

# Benzimidazolium Salts Bearing 2-methyl-1,4-benzodioxane Group: Synthesis, Characterization, Computational Studies, *In vitro* Antioxidant and Antimicrobial Activity

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**Abstract:** This study contains the synthesis of the 1-(2-methyl-1,4-benzodioxane)benzimidazole and 2-methyl-1,4-benzodioxane substituted benzimidazolium salts. The benzimidazolium salts were synthesized from the reaction of the 1-(2-methyl-1,4-benzodioxane)benzimidazole and various aryl chlorides. All compounds were characterized using <sup>1</sup>H NMR, <sup>13</sup>C NMR, FTIR spectroscopy, and elemental analysis techniques. The antioxidant properties of benzimidazolium salts were examined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and hydrogen peroxide scavenging ability assays. The compounds showed a moderate inhibitory effect on DPPH radical (The percent inhibition = 29.53-39.75). Also, the compounds exhibited significant H<sub>2</sub>O<sub>2</sub> radical scavenging activity. Antimicrobial activities of the compounds were examined against nine bacterial strains and *Candida albicans*. All compounds displayed marked antimicrobial activity against tested microorganisms, particularly against *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *C. albicans*. From the computational perspective, benzimidazolium salts were also optimized at B3LYP / DMol3// DFT level using the Discovery Studio 2020 program. HOMO–LUMO analysis and molecular electrostatic potential surface (MESP) were exerted to examine the effects of benzimidazolium salts' electronic and structural properties.

**Keywords:** antimicrobial activity; antioxidant; benzimidazolium salts; DPPH; hydrogen peroxide; computational study.

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## 1. Introduction

The *N*-heterocyclic carbene (NHC) precursors constitute an important class of ligands for organic and organometallic chemistry [1]. In particular, the unique electronic and structural properties of NHCs enhance their reputation [2, 3]. NHC ligands can be formed of 4-membered [4], 5-membered [5-7], 6-membered [8], and 7-membered [9]. However, the most used are NHCs containing 5-membered such as imidazole [5], imidazoline [6], and benzimidazole [7].

The benzimidazole is a bicyclic heteroaromatic molecule that is a structural isostere of naturally occurring nucleotides. Therefore, it has been extensively utilized as a useful scaffold in medicinal chemistry [10]. Recently, the biological activities of different benzimidazole derivatives have been investigated [11,12]. The benzimidazolium salts are the important benzimidazole derivatives. The benzimidazolium salts have been found to display many biological activities such as antimicrobial [13], antibiofilm [14], antifungal [15], antiviral [16], antioxidant [17], anti-inflammatory [18], anticancer [19,20] and cytotoxicity [21]. In addition to these studies, benzimidazolium salts' enzyme inhibition effects have recently been remarkable [22-27].

The development of bacterial resistance against macrolides,  $\beta$ -lactam antibiotics, vancomycin, and quinolones causes widespread health problems [28, 29]. There is a need to discover new antibacterial drugs with a different structure to combat these problems than the existing antibiotics [28]. Thus, the research of new antibacterial substances with new mechanisms of action is to be always important [29]. There has been much recent interest in the therapeutic potential of benzimidazole as antibacterial agents. Therefore, this situation has led us to screen the antimicrobial effects of 1-(2-methyl-1,4-benzodioxane)benzimidazole and their benzimidazolium salts.

The term structure-activity relationship (SAR) provides an understanding of the relationship between a chemical molecule structure and its biological activity. When the SAR of the compounds is examined, we believe that the electronic and structural properties of the 2-methyl-1,4-benzodioxane substituent on benzimidazole salts are important. In particular, it could be said that the benzodioxane group exhibits antioxidant and antimicrobial activity due to both weak interactions and chemical interactions. One of the most important reasons for using this group is the presence of hydrophilic dioxane moiety and hydrophobic benzene rings on the same structure. Also, the second groups (different aryl groups) on the benzimidazole are important in terms of SAR. Aryl groups perform both hydrophobic interactions and providing an electronic contribution to the molecules.

Recently, new studies on the biological activities of *N*-heterocyclic compounds such as thiadiazole, triazole, imidazolium, and benzimidazolium have been published [30-32]. We have previously carried out studies on the biological activities of benzimidazolium salts [33,34] and their Ag(I)NHC complexes [27,33,35-38] and Pd(II)NHC complexes [39-43]. Here, we have described the synthesis and characterization of new 2-methyl-1,4-benzodioxane-substituted benzimidazolium salts. The antioxidant and antimicrobial activities of all compounds have been investigated using antioxidant assays and the agar-well diffusion method. In the meantime, computational studies were carried out to illuminate their activities at the atomic level.

## 2. Materials and Methods

All synthesis, including benzimidazolium salts bearing 2-methyl-1,4-benzodioxane group **2a-f** were prepared in the air atmosphere using standard Schlenk techniques. The solvents and all other reagents were commercially available from Merck, Sigma-Aldrich, and Scharlau chemical company and used without further purification. Melting points were identified in glass capillaries under air with an Electrothermal-9200 melting point apparatus. FT-IR spectra were saved in the range 400-4000  $\text{cm}^{-1}$  on Perkin Elmer Spectrum 100 FT-IR spectrometer. Proton ( $^1\text{H}$ ) and Carbon ( $^{13}\text{C}$ ) NMR spectra were recorded using either a Bruker AC300P FT spectrometer operating at 300.13 MHz ( $^1\text{H}$ ) and 75.47 MHz ( $^{13}\text{C}$ ) in DMSO- $d_6$

with tetramethylsilane as an internal reference. Elemental analyses were performed by Inonu University Scientific and Technological Research Center (Malatya, TURKEY).

