




## Research Article

# Indole-3-carbaldehyde Semicarbazone Derivatives: Synthesis, Characterization, and Antibacterial Activities

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Four indole-3-carbaldehyde semicarbazone derivatives, 2-((5-bromo-1H-indol-3-yl)methylene)hydrazinecarboxamide (**1**), 2-((5-chloro-1H-indol-3-yl)methylene)hydrazinecarboxamide (**2**), 2-((5-methoxy-1H-indol-3-yl)methylene)hydrazinecarboxamide (**3**), and 2-((4-nitro-1H-indol-3-yl)methylene)hydrazinecarboxamide (**4**) were synthesized and characterized by ESI-MS and spectroscopic (FT-IR, <sup>1</sup>H NMR, and <sup>13</sup>C NMR) techniques. The two-dimensional NMR (in acetone-d<sub>6</sub>) spectral data revealed that the molecules **1** and **2** in solution are in the *cisE* isomeric form. This evidence is supported by DFT calculations at the B3LYP/6-311++G(d,p) level of theory where it was shown that the corresponding most stable conformers of the synthesized compounds have a *cisE* geometrical configuration, in both the gas and liquid (acetone and DMSO) phases. The *in vitro* antibacterial activity of compounds **1–4** was determined against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria. Among all the tested semicarbazones, **1** and **2** exhibited similar inhibitory activities against *Staphylococcus aureus* (MIC = 100 and 150 µg/mL, respectively) and *Bacillus subtilis* (MIC = 100 and 150 µg/mL, respectively). On the other hand, **3** and **4** were relatively less active against the tested bacterial strains compared with **1**, **2**, and tetracycline.

## 1. Introduction

Diseases caused by bacteria have gained considerable attention because of their resistance to the standard antibacterial drugs [1]. Bacterial resistance is a natural process

that occurs in all microorganisms and is enhanced due to the inappropriate use of drugs intended for the control of bacterial infections [2], leading to increased mortality in humans [3]. On the other hand, the World Health Organization has reported that the Gram-negative strains

*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* are critical-risk microorganisms, priority 1 [4]. *Pseudomonas aeruginosa* is one of the main pathogens involved in nosocomial infections and immunosuppressed patients [5–8]. Besides, this bacterium is responsible for bloodstream infections [9]. *Escherichia coli* is a type of bacteria that belongs to the *Enterobacteriaceae* family. These bacteria live in our intestines, but some strains can cause diarrheal infections, meningitis, and urinary tract infections when people eat contaminated food [10]. On the other hand, *Staphylococcus aureus* is reported as a high-risk microorganism, priority 2 (WHO) [4], and is considered one of the most important pathogens of the human being, which is associated with skin infections, pneumonia, osteomyelitis, and endocarditis, among others [11–13]. Recent studies carried out against *Staphylococcus aureus*, *E. coli*, and *P. aeruginosa* bacteria have shown that the mode of action of some semicarbazone and thiosemicarbazone derivatives is due to their ability to cause the rupture of the cell membrane [14].

In recent years, interest in the synthesis of semicarbazones ( $R^1C=N-NH-(C=O)-NHR^2$ ) has increased due to the facility to replace the  $R^1$  and  $R^2$  substituent groups by alkyl, aryl, or heterocyclic derivatives, thus leading to a broad spectrum of new ligands with donor atoms (N,O), capable of coordinating to metal centres [15–17]. In addition to this, semicarbazones possess a variety of biological properties including antibacterial [18, 19], anticonvulsant [20], anti-tubercular [21], and anticancer [15, 16, 22] activities.

Recently, it has been reported that some of 1-(2-oxo-2-phenyl-ethyl)-2-phenyl-1H-indol-3-yl) methylene) semicarbazone derivatives exert good antifungal activity against *Candida albicans* and *C. rugosa* strains and exhibited moderate antibacterial activity (MIC = 37.5–150  $\mu\text{g/mL}$ ) against *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, and *Klebsiella pneumoniae* strains [19].

This paper reports the synthesis, spectral characterization, and *in vitro* antibacterial activity of four indole-3-carbaldehyde semicarbazone derivatives, 2-((5-bromo-1H-indol-3-yl)methylene)hydrazinecarboxamide (1), 2-((5-chloro-1H-indol-3-yl)methylene)hydrazinecarboxamide (2), 2-((5-methoxy-1H-indol-3-yl)methylene)hydrazinecarboxamide (3), and 2-((4-nitro-1H-indol-3-yl)methylene)hydrazinecarboxamide (4). Theoretical IR data (as comparative data) and energy values to determine the most stable conformers of the synthesized compounds 1–4 have been obtained with DFT calculations, using the B3LYP functional with the 6-311++G(d,p) basis set in both gas and liquid phases (acetone and DMSO). It is known that the level of theory employed in this work provides reliable molecular structures [23].

## 2. Materials and Methods

**2.1. Chemicals and Instrumentation.** All reagents and solvents were purchased from Merck and Sigma-Aldrich and used without further purification. The tested bacterial strains were Gram-positive (*S. aureus* ATTC25923 and *B. subtilis* ATTC11774) and Gram-negative (*E. coli* ATTC25922 and

*P. aeruginosa* ATTC278533), which were obtained from the biology laboratory of the Department of Sciences, Universidad Nacional Santiago Antúnez de Mayolo. Antibacterial assays were carried out using the microdilution method according to the M07-A8 protocol followed at the Clinical & Laboratory Standards Institute [24].

Infrared (IR) spectra were recorded using a Nicolet iS10 Fourier Transform Infrared (FT-IR) spectrometer equipped with an attenuated total reflectance accessory using a diamond crystal. The measurements were obtained in absorbance mode, recorded for 32 scans at a resolution of  $4\text{ cm}^{-1}$ . All the measurements were carried out with a baseline automatic correction.

NMR spectra ( $^1\text{H}$  and  $^{13}\text{C}$ ) were recorded on an Agilent instrument (500 MHz for  $^1\text{H}$  or 126 MHz for  $^{13}\text{C}$ ) or a Bruker AVANCE™ spectrometer (600 MHz for  $^1\text{H}$  or 150 MHz for  $^{13}\text{C}$ ), using DMSO- $d_6$  or acetone- $d_6$ , as dissolution medium. All the  $^1\text{H}$  NMR spectra were obtained with the instrumental settings such as number of scans (8 and 8–16), acquisition time (2.044 and 1.363 s), and resolution (0.489 and 0.734 Hz), for the Agilent and Bruker AVANCE™ spectrometers, respectively. The  $^{13}\text{C}$  NMR spectra were obtained with the following parameters: number of scans (2500–10000 and 2–4), acquisition time (1.0486 s and 0.456 s), and resolution (0.953 and 2.211 Hz), for the Agilent and Bruker AVANCE™ spectrometers, respectively. The chemical shifts were measured in ppm relative to tetramethylsilane (SIME $_4$ ). The coupling constant ( $J$ ) is expressed in Hertz (Hz) while the splitting of proton resonances is defined as s = singlet, d = doublet, t = triplet, and m = multiplet. ESI mass spectra of the synthesized compounds were recorded on the Waters-Quattro Premier XE™ tandem quadrupole and VG Micromass ZAB-2F mass spectrometers, using methanol as the sample dissolution medium.

