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Synthesis, Structural Analysis, Antimicrobial Activity and The Molecular Electrostatic Potential Surface (MEP) of 2/3/4-Chloro Benzamide-Spiro[Benzo[B]Thiophene-Dioxolane] Derivatives

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Abstract: In this research, 2-amino-4,7-dihydro-5H-spiro[benzo[b]thiophene-6,2'-[1,3]dioxolane]-3-carbonitrile (ST) was synthesized using the Gewald method, starting with 1,4-dioxaspiro[4,5]decan-8-one ketone. The structures of compounds were characterized through FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectra. The antimicrobial properties of the compounds were examined by the disk diffusion process. The compounds (N1-3) did not exhibit effectiveness against the *E. Coli (ATCC)* and *S. Aureus (ATCC)* bacteria. The molecular electrostatic potential surface (MEP) of all compounds was calculated via DFT calculations based on the optimized geometries at the B3LYP/6-31G (d,p) level of theory. Negative potential regions were located over the oxygen and nitrogen atoms, whereas positive potential regions were identified over the oxygen and sulfur atoms. Conceptually, computations of the molecular structures of the compounds were carried out using molecular modeling software, specifically GaussView 5.0 and the GAUSSIAN 09 package programs. Additionally, computations were performed for the HOMO and LUMO molecular orbitals of isolated molecules in the gas phase. Molecular electrostatic potential (MEP) surfaces were used to visualize potential interactions between receptors and ligands over the steady-state geometries of the molecules and to highlight the electrophilic and nucleophilic regions of the molecules.

Key words: Amino Thiophene, Spiro Compounds, Benzamide Derivatives, Molecular Electrostatic Potential Surface

1. Introduction

Amides, which constitute a notable subgroup in the field of organic chemistry and were originally thought to exist exclusively in living organisms, gained new importance in synthetic chemistry with the synthesis of urea by the German chemist Friedrich Wöhler in 1828 [1]. Subsequent research during that period led to numerous significant discoveries related to amides. The ability to synthesize amide compounds under controlled laboratory conditions and to gain a detailed understanding of their structures also provided opportunities to elucidate the fundamental processes within biological systems.

Amides are typically compounds containing one or more amino groups attached to a carbonyl group. The synthesis of amide compounds, such as those derived from

structurally diverse amino or carboxylic acid derivatives, as used in this study, often requires specific conditions depending on the desired structure and intended applications of the amides [2,3]. The chemical structure of amides not only plays a crucial role during their synthesis but also holds significant importance in the biological context.

The structural diversity of amides makes them important in various fields, including biochemistry [4], pharmaceutical chemistry [5, 6], polymer science [7, 8], and materials science [9]. Amide groups, particularly those found in essential compounds like amino acids, play a critical role in biological systems and are frequently employed in the design of drug molecules to enhance efficacy or achieve specific targets. In a study conducted in 2020, it has been demonstrated that [1+1] condensed furan and thipohene-based cycloheterophane amide derivatives are effective against *S. aureus, B. cereus, E. coli, Listeria monocytogenes, Salmonella typhimurium* bacterial strains, and *C. albicans* yeast culture [10]. Bondock et al synthesized heterocyclic amide derivatives and determined that these compounds exhibited high efficacy, in vitro assays. Additionally, some derivatives exhibited antifungal activity [11]. A study of comparing the ligand and metal complexes of benzo[b]thiophene-2-carbohydrazide, reported that antimicrobial activity was observed with the ligand, but an increase in antimicrobial activity was noted with them metal complex [12]. Different types of amides can exhibit various therapeutic effects, including antibacterial [13], antiviral [14], anticancer [15], antiparkinson [16], and analgesic [17] properties.

In this study, the amine compound used in the synthesis of amide derivatives is 2-amino-4,7-dihydro-5H-spiro[benzo[b]thiophene-6,2'-[1,3]dioxolane]-3-carbonitrile (ST). This compound is structurally a thiophene ring. Thiophene compounds have played significant roles in many studies due to their high chemical reactivity. It is known that some mushrooms and a perennial plant species contain the thiophene rings. These biologically active compounds frequently appear in pharmacological studies with different properties such as antioxidant [18], antibacterial [19], antitumor [20] etc. Due to the effects originating from thiophene, the 2-aminothiophene compound has been preferred to include in our target compounds.