### 2.1. Synthesis.

#### 2.1.1. Synthesis of 1-(2-methyl-1,4-benzodioxan)benzimidazole, 1.

For the synthesis of 1-(2-methyl-1,4-benzodioxan)benzimidazole has used the method described in the literature [23]. For this, the benzimidazole (2.36 g, 20 mmol) and KOH (1.23 g, 22 mmol) was added in ethanol (25 mL) and stirred at room temperature for 1 hour. Then was added 2-bromomethyl-1,4-benzodioxane (4.58 g, 20 mmol). The reaction mixture was stirred for 12 h. at 80 °C temperatures. The solvents were evaporated under vacuum to afford the product as a viscous liquid. The crude product was crystallized from ethyl alcohol/diethyl ether mixture. Yield: 80% (4.26 g); m.p.: 100-101 °C;  $\nu_{\text{CN}}$ : 1501  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$ : 3.96 (dd, 2H,  $J$ : 9.6 Hz - $\text{NCH}_2\text{CHCH}_2\text{O}_2\text{C}_6\text{H}_4$ ); 4.54 (dd, 2H,  $J$ : 9.6 Hz, - $\text{NCH}_2\text{CHCH}_2\text{O}_2\text{C}_6\text{H}_4$ ); 4.57 (m, 1H, - $\text{NCH}_2\text{CHCH}_2\text{O}_2\text{C}_6\text{H}_4$ ); 6.81-8.22 (m, 8H, Ar- $H$ ); 12.45 (s, 1 H, - $\text{NCHN}$ -).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ),  $\delta$  47.1 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 64.2 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 72.5 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 111.1-117.3-120.4-120.6-122.0-122.2-134.4 and 143.6-144.4 (Ar-C).

#### 2.1.2. Synthesis of 1-(2-methyl-1,4-benzodioxane)-3-(2-methylbenzyl)benzimidazolium chloride, 2a.

For the synthesis of **2a**, 1-(2-methyl-1,4-benzodioxane)benzimidazole (1,33 g, 5 mmol) and 2-methylbenzyl chloride (0.70 g, 5 mmol) was added in DMF (4 mL). Yield: 83% (1.69 g), m.p.: 147-148 °C;  $\nu_{\text{CN}}$ : 1560  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ),  $\delta$ : 2.34 (s, 3H, - $\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 4.33 and 4.54 (dd, 2H, - $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -,  $J$ : 9.3 Hz); 4.93 (m, 1H, - $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 4.92 and 5.40 (dd, 2H, - $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -,  $J$ :9.6 Hz); 5.82 (s, 2H,  $\text{CH}_2\text{C}_6\text{H}_5$ ); 6.69-7.70 (m, 12H, Ar- $H$ ); 11.48 (s, 1H, 2- $\text{CH}$ ).  $^{13}\text{C}$  NMR (300 MHz, DMSO- $d_6$ ),  $\delta$ : 21.7 (- $\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 47.7 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 51.8 (- $\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 64.8 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 72.2 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 113.4, 113.8, 114.1, 117.4, 117.7, 122.1, 127.2, 127.8, 128.3, 129.3, 129.4, 129.5, 130.7, 132.3, 132.5, 141.4, 142.4 and 142.8 (Ar-C); 143.4 (2- $\text{CH}$ ). Anal. Calc. for  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2$ : C: 71.84, H: 5.70, N: 6.88. Found C: 71.79, H: 5.72, N: 6.86.

#### 2.1.3. Synthesis of 1-(2-methyl-1,4-benzodioxane)-3-(3-methylbenzyl)benzimidazolium chloride, 2b.

The synthesis of **2b** was performed in the same method as that described for **2a**, but 3-methylbenzyl chloride (0.70 g, 5 mmol) was used instead of 2-methylbenzyl chloride. Yield: 72% (147 g); m.p.: 140-141 °C;  $\nu_{\text{CN}}$ : 1558  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ),  $\delta$ : 2.33 (s, 3H, - $\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 4.34 and 4.54 (dd, 2H, - $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -,  $J$ : 9.3 Hz); 5.00 (m, 1H, - $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 4.95 and 5.60 (dd, 2H, - $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -,  $J$ :9.6 Hz); 5.76 (s, 2H, - $\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 6.83-7.70 (m, 12H, Ar- $H$ ); 11.40 (s, 1 H, 2- $\text{CH}$ ).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ),  $\delta$ : 21.4 (- $\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 47.7 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 51.8 (- $\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 64.8 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 72.2 (- $\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}$ -); 113.4, 114.1, 117.4, 122.1, 125.2, 126.6, 127.2, 128.7, 128.9, 129.3, 130.1, 130.8, 131.8, 132.1, 132.5, 139.5 and 141.4 (Ar-C); 142.9 (2- $\text{CH}$ ). Anal. Calc. for  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2$ : C: 71.84, H: 5.70, N: 6.88. Found C: 71.78, H: 5.72, N: 6.85.

2.1.4. Synthesis of 1-(2-methyl-1,4-benzodioxane)-3-(4-methylbenzyl)benzimidazolium chloride, 2c.

The synthesis of **2c** was performed in the same method as that described for **2a**, but 4-methylbenzyl chloride (0.70 g, 5 mmol) was used instead of 2-methylbenzyl chloride. Yield: 75% (1.53 g); m.p.: 138-139 °C;  $\nu_{\text{CN}}$ : 1560  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ),  $\delta$ ; 2.32 (s, 3H,  $-\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 4.35 and 4.54 (dd, 2H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ,  $J$ : 9.3 Hz); 4.97 (m, 1H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 4.92 and 5.47 (dd, 2H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ,  $J$ : 9.6 Hz); 5.70 (s, 2H,  $-\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 6.68-8.95 (m, 12H, Ar- $H$ ); 11.46 (s, 1H, 2-CH).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ),  $\delta$ ; 21.2 ( $-\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 47.7 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 51.7 ( $-\text{CH}_2\text{C}_6\text{H}_4(\text{CH}_3)$ ); 64.8 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 72.2 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 111.2, 113.4, 114.0, 117.4, 118.4, 121.7, 122.1, 124.6, 127.2, 128.3, 129.1, 130.2, 131.0, 132.5, 138.7, 139.6, 141.4 and 142.9 (Ar- $C$ ); 143.2 (2-CH). Anal. Calc. for  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2$ : C: 71.84, H: 5.70, N: 6.88. Found C: 71.87, H: 5.68, N: 6.86.