### 2.2. Experimental Procedures

**2.2.1. General Method.** A solution of sodium acetate (164 mg, 2 mmol) in water (10 mL) was added drop by drop into a hot solution of hydrochlorinated semicarbazide (110 mg, 1 mmol) in methanol: water (100 mL, v/v, 1 : 1). To the resulting mixture was added the respective indole-3-carboxaldehyde derivative (2 mmol) in ethanol (10 mL). The reaction mixture was refluxed for 3 h and stirred for 24 h at room temperature. After the slow evaporation of the solvent at room temperature, the solid product was filtered, washed several times with hot water and cold ethanol, dried *in vacuo*, and then recrystallized from a methanol:acetone mixture (10 mL, v/v, 1 : 1).

2-((5-Bromo-1H-indol-3-yl)methylene)hydrazinecarboxamide (1). Light yellow solid; yield: 0.146 g (56%); M.P: 221°C–223°C; FT-IR ( $\nu$ ,  $\text{cm}^{-1}$ ): 3428.58, 3379.89, 3332.36, 3225.74 (N–H), 3105.83 ( $C_{ar}$ -H), 1615.14 (C=O), 1536.33 (C=N), and 1099.51 ( $C_{ar}$ -Br); MS/ESI ( $m/z$ ): calculated for  $C_{10}H_9N_4O^{79}\text{Br}$  280.12, found: 279.12 [M-H] $^-$ , calculated for  $C_{10}H_9N_4O^{81}\text{Br}$  282.11, found: 281.189 [M-H] $^-$ ;  $^1\text{H}$  NMR (500 MHz, acetone- $d_6$ ),  $\delta$  ppm: 10.81 (s, 1H, NH-indole),

9.19 (d, 1H,  $J = 3.4$  Hz, =N–NH), 8.37 (d, 1H,  $J = 2.2$  Hz, H4), 8.18 (s, 1H, CH=N), 7.75 (d, 1H,  $J = 3.3$  Hz, H2), 7.45 (d, 1H,  $J = 8.5$  Hz, H7), 7.33 (dd, 1H,  $J = 8.5, 2.2$  Hz, H6), and 5.98 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (126 MHz, acetone-d<sub>6</sub>),  $\delta$  ppm: 157 (C=O); 137.2 (CH=N); 136.2 (C8, Ar), 129.8 (C2, Ar), 126.3 (C9, Ar), 125.4 (C6, Ar), 123.9 (C4, Ar), 113.5 (C7, Ar), 113.3 (C5–Br, Ar), and 112.2 (C3, Ar).

2-((5-Chloro-1H-indol-3-yl)methylene)hydrazinecarboxamide (2). Colorless solid; yield: 0.144 g (61%); M.P: 210°C–211°C; FT-IR ( $\nu$ , cm<sup>-1</sup>): 3453.02, 3385.82, 3335.47, 3229.61 (N–H), 3107.59 (C–H<sub>ar</sub>), 1615 (C=O), 1536.66 (C=N), and 1102.97 (C<sub>ar</sub>-Cl); MS/ESI ( $m/z$ ): calculated for C<sub>10</sub>H<sub>9</sub>N<sub>4</sub>O<sup>35</sup>Cl 236.17, found: 235.08 [M–H]<sup>-</sup>, calculated for C<sub>10</sub>H<sub>9</sub>N<sub>4</sub>O<sup>37</sup>Cl 238.16, found: 237.09 [M–H]<sup>-</sup>; <sup>1</sup>H NMR (500 MHz, acetone-d<sub>6</sub>),  $\delta$  ppm: 10.75 (s, 1H, NH-indole), 9.13 (br, 1H, =N–NH), 8.21 (d, 1H,  $J = 3.1$  Hz, H4), 8.18 (s, 1H, CH=N), 7.75 (d, 1H,  $J = 3.1$  Hz, H2), 7.48 (d, 1H,  $J = 9.3$  Hz, H7), 7.20 (dd, 1H,  $J = 9.3, 3.1$  Hz, H6), and 6.01 (s, 2H, NH<sub>2</sub>). <sup>13</sup>C NMR (126 MHz, acetone-d<sub>6</sub>),  $\delta$  ppm: 156.8 (C=O); 137.1 (C=N); 135.8 (C8, Ar), 130.0 (C2, Ar), 125.8 (C5–Cl, Ar), 125.6 (C9, Ar), 122.9 (C6, Ar), 120.9 (C4, Ar), 113.1 (C7, Ar), and 112.2 (C3, Ar).

2-((5-Methoxy-1H-indol-3-yl)methylene)hydrazinecarboxamide (3). Orange solid; yield: 0.148 g (64%); M.P: 185°C–186°C; FT-IR ( $\nu$ , cm<sup>-1</sup>): 3476.01, 3306.70 (N–H), 1681.33 (C=O), and 1579.51 (C=N); EMAR-ES ( $m/z$ ): calculated for C<sub>11</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub> 232.09, found: 255.10 [M + Na]<sup>+</sup>; <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>),  $\delta$  ppm: 11.31 (s, 1H, NH-indole), 9.87 (s, 1H, =N–NH), 8.07 (s, 1H, CH=N), 7.65 (s, 1H, H4), 7.56 (d, 1H,  $J = 2.3$  Hz, H2), 7.32 (d, 1H,  $J = 8.8$  Hz, H7), 6.83 (dd, 1H,  $J = 8.8, 2.5$  Hz, H6), 6.17 (s, 2H, NH<sub>2</sub>), and 3.79 (s, 3H, OCH<sub>3</sub>). <sup>13</sup>C NMR (150 MHz, DMSO-d<sub>6</sub>),  $\delta$  ppm: 157.4 (C=O); 154.7 (C5–OCH<sub>3</sub>); 138.2 (CH=N); 132.4 (C9, Ar), 129.9 (C8, Ar), 125.0 (C2, Ar), 112.9 (C4, Ar), 112.6 (C6, Ar), 111.8 (C3, Ar), 103.8 (C7, Ar), and 55.7 (OCH<sub>3</sub>).

