text{NH}_2$), 2576.98w ($-\text{SH}$), 1719.60 (aryl) and 2265.47 (N = C-S). Proton spectrum (δ ^1H): 7.49 (CDCl_3), 7.99 (Ar-H, J = 0.96), 3.55 (SH) and 2.46 (NH_2). δ ^{13}C : 126.73, 128.36, 129.28 (Ph-C), 129.71 (C-NH) and 143.00 (C-NH). Analytical Calculated: for $\text{C}_{13}\text{H}_{12}\text{N}_2\text{S}$: C = 68.39; H = 5.31; N = 12.27; Found: C = 68.32; H = 5.19; N = 12.15.

2.2. Synthesis Of 2-Amino-6-Phenylbenzothiazole (2)

A freshly prepared solution of bromine gas in 20 mL chloroform at the temperature of 0°C to -5°C was added to a well cooled solution of diphenylthiourea (2 g) in 20 mL chloroform. The solid obtained after evaporation of the solvent was washed with water and recrystallized to obtain cream colour crystal with mp 158 °C and yield of 64% (1.28 g). FT-IR (KBr) cm^{-1} gave absorption bands at 1489.10 (NH_2), 1614.47 (Aryl), 2067.67 (arylthiocyanate) and 2268.6 (N = C-S). Proton spectrum (δ ^1H): 7.57 (CDCl_3), δ 2.59 (NH_2), δ 3.73 (CHX3 of benzothiazole H1) and δ 8.52 (Ph-H). δ ^{13}C : 39.77 (CDCl_3), 40.27-39.27 (Benzothiazole-C), 80.12 (Ph-C). Analytical Calculated: for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{S}$: C = 68.99; H=4.46; N=12.38; Found: C = 68.43; H = 4.41; N = 12.57.

2.3. General Synthesis Of Benzothiazole Derivatives Of Sulphonamide

A solution of 2-amino-6-phenylbenzothiazole (0.01 mol) in 25 mL of chloroform was added to a well stirred of sulphonyl chloride (0.01 mol) in 40 mL chloroform. The reaction mixture was refluxed for 30 min. The chloroform was removed by evaporation and the residue treated with 30% dilute HCl. The reaction mixture was boiled to 45 °C, decolourised with activated charcoal and filtered while hot. 0.2 M NaHCO_3 was added to the filtrate while stirring and the completion of the reaction was ascertained by litmus paper showing neutral. The crude white product was filtered upon cooling, washed with methanol and recrystallized with 1:1 chloroform/methanol mixture.

2.4. Synthesis Of N-(6-Biphenyl-1,3-Benzo[D]Thiazole-2-Yl)Benzenesulphonamide (3a)

Molecular formula $\text{C}_{19}\text{H}_{14}\text{N}_2\text{O}_2\text{S}_2$, weight 366.46(cal.), mp 234 °C and yield 68% FT-IR (KBr) cm^{-1} : 1410.01 ($-\text{SO}_2$), 1591.33 ($-\text{NH}$), 1641.48 (Aryl) and 2023.40 (N = C-S). δ ^1H : 2.51 (CDCl_3), 12.63 ($-\text{NH}-$), 3.60 (2 CHd J = 2.02), 3.62 (1 CHs J=2.02), 7.63, 7.60, 7.59, 7.57, 7.55 (5 CH. J = 3.01), 7.83, 7.81 (2 CH. J = 2.01), 8.06, 8.04, 8.02 (3CH J=1.00). δ ^{13}C : 44.27 (phenothiazole carbons) 132.85-126.91 (Ph-C), 141.16 (C-N), and 170.70 (C- SO_2). Analytical Calculated: for $\text{C}_{19}\text{H}_{14}\text{N}_2\text{O}_2\text{S}_2$: C = 62.69; H = 3.86; N = 7.47; Found: C = 62.71; H = 4.01; N=7.46.

2.5. Synthesis Of N-(6-Biphenyl-1,3-Benzo[D]Thiazole-2-Yl)-4-Methylbenzenesulphonamide (3b)

Molecular formula $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_2\text{S}_2$, weight 380.48, mp 210-212°C FT-IR (KBr) cm^{-1} : 1440.87 ($-\text{SO}_2$), 1487.17 ($-\text{NH}-$), 1647.26 (Ar) and 2108.27 (N = C-S). δ ^1H : 2.51 (CDCl_3), 2.37-2.29 (J = 6.75, -CH3), 3.54- 3.56 (J = 10.34, of 3X CH), 4.31 (J = 0.81 CHX2), 7.36, 7.38 (J = 0.11 CHX2), 7.96, 7.94, 7.92 (J = 2.28 CHX3), 7.70, 7.67 (J = 4.35 CHX2). δ ^{13}C : 21.41 (CH3-C), 44.23-72.62 (phenothiazole-C) 126.79-143.08 (Ph-C), 170.53 (C- SO_2) and 170.67 (C-N). Analytical Calculated: for $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_2\text{S}_2$: C=63.13; H=4.25; N = 7.36; Found: C = 63.02; H = 4.93; N=7.21.

