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Efficient Synthesis of Two Chloramphenicol Derivatives as Antibacterial Agents

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Abstract: Several protocols have been used to prepare some chloramphenicol analogs using different reagents, which can be expensive and difficult to handle. The aim of this investigation was to synthesize six chloramphenicol analogs (compounds **2** to **7**) using some chemical strategies to evaluate their biological activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Klebsiella pneumoniae* with the minimum inhibitory concentration method. The results indicate that protocols used to synthesize chloramphenicol derivatives do not require special conditions such as different pH and higher temperatures to give a good yielding. Besides, only compounds **4** and **6** decreased the bacterial growth of either Gram-positive or Gram-negative bacteria. However, the biological activity of compound 4 was higher compared with both chloramphenicol and compound **6**.

Keywords: synthesis; chloramphenicol; derivatives; Gram-positive; Gram-negative.

1. Introduction

Infectious diseases are one of the leading causes of death worldwide [1-3]; it is important to mention that several drugs have been used to treat infectious diseases, such as penicillins [4], cephalosporins [5], aminoglycosides [6, 7], fluoroquinones [8], sulphas [9]. However, some of these drugs can produce bacterial resistance [10-12]; in this way, some studies showed that chloramphenicol could induce bacterial resistance in both Gram-positive and Gram-negative bacteria [13-17]. In the search for a therapeutic alternative to reduce bacterial resistance produced by some bacteria, several antibacterial agents have been developed. For example, a series of derivatives of fluorine-chloramphenicol with antibacterial activity on Gram-negative bacteria were synthesized [18]. Other data showed that some aminoacyl-chloramphenicol analogs could cause decreased bacterial growth of *Escherichia coli* [19]. In addition, a report showed the synthesis of different chloramphenicol-amine derivatives with antibacterial activity on *Escherichia coli* [20]. Besides, some carbonyl-chloramphenicol derivatives were prepared as antibacterial agents against *Staphylococcus*

aureus [21]. Recently, a 3-acylchloramphenicol derivative was synthesized from dichloroacetyl and chloramphenicol as a potential antimicrobial agent [22]. It is important to mention that all these methods require special conditions such as different pH and high temperatures. Analyzing these data, the objective of this research was to synthesize some derivatives of chloramphenicol. In addition, the biological activity exerted by chloramphenicol derivatives on *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Klebsiella pneumoniae* was evaluated using the minimum inhibitory concentration method.

2. Material and Methods

2.1. General methods.

All reagents were acquired from Sigma-Aldrich company. NMR spectra were recorded on a Varian VXR300/5 FT apparatus (300 MHz/$CDCl_3$) using tetramethylsilane as an internal standard. Electron Ionization mass spectrometry was recorded on a Finnigan PolarisQ ion trap mass spectrometer. The melting point was recorded on an electrothermal-900 model apparatus. The infrared spectrum was recorded on a thermo-scientific iSOFT/IR device. Elemental analysis was determined using a PerkinElmer apparatus (Ser. II CHNS / 02400).

2.2. 4-[(2,2-dichloroacetyl)amino]-3-(4-nitrophenyl)-2,6-dioxabicyclo[5.3.1]undeca-1(10),7(11),8-triene-9-carboxylic acid (2).

In a round bottom flask (10 mL), chloramphenicol (200 mg, 0.62 mmol), 3,5-dinitrobenzoic acid (135 mg, 0.64 mmol) and potassium carbonate anhydrous (90 mg, 0.65 mmol) in dimethyl sulfoxide (5 mL) was stirring for 72 h at 90 ^oC. Then the solvent is evaporated on a rotary evaporator and the product is separated using the methanol:hexane (4:1) system; yielding 76 % of product; m.p. 102-104 ^oC; IR (V_{max}, cm^{-1}) 1712, 1632, 1540 and 1242: ^1H NMR (300 MHz, $CDCl_3$-*d*) δ_H: ^1H NMR (300 MHz, $CDCl_3$-*d*) δ_H: 3.32-5.04 (m, 5H), 6.02, (m, 1H), 6.22 (m, 1H), 6.60 (m, 1H), 6.66 (broad, 2H), 6.68 (m, 1H), 7.90-7.96 (m, 4H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 40.82, 64.42, 78.54, 80.44, 102.10, 111.66, 112.52, 122.94, 125.50, 128.14, 145.30, 146.40, 154.36, 157.56, 161.60, 168.10 ppm. EI-MS m/z: 440.01. Anal. Calcd. for $C_{18}H_{14}Cl_2N_2O_7$. C, 49.00; H, 3.20; Cl, 16.07; N, 6.35; O, 25.38. Found: C, 49.00; H, 3.18.