2-((4-Nitro-1H-indol-3-yl)methylene)hydrazinecarboxamide (4). Orange solid; yield: 0.187 g (76%); M.P: >250°C; FT-IR ( $\nu$ , cm<sup>-1</sup>): 3424.33, 3185.67 (N–H); 1675.96 (C=O), 1573.72 (C=N), and 1509.86 (C=N–NO<sub>2</sub>); EMAR-ES ( $m/z$ ): calculated for C<sub>10</sub>H<sub>9</sub>N<sub>5</sub>O<sub>3</sub> 247.06, found: 270.10 [M + Na]<sup>+</sup>; <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>),  $\delta$  ppm: 12.35 (s, 1H, NH-indole), 10.04 (s, 1H, =N–NH), 8.23 (s, 1H, CH=N), 8.18 (s, 1H, H2), 7.84 (d, 1H,  $J = 8.0$  Hz, H5), 7.78 (d, 1H,  $J = 7.7$  Hz, H7), 7.33 (t, 1H,  $J = 7.9$  Hz, H6), and 6.25 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (150 MHz, DMSO-d<sub>6</sub>),  $\delta$  ppm: 157.3 (C=O); 136.30 (CH=N); 142.8 (C4–NO<sub>2</sub>, Ar), 139.5 (C8, Ar), 131.7 (C2, Ar), 121.5 (C6, Ar), 118.6 (C5, Ar), 117.5 (C7, Ar), 116.2 (C3, Ar), and 110.9 (C9, Ar).

2.2.2. *Antibacterial Tests.* The in vitro antibacterial activity of the semicarbazone derivatives **1–4** was investigated against the standard strains of Gram-positive (*Staphylococcus aureus* ATCC25923 and *Bacillus subtilis* ATCC11774) and Gram-negative (*Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853) bacteria. In order to compare the results, tetracycline was used as standard drug. The antibacterial assays were carried out by the dilution method. To prepare the initial inoculum (IIB), two fresh

bacterial colonies were suspended for 14 h in a solution of NaCl 0.8% until obtaining an optical density by 0.08–0.1 at a wavelength of 620 nm. Tetracycline (Sigma) was used as the standard antibiotic. Each assay was performed in triplicate, and reproducibility was evaluated twice.

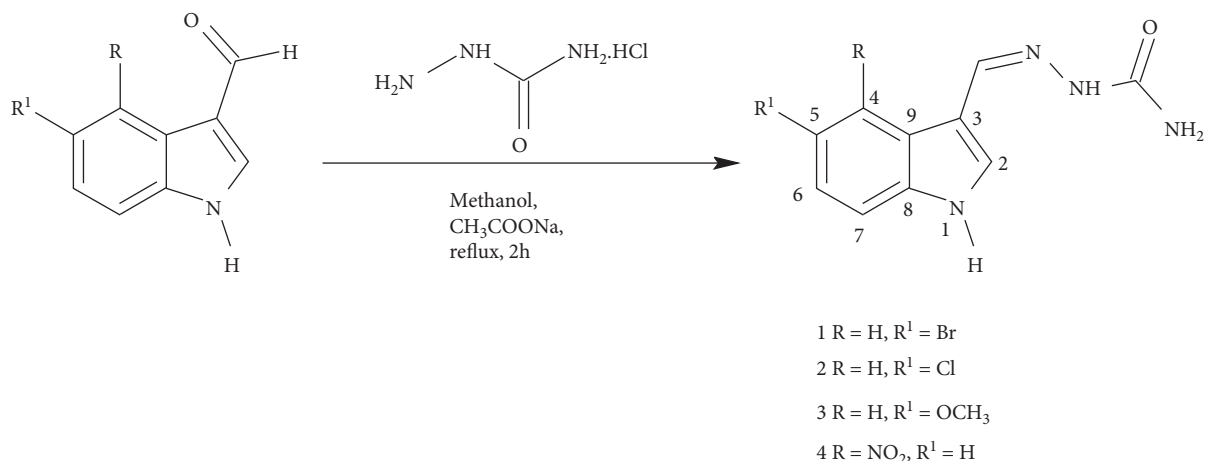
The compounds were dissolved in DMSO at 50  $\mu\text{g}/\mu\text{L}$  as stock solution, which was diluted with Müller–Hinton broth II (MHBII, Difco) at concentrations of 12.5, 25, 50, 100, 150, and 200  $\mu\text{g}/\text{mL}$ . Serial dilutions were placed inside the 96-well microplates (100  $\mu\text{L}/\text{well}$ ) and were inoculated with 10  $\mu\text{L}$  of solution of IIBd (IIB diluted in MHBII, 1:20). Positive growth controls were prepared using the dilutions of the compounds inoculated with bacteria-free MHBII. The microplates were incubated for 24 h at 35°C. Tetrazolium salt was used as a growth indicator [25], and 10  $\mu\text{L}$  of a solution of tetrazolium violet 0.1% (TV, Sigma T0138) was added to each microplate. Then, these microplates were incubated under darkness for 4 h at 35°C. The TV indicator is reduced to a violet precipitate when there is bacterial respiratory activity. Minimum inhibitory concentration (MIC) value is considered as the lowest extract concentration that inhibits bacterial growth, which is reflected by the absence of the violet precipitate.

2.2.3. *Computational Details.* The quantum chemical calculations were carried out using the Gaussian 09 and D01 software packages [26]. The geometries of the synthesized compounds were optimized using B3LYP functional and the 6-311++G(d,p) basis set without symmetry restrictions [27–29]. Harmonic vibrational frequencies were obtained at the same level without scaling to verify that all the stationary points are minimal. The computed energies and enthalpies for the most stable species studied were calculated in both the gas and liquid phases (acetone and DMSO solvents), using the polarizable continuum model [29, 30]. The conformational analysis of these species was also explored, and the corresponding population distribution values were determined using the Boltzmann distribution [27]. For more details, see Table S1 of Supporting Information.

### 3. Results and Discussion

3.1. *Synthesis and Characterization.* Compounds **1–4** were prepared according to literature [31], as shown in Scheme 1. The semicarbazone derivatives were obtained in satisfactory yields (56–76%) and characterized by FT-IR, ESI mass spectrometry, and NMR (<sup>1</sup>H, <sup>13</sup>C) spectroscopy. Spectroscopic data obtained for the synthesized semicarbazones are in agreement with the proposed structures. All the synthesized compounds were recrystallized from a methanol: acetone mixture (10 mL, v/v, 1:1), before characterization.

3.2. *Infrared Spectra.* The IR spectra of the compounds **1–4** showed absorption bands in the range of 3200–3450 cm<sup>-1</sup> which are assigned to the N–H groups of the indole ring and terminal amine group (–NH<sub>2</sub>) [27, 32]. The absorption bands corresponding to the carbonyl group (C=O) appeared at 1639–1681 cm<sup>-1</sup> [27, 32, 33]. This observed band indicates



Scheme 1: Synthesis of the indole-3-carbaldehyde semicarbazone derivatives (1–4).

the presence of the keto tautomer in the solid state [34]. An intense sharp band was observed at  $1536\text{--}1580\text{ cm}^{-1}$  due to vibration of the imine  $\text{C}=\text{N}$  stretching frequency [27]. For compounds 3 and 4, the presence of the  $\text{OCH}_3$  and  $\text{NO}_2$  substituents in the C5 and C4 positions, of the indole moiety, generated shifts of the  $\nu(\text{C}=\text{N})$  bands to lower frequencies ( $37\text{--}43\text{ cm}^{-1}$ ). A good linear correlation of vibrational frequencies (given as wave number  $\sigma$ , in  $\text{cm}^{-1}$ ) is obtained between these experimental IR data and the corresponding to B3LYP/6-311++G(d,p) theoretical values (see Supporting Information).