In the front molecular orbital analysis, certain reactivity descriptors were utilized, investigated through the DFT approach and B3LYP/6-31G (d,p) level of theory. These descriptors provide important information for determining the chemical reactivity, stability, and behavior of the molecular structure. The ionization potential **(IP)** for the tendency to lose electrons, the electron affinity **(EA)** for the tendency to gain electrons, and the electronegativity **(χ)** values for the ability to attract electrons are examined in a molecule. The resistance a molecule exhibits in reactions is quantified by chemical hardness **(η)**, and sensitivity is determined by chemical softness **(S)**. Chemical potential gauges a molecule's capacity to either lose or gain electrons. A higher global electrophilicity ındex **(ω)** value signifies a more pronounced electrophilic character, while molecular electrostatic potential **(MEP)** specifies electron density around a molecule's nuclei.

2. Material and Method

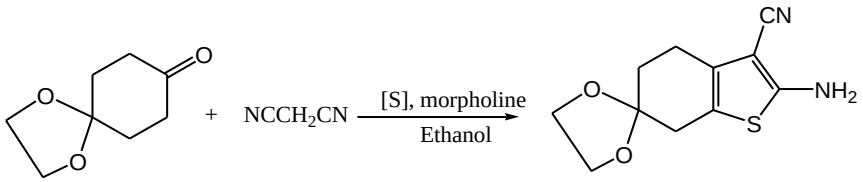
Melting points of all samples were measured using the open capillary method with a Gallenkamp apparatus and were reported without any corrections. Fourier Transform Infrared (FT-IR) spectra, including the Attenuated Total Reflectance (ATR) technique, were obtained from a Thermo Nicolet 6700 Spectrometer in the Chemistry Department of Hitit University. Nuclear magnetic resonance spectra were obtained at Giresun University using a Bruker AVANCE III spectrometer, 400 MHz for ^{1}H-NMR and 100

MHz for ^{13}C/APT-NMR, in $CDCl_3$ (with TMS as the internal standard). The mass analyses of the compounds were determined using Liquid Chromatography-Mass Spectrometry (LC-MS/MS) method with an AB Sciex 3200 Q Trap Mass Spectrometer located at Hitit University Scientific Technical Application and Research Center. The chemicals obtained from Sigma-Aldrich were of high purity, and therefore, no purification process was deemed necessary. The course of reactions was observed by Thin-Layer Chromatography (TLC) on silica gel aluminum sheets with a UV indicator at 254 nm. Antimicrobial activity assays were performed at Amasya University Central Research and Application Laboratory.

2.1. Synthesis Procedures

2.1.1. 2-amino-4,7-dihydro-5H-spiro[benzo[b]thiophene-6,2'-[1,3]dioxolane]-3-carbonitrile (ST)

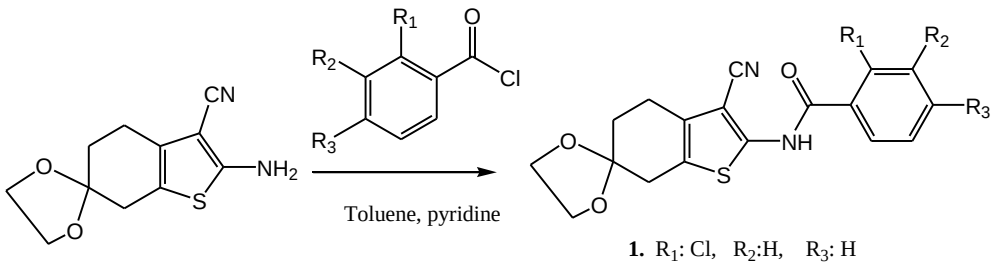
The title compound coded as **ST** was synthesized according to the relevant literature studies [21-23] from 1,4-dioxaspiro[4.5]decan-8-one (0.01 mmol), malononitrile, sulfur (0.01 mmol), and morpholine (0.01 mmol), and used for the synthesis of the chloro benzamide-thiophene compounds **(Scheme 1)**.



Scheme 1. Synthesis of **ST**

2.1.2. 2/3/4-chloro-N-(3-cyano-4,7-dihydro-5H-spiro[benzo[b]thiophene-6,2'-[1,3]dioxolan]-2-yl)benzamide] derivatives (N1-3)

The reaction mixture containing **ST** (1.0 mmol), toluene (1.0 mmol), and pyridine (1.0 mmol) was stirred for approximately 30 minutes at room temperature in a dry nitrogen atmosphere. Subsequently, 2-chlorobenzoyl chloride, 3-chlorobenzoyl chloride or 4-chlorobenzoyl chloride (1.0 mmol) were slowly introduced drop by drop to the reaction flask. The obtained mixture was stirred at 110°C for 6-8 h. It was allowed to cool to room temperature, and then water (20 ml) was added. The product **(N1-3)** was extracted into the organic phase with ethyl acetate (2x20 mL) and then obtained in solid form after the removal of the solvent **(Scheme 2)**.



