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Synthesis, characterization and antimicrobial activities of derivative of 2-mercaptobenzimidazole with 2-bromomethylmesitylene

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Abstract
The chemistry of heterocyclic compounds was used to prepare 2-mercaptobenzimidazole and its derivatives for obtaining novel biologically active ingredients such as anthelmintic, anti-HIV, antifungal, antibacterial, CNS depressant, anti-inflammatory, and analgesic activities. In this present study, a derivative of 2-mercaptobenzimidazole has been prepared and characterized. FTIR, ^{1}H NMR and ^{13}C NMR have strongly supported the formation 2-((2,4,6-trimethylbenzyl)thio)-1H-benzo[d]imidazole and the synthetic route has been validated by comparing the results with theoretical FTIR, ^{1}H NMR and ^{13}C NMR characterization. The synthesised compound have very potential antimicrobial activity against *Staphylococcus aureus* bacterium and *Aspergillus niger* fungi.

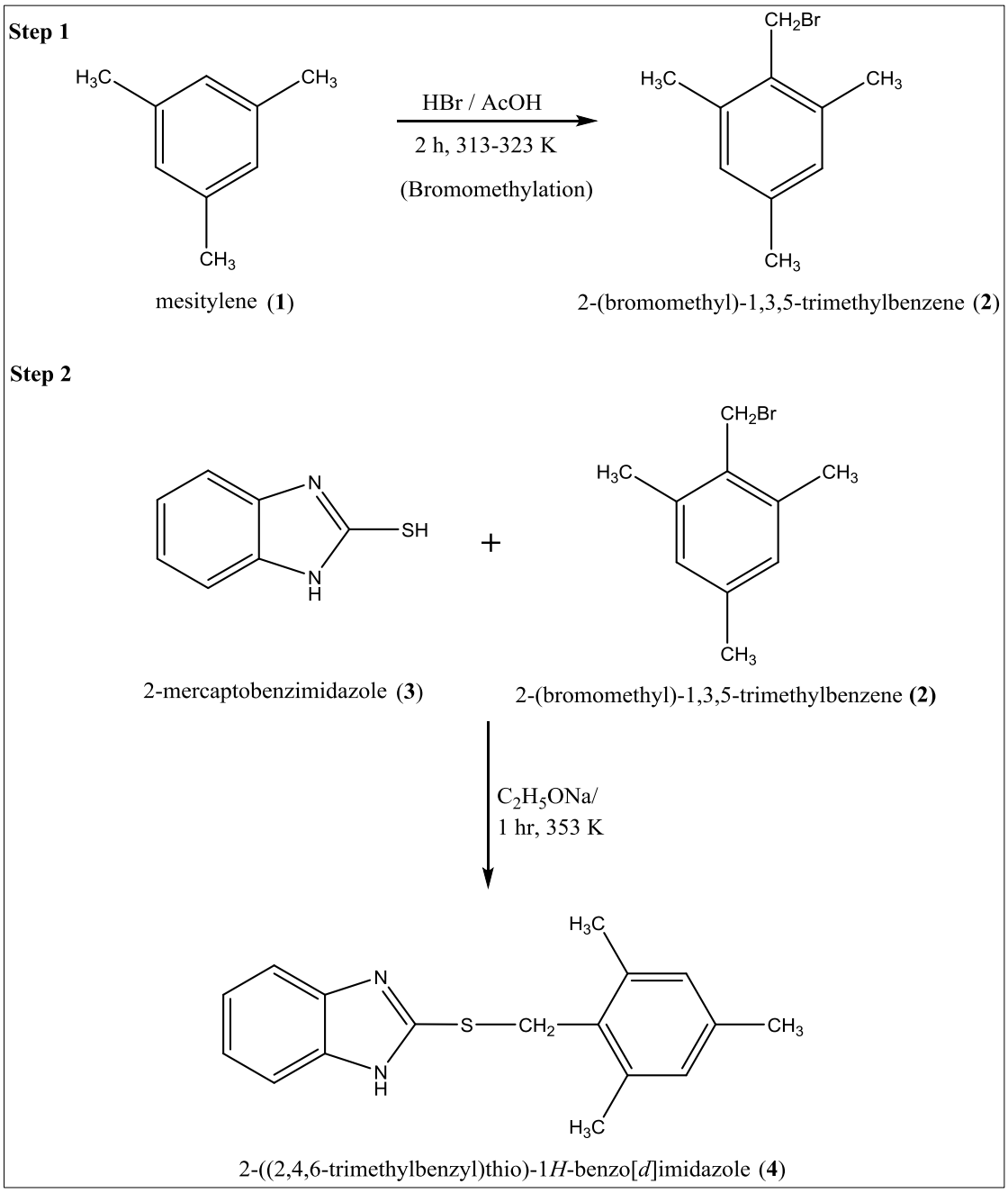
Keywords: Heterocyclic compounds, 2-mercaptobenzimidazole, mesitylene, antimicrobial studies.