2.3. 2,2-dichloro-N-[8-fluoro-3-(4-nitrophenyl)-2,6-dioxabicyclo[5.3.1]undeca-1(10),7(11), 8-trien-4-yl]acetamide (3).

In a round bottom flask (10 mL), chloramphenicol (200 mg, 0.62 mmol), 3,5-dinitrofluoro benzene (120 mg, 0.64 mmol) and potassium carbonate anhydrous (90 mg, 0.65 mmol) in dimethyl sulfoxide (5 mL) was stirring for 72 h at 90 ^oC. Then the solvent is evaporated on a rotary evaporator and the product is separated using the methanol:hexane:water (3:1:1) system; yielding 51% of product; m.p. 92-94 ^oC; IR (V_{max}, cm^{-1}) 1632, 1542 and 1242: ^1H NMR (300 MHz, $CDCl_3$-d) δ_H: ^1H NMR (300 MHz, $CDCl_3$-d) δ_H: 3.72-5.60 (m, 5H), 6.22, (m, 1H), 6.50-6.96 (m, 2H), 7.60 (broad, 1H), 7.90-7.96 (m, 4H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 42.90, 64.42, 80.34, 83.12, 100.60, 110.56, 116.90, 122.82, 125.50, 144.64, 146.57, 149.12, 150.86, 151.30, 161.60 ppm. EI-MS m/z: 414.01. Anal. Calcd. for $C_{17}H_{13}Cl_2N_2O_5$. C, 49.18; H, 3.16; Cl, 17.08; F, 4.58; N, 6.75; O, 19.27. Found: C, 49.15; H, 3.14.

2.4. 2,2-dichloro-N-[2-(4-nitrophenyl)-6,9-dioxo-1,5-dioxonan-3-yl]acetamide (4).

In a round bottom flask (10 mL), chloramphenicol (200 mg, 0.62 mmol), succinic acid (100 mg, 0.84 mmol), N,N´-dicyclohexylcarbodiimide (160 mg, 0.77 mmol) in ethanol (5 mL) was stirring for 72 h at room temperature. Then the solvent is evaporated on a rotary evaporator and the product is separated using the methanol:hexane:benzene (3:1:1) system; yielding 65% of product; m.p. 118-120 ^{o}C; IR (V_{max}, cm^{-1}) 1750, 1630 and 1542: ^{1}H NMR (300 MHz, $CDCl_3$-d) δ_H: ^{1}H NMR (300 MHz, $CDCl_3$-d) δ_H: 2.22-6.22 (m, 7H), 6.26 (m, 1H), 7.74-7.80 (m, 4H), 7.96 (broad, 1H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 29.70, 30.44, 49.16, 64.40, 69.50, 79.73, 122.85, 126.18, 144.77, 146.90, 161.80, 170.12, 171.22 ppm. EI-MS m/z: 404.01. Anal. Calcd. for $C_{15}H_{14}Cl_2N_2O_7$. C, 44.46; H, 3.48; Cl, 17.50; N, 6.91; O, 27.64. Found: C, 44.43; H, 3.45.

2.5. 4-{4-[2-(2,2-Dichloro-acetylamino)-1,3-dihydroxy-propyl]-phenoxy}-benzoic acid (5).

In a round bottom flask (10 mL), chloramphenicol (200 mg, 0.62 mmol), 4-hydroxibenzoic acid (85 mg, 0.61 mmol), and potassium carbonate anhydrous (90 mg, 0.65 mmol) in dimethyl sulfoxide (5 mL) was stirring for 72 h at 90 ^{o}C. Then the solvent is evaporated on a rotary evaporator and the product is separated using the methanol:hexane (4:1) system; yielding 74% of product; m.p. 78-80 ^{o}C; IR (V_{max}, cm^{-1}) 3400, 1712, 1682 and 1240 : ^{1}H NMR (300 MHz, $CDCl_3$-d) δ_H: 4.06-5.02 (m, 4H), 6.20 (m, 1H), 6.35 (broad, 4H), 6.66-8.10 (m, 8H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 57.70, 63.04, 64.44, 72.32, 114.82, 117.22, 124.20, 128.12, 133.86, 141.20, 154.92, 158.30, 163.22, 168.40 ppm. EI-MS m/z: 413.04. Anal. Calcd. for $C_{18}H_{17}Cl_2NO_6$. C, 52.19; H, 4.14; Cl, 17.12; N, 3.38; O, 23.17. Found: C, 52.16; H, 4.10.

2.6. (2E)-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-2-(phenylhydrazono)-acetamide (6).