2.1.5. Synthesis of 1-(2-methyl-1,4-benzodioxane)-3-(2,4,6-trimethylbenzyl)benzimidazolium chloride, 2d.

The synthesis of **2d** was performed in the same method as that described for **2a**, but 2,4,6-trimethylbenzyl chloride (0.85 g, 5 mmol) was used instead of 2-methylbenzyl chloride. Yield: 70% (1.52 g); m.p.: 203-204 °C;  $\nu_{\text{CN}}$ : 1567  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ),  $\delta$ ; 2.27 and 2.31 (s, 9H,  $-\text{CH}_2\text{C}_6\text{H}_2(\text{CH}_3)_3$ ); 4.07 and 4.45 (dd, 2H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ,  $J$ : 9.6 Hz); 4.75 (m, 1H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 4.78 and 4.99 (dd, 2H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ,  $J$ : 9.6 Hz); 5.70 (s, 2H,  $-\text{CH}_2\text{C}_6\text{H}_2(\text{CH}_3)_3$ ); 6.58-8.21 (m, 10H, Ar- $H$ ); 9.62 (s, 1H, 2-CH).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ),  $\delta$ ; 19.8 and 21.2 ( $-\text{CH}_2\text{C}_6\text{H}_2(\text{CH}_3)_3$ ); 45.6 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 47.0 ( $-\text{CH}_2\text{C}_6\text{H}_2(\text{CH}_3)_3$ ); 64.8 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 71.0 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 114.3, 114.7, 114.9, 117.4, 117.7, 122.3, 126.1, 126.5, 127.4, 130.0, 131.0, 131.9, 132.2, 138.6, 139.1, 139.3, 141.2 and 142.1 (Ar- $C$ ); 143.2 (2-CH). Anal. Calc. for  $\text{C}_{26}\text{H}_{27}\text{ClN}_2\text{O}_2$ : C: 71.80, H: 6.26, N: 6.44. Found C: 71.84, H: 6.24, N: 6.47.

2.1.6. Synthesis of 1-(2-methyl-1,4-benzodioxane)-3-(2,3,5,6-tetramethylbenzyl)benzimidazolium chloride, 2e.

The synthesis of **2e** was performed in the same method as that described for **2a**, but 2,3,5,6-tetramethylbenzyl chloride (0.92 g, 5 mmol) was used instead of 2-methylbenzyl chloride. Yield: 70% (1.57 g); m.p.: 222-223 °C;  $\nu_{\text{CN}}$ : 1561  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ),  $\delta$ ; 2.25 and 2.30 (s, 12H,  $-\text{CH}_2\text{C}_6\text{H}(\text{CH}_3)_4$ ); 4.31 and 4.52 (dd, 2H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ,  $J$ : 9.3 Hz); 4.85 (m, 1H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 5.61 and 5.72 (dd, 2H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ,  $J$ : 9.3 Hz); 5.89 (s, 2H,  $-\text{CH}_2\text{C}_6\text{H}(\text{CH}_3)_4$ ); 6.52-7.97 (m, 9H, Ar- $H$ ); 10.06 (s, 1H, 2-CH).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ),  $\delta$ ; 16.1 and 20.6 ( $-\text{CH}_2\text{C}_6\text{H}(\text{CH}_3)_4$ ); 47.7 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 48.0 ( $-\text{CH}_2\text{C}_6\text{H}(\text{CH}_3)_4$ ); 65.7 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 71.9 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 112.9, 113.8, 114.6, 117.1, 117.7, 121.9, 122.1, 126.9, 127.2, 127.8, 131.0, 131.8, 132.5, 133.5, 133.9, 135.1, 141.2 and 142.0 (Ar- $C$ ); 143.0 (2-CH). Anal. Calc. for  $\text{C}_{27}\text{H}_{29}\text{ClN}_2\text{O}_2$ : C: 72.23, H: 6.51, N: 6.24. Found C: 72.28, H: 6.47, N: 6.26.

2.1.7. Synthesis of 1-(2-methyl-1,4-benzodioxane)-3-(naphthalenomethyl)benzimidazolium chloride, 2f.

The synthesis of **2f** was performed in the same method as that described for **2a**, but naphthalenomethyl chloride (0.89 g, 5 mmol) was used instead of 2-methylbenzyl chloride. Yield: 68% (1.51 g); mp.: 239-240 °C;  $\nu_{\text{CN}}$ : 1563  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ),  $\delta$ ; 4.14 and 4.53 (dd, 2H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ,  $J$ : 9.3 Hz); 4.85 (m, 1H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 4.88 and 5.06 (dd, 2H,  $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ,  $J$ : 11.7 Hz); 5.23 (s, 2H,  $-\text{CH}_2\text{C}_{10}\text{H}_7$ ); 6.81-8.56 (m, 12H, Ar- $H$ ); 9.98 (s, 1H, 2- $\text{CH}$ ).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$ ; 47.3 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 50.2 ( $-\text{CH}_2\text{C}_{10}\text{H}_7$ ); 64.9 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 68.9 ( $-\text{CH}_2\text{CH}(\text{O})\text{CH}_2\text{O}-$ ); 114.5, 114.7, 115.8, 117.6, 122.2, 127.1, 127.3, 128.8, 129.2, 129.3, 131.1, 131.5, 132.2, 133.8, 136.4, 138.0 and 142.3 (Ar- $\text{C}$ ); 144.2 (2- $\text{CH}$ ). Anal. Calc. for  $\text{C}_{27}\text{H}_{23}\text{ClN}_2\text{O}_2$ : C: 73.21, H: 5.23, N: 6.32. Found C: 73.18, H: 5.25, N: 6.34.