**3.3. NMR Spectra.** The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of the synthesized compounds were recorded in acetone- $d_6$  and DMSO- $d_6$ , respectively. In the  $^1\text{H}$  NMR spectra of compounds 1–4, the signal of the  $\text{HC}=\text{N}$  proton appeared as a singlet at  $\delta = 8.07\text{--}8.23$ , while the signal of the  $=\text{N}-\text{NH}$  appears as a broad singlet at  $\delta = 9.13\text{--}10.04$ . These results are similar with the chemical shifts reported for other benzaldehyde and phenoxyphenyl semicarbazone derivatives with the OH,  $\text{CH}_3$ , Br, and  $\text{NO}_2$  substituents at the phenyl ring [31, 35]. The resonance lines of the protons corresponding to the indole ring were observed at  $\delta = 6.83\text{--}8.37$ . For compounds 1 and 2, the aromatic proton signals of the indole fragment bound to the  $\text{HC}=\text{N}$  group were affected by the presence of the *bromo* and *chloro* substituents on the C5 position of the indole moiety. These signals are shifted downfield for the protons on the C4 (0.95 and 0.79 ppm, respectively), C6 (0.20 and 0.07 ppm, respectively), and C7 (0.77 and 0.74 ppm, respectively) positions, compared to the unsubstituted indole moiety [36]. For compound 3, the presence of the *methoxy* substituent group on the C5 position of the indole moiety affected also the resonance signals of the aromatic protons. These signals are shifted upfield for the protons on the C2 (0.24 ppm), C6 (0.30 ppm), and C7 (0.90 ppm) positions, while for the proton in the C4 position, this is shifted 0.23 ppm upfield, with respect to the unsubstituted indole moiety. For compound 4 with the *nitro* substituent group on the C4 position of the indole moiety, the signals of the aromatic protons on the C2, C5, and C6 positions are shifted 0.38, 0.65,

and 0.20 ppm downfield, respectively, while the signal of the proton on the C7 position is shifted 0.44 ppm upfield, with respect to the unsubstituted indole ring. For compounds 1, 2, and 3, the presence of the *bromo*, *chloro*, and *methoxy* substituents on the C5 position affected the resonance signals of the NH indole proton. These signals are shifted upfield (0.78, 0.84, and 0.28 ppm, respectively) while for compound 4, the signal of the NH indole proton is shifted 0.76 ppm downfield compared to the unsubstituted indole moiety [36]. These results are in agreement with the chemical shifts observed for other acylhydrazone derivatives, ((*E*)-*N'*-((5-bromo-1H-indol-3-yl)methylene)isonicotinohydrazide, (*E*)-*N'*-((4-nitro-1H-indol-3-yl)methylene) isonicotinohydrazide, and melatonin [24, 34].

The  $\text{NH}_2$  protons of the thioamide group appeared as a broad singlet in the region  $\delta = 5.98\text{--}6.25$ , in agreement with the chemical shifts found for other semicarbazone derivatives with terminal amine groups [31].

In the  $^{13}\text{C}$  NMR spectra of compounds 1–4, the carbon resonance signals of the  $\text{C}=\text{N}$  group appear at  $\delta = 136.3\text{--}138.5$ . These results are similar to the chemical shifts found for other carbazone derivatives with imidazole fragment [27]. The signals observed at  $\delta = 156.5\text{--}157.4$  are characteristic for the CO carbonyl group present in all compounds. The aromatic carbons of the indole ring were observed at  $\delta = 103.8\text{--}142.8$  ppm, and these chemical shifts are in agreement with those found for other indole and carbazone derivatives [27, 32, 37]. On the other hand, the  $\text{OCH}_3$  methoxy carbon signal for compound 3 appeared at 55.7 ppm [37].

The two-dimensional  $^1\text{H}-^1\text{H}$  NOESY spectra recorded in acetone- $d_6$  for compounds 1 (Figure 1) and 2 show coupling between the imino proton ( $-\text{CH}=\text{N}$ ) and the hydrazine proton ( $=\text{N}-\text{NH}$ ). These results are in agreement with the *E* configurational isomer found for other semicarbazone derivatives, whose chemical shifts of the hydrazine protons are in the range of 9.13–10.04 ppm [38, 39]. In addition, in the two-dimensional  $^1\text{H}-^1\text{H}$  NOESY spectrum recorded for the compound 1, no coupling is observed between the hydrogens of the ( $\text{NH}_2$ ) terminal amine and  $=\text{N}-\text{NH}$  hydrazine groups, which confirms the existence of the *cis* conformational isomer.