Scheme 2. Synthetic prodecure of ST-benzamide derivatives **(N1-3)**

2-chloro benzamide derivative of ST (N1) As started in headline 2.1.2, it was prepared on the basis of items ST and 2-chlorobenzoyl chloride. FT-IR ATR, cm^{-1}: 3272, 3214 NH, 2984-2867 aliphatic C-H, 2224 CN, 1670 CO. ^{1}H-NMR spectral data (ppm, $CDCl_3$) : 9.60 (s, 1H, NH), 7.94 (d, 1H, Ar-H) 7.52 (d and m, 3H, Ar-H), 4.06 (s, 4H, OCH_2), 2.91 (s, 2H, CH_2), 2.85 (t, 2H, CH_2), 1.96 (t, 2H, CH_2). ^{13}C-NMR APT (ppm, $CDCl_3$): 162.19, 147.17, 133.10, 131.64, 130.77, 130.34, 127.60, 126.29, 114.05, 108.00, 64.80, 34.52, 31.00, 22.77.

3-chloro benzamide derivative of ST (N2) As started in headline 2.1.2, it was prepared on the basis of items ST and 3-chlorobenzoyl chloride. FT-IR ATR cm^{-1}: 3247, 3207 NH, 2944-2880 aliphatic C-H, 2225 CN, 1669 CO. ^{1}H-NMR spectral data (ppm, $CDCl_3$): 9.43 (s, 1H, NH), 7.96 (s, 1H, Ar-H) 7.83 (d, 1H, Ar-H), 7.58 (d, 1H, Ar-H) 7.46 (t, 1H, Ar-H) 4.06 (s, 4H, OCH_2), 2.89 (s, 2H, CH_2), 2.81 (t, 2H, CH_2), 1.98 (t, 2H, CH_2). ^{13}C-NMR APT (ppm, $CDCl_3$): 162.94, 147.55, 134.99, 133.43, 132.70, 130.43, 130.00, 128.12, 125.85, 114.20, 108,00, 64.79, 34.10, 30.85, 22.67.

4-chloro benzamide derivative of ST (N3) As started in headline 2.1.2, it was prepared on the basis of items ST and 4-chlorobenzoyl chloride. FT-IR ATR cm^{-1}: 3265, 3202 NH, 2989-2890 aliphatic C-H, 2222 CN, 1665 CO. ^{1}H-NMR spectral data (ppm, $CDCl_3$): 9.27 (s, 1H, NH), 7.90 (d, 2H, Ar-H) 7.51 (d, 2H, Ar-H), 4.06 (s, 4H, OCH_2), 2.90 (s, 2H, CH_2), 2.82 (t, 2H, CH_2), 1.99 (t, 2H, CH_2). ^{13}C-NMR APT (ppm, $CDCl_3$): 163.05, 147.92, 139.27, 130.23, 129.28, 129.15, 126.11, 114.46, 107.99, 64.63, 34.38, 30.98, 22.53.

2.2. Theoretical Calculations

All compounds underwent quantum chemical calculations using the Gaussian 09 program package, employing density functional theory (DFT) [24]. The full optimization of the molecular geometries of all compounds was performed using the Becke-3-Lee-Yang-Parr (B3LYP) functional and 6-31 G (d,p) basis set. [25, 26].

2.2.1. Frontier Molecular Orbital Analysis

The highest occupied molecular orbital is known as HOMO, and the lowest unoccupied molecular orbital is known as LUMO. HOMO orbitals, being occupied, have an electron-donating tendency, while LUMO orbitals, being unoccupied, have an electron-accepting tendency. These concepts facilitate the understanding of chemical reactivity and play a crucial role in explaining the mechanisms of chemical reactions. Ionization potential is directly related to HOMO energy, while electron affinity to LUMO energy. Fig. 1 and Table 1 illustrate the computed E_{HOMO} and E_{LUMO} energy values, $\Delta E_{HOMO-LUMO}$ band gaps, and other descriptors for all compounds. As indicated by Figure 1 and Table 1, the energy band gaps ($\Delta E_{HOMO-LUMO}$) of 1, 2 and 3 were found to be 2.5269, 2.4969 and 2.5377 eV at B3LYP/6-31G (d,p) level of theory. The electrophilicity index (ω) is helpful in predicting the formation of chemical bonds in biomolecules, thus aiding in understanding of the mechanisms of chemical reactions.