## 2.2. DPPH assay.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to determine of antioxidant activity of the tested compounds [44]. The tested compounds were dissolved in dimethyl sulfoxide (DMSO, 1  $\mu\text{g}/\text{ml}$ ). 50  $\mu\text{l}$  of the compounds were mixed with DPPH solution in methanol (0.1 mM). The absorbance values were read at 517 nm after 2h. As the standard, butylated hydroxytoluene (BHT) was used. The percentage inhibition was calculated by using a formula:

%Antioxidant activity (%Inhibisyon) =  $100 \times (1 - \text{Absorbance of test compound} / \text{Absorbance of control compound})$  (I)

## 2.3. $\text{H}_2\text{O}_2$ scavenging activity.

$\text{H}_2\text{O}_2$  scavenging effects of the synthesized compounds were measured according to Pick and Keisari [45]. 43 mM  $\text{H}_2\text{O}_2$  was produced in phosphate buffer (0.1 M, pH 7.4). Compounds (10  $\mu\text{g}/\text{ml}$ ) in 3.4 ml phosphate buffer were mixed with  $\text{H}_2\text{O}_2$  solution. Absorbance was read at 230 nm after 10 minutes. The percentage of  $\text{H}_2\text{O}_2$  scavenging activities of the compounds were calculated according to formula (I).

## 2.4. Evaluation of antimicrobial activity.

The eleven microorganisms were tested: *Bacillus cereus* FMC 19, *Aeromonas hydrophila* ATCC 7965, *Escherichia coli* ATCC 25922, *E. coli* O157:H7 932, *Listeria monocytogenes* 1/2B, *Klebsiella pneumoniae* FMC 5, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* NRRLE 4463, *Yersinia enterocolitica* ATCC 1501 and *Candida albicans* ATCC 1223.

The antimicrobial activities of seven compounds (1 and 2a-f) were tested by the agar-well diffusion assay [46]. The compound solutions (10 mg/ml) were prepared in DMSO. The solution was sterilized through a membrane filter (0.2  $\mu\text{m}$ ). The bacteria were grown in nutrient broth at 35 °C for 18 h, while malt extract for *C. albicans* at 25 °C for 18 h was used. Cultures were adjusted to 0.5 McFarland standard turbidity. Inoculated Mueller Hinton agar or malt extract agar was poured into Petri dishes. The Petri dishes were stored at 4 °C for 1 h. The holes were made in the agar by sterile cork borers ( $\text{Ø} = 6$  mm). The solutions of compounds (50  $\mu\text{l}$ ) were added to the holes. DMSO was used as a control. The bacteria were incubated at 35°C for 18-24 h (*Y. enterocolitica* and *C. albicans* except). Inhibition zones were measured in millimeters. The standard antibiotics Tetracycline (10 mg/ml) (Sigma T3258-56) and Natamycin (30 mg/ml) (Delvocid DMS) were applied as positive control.

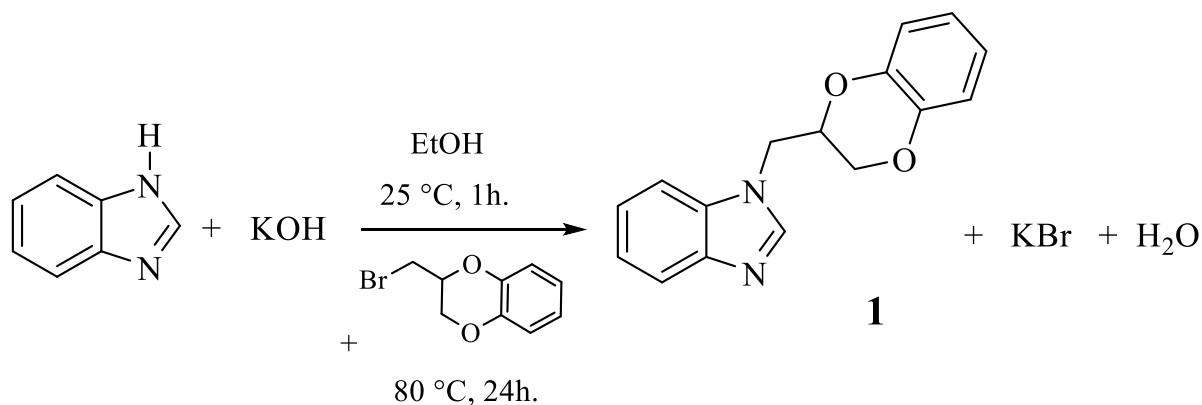
### 2.5. Computational studies.

All density functional theory (DFT) calculations were exerted with the Discovery Studio (DS) 2020 program [47] to support the proposed activities for the antioxidant and antimicrobial activities of all compounds. The geometry of benzimidazolium salts was fully optimized with Becke3 (B3) Lee–Yang–Parr (LYP) method (B3LYP) using the DMol3 basis set in the gas phase. The HOMO–LUMO analysis and molecular electrostatic potential surface (MESP) are employed to obtain important insights into the atomic level compounds.