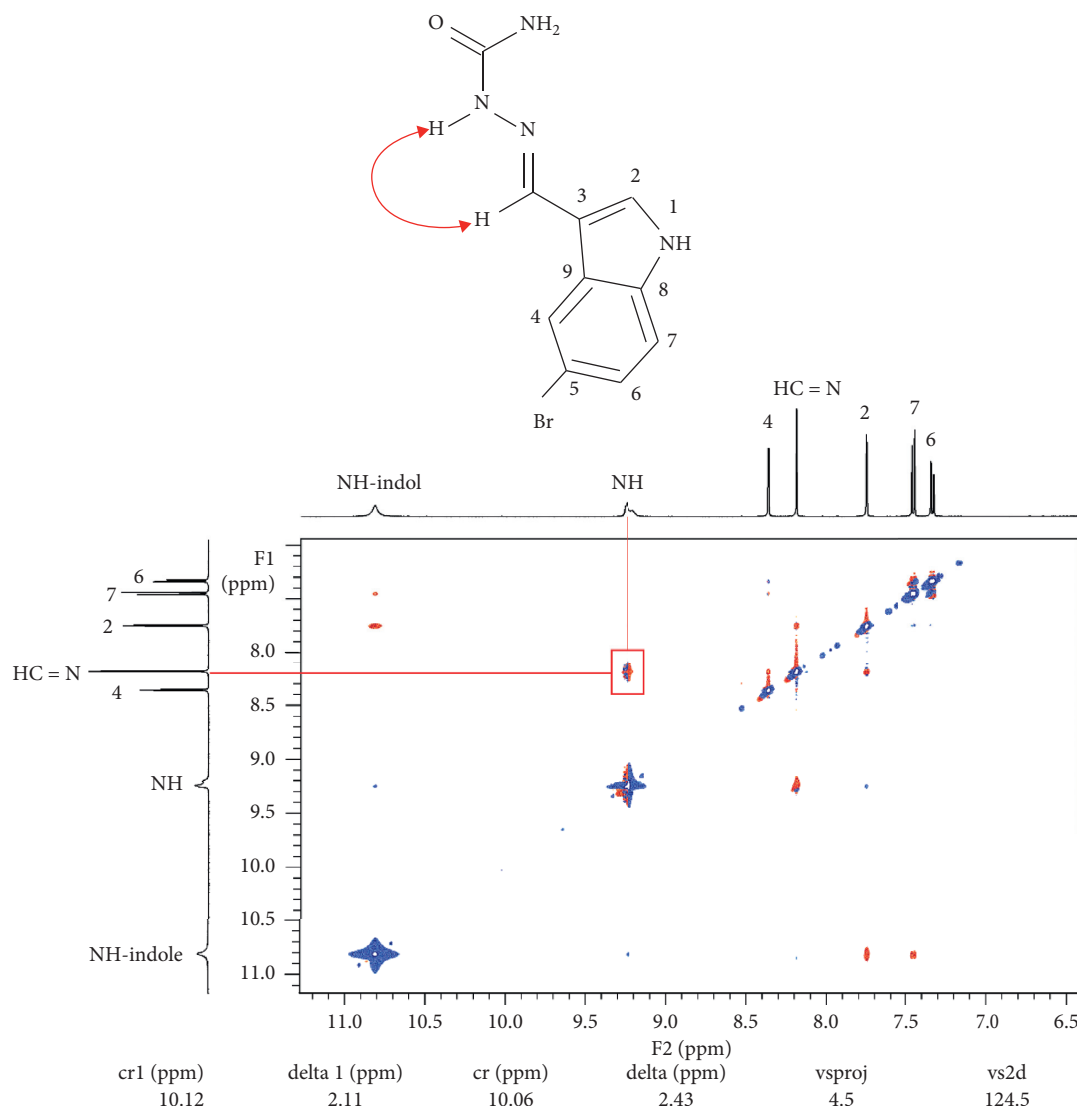


FIGURE 1: Two-dimensional  $^1\text{H}$ - $^1\text{H}$  NOESY NMR spectrum (500 MHz) recorded in acetone- $d_6$  for the compound **1**.

**3.4. DFT Calculations.** The synthesized compounds **1–4** present an indole ring, a side chain at the C3 position (carbaldehyde semicarbazone moiety), and substituents C5-Br (**1**), C5-Cl (**2**), C5-OCH<sub>3</sub> (**3**), and C4-NO<sub>2</sub> (**4**) (see Scheme 1). The *Z/E* isomerism was considered with respect to the C=N bond and *cis/trans* conformers with respect to the CONH amide group [40]. For each studied compound and from its isomeric-conformational analysis, the theoretical calculations have shown that the most stable conformer has a *cisE* geometrical configuration, where the rotation (around C3-CN bond) of side chain is characterized by the dihedral angle  $\Phi$  (see Figure 2). This conformer represents more than 97% of the corresponding conformational population, in both the gas and liquid phases (acetone and DMSO) (see Table 1). Thus, the rest of the conformers considered (*trans E*, *cis Z*, and *trans Z*) display high enthalpy differences relative to the corresponding most stable one (greater than 4 kJ mol<sup>-1</sup>). It is important to

mention that the *cisE* configuration obtained for the most stable **1** and **2** conformers is supported by the two-dimensional  $^1\text{H}$ - $^1\text{H}$  NOESY spectra.

In the most stable conformers, **1–3**, the side chain and the indole moiety present an almost coplanar geometry because the dihedral angle  $\Phi$  is close to 180°, while the side chain of compound **4** is out of the indole plane ( $\Phi = 156^\circ$ ). This result would be related with the inductive and resonant effects of the substituent group NO<sub>2</sub> (strong  $\pi$ -withdrawing) which would reflect also the presence of a high dipolar moment ( $\mu = 9.8$  D) of the most stable conformer **4**. The presence of the OCH<sub>3</sub> ( $\pi$ -donating) and Cl and Br (both  $\pi$ -donating/withdrawing) substituent groups for compounds **1**, **2**, and **3**, respectively, causes a  $\pi$ -electron delocalization on their side chains due to the following obtained dipolar moments:  $\mu$  (**3**) <  $\mu$  (**1**)  $\approx$   $\mu$  (**2**) <  $\mu$  (**4**) (see Table 2). This behavior was observed in the gas phase and in both acetone and DMSO solvents.

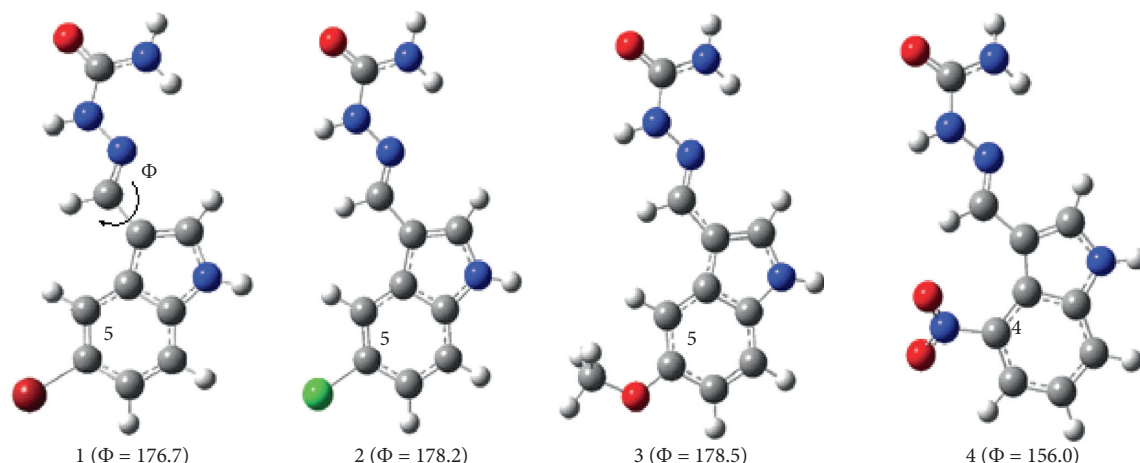


FIGURE 2: Molecular geometry of the most stable *cisE* conformers of 1–4 optimized at the B3LYP/6–311++G(d,p) level of theory. Dihedral angle  $\Phi$  in degrees.

TABLE 1: Computational results, at the B3LYP/6–311++G(d,p) level of theory, for the synthesized compounds 1–4.