2.6. Antibacterial Sensitivity Test Of Compounds

The antibacterial activity of the synthesised compounds was determined using *in vitro* agar well diffusion technique as described by Adeniyi and Ayepola [12]. 0.1 mL culture of each bacteria strain (equivalent to 108 CFU/mL) was used to inoculate the agar plates. The inoculated agar plates were appropriately labelled after drying and uniformed wells were bored in it using a plastic-cork borer of 6 mm diameter. 200 μ L of each test compound (20 mg/ml) was delivered into different wells with micropipette. Ampicillin was used as standard and was tested in the same manner. The plates were incubated at 37°C for 24 h and thereafter the diameters of zones of growth inhibition were measured with metre rule to the nearest millimetre.

Minimum inhibitory concentration (MICs) was determined by broth dilution technique as reported by Bonjar [13]. Different concentrations (12.5, 25, 50, 75, 100, 125, 150, 175, 200 μ g/mL) of the test solution were evaluated. The lowest concentration that arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC).

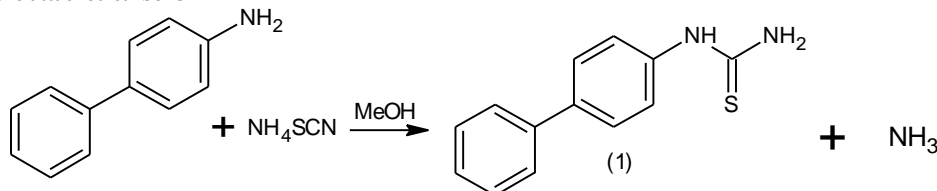
3. Results And Discussion

3.1. Chemistry

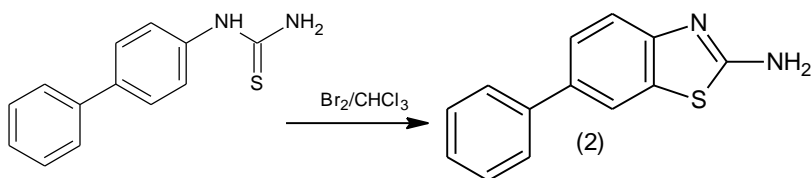
The synthesis of both N-(biphenyl-4-yl)thiourea (1) and 2-amino-6-phenylbenzothiazole (2) was successfully achieved by following the reaction protocol of Saeed and coworkers [14]. The reaction of biphenylamine with ammonium thiocyanate at room temperature gave N-(biphenyl-4-yl)thiourea (1) (Scheme 1). Cyclization of the synthesised thiourea using freshly prepared mixture of bromine gas in chloroform gave a cream colour crystalline solid 2-amino-6-phenylbenzothiazole (2) (Scheme 2).

Further reaction of the 2-amino-6-phenylbenzothiazole (2) with benzenesulphonyl chloride and p-toluenesulphonyl chloride was used to obtain N-(6-phenyl-1, 3-benzothiazole-2-yl) benzenesulphonamide (3a) and N-(6-phenyl-1, 3-benzothiazole-2-yl) -4-methylbenzene sulphonamide (3b), respectively (Scheme 3). The structures of the synthesised compounds were confirmed by spectroscopic data of IR, ^1H and ^{13}C NMR as well as elemental analytical data.