2.2.2. Molecular Electrostatic Potential Surface

Molecular electrostatic potential surface (MEP) is a concept that illustrates the electron density and electrical interactions surrounding a molecule. In simpler terms, MEP demonstrates how a molecule distributes electron density in space and how this distribution affects the electrical potential on the molecular surface. In our study, MEP potentials of all molecules were modeled using DFT calculations. For geometry optimization in DFT calculations, the B3LYP / 6-31G (d,p) level of theory was employed. The potential values of the molecules in the MEP diagrams provided in Figure 2 are

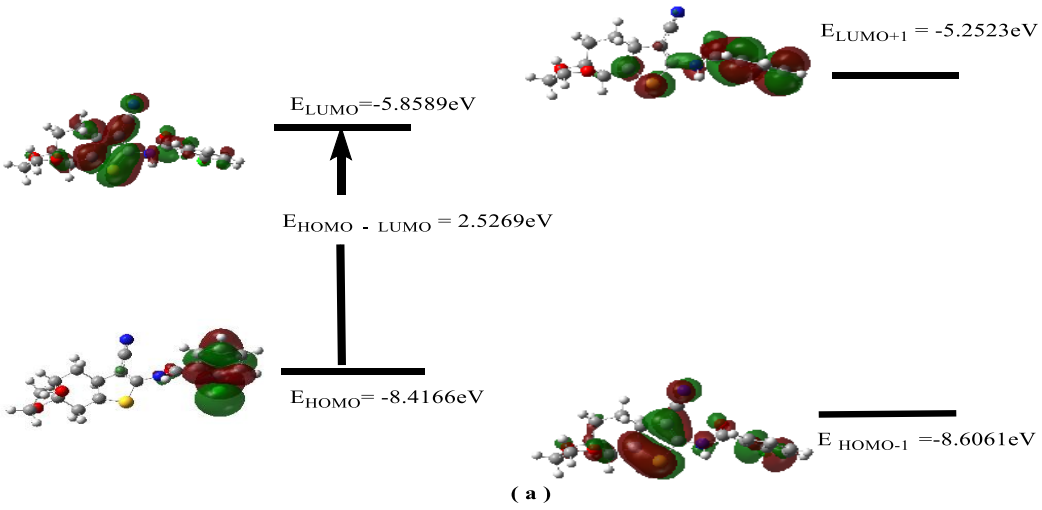
observed to decrease from blue to red. As seen in Figure 2, the negative potential regions are located over the oxygen and nitrogen atoms, whereas positive potential regions are found over the oxygen and sulfur atoms.

Additionally, chemical reactivity descriptors such as chemical potential (μ), chemical hardness (η) and chemical softness (S) were also calculated in this study and shown in **Table 1**. The corresponding values are calculated using the following formulas [27-30].

$$I = -E_{HOMO} \qquad A = -E_{LUMO} \qquad \eta = \frac{1}{2}(E_{LUMO} - E_{HOMO})$$

$$S = \frac{1}{2\eta} \qquad \chi = \frac{1}{2}(I + A) \qquad \omega = \frac{\mu^2}{2\eta}$$