In a round bottom flask (10 mL), chloramphenicol (200 mg, 0.62 mmol), phenylhydrazine hydrochloride (100 mg, 0.69 mmol), copper(II) chloride anhydrous (100 mg, 0.74 mmol) in methanol (5 mL) was stirring for 72 h at room temperature. Then the solvent is evaporated on a rotary evaporator and the product is separated using the methanol:water (4:1) system; yielding 74% of product; m.p. 156-158 ^{o}C; IR (V_{max}, cm^{-1}) 3400. 3320, 1630 and 1540: ^{1}H NMR (300 MHz, $CDCl_3$-d) δ_H: 3.62 (m, 2H), 4.00-5.00 (m, 4H), 6.00 (broad, 5H), 6.44 (m, 1H), 6.80-7.26 (m, 5H), 7.60-7.80 (m, 4H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 59.90, 63.02, 72.32, 115.60, 121.10, 122.54, 126.94, 128.22, 128.44, 146.68, 149.12, 150.30, 158.60 ppm. EI-MS m/z: 358.12. Anal. Calcd. for $C_{17}H_{18}N_4O_5$. C, 56.98; H, 5.06; N, 15.63; O, 22.32. Found: C, 56.95; H, 5.04.

2.7. (2E)-2-hydrazinylidene-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]acetamide (7).

In a round bottom flask (10 mL), chloramphenicol (200 mg, 0.62 mmol), hydrazine hydrochloride (30 µL, 0.95 mmol), copper(II) chloride anhydrous (100 mg, 0.74 mmol) in methanol (5 mL) was stirring for 72 h at room temperature. Then the solvent is evaporated on a rotary evaporator and the product is separated using the methanol:water (4:1) system; yielding 78% of product; m.p. 158-160 ^{o}C; IR (V_{max}, cm^{-1}) 3400, 3380, 3320, 1542: ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ_H: 4.00-5.00 (m, 4H), 5.18 (broad, 5H), 6.00 (m, 1H), 7.60-7.80 (m, 4H) ppm. ^{13}C

NMR (300 Hz, $CDCl_3$) δ_C: 59.90, 63.02, 72.32, 122.54, 126.94, 129.70, 146.72, 150.36, 158.24 ppm. EI-MS m/z: 282.09. Anal. Calcd. for $C_{11}H_{14}N_4O_5$. C, 46.81; H, 5.00; N, 19.85; O, 28.34. Found: C, 46.78; H, 5.00.

2.8. Biological evaluation.

2.8.1. Bacteria strains.

Staphylococcus aureus (ATCC 33591, MRSA), *Streptococcus pneumoniae* (ATCC 6303,) *Escherichia coli* (ATCC 14035), and *Klebsiella pneumoniae* (ATCC 4352) were acquired from the strain bank from Laboratory of Pharmacochemistry, Faculty of Chemical-Biological Sciences of the Autonomous University of Campeche.

2.9. Drugs.

Compounds **2** to **7** were evaluated, and chloramphenicol was used as a control. It is important to mention that the compounds used in this study were dissolved in methanol (stock solution), and all dilutions were carried out with distilled water.

2.10. Antimicrobial activity.

The biological activity produced by compounds **2** to **7** on *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Klebsiella pneumoniae* was carried out using the minimum inhibitory concentration (MIC) [23]. In this way, the bacteria were incubated in specific mediums; Staphylococcus 110 agar (*Staphylococcus aureus*), chocolate agar (*Staphylococcus aureus*), and Mc-Conkey (*Escherichia coli* and *Klebsiella pneumoniae*) for 24 h at 37 °C to determine the bacterial growth. This stage was carried out using 12 tubes containing 2 mg/2 ml of culture medium (soybean trypticase); From the first tube, a 1 mg/ml aliquot of chloramphenicol (control) and either of compounds **2** to **7** (1 mg/mL) was added, and the resulting solution was shaken; then, in the next 11 tubes, different dilutions of any of compounds **2** to **7** at a dose of 0.5 to 0.0004 mg/mL were added with constant stirring (Table 1). Then, each tube was inoculated with 0.1 ml of the bacterial suspension, whose concentration corresponded to the McFarland scale (9×10^8 cells/mL). Following, all the tubes were incubated at 37 ^oC for 24 h. Finally, a loop was taken from each of them, inoculated into the appropriate cultures for different bacterial organisms, and incubated for 24 h at 37 ^oC.

2.11. Theoretical analysis.

The interaction of chloramphenicol and compounds **2** to **7** with either *Staphylococcus aureus* or *Escherichia coli* was evaluated using both 5fpo and 3zbi proteins surface as theoretical models [24, 25]. In addition, to evaluate the binding energy involved in the interaction of compounds **2** to **7** with either 5fpo or 3zbi proteins surface, chloramphenicol was used as a control on a docking Server software [26, 27].