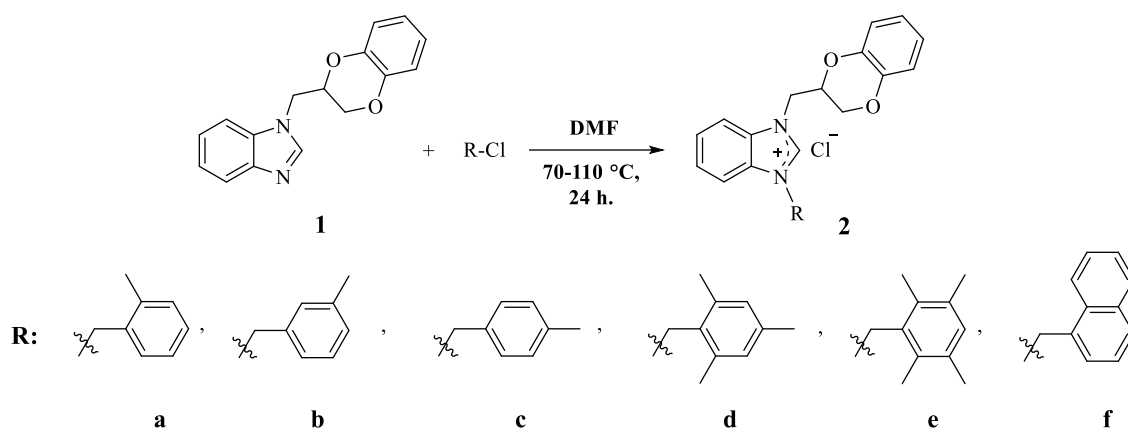
## 3. Results and Discussion

### 3.1. Synthesis and characterization study.

The 1-(2-methyl-1,4-benzodioxane)benzimidazole **1** was synthesized using the benzimidazole 2-bromomethyl-1,4-benzodioxane, and it was obtained in yield 80%. This compound has been illustrated in Scheme 1. The 2-methyl-1,4-benzodioxane substituted benzimidazolium salts **2a-f** were synthesized using the common alkylation assay and were obtained in moderate yields between 68-83%. The air and moisture stable benzimidazolium salts were soluble in polar solvents such as water, alcohol, and dimethyl sulfoxide. But all compounds were less-soluble in halogenated solvents such as dichloromethane and chloroform. The benzimidazolium salts **2a-f** have been illustrated in Scheme 2. The synthesized salts' structures were characterized by using FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR spectroscopic methods, and elemental analysis techniques. In the <sup>1</sup>H NMR spectra, the benzimidazolium salts were detected by a characteristic proton peak at the 2-position (NCHN) of the benzimidazolium salts **2a-f**, which appeared highly downfield shifted singlets at  $\delta$  11.48, 11.40, 11.46, 9.62, 10.06 and 9.98 ppm for **2a-f**, respectively. Multiplets in the range  $\delta$  between 6.52 and 8.95 were attributed to aromatic protons. In the <sup>13</sup>C NMR spectra of the benzimidazolium salts **2a-f**, a carbon signal in the range  $\delta$  142.9-143.4 was attributed to NCHN carbon at a lower area compared to other aromatic carbons at 143.4, 142.9, 143.2, 143.2, 143.0, and 144.2 for **2a-f**, respectively. FT-IR spectra of the benzimidazolium salts showed a band at 1501, 1560, 1558, 1560, 1567, 1561, and 1563 cm<sup>-1</sup> corresponding to the vibrations of C=N of the 1-(2-methyl-1,4-benzodioxane) benzimidazole **1** and benzimidazolium salts **2a-f**, respectively. All spectroscopic data of the compounds are consistent with the literature [23-25,29,33].



Scheme 1. 1-(2-methyl-1,4-benzodioxane) benzimidazole, **1**.



**Scheme 2.** Synthesis of novel 2-methyl-1,4-benzodioxane-substituted benzimidazolium salts **2a-f**.

### 3.2. *In vitro* antioxidant activity study.

Newly synthesized compounds experimented with free radical scavenging ability. BHT was used as a standard compound. The results of the DPPH radical scavenging activities of the compounds are summarized in Table 1 and Fig. 1. We found that all of the compounds showed moderate free radical-scavenging activities. All compounds exhibited DPPH scavenging activity in the range of 30.85 - 39.75% as compared to BHT (91.47%). The maximum free radical scavenging activity was found as 39.75% for compound **2f** at 1 mg/ml, while the least activity was calculated for **2a** (30.85%). This study reveals that substitution with electron-donating groups, which may lead to free radical quenching, increases the antioxidant potential. It can be said that the presence of the 2-methyl-1,4-benzodioxane substituent on the compounds contributes to this effect, too. Also, it is observed that the effect of inhibition increases as the methyl number increases of benzyl substituent groups on the benzimidazolium salts. In a similar study, Haque *et al.* worked on para-xylyl linked bis-benzimidazolium salts' antioxidant activities and respective dinuclear Ag-NHC complexes by using DPPH assay and found their %inhibition values in the range of 6.37–21.00 [15]. The %inhibition values which we get in our work are higher than this work.

### 3.3. $\text{H}_2\text{O}_2$ scavenging activity study.

All synthesized compounds tested for hydrogen peroxide scavenging activity. The activities of test compounds to scavenge  $\text{H}_2\text{O}_2$  are given in Table 1 and Fig. 1, and compared with that of BHA, BHT, and Gallic acid as standard compounds. All compounds exhibited strong hydrogen peroxide scavenging activity ranges between 121.16 and 143.54% at 10  $\mu\text{g/ml}$ . BHA, BHT, and Gallic acid exhibited 18.52 6.67 and 30.09% inhibition, respectively, at the same concentration. The synthesized compounds have more potential  $\text{H}_2\text{O}_2$  scavenging activity compared with those of the standards. These activities may be due to the presence of electron-donating methyl groups on the benzene ring.