1. Introduction
2-Mercaptobenzimidazole derived from benzimidazole with thiol group in the 2-position. It possesses other chemical names such as, o-phenylen thiourea, benzimidazol-2-thion with formula of $C_7H_6N_2S$ [1, 2]. O-phylenediamine and carbon disulphide reacts in presence of aqueous ethanolic KOH to form 2-mercaptobenzimidazole. Some characteristic of 2-mercaptobenzimid- azole are containing of thioamide group (-N-C=S), therefore it is considered one of thioamide compounds for its ability to react under special conditions to give derivatives having substituent at either nitrogen or sulfur atoms [3, 4], 2-mercaptobenzimidazole possess the form dimer, because it has (C=S) group, this preferable product is the dime r [5], it is known to exist in two tautomerism forms, the thiol and thione [6,7].
Various derivatives of 2-mercaptobenzimidazole have been synthesized by several investigators and have been reported to exhibit a wide range of biological activities such as antimicrobial [8], antihistamine [9], neutropic [10] and analgesic [11] activities. Although a great deal of the scientific literature concerning 2-mercaptobenzimidazole is in the area of medicinal chemistry, 2-mercaptobenzimidazole is also used in non-biological application, it serve as plant growth regulators [12] and used as corrosion inhibitor for mild steel in sulfuric acid solution [13], stainless steel in aqueous solutions of NaCl [14], mild steel and zinc in phosphoric acid [15, 16]. Also, it is widely used as an accelerator in rubber processing [17], and anti-oxidant for rubber and plastics [18]. Mercaptobenzimidazole and its derivatives display insecticidal properties [19], it is also a well-known analytical reagent for mercury, and have been used for the determination Fe (II), Cu (II), and Cd (II) metal ions in sewage water and industrial waste waters samples [20, 21].
Thus this present work concentrate on the preparation of mesitylene based substitution with 2-mercaptobenzimidazole and its applicability towards antimicrobial characters.

2. Experimental Methods
2.1 Chemicals
All the chemicals used in this work were of analytical/guaranteed reagent (AR/GR) grade and most of them are purchased from Merck, USA. All other chemicals were used as purchased without any further purification.

2.3 Synthesis of 2-((2, 4, 6-trimethylbenzyl) thio)-1H-benzo[d]imidazole (4)
A mixture of 2-mercaptobenzimidazole (3) (0.189 g, 1 mmol) and (2) (0.213 g, 1 mmol) in sodium ethoxide were heated with stirring at 353 K for 1 hour.

The formed compound was filtered and dried. The dried compound (4) was then dissolved in chloroform and allowed to undergo the process of slow evaporation. Fine white crystals of (4) were obtained and the same have undergone for characterization.