| Conformers              | Compounds   |                     |             |                     |             |                     |             |                     |
|-------------------------|-------------|---------------------|-------------|---------------------|-------------|---------------------|-------------|---------------------|
|                         | 1           |                     | 2           |                     | 3           |                     | 4           |                     |
|                         | <i>cisE</i> | Others <sup>a</sup> | <i>cisE</i> | Others <sup>a</sup> | <i>cisE</i> | Others <sup>a</sup> | <i>cisE</i> | Others <sup>a</sup> |
| Gas phase               |             |                     |             |                     |             |                     |             |                     |
| $\Delta H^b$            | 0           | >5                  | 0           | >5                  | 0           | >4                  | 0           | >11                 |
| $\mu^c$                 | 6.6         | $\leq 8.6$          | 6.6         | $\leq 7.8$          | 5.0         | $\leq 6.4$          | 9.8         | $\leq 10.6$         |
| Population <sup>d</sup> | 98.4        | $\leq 1.6$          | 98.3        | $\leq 1.7$          | 97.1        | $\leq 2.9$          | 99.3        | $\leq 0.7$          |
| DMSO                    |             |                     |             |                     |             |                     |             |                     |
| $\Delta H^b$            | 0           | >8                  | 0           | $\geq 9$            | 0           | >7                  | 0           | >10                 |
| $\mu^c$                 | 8.9         | $\leq 12.4$         | 9.0         | $\leq 11.8$         | 6.6         | $\leq 9.4$          | 13.9        | $\leq 16.2$         |
| Population <sup>d</sup> | 99          | $\leq 0.9$          | 99.1        | $\leq 0.7$          | 98.8        | $\leq 1.1$          | 99.5        | $\leq 0.3$          |
| Acetone                 |             |                     |             |                     |             |                     |             |                     |
| $\Delta H^b$            | 0           | >8                  | 0           | $\geq 9$            | 0           | >7                  | 0           | >10                 |
| $\mu^c$                 | 8.8         | $\leq 12.2$         | 8.9         | $\leq 11.6$         | 6.6         | $\leq 9.2$          | 13.8        | $\leq 15.8$         |
| Population <sup>d</sup> | 99          | $\leq 0.9$          | 99.1        | $\leq 0.8$          | 98.8        | $\leq 1.1$          | 99.4        | $\leq 0.5$          |

<sup>a</sup>*trans E*, *cis Z*, and *trans Z* conformers. <sup>b</sup>Relative enthalpy of stable conformer, in  $\text{kJ}\cdot\text{mol}^{-1}$ . <sup>c</sup>Electric dipolar moment, in D (debye). <sup>d</sup>Equilibrium molar fractions or conformational population in %.

TABLE 2: Minimum inhibitory concentration (MIC) values obtained for the compounds 1–4 and tetracycline against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains.

| Compound     | MIC ( $\mu\text{g}/\text{mL}$ ) |                      |                  |                    |
|--------------|---------------------------------|----------------------|------------------|--------------------|
|              | <i>E. coli</i>                  | <i>P. aeruginosa</i> | <i>S. aureus</i> | <i>B. subtilis</i> |
| 1            | >200                            | >200                 | 100              | 100                |
| 2            | >200                            | >200                 | 150              | 150                |
| 3            | >200                            | >200                 | >200             | >200               |
| 4            | >200                            | >200                 | >200             | >200               |
| Tetracycline | <12.5                           | <12.5                | <12.5            | 12.5               |

**3.5. Antibacterial Activity.** The *in vitro* antibacterial activities of the compounds 1–4 were studied along that of tetracycline (standard antibacterial drug). The microorganisms used in this work included *S. aureus* and *B. subtilis* (as Gram-positive bacteria) and *E. coli* and *P. aeruginosa* (as Gram-negative bacteria), and the results are presented in Table 2.

Comparing the bacterial activities of the semicarbazone derivatives and the standard drug, it became evident that compounds 1 and 2 exhibited moderate inhibitory activities against *S. aureus* (MIC = 100 and 150  $\mu\text{g}/\text{mL}$ , respectively) and *B. subtilis* (MIC = 100 and 150  $\mu\text{g}/\text{mL}$ , respectively) [41, 42], as compared to tetracycline [43] (in this work). This same effect was obtained when comparing with the indole-2-carbaldehyde-semicarbazone derivative, 2-((3-chloro-1H-indol-yl)methylene)-1-(3,5-diamine-4-propionate-2-carbonylthiophene) hydrazine, assayed against *Staphylococcus aureus* and *Bacillus subtilis* (MIC = 5 and 6  $\mu\text{g}/\text{mL}$ , respectively) [44, 45]. The results found in this work indicate that the position of Br and Cl atoms on the indole rings plays an important role in inhibiting bacterial growth. The presence of bromine on the fifth position of indole may have a contribution in increasing the lipophilic character of the compound 1, facilitating transport across the microorganism cell membrane and increasing antimicrobial activity [46]. This antimicrobial behavior was also observed for 3-imino-[4-benzylidene-2-phenyl-imidazole-5-one-1-(4-benzoylhydrazono)]-X-indole-

2-one (X = Cl, F, Br) derivatives assayed against *Staphylococcus aureus* (X = Cl, F: MIC = 14  $\mu\text{g}/\text{mL}$ ; X = Br: MIC = 12  $\mu\text{g}/\text{mL}$ ) [47]. On the other hand, the compounds **3** and **4** with the OCH<sub>3</sub> and NO<sub>2</sub> substituents on the C5 and C4 positions of the indole moiety, respectively, were relatively less active (MIC = > 200  $\mu\text{g}/\text{mL}$ ) against the tested bacterial strains. The antibacterial results obtained for compound **4** are in agreement with those obtained for the carbohydrazide derivative, with the NO<sub>2</sub> substituent group on its indole ring, against *S. aureus* bacterial strain [48].

#### 4. Conclusions

In the present study, four semicarbazone derivatives with different substituent groups on the indole moiety were synthesized and characterized by ESI-MS and standard spectroscopic techniques. The two-dimensional NMR (in acetone-d<sub>6</sub>) spectral data revealed that **1** and **2** in solution exist in the *cisE* isomeric form. This evidence was also confirmed by DFT calculations carried out for all the synthesized compounds. The results of the antibacterial activity showed that compounds **1** and **2** (with the Br and Cl substituents, respectively) exhibited moderate bioactivity against *S. aureus* and *B. subtilis* (as Gram-positive bacteria) while compounds **3** and **4** (with the OCH<sub>3</sub> and NO<sub>2</sub> substituents, respectively) were relatively less active against the tested bacterial strains compared with tetracycline.

#### Data Availability

The data used to support the findings of this study are included within the article, except for the computational results, which are found in the Supporting Information (Table S1). These data will be available on request to bona fide researchers.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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#### Supplementary Materials

Table S1: computational results, at B3LYP/6-311++G(d,p) for **1** to **4** synthesized compounds. Relative enthalpies ( $\Delta H$ ) of stable conformers and their equilibrium molar fractions (populations). Compound **1**—<sup>1</sup>H NMR spectra—<sup>13</sup>C NMR spectra—two-dimensional NMR spectroscopy (<sup>1</sup>H-<sup>1</sup>H DQF COSY)—two-dimensional NMR spectroscopy (<sup>1</sup>H-<sup>1</sup>H

NOESY)—two-dimensional NMR spectroscopy (<sup>1</sup>H<sup>13</sup>C HSQC)—two-dimensional NMR spectroscopy (<sup>1</sup>H<sup>13</sup>C HMBSC)—mass spectra—infrared spectra. Compound **2**—<sup>1</sup>H NMR spectra—pure shift <sup>1</sup>H NMR—<sup>13</sup>C NMR spectra—two-dimensional NMR spectroscopy (<sup>1</sup>H-<sup>1</sup>H DQF COSY)—two-dimensional NMR spectroscopy (<sup>1</sup>H-<sup>1</sup>H NOESY)—mass spectra—infrared spectra. Compound **3**—<sup>1</sup>H NMR spectra—<sup>13</sup>C NMR spectra—mass spectra—infrared spectra. Compound **4**—<sup>1</sup>H NMR spectra—<sup>13</sup>C NMR spectra—mass spectra—infrared spectra. (Supplementary Materials)