### 3.4. Antimicrobial activity study.

The antimicrobial effects of the tested compounds were examined against nine bacteria and yeast. Compounds showed varying degrees of antimicrobial effect against all of the tested microorganisms. Especially, they showed strong activity against *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *C. albicans* (growth inhibition zones  $\geq 18$  mm) (Table 2 – Fig. 2). Among all compounds, the 1-(2-methyl-1,4-benzodioxan)benzimidazole **1** exerted the least

antibacterial effect against all the studied bacteria, with the inhibition zones ranging from 9.0 mm to 14 mm. This compound had no inhibitory activity against *A. hydrophila*, *E. coli*, and *S. typhimurium*. While compound **2e** having tetramethyl groups exhibited the strongest antibacterial activity against one or more tested bacteria (12-26 mm), the tetracycline exhibited antibacterial effect against all the studied bacteria with the inhibition zones ranging from 16 mm to 27 mm. All compounds except **1** exhibited strong activity against *L. monocytogenes* and *P. aeruginosa* (inhibition zones  $\geq 18$  mm) (Table 2 – Fig. 2-3). Compound **1** was inactive against *A. hydrophila*, *E. coli*, and *S. typhimurium*. Compounds **2b** and **2d** showed the same inhibitor activity, while compound **2e** had stronger activity against *L. monocytogenes* than standard tetracycline. Also, compound **2f** containing naphthalenomethyl substituent showed the same inhibitor activity while compounds **2d** and **2e** containing three and tetramethyl groups had stronger activity against *P. aeruginosa* compared with standard tetracycline (Table 2 – Fig. 2).

The compounds **2a-f** exhibited weak activity against *A. hydrophila* and *S. typhimurium* (10-13 mm and 9-12 mm, respectively). While the compound **2e** containing tetramethyl groups exhibited moderate activity, other tested compounds exhibited weak activity against *Y. enterocolitica* (0.9-11 mm). All compounds except **1** exhibited a moderate or weak effect on *E. coli* (10-14 mm). *E. coli* O157:H7 and *K. pneumoniae* are sensitive to all of the test compounds with the inhibition zones ranging from 0.9 mm to 19 mm and from 10 mm to 19 mm, respectively (Table 2 – Fig. 2). When the antibacterial activity of all compounds examined, the compounds **2d** and **2f** exhibited higher antibacterial activity (14 mm) than compounds **2a**, **2b**, **2c**, **2e** (13 mm), and compound **1** (10 mm) against *B. cereus* (Table 2 – Fig. 3). When the benzimidazolium salts (**2a-f**) containing aromatic groups compared with similar benzimidazolium salts [33] containing aliphatic groups exhibited higher activity against *Gram* (+) and *Gram* (-) bacteria.

The compounds **1** and **2a-f** had a more potential effect with either high or equal inhibition zones than standard natamycin against *C. albicans* (Table 2 - Fig. 4). According to the results, the tested compounds' antimicrobial activities against bacteria and yeast vary due to structural differences between the microorganisms and compounds. On the other hand, the existence of the electron-donating methyl groups existing on the benzene ring generally has increased the antibacterial activity. The many benzimidazole-based compounds have exhibited biological activity due to bearing functional groups at the 1, 2, and/or 5, 6 positions [48]. It has been previously reported that *N*-morpholinoethylbenzimidazole and 2-morpholinoethyl-substituted benzimidazolium salts have exhibited strong antimicrobial activity against *E.coli* and *S. aureus* [13].

**Table 1.** Inhibitory effect of the compounds **1** and **2a-f** on DPPH radical and H<sub>2</sub>O<sub>2</sub>.

Compounds	DPPH	H <sub>2</sub> O <sub>2</sub>
	% Inhibition*	% Inhibition
<b>1</b>	29.53	136.46
<b>2a</b>	30.85	143.54
<b>2b</b>	32.27	121.50
<b>2c</b>	31.14	121.16
<b>2d</b>	33.01	127.76
<b>2e</b>	34.33	122.38
<b>2f</b>	39.75	124.86
<b>BHT</b>	91.47	6.67
<b>BHA</b>	-	18.52
<b>Gallic acid</b>	-	30.09