$$\mu = -\frac{1}{2}(I + A) = \frac{1}{2}(E_{HOMO} + E_{LUMO})$$



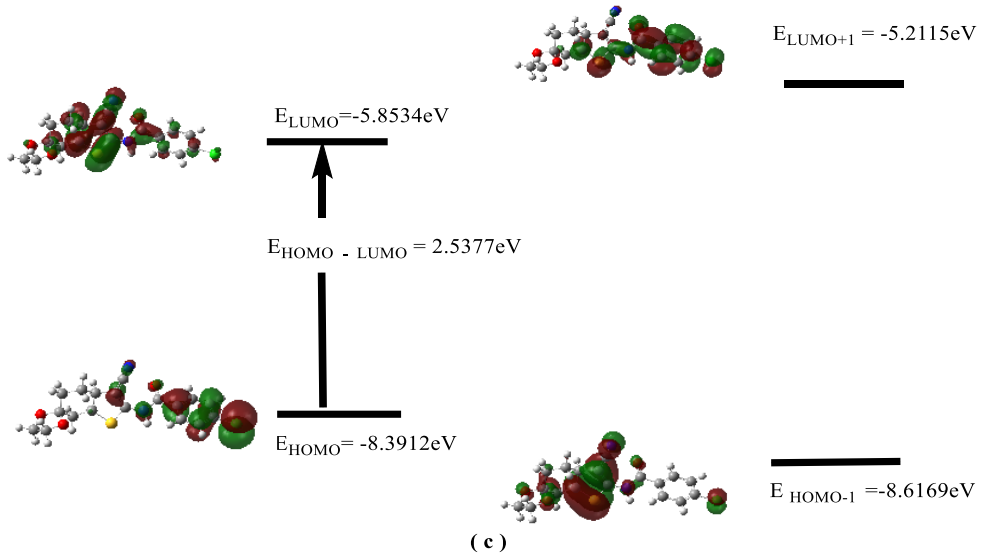
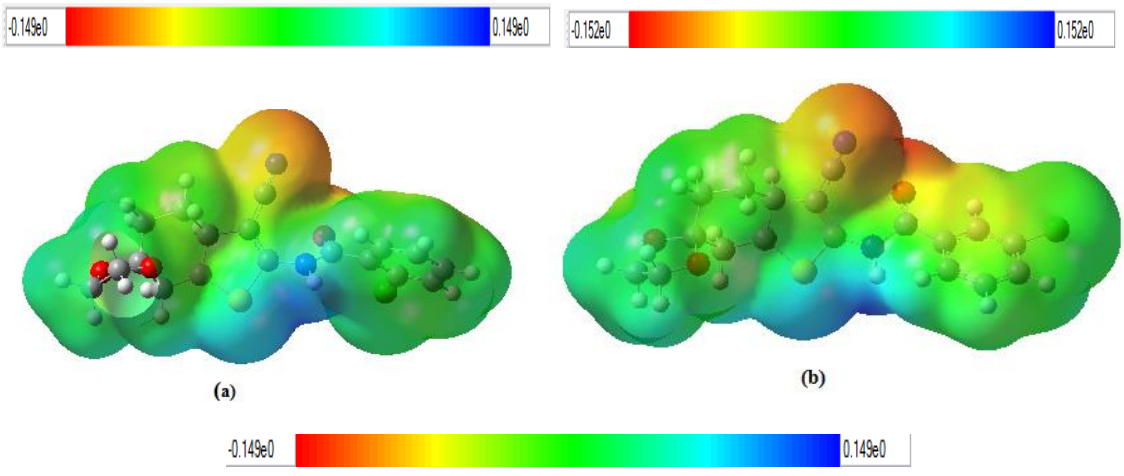


Figure 1. HOMO-LUMO energies of all compounds.

Table 1. Chemical reactivity descriptor result of all compounds

Molecular Properties (eV)	**1**		**2**		**3**	
	a.u.	**ev**	**a.u.**	**ev**	**a.u.**	**ev**
E_{HOMO}	-0.3083	-8.4166	-0.3084	-8.3885	-0.3085	-8.3912
E_{LUMO}	-0.2154	-5.8589	-0.2165	-5.8888	-0.2152	-5.8534
$\Delta E_{HOMO-LUMO}$	0.0929	2.5269	0.0918	2.4969	0.0933	2.5377
E_{HOMO-1}	-0.3164	-8.6061	-0.3167	-8.6142	-0.3168	-8.6169
E_{LUMO+1}	-0.1931	-5.2523	-0.2007	-5.4590	-0.1916	-5.2115
Ionization Potential **(IP)**	0.3083	8.4166	0.3084	8.3885	0.3085	8.3912
Electron Affinity **(EA)**	0.2154	5.8589	0.2165	5.8888	0.2152	5.8534
Chemical Hardness **(η)**	0.0465	1.2788	0.04595	1.2498	0.0466	1.2689
Electronegativity **(χ)**	0.2618	7.1377	0.2625	7.1387	0.2618	7.1223
Chemical Potential **(μ)**	-0.2618	-7.1377	-0.2625	-7.1387	-0.2618	-7.1223
Softness **(S) ev^{-1}**	10.75	0.3909	10.88	0.4000	10.73	0.3940
Electrophilicity index **(ω)**	0.737	19.919	0.749	20.388	0.735	19.988



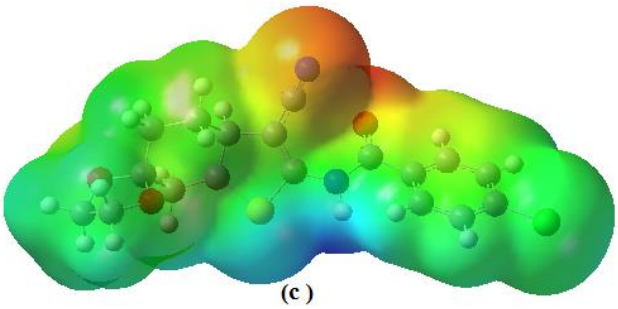


Figure 2. Molecular electrostatic potential surface (MEP) results of all compounds