Scheme 1 Synthesis of 2-((2,4,6-trimethylbenzyl)thio)-1H-benzo[d]imidazole (4)

2.4 Spectral Characterizations

The powdered sample of (4) was characterized with FTIR spectrometer JASCO 4600 (Japan), with a scan range from 400 to 4000 cm^{-1} and the spectra was collected and processed using the JASCO-Spectra manager software. In addition to that the theoretical IR data for all product compounds were calculated using Gaussian 16 and viewed by Gauss View 6. Both experimental as well as theoretical data were compared, and discussed. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance III, 400 MHz, 9.4 Tesla super-conducting Magnet, NMR spectrometer at at School of Chemistry, Madurai Kamaraj University, Madurai, Tamilnadu, India, using $CDCl_3$ as solvent. Chemical shifts were reported as δ (ppm) with reference to the solvents (TMS as an internal standard).

2.5 Antibacterial and Antifungal Activities

Agar Well Difussion method has been adopted to study the antibacterial and antifungal activity of the synthesised compound. The sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

2.5.1 Agar-Well Diffusion Method

a. Nutrient Agar Medium

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100 mL of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121 °C for 15 minutes. The

autoclaved medium was mixed well and poured onto 100 mm petri plates (25-30 mL/plate) while still molten.

b. Nutrient broth

Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100 mL distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121 ºC) for 15 minutes. *Staphylococcus aureus* and *Bacillus subtilis* was inoculated in a nutrient broth and incubated for 24 hours in a bacteriological incubator.

c. Procedure

Petri plates containing 20 mL nutrient agar medium were seeded with 24 hour culture of bacterial strains *Staphylococcus aureus* and *Bacillus subtilis*; and fungal strains *Candida albicans* and *Aspergillus niger*. Wells were cut and different concentration of 500 µg/mL, 250 µg/mL, 100 µg/mL and 50 µg/mL were added. The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin (bacterial) and Amphotericin B (fungal) were used as a positive control. The zone of inhibition values was calculated using Graph Pad Prism 6.0 software (USA).

3. Results and Discussion

3.1 Physical Properties

Chemical Formula: $C_{17}H_{18}N_2S$; Appearance: White crystalline powder; M.P. 177 °C; IR (KBr) ν_{max}: 3011, 1613, 1580, 1424, 1393, 1352, 1269, 1224, 1195, 1010, 981, 849, 749, 693, 598, 539, 437 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 – 6.84 (m, 6H, Ar-H), 4.61 (s, 2H, CH_2), 2.37 – 2.18 (m, 9H, 2',4',6' CH_3); ^1H NMR Predicted (300 MHz, $CDCl_3$): δ 7.44 – 6.67 (m, 6H, Ar-H), 4.46 (s, 2H, CH_2), 2.29-2.27 (m, 9H, 2',4',6' CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 151, 137.88, 137.77, 129.37, 128.86, 122.60, 77.68, 77.26, 76.84, 32.38, 21.19, 19.79; ^{13}C NMR Predicted (300 MHz, $CDCl_3$) δ 149.7, 139.4, 138.9, 136.6, 135.3, 127.9, 123.0, 115.2, 33.1, 21.9, 19.0; Elemental Analysis (%) (calc.): C, 72.30; H, 6.42; N, 9.92; S, 11.35.

3.2 Spectral Characterizations

Derivative (4) of 2-mercaptobenzimidazole (3) with 2-bromomethylmesitylene (2) has been synthesised and characterized by Fourier Transform infrared spectroscopy. The corresponding products' FTIR spectrum is shown in Fig. 1. In the wide range of wavenumbers 4000 cm^{-1}-400 cm^{-1}, the spectrum provides seventeen distinct peaks which are caused by various vibrational modes.