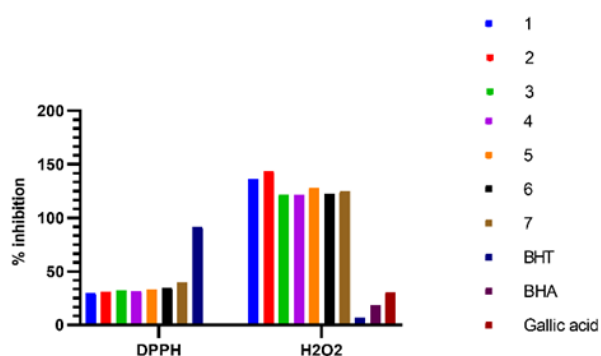


**Table 2.** Antimicrobial activities of compounds 1, 2a-f, and reference drug against the tested microorganisms.

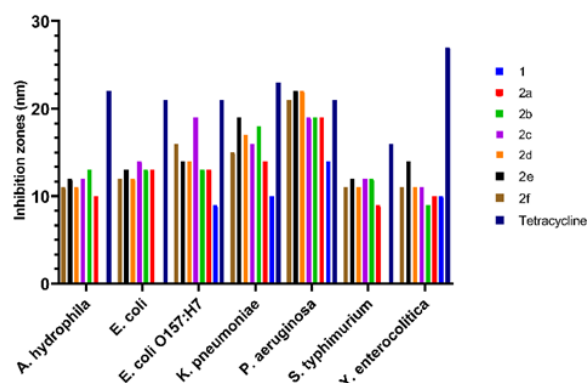
Organisms	Compounds								Tetracycline (10 µg/ml)
	1	2a	2b	2c	2d	2e	2f		
Gram (-)									
<i>A. hydrophila</i>	-	10	13	12	11	12	11		22.0
<i>E. coli</i>	-	13	13	14	12	13	12		21.0
<i>E. coli</i> O157:H7	9*	13	13	19	14	14	16		21.0
<i>K. pneumoniae</i>	10	14	18	16	17	19	15		23.0
<i>P. aeruginosa</i>	14	19	19	19	22	22	21		21.0
<i>S. typhimurium</i>	-	9	12	12	11	12	11		16.0
<i>Y. enterocolitica</i>	10	10	9	11	11	14	11		27.0
Gram (+)									
<i>B. cereus</i>	10	13	13	13	14	13	14		26.0
<i>L. monocytogenes</i>	11	18	21	20	21	26	19		21.0
Yeast									Natamycin (30 µg/ml)
<i>C. albicans</i>	28	19	24	31	26	26	23		24.0

\*: inhibition zones include the diameter of the hole (6 mm). Sample amount 50 µl.

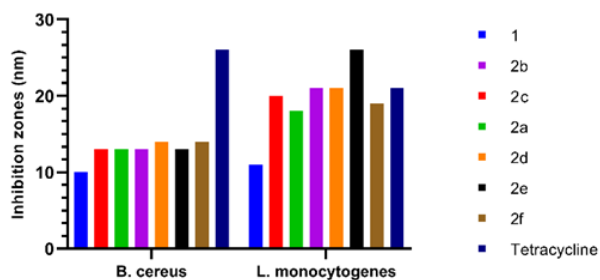
-: Not active



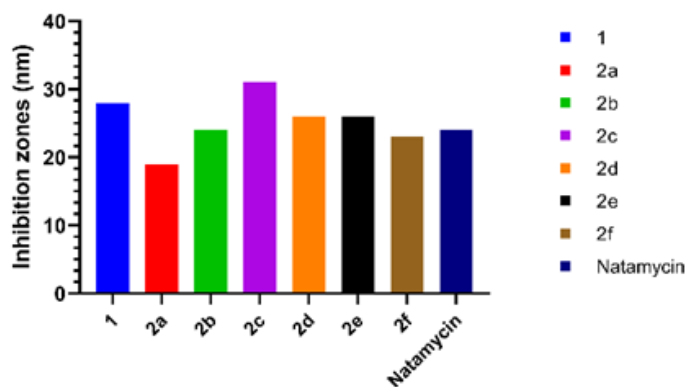
**Figure 1.** Inhibitory effect of the compounds 1 and 2a-f on DPPH radicals and H<sub>2</sub>O<sub>2</sub>.



**Figure 2.** Antibacterial activities of the compounds 1, 2a-f and reference drug (Tetracycline) against the tested Gram (-) bacteria.



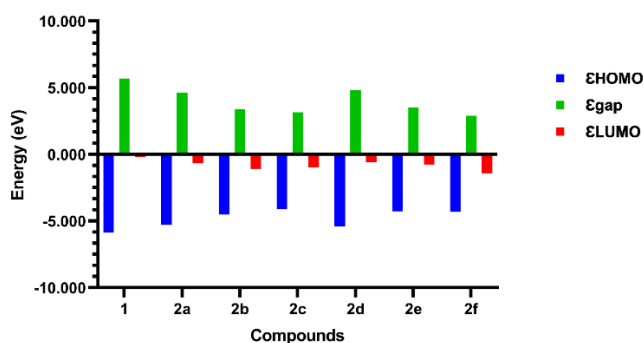
**Figure 3.** Antibacterial activities of the compounds 1, 2a-f and reference drug (Tetracycline) against the tested Gram (+) bacteria.



**Figure 4.** Anticandidal activities of the compounds 1, 2a-f and reference drug (Natamycin) against the *C. albicans*

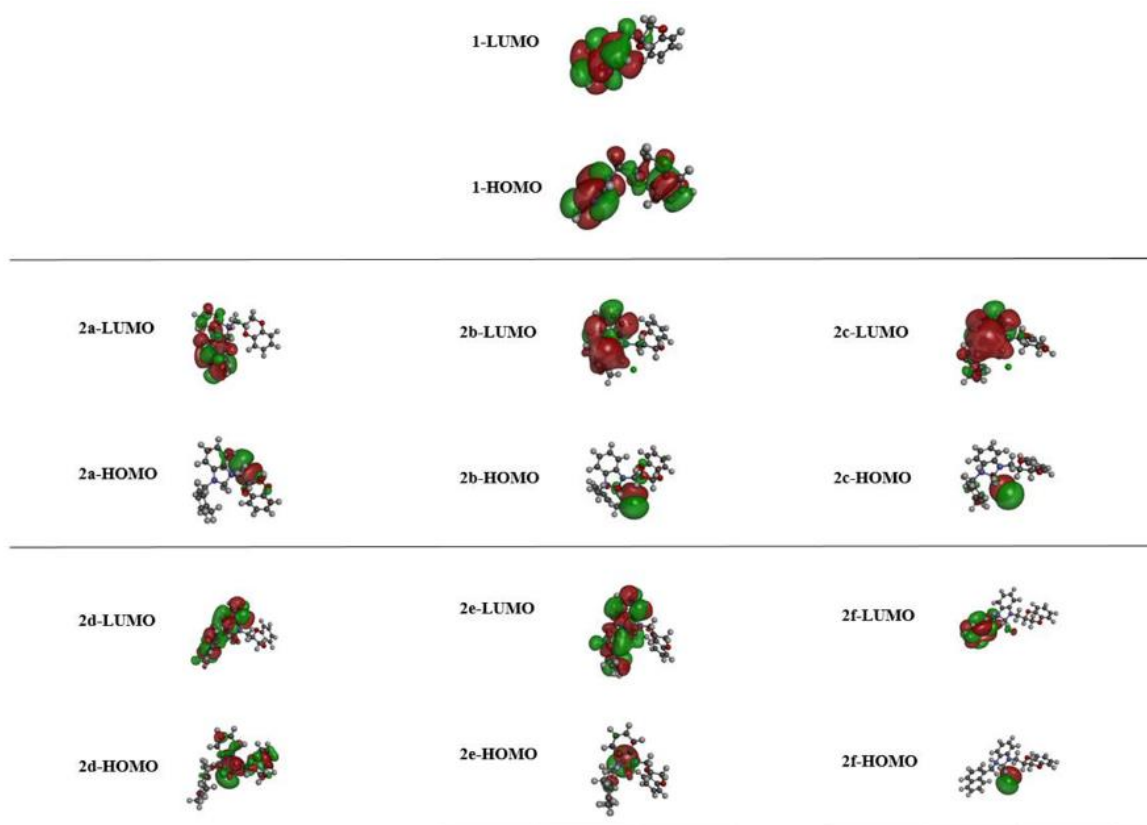
### 3.5. Computational results.