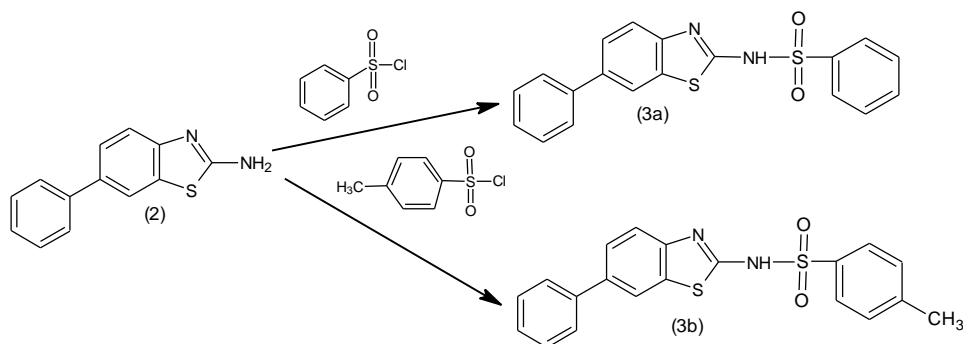
The infrared spectra of the compounds gave the absorption bands of -N-S-C- at 2023.40 – 2265.47 cm^{-1} , while the absorption bands of -SO₂- were observed at 1410.01-1440.01 cm^{-1} as expected. The vibration frequencies of 1614.47-1719.60 were attributed to the C=C aromatic absorption bands. In the ^1H NMR spectrum, all the three benzothiazole protons resonated at δ 3.54-3.73 while protons of the phenyl moiety attached to benzothiazole appeared at downfield region of δ 7.67-8.52 for compounds (3a) and (3b). The carbon NMR ^{13}C indicated the signals for phenothiazole at δ 39.27-40.27 for compound (2) while the introduction of benzenesulphonyl chloride and para-toluenesulphonyl chloride to obtain compounds (3a) and (3b) shifted the phenothiazole carbons to δ 44.23-72.62. The carbon attached to -SO₂- were observed at downfield region at δ 170.67-170.70 probably due to the high electronegative effect of -SO₂- on the attached carbons.



Scheme 1: Synthesis of N-biphenyl-4-yl thiourea (1)



Scheme 2: Synthesis of 2-Amino-6-phenylbenzothiazole (2)



Scheme 3: Synthesis of benzothiazole derivatives of sulphonamide (3a & 3b)

3.2. Antibacterial Activity

The antibacterial activities tested on *S. aureus* as Gram positive bacteria and *E. coli* as Gram negative bacteria are presented in Table 1 and Table 2. The screening of the synthesised compounds showed that some compounds inhibited the growth of both Gram negative and Gram positive bacteria organisms, only compound (2) showed inactive to the Gram negative organism *Escherichia coli*. Compound (3b) gave the best minimum inhibition concentration amongst the synthesised compounds, while the standard drug ampicillin showed better activities than the synthesised compounds. The possible mechanism of action of the synthesized compounds (3a and 3b) could be the competitive inhibition of the enzyme dihydropteroate synthase that catalyze the reaction of p-aminobenzoic acid with 7,8-dihydro-6-hydroxymethylpterin-pyrophosphate to form dihydropteroic acid which is one of the steps in the formation of dihydrofolic acid [15]. The mechanism of action could be as well via the inhibition of carbonic anhydrase activities. Sulphonamides inhibits carbonic anhydrase and dihydropteroate synthase activities [5]. The mechanism of action of the thiourea (1) and benzothiazole (2) derivative could be through the inhibition of protein tyrosine kinases [5]. The compounds had reduced activities against the Gram negative organism (*E. coli*) and this could be attributed to the presence of efflux pump that reduce intracellular concentration of drug in Gram negative organism [16,17].

Table 1: Zone Of Inhibition Of Synthesized Compounds

Organism	Zone of inhibition (mm)				
	Compd 1	Compd 2	Compd 3a	Compd 3b	Ampicillin
<i>Staphylococcus aureus</i>	16.00	17.00	28.00	18.00	32.00
<i>Escherichia coli</i>	8.00	-	17.00	20.00	24.00

Compd = Compound

Table 2: Minimum Inhibitory Concentration Of The Synthesized Compounds

Organisms	Minimum inhibition concentration ($\mu\text{g}/\text{mL}$)				
	Compd 1	Compd 2	Compd 3a	Compd 3b	Ampicillin
<i>Staphylococcus aureus</i>	>75.00	<75.00	50.00	<50.00	25.00
<i>Escherichia coli</i>	>100.00	-	>50.00	<50.00	12.50

Compd = Compound

4. Conclusion

Benzothiazole derivatives of sulphonamides with anti-bacteria properties were synthesised from thiourea with good yields. The synthesis of the targeted benzene sulphonamide and para-toulenesulphonamide were successfully achieved by cyclization of thiourea (1) to obtained benzothiazole (2) which reacted with benzenesulphonyl chloride and para-toulenesulphonyl chloride to give benzenesulphonamide (3a) and para-toulenesulphonamide (3b). The compounds showed good antibacterial properties on *Staphylococcus aureus* and *Escherichia coli* except 2-amino-6-phenylbenzothiazole that showed no activity against *Escherichia coli*. We recommend further studies to evaluate the toxicity and mechanism of action of the antibacterial activities of the compounds.

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