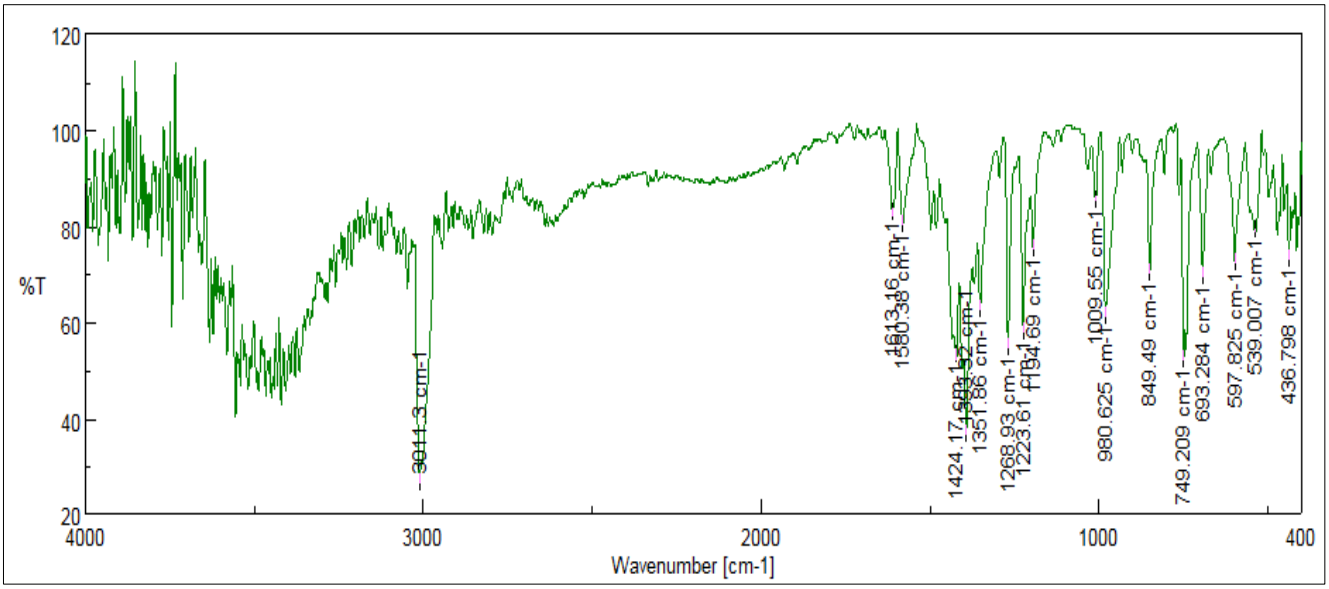


Fig 1: FTIR spectrum of 2-((2,4,6-trimethylbenzyl)thio)-1H-benzo[d]imidazole (4)

The general characteristic peaks such as for CH, CC, CN, CS, NH, CCH, HCH, etc., are found as per the standard values when compared with the theoretical data for the same product. The characteristic stretching peak for imide NH was found at 3011.3 cm^{-1}. The significant bond formation between the reactants are confirmed by the spectral peaks 849.5 cm^{-1}, 980.6 cm^{-1}, 1194.7 cm^{-1}, 1223.6 cm^{-1} and 1268.9 cm^{-1} which are the responsible peaks for HCS bending vibrations and HCSC torsional vibrations. Suppose the product hasn't formed, these both the vibrational modes would not be appeared. Therefore the formation of specified compound IMM1 (4) has been confirmed. The later studies of ^1H NMR and ^{13}C NMR are also give good instrumental support for this successful synthetic route.

The synthesised compound (4) has been characterized by the both ^1H NMR and ^{13}NMR and their spectral responses were depicted in Figs. 2 and 4 respectively. Both the figures were shown with theoretical calculations which were done by Gaussian 03 software. Along with this, Figs. 3 and 5 are presented to get an idea about the reactants if they were supposed to be existed as it is. The aromatic protons were noted within the range δ 7.523 – 6.835 ppm. The main significant singlet spectral peak at δ 4.608 reveals the formation of new bond with thiol sulphur of reactant (3). All other non-bonded aliphatic methyl groups on mesitylene compound shown their ^1H NMR multiplicities at the range δ 2.367 – 2.179. These experimental reports are in good agreement with theoretical predictions. Since the imide proton is a labile in nature, it does not identified through this ^1H NMR study. This is the reason that the theoretical study predicted its value as uncertainty and hence it is indicated as red text.