In this study, besides the experimental studies of benzimidazolium salts, theoretical calculations were applied to elucidate the compounds' relevant parts in the applied activity techniques. Therefore, the benzimidazolium salt structures are geometrically and energetically optimized at the DFT / B3LYP / DMol3 level to predict the boundary molecular orbitals and electronic structure benzimidazolium salts using DS program. The highest occupied molecular orbital (HOMO) energy,  $\epsilon_{\text{HOMO}}$ , the lowest unoccupied molecular orbital (LUMO) energy,  $\epsilon_{\text{LUMO}}$ , energy gap,  $\epsilon_{\text{gap}}$  values, and views for benzimidazolium salts, obtained by the same method are displayed in Figure 5 and Figure 6, respectively. Red and green colors exhibit the positive and negative phases, respectively. The HOMO-LUMO energy gap values of benzimidazolium salt, as remarked bold red color in Figure 5, are 5.694, 4.635, 3.403, 3.169, 4.819, 3.521, and 2.893 eV, respectively. These results show us why which compound exhibits better activity than other compounds.

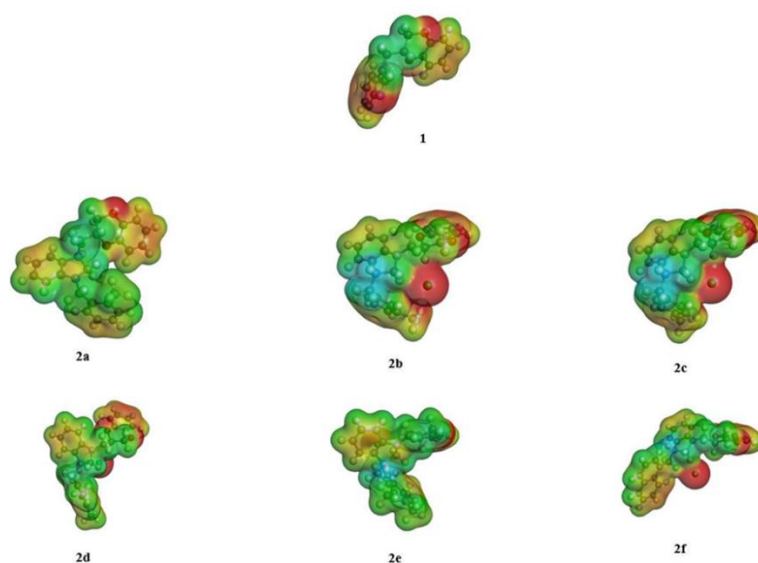


**Figure 5.** The calculated  $\epsilon_{\text{HOMO}}$ ,  $\epsilon_{\text{LUMO}}$ , and  $\epsilon_{\text{gap}}$  values of all compounds, respectively.

Furthermore, the molecular electrostatic potential surface (MESP) was calculated to investigate benzimidazolium salts' electron density. Generated MEPS map shows the relation between molecular structure and physicochemical property of benzimidazolium salts (Figure 7). Different colors show and visualize different electrostatic potentials at the surface of the structure. The region of attractive potential appears in red. Those of repulsion potential appear in blue. Thus, the Cl and O atoms regions have higher positive electrostatic potential and participate in nucleophilic reactions. N atoms with higher electronegative values have higher electron density around them and participate in electrophilic attacks.



**Figure 6.** The pictorial image of HOMO and LUMO frontier molecular orbitals for benzimidazolium salts is calculated using the B3LYP method with a DMol3 basis set.



**Figure 7.** The electron density isosurface for benzimidazolium salts calculated by B3LYP method with DMol3 basis set.

#### 4. Conclusions

In conclusion, this study contains the synthesis and characterization of the N-substituted benzimidazole and six new benzimidazolium salts. DPPH and H<sub>2</sub>O<sub>2</sub> scavenging abilities of all compounds were examined. All compounds exhibited moderate free radical-scavenging activity but strong hydrogen peroxide scavenging activity. Synthesized compounds exhibited varying degrees of antimicrobial activity against all tested microorganisms, especially against *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *C. albicans*. The antioxidant and

antimicrobial activities of the synthesized benzimidazolium salts are very promising. Besides these applied studies, computational applications HOMO–LUMO analysis and molecular electrostatic potential surface (MESP) were probed using DS 2020. Obtained results from the present study will contribute to further study in this field. Further optimizations and detailed SAR studies are needed.

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## Conflicts of Interest

The authors declare no conflict of interest.

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