#### References

- [1] S. T. Odonkor and K. K. Addo, "Bacteria resistance to antibiotics: recent trends and challenges," *International Journal of Biological & Medical Research*, vol. 2, no. 3, pp. 1204–1210.
- [2] R. K. Fukuda, *Antimicrobial Resistance Global Report on Surveillance*, World Health Organization, Geneva, Switzerland, 2014.
- [3] Y. Didem, A. Oztekin, D. g. Aylin et al., "Synthesis and antimicrobial activity evaluation of some benzimidazole and indole derivatives," *African Journal of Microbiology Research*, vol. 7, no. 17, pp. 1708–1715, 2013.
- [4] World Health Organization, "Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics," *World Health Organization*, vol. 43, no. 148, pp. 348–365, 2013.
- [5] A. Elabbadi, S. Pont, C. Verdet et al., "An unusual community-acquired invasive and multi systemic infection due to ExoUharboring *Pseudomonas aeruginosa* strain: Clinical disease and microbiological characteristics," *Journal of Microbiology, Immunology and Infection*, 2019, In press.
- [6] V. M. Paz-Zarza, S. Mangwani-Mordani, A. Martínez-Maldonado, D. Álvarez-Hernández, S. G. Solano-Gálvez, and R. Vázquez-López, "Pseudomonas aeruginosa: patogenicidad y resistencia antimicrobiana en la infección urinaria," *Revista chilena de infectología*, vol. 36, no. 2, pp. 180–189, 2019.
- [7] A. C. Ossa-Giraldo, L. M. Echeverri-Toro, Z. M. Santos et al., "Factores de riesgo para infección por *Pseudomonas aeruginosa* multi-resistente en un hospital de alta complejidad," *Revista chilena de infectología*, vol. 31, no. 4, pp. 393–399, 2014.
- [8] M. Bassetti, A. Vena, A. Croxatto, E. Righi, and B. Guery, "How to manage Pseudomonas aeruginosa infections," *Drugs in Context*, vol. 7, pp. 1–18, 2018.
- [9] S. S. Magill, J. R. Edwards, W. Bamberg et al., "Emerging infections program healthcare-associated, T. antimicrobial use prevalence survey. Multistate point-prevalence survey of health care-associated infections," *New England Journal of Medicine*, vol. 370, no. 13, pp. 1198–1208.
- [10] S. Makvana and L. R. Krilov, "Escherichia coli infections," *Pediatrics in Review*, vol. 36, no. 4, pp. 167–171, 2015.
- [11] J. Bien, O. Sokolova, and P. Bozko, "Characterization of virulence factors of *Staphylococcus aureus*: novel function of known virulence factors that are implicated in activation of airway epithelial proinflammatory response," *Journal of Pathogens*, vol. 2011, Article ID 601905, 13 pages, 2011.
- [12] A. F. Shorr, "Epidemiology of staphylococcal resistance," *Clinical Infectious Diseases*, vol. 45, no. Supplement\_3, pp. S171–S176, 2007.
- [13] E. Gonçalves, R. Carvalhal, R. Mesquita et al., "Detection of staphylococcus aureus (MRSA/MSSA) in surfaces of dental medicine equipment," *Saudi Journal of Biological Sciences*, 2019, In press.