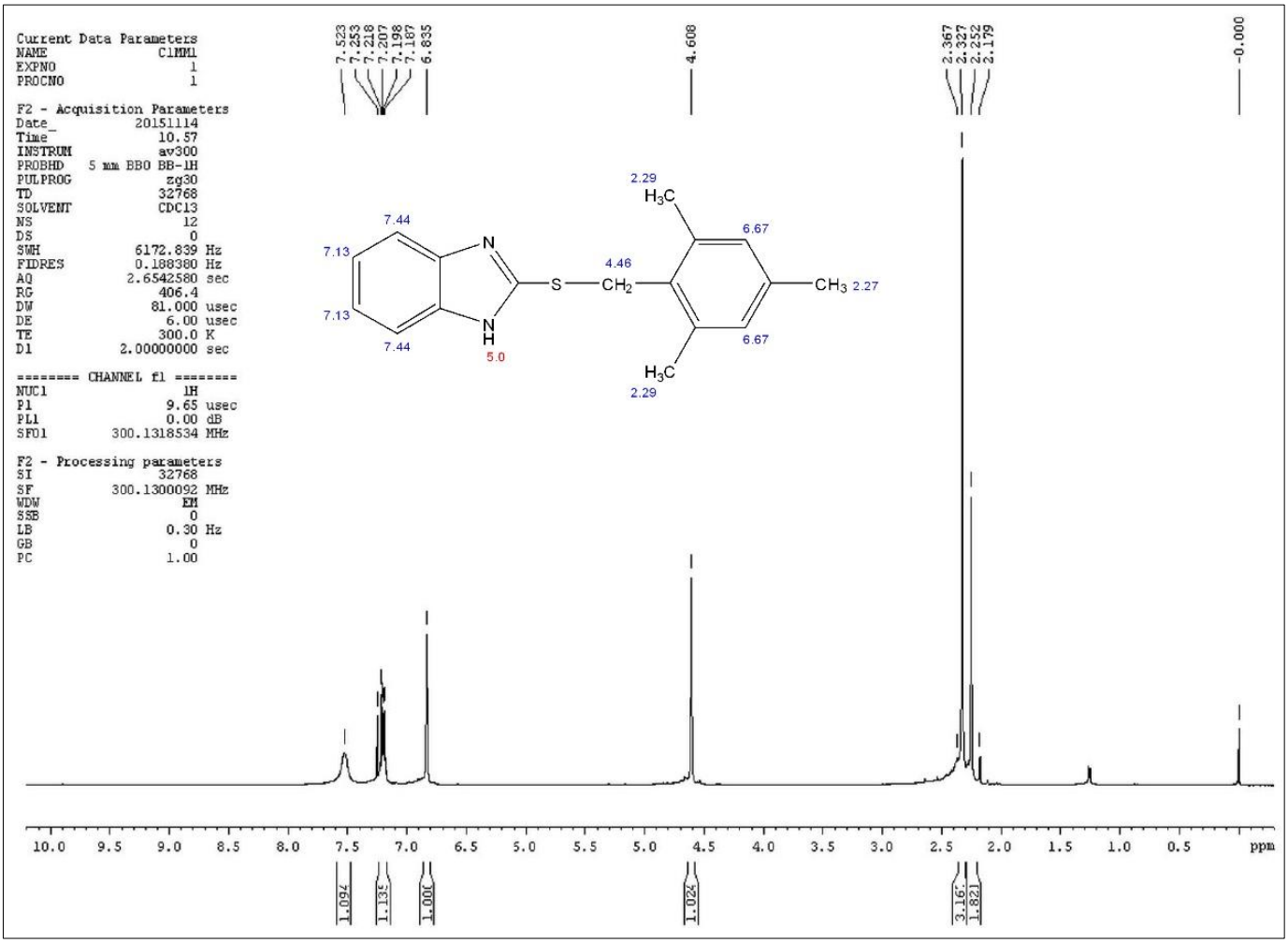


Fig 2: ^{1}H NMR spectrum of 2-((2,4,6-trimethylbenzyl)thio)-1H-benzo[d]imidazole (**4**) and inset image with theoretical δ values