2.3. Antimicrobial Activity with Disk Diffusion Test

The vulnerability of bacteria to antibiotics was determined on MH (Mueller Hinton) agar using Kirby-Bauer disk diffusion technique, in accordance with the guidelines outlined in CLSI January-2011 (Clinical and Laboratory Standards Institute) documents M02 and M07. MH (Mueller Hinton) agar was used for sterile, disposable, 15 cm diameter petri plates with a medium at a height of 4 mm. A few bacterial colonies that grew as pure colonies on culture plates were collected using a sterile extract and inoculated into MH broth, incubated at 37°C for 1-2 hours. After turbidity was developed, a typical turbidity was established by calibrating it to McFarland 0.5 (108 microorganisms/ml). Extensive sowing was performed from this suspension on MH agar medium using a sterile swab. Dilutions of the substances to be investigated were prepared with DMSO at concentrations of 2-1-0.5 and 0.25 mg/ml, respectively, and paper discs impregnated with 20µl of these dilutions were placed in the medium. 2 mg/ml streptomycin antibiotic was used for positive control. Petri dishes were incubated at 35-37ºC for 18–24 h and then inhibition zone diameters were measured. The results are shown in Table 2, in which inhibition zones are provided in millimeters and expressed as mean ±SE.

Table 2. The results of disk diffusion test

Bacterial İsolates	Mg/mL	Inhibition zone diameters (mm)			
E.Coli ATCC 25922		1	2	3	*Control Streptomycin*
	2	-	-	-	17
	1	-	-	-	
	0.5	-	-	-	
	0.25	-	-	-	
S.Aureus ATCC 25923	2	-	-	-	19
	1	-	-	-	
	0.5	-	-	-	
	0.25	-	-	-	

3. Results

This study has been occurred in two stages. In the first stage, we synthesized 2-amino-4,7-dihydro-5H-spiro[benzo[b]thiophene-6,2'-[1,3]dioxolane]-3-carbonitrile as the starting 1,4-dioxaspiro[4.5]decan-8-one. In the second stage, we prepared amid

compounds **(N1-3)** derivatives. Amit peaks were confirmed by FT-IR, which showed peaks at 3272, 3214 for compound 1, 3247, 3207 for compound 2, and 3265, 3202 for compound 3. Aliphatic C-H peaks were observed between 2989 and 2867 for compounds **(N1-3)**. CN peaks were observed at 2224 cm^{-1}, 2225 cm^{-1} and 2222 cm^{-1} and amide carbonyl peaks were observed 1670 cm^{-1}, 1669 cm^{-1}, 1665 cm^{-1} respectively for compounds **N1**, **N2** and **N3**.

The ^{1}H-NMR spectra of amide compounds have been carried out in $CDCl_3$ at room temperature. The peaks at 9.60, 9.43, and 9.27 ppm were attributed to NH protons. Aromatic protons were observed at 7.94-7.52 ppm for compound **N1**, 7.96-7.46 ppm for compound **N2** and 7.90-7.51 ppm for compound **N3**. Peaks of the amine compound were observed where expected and with expected cleavages. C peaks, observed in the ^{13}C-NMR APT spectra, confirmed the expected structure.

4. Conclusion

E. coli, which is an element of intestinal flora, and *S. aureus Streptomycin* in human skin have been found to have no effect according to the antibiotic reference. In determining and relating the areas of these compounds, it can be said that at least they will not disrupt the intestinal flora or harm the human skin. Additionally, frontier molecular orbital energies, chemical parameters and molecular electrostatic potentials (MEPs) were investigated with DFT approach 6-31G (d,p) level of theory. The *3-chloro benzamide derivative of ST,* **N2**, which has a low ionization potential (8.3885 eV), exhibits high activity.

Authorship contribution statement

N. Çolak: Project Administration, Conceptualization, Methodology; **F. Şahin**: Investigation, Visualization ; **G. Erten**: Original Draft Writing, Review and Editing; **S. M. Muhammet**: Original Draft Writing, Data Curation, Formal Analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics Committee Approval and/or Informed Consent Information

As the authors of this study, we declare that we do not have any ethics committee approval and/or informed consent statement.

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