- [14] M. Singh, S. K. Singh, M. Gangwar, G. Nath, and S. K. Singh, "Design, synthesis and mode of action of novel 2-(4-aminophenyl)benzothiazole derivatives bearing semicarbazone and thiosemicarbazone moiety as potent antimicrobial agents," *Medicinal Chemistry Research*, vol. 25, no. 2, pp. 263–282, 2016.
- [15] Z. Afrasiabi, E. Sinn, W. Lin, Y. Ma, C. Campana, and S. Padhye, "Nickel (II) complexes of naphthaquinone thiosemicarbazone and semicarbazone: synthesis, structure, spectroscopy, and biological activity," *Journal of Inorganic Biochemistry*, vol. 99, no. 7, pp. 1526–1531, 2005.
- [16] É. A. Enyedy, G. M. Bognár, N. V. Nagy, T. Jakusch, T. Kiss, and D. Gambino, "Solution speciation of potential anticancer metal complexes of salicylaldehyde semicarbazone and its bromo derivative," *Polyhedron*, vol. 67, pp. 242–252, 2014.
- [17] S. Datta, D. Kumar Seth, S. Halder et al., "Mononuclear palladium and heterodinuclear palladium-ruthenium complexes of semicarbazone ligands. Synthesis, characterization, and application in C-C cross-coupling reactions," *RSC Advances*, vol. 2, no. 12, pp. 5254–5264, 2012.
- [18] M. J. Ahsan, M. Amir, M. A. Bakht, J. G. Samy, M. Z. Hasan, and M. S. Nomani, "Synthesis and antimicrobial activity of N1-(3-chloro-4-fluorophenyl)-N4-substituted semicarbazone derivatives," *Arabian Journal of Chemistry*, vol. 9, pp. S861–S866, 2016.
- [19] S. V. Laxmi and B. Rajitha, "Synthesis and antimicrobial activity of newer indole semicarbazones," *Medicinal Chemistry Research*, vol. 21, no. 1, pp. 85–90, 2012.
- [20] S. N. Pandeya, "Semicarbazone-a versatile therapeutic pharmacophore for fragment based anticonvulsant drug design," *Acta Pharmaceutica*, vol. 62, no. 3, pp. 263–286, 2012.
- [21] D. Sriram, P. Yogeewari, and R. Thirumurugan, "Antituberculous activity of some aryl semicarbazone derivatives," *Bioorganic & Medicinal Chemistry Letters*, vol. 14, no. 15, pp. 3923–3924, 2004.
- [22] S. M. M. Ali, M. A. K. Azad, M. Jesmin et al., "In vivo anticancer activity of Vanillin semicarbazone," *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 6, pp. 438–442, 2012.
- [23] H. P. Ebrahimi, J. S. Hadi, T. A. Alsalm, T. S. Ghali, and Z. Bolandnazar, "A novel series of thiosemicarbazone drugs: from synthesis to structure," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 137, pp. 1067–1077, 2015.
- [24] M. A. Wikler, F. R. Cockerill, K. Bush et al., *Methods Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, vol. 29, Clinical and Laboratory Standards Institute, M07-A08. Approved Standard, Pennsylvania, PA, USA, 8th edition, 2009.
- [25] C. Tamariz-Angeles, P. Olivera-Gonzales, and M. Santillán-Torres, "Antimicrobial, antioxidant and phytochemical assessment of wild medicinal plants from Cordillera Blanca (Ancash, Peru)," *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, vol. 17, no. 3, pp. 270–285, 2018.
- [26] W. C. M. J. Frisch, G. W. Trucks, H. B. Schlegel et al., *Gaussian 09*, Gaussian, Inc., Wallingford, CT, USA, 2009.
- [27] P. F. M. Oliveira, B. Guidetti, A. Chamayou et al., "Mechanochemical synthesis and biological evaluation of novel isoniazid derivatives with potent antitubercular activity," *Molecules*, vol. 22, no. 9, 2017.
- [28] J. B. P. Da Silva, F. Hallwass, A. G. Da Silva et al., "Intermolecular interaction of thiosemicarbazone derivatives to solvents and a potential *Aedes aegypti* target," *Journal of Molecular Structure*, vol. 1093, pp. 219–227, 2015.
- [29] A. D. Shutalev, A. A. Fesenko, A. N. Yankov, V. A. Tafeenko, and V. V. Chernyshev, "14-Membered cyclic bis-semicarbazones: stereoselective synthesis and structural features," *Journal of Molecular Structure*, vol. 1150, pp. 349–357, 2017.
- [30] T. K. Venkatachalam, G. K. Pierens, and D. C. Reutens, "Synthesis, NMR structural characterization and molecular modeling of substituted thiosemicarbazones and semicarbazones using DFT calculations to prove the syn/anti isomer formation," *Magnetic Resonance in Chemistry*, vol. 52, no. 3, pp. 98–105, 2014.
- [31] S. U. Qazi, S. U. Rahman, A. N. Awan et al., "Semicarbazone derivatives as urease inhibitors: synthesis, biological evaluation, molecular docking studies and in-silico ADME evaluation," *Bioorganic Chemistry*, vol. 79, pp. 19–26, 2018.
- [32] V. Mashayekhi, K. Haj Mohammad Ebrahim Tehrani, S. Amidi, and F. Kobarfard, "Synthesis of novel indole hydrazone derivatives and evaluation of their antiplatelet aggregation activity," *Chemical and Pharmaceutical Bulletin*, vol. 61, no. 2, pp. 144–150, 2013.
- [33] P. Choppara, Y. V. Prasad, C. V. Rao et al., "Design, synthesis of novel N prenylated indole-3-carbazones and evaluation of in vitro cytotoxicity and 5-LOX inhibition activities," *Arabian Journal of Chemistry*, vol. 12, no. 8, pp. 2328–2335, 2019.
- [34] M. R. P. Kurup, B. Varghese, M. Sithambaresan, S. Krishnan, S. R. Sheeja, and E. Suresh, "Synthesis, spectral characterization and crystal structure of copper(II) complexes of 2-benzoylpyridine-N(4)-phenylsemicarbazone," *Polyhedron*, vol. 30, no. 1, pp. 70–78, 2011.
- [35] A. Shafiee, A. Rineh, A. Kebriaeezadeh, A. Foroumadi, V. Sheibani, and M. R. Afarinesh, "Synthesis and anticonvulsant activity of 4-(2-phenoxyphenyl)semicarbazones," *Medicinal Chemistry Research*, vol. 18, no. 9, pp. 758–769, 2009.
- [36] M. C. Rodríguez-Argüelles, E. C. López-Silva, J. Sanmartín, P. Pelagatti, and F. Zani, "Copper complexes of imidazole-2-, pyrrole-2- and indol-3-carbaldehyde thiosemicarbazones: inhibitory activity against fungi and bacteria," *Journal of Inorganic Biochemistry*, vol. 99, no. 11, pp. 2231–2239, 2005.
- [37] P. Pavlát, A. Halam, T. Weidlich, B. Fiala, and V. Berkárek, "Medium effect on <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of melatonin," *Acta Universitatis Palackianae Olomucensis Facultas Rerum Naturalium, CHEMICA*, vol. 38, 1999.
- [38] S. Saravanan, S. Athimoolam, and S. Muthusubramanian, "Synthesis and characterization of 3-aryl-3-[4-aryl-1,2,3-selenadiazol-5-yl]-2-phenyl-2-propenenitrile," *Arkivoc*, vol. 2007, no. 1, pp. 22–33, 2007.
- [39] K. Jakusová, M. Gáplovský, J. Donovalová et al., "Effect of reactants' concentration on the ratio and yield of E,Z isomers of isatin-3-(4-phenyl)semicarbazone and N-methylisatin-3-(4-phenyl)semicarbazone," *Chemical Papers*, vol. 67, no. 1, pp. 117–126, 2013.
- [40] G. Palla, G. Predieri, P. Domiano, C. Vignali, and W. Turner, "Conformational behaviour and isomerization of -acyl and -aroylhydrazones," *Tetrahedron*, vol. 42, no. 13, pp. 3649–3654, 1986.
- [41] S. N. Pandeya, T. Srivastava, M. Gangwar, B. Bihari, and G. Nath, "Synthesis and antimicrobial activities of 1-Naphthylamine based acetophenone semicarbazones," *Medicinal Chemistry in Drug Discovery*, vol. 3, no. 2, pp. 94–102, 2012.
- [42] F. S. Cunha, J. M. R. Nogueira, and A. P. De Aguiar, "Synthesis and antibacterial evaluation of 3,5-Diaryl-1,2,4-oxadiazole



- derivatives," *Journal of the Brazilian Chemical Society*, vol. 29, no. 11, pp. 2405–2416, 2018.
- [43] A. A. Jadhav, V. P. Dhanwe, P. G. Joshi, and P. K. Khanna, "Solventless synthesis of new 4,5-disubstituted 1,2,3-selenadiazole derivatives and their antimicrobial studies," *Cogent Chemistry*, vol. 2, no. 1, pp. 1–12, 2016.
- [44] M. Sayed, A. M. Kamal El-Dean, M. Ahmed, and R. Hassanien, "Synthesis of some heterocyclic compounds derived from indole as antimicrobial agents," *Synthetic Communications*, vol. 48, no. 4, pp. 413–421, 2018.
- [45] A. Kumari and R. K. Singh, "Medicinal chemistry of indole derivatives: current to future therapeutic prospectives," *Bioorganic Chemistry*, vol. 89, p. 103021, 2019.
- [46] P. Pakravan, S. Kashanian, M. M. Khodaei, and F. J. Harding, "Biochemical and pharmacological characterization of isatin and its derivatives: from structure to activity," *Pharmacological Reports*, vol. 65, no. 2, pp. 313–335, 2013.
- [47] A. Patel, S. Bari, G. Talele, J. Patel, and M. Sarangapani, "Synthesis and antimicrobial activity of some new isatin derivatives," *Iranian Journal of Pharmaceutical Research*, vol. 4, pp. 249–254, 2006.
- [48] B. Narayana, B. V. Ashalatha, K. K. Vijaya Raj, and B. K. Sarojini, "Synthesis and studies on antimicrobial, antiinflammatory and antiproliferative activities of heterocycles derived from 4-/5-/6-/7-nitro/5fluoro/chloro/bromoindole-2-carbohydrazides," *Indian Journal of Chemistry-Sect. B Organic and Medicinal Chemistry*, vol. 48, no. 12, pp. 1794–1805, 2009.