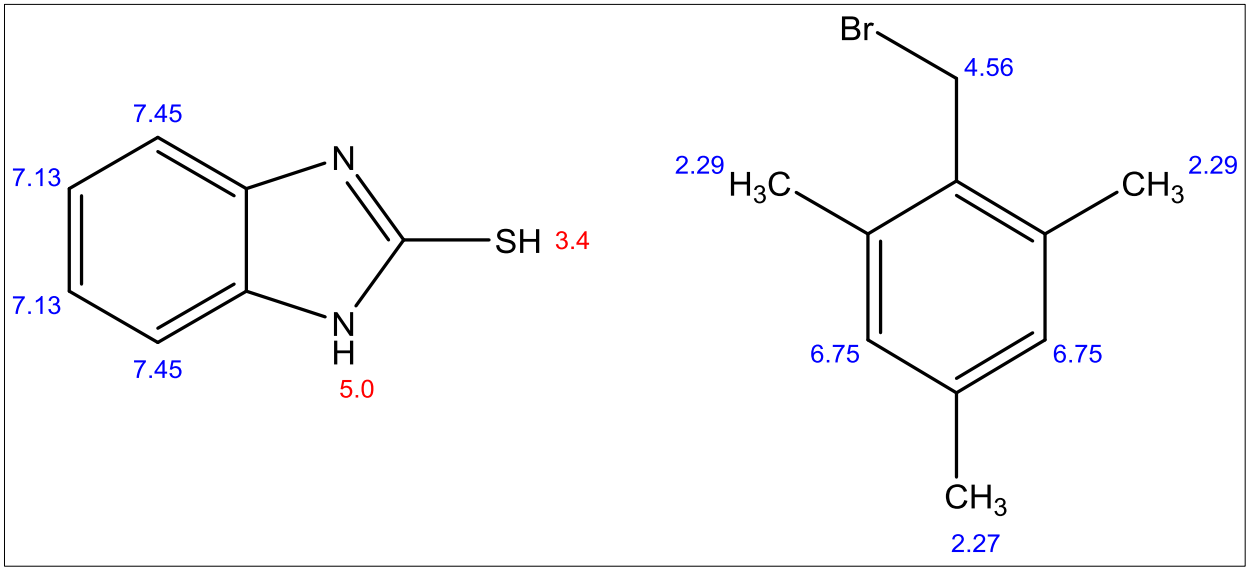


Fig 3: Theoretical ^{1}H NMR spectral response of (3) and (2) in the same environment

In ^{13}C NMR, aromatic carbons are obtained in the range δ 151.01–122.6 and alphatic carbons in the range δ 32.38–19.79. The solvent $CDCl_3$ carbon responded to the ^{13}C NMR along with the product and the values were found between δ 77.68-76.83 but not interfered with the products' ^{13}C NMR spectral peaks. After formation of compound (4), the imidazole ring carbon's NMR response changed drastically (exp.151.01 and calc. 149.7) when compare with its unreacted one (168.2). Similarly the bromomethyl carbon on reactant (3) gave its ^{13}C NMR response as increased after formed the product with (2). And also the bromomethyl attached ring carbon at (2) presented with much higher δ value (137.875) after the reaction from its initial value of δ 131 in the reactant (2). Thus the above spectral data reveals the formation of compound (4) and validating the Scheme 1 as better yielding route for the synthesis of compound (4).

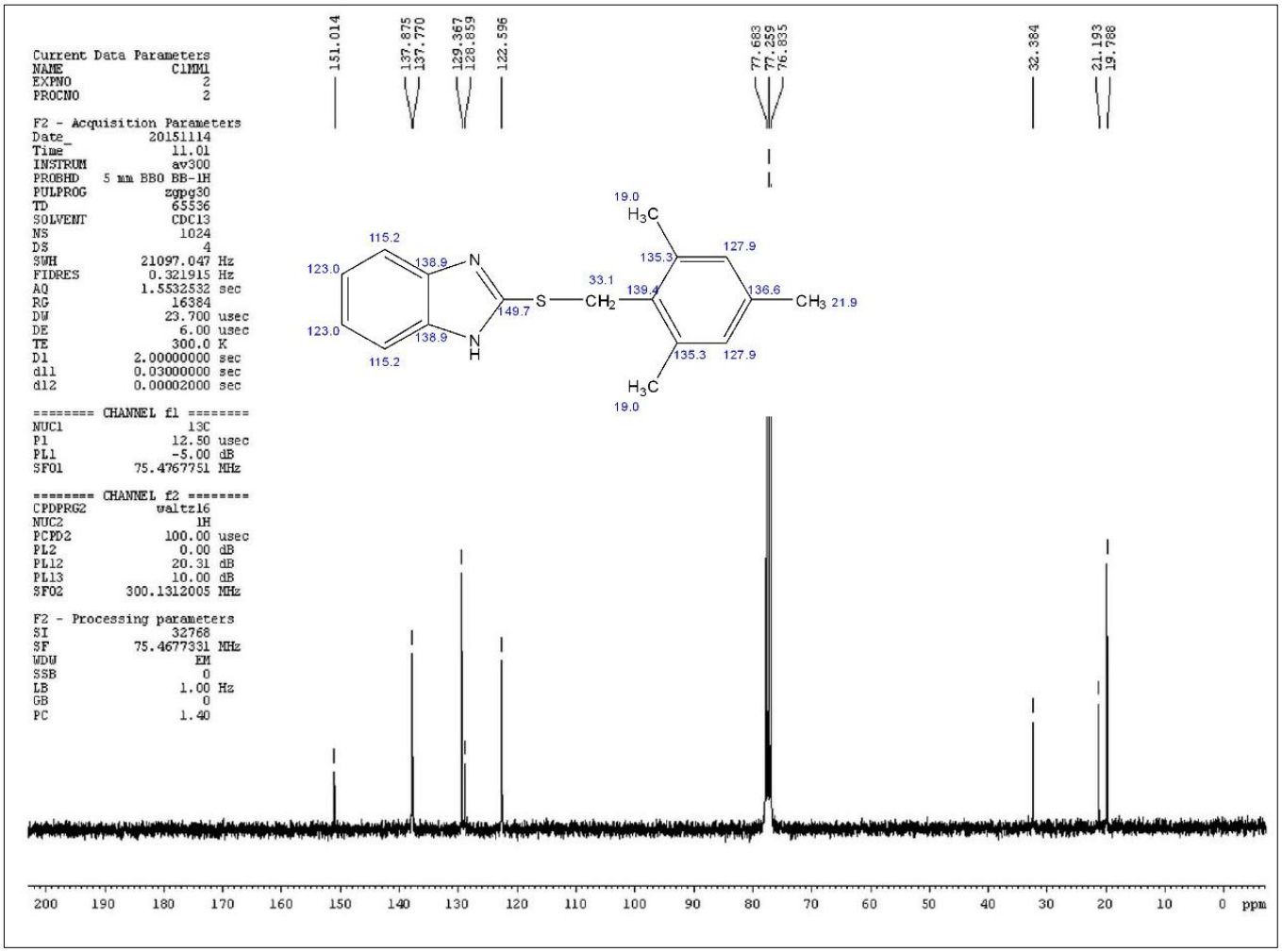


Fig 4: ^{13}C NMR spectrum of 2-((2,4,6-trimethylbenzyl)thio)-1H-benzo[d]imidazole (**4**) and inset image with theoretical δ values

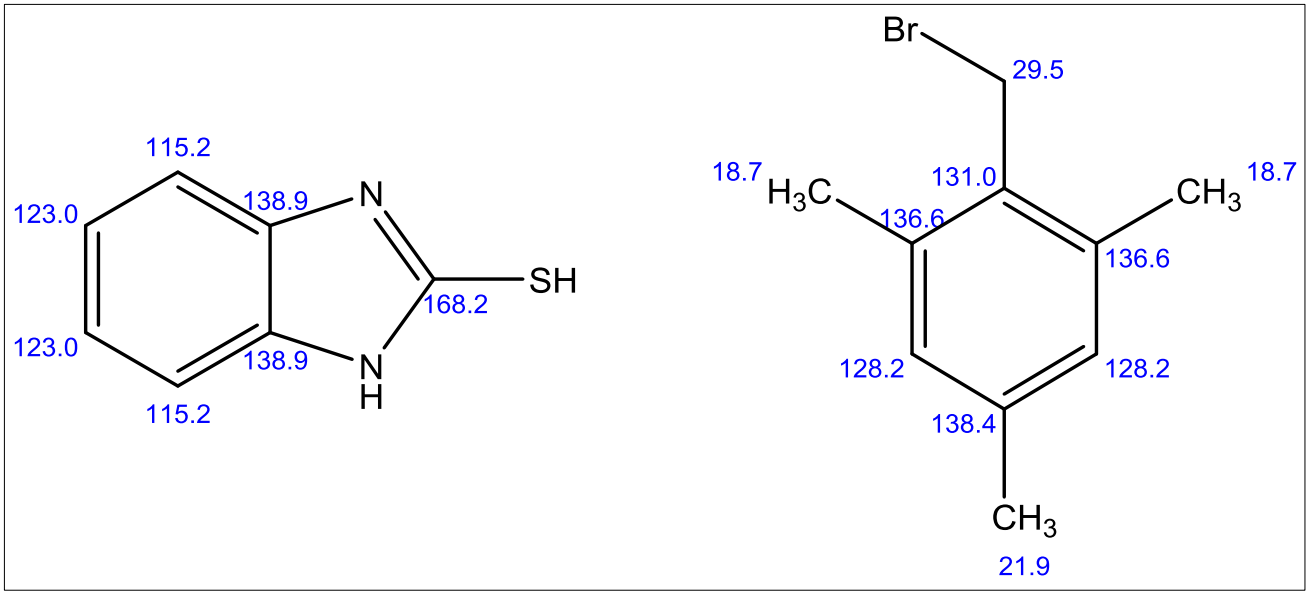


Fig 5: Theoretical ^{13}C NMR spectral response of (3) and (2) in the same environment

3.2 Antimicrobial Activities

In the antibacterial study, the bacterial strains *Bacillus subtilis* and *Staphylococcus aurous* and the antifungal strains *Candida albicans* and *Aspergillus niger* were used to test the antibacterial proficiency of synthesized compound and their data are exemplified in Table 1 and experimental petri-plates after the inhibitory studies are shown in Fig. 6. Out of four different concentrations of each compounds, 500 µg/mL concentration of the compound gives response towards all strains and with all remaining concentrations, it gives response towards *Staphylococcus aurous* and *Aspergillus niger*. In account with 500 µg/mL concentration, the compound could control the fungal strain *Aspergillus niger* than all others when compared with standard drugs.

Table 1: Antifungal responses of the synthesised compound (4)

S. No.	Microbe	Zone of inhibition(mm)				
		500 µg/mL	250 µg/mL	100 µg/mL	50 µg/mL	Std*
1.	*Bacillus subtilis*	3.5	0	0	0	12
2.	*Staphylococcus aureus*	16	9	5.5	0	13.5
3.	*Candida albicans*	2.5	0	0	0	8
4.	*Aspergillus niger*	11	5.5	2.5	1.5	17

** Gentamicin (bacterial); Amphotericin B (fungal)*

Bacillus subtilis ***Staphylococcus aureus*** ***Candida albicans*** ***Aspergillus niger***

Fig 6: Antimicrobial effects of synthesised compound (4)

4. Conclusion

2-((2,4,6-trimethylbenzyl)thio)-1H-benzo[d]imidazole has been synthesised as per the represented scheme. Further, the product was confirmed by the characterizations FTIR, ^{1}H NMR and ^{13}C NMR spectral studies. This 2-mercaptobenzimidazole derivative has good antibacterial and antifungal efficiency against the studied strains especially against *Staphylococcus aurous* and *Aspergillus niger*, the derivative (4) depict excellent antimicrobial activities. Therefore the synthesised compound could be useful in the field of needy environment.

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