2.12. Statistical analysis.

Statistical analysis The obtained values are expressed as average ± SE, using each heart as its control. The data obtained were put under variance (ANOVA) analysis using the

Bonferroni correction factor [28]. The differences were considered significant when p was equal to or smaller than 0.05.

3. Results and Discussion

Some chloramphenicol derivatives have been synthesized to treat different infectious diseases; however, the methods used involve some reagents that may be dangerous or require special conditions such as differences in pH or higher temperatures [18-22]. Analyzing these data, in this research, some chloramphenicol analogs (compounds **2** to **7**) were prepared using some chemical strategies as follows.

3.1. Synthesis of two dioxabicyclo (2 and 3).

The are several reports in the literature on the synthesis of dioxabicyclo derivatives which use some protocols that involve different reagents such as phenylmagnesium bromide [29], phenylpropanoid derivative [30], osmium tetroxide [31], D-proline [32], dianhydromannitol ditosylate [33] and others. In this research, two dioxabicyclo derivatives (**2** and **3**) were prepared *via* reaction of chloramphenicol with either 3,5-dinitrobenzoic acid or 3,5-dinitroflurobenzene to form **2** or **3** in the presence of dimethyl sulfoxide (Figure 1).

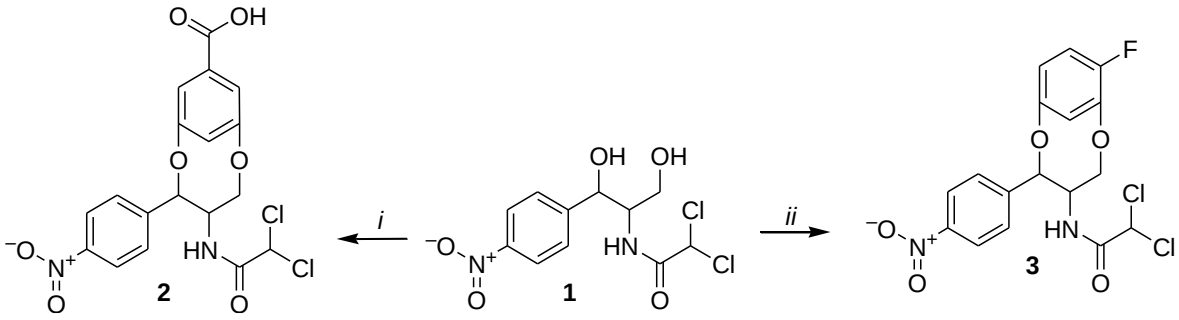

Figure 1. Synthesis of two dioxabicyclo derivatives (**2** and **3**). Reagents and Conditions: *i* = 3,5-dinitrobenzoic acid, potassium carbonate anhydrous, dimethyl sulfoxide, 72 h, 90 °C; *ii* = 3,5-dinitrofluoro benzene, potassium carbonate anhydrous, dimethyl sulfoxide, 72 h, 90 °C.

The ^{1}H NMR spectrum from **2** showed several bands at 3.32-6.02 and 6.60 and 6.68 ppm for 2,6-Dioxa-bicyclo[5.3.1]undeca-1(10),7(11),8-triene fragment; at 6.22 ppm for methylene bound to amide group; at 6.66 ppm for both carboxyl and amide groups; at 7.90-7.96 ppm for phenyl group bound to nitro group. ^{13}C NMR spectra display chemical shifts at 40.82, 78.54-112.52, 128.14, and 154.36-157.56 ppm for 2,6-Dioxa-bicyclo[5.3.1]undeca-1(10),7(11),8-triene fragment; at 64.42 ppm for ppm for methylene linked to amide group; at 122.94-125.50 and 145.30-146.40 ppm for phenyl group bound to the nitro group; at 161.60 ppm for amide group; at 168.10 ppm for carboxyl group. Besides, the mass spectrum from **2** showed a molecular ion (m/z) 440.01.

On the other hand, the ^{1}H NMR spectrum from **3** showed several bands at 3.72-5.60, and 6.50-6.96 ppm for 2,6-Dioxa-bicyclo[5.3.1]undeca-1(10),7(11),8-triene fragment; at 6.22 ppm for methylene linked to amide group; at 7.90-7.96 ppm for phenyl group bound to nitro group. ^{13}C NMR spectra display chemical shifts at 42.90, 80.34-116.90, and 149.12-151.30 ppm for 2,6-Dioxa-bicyclo[5.3.1]undeca-1(10),7(11),8-triene fragment; at 64.42 ppm for methylene bound to amide group; at 122.82-146.57 ppm from phenyl group linked to nitro group. In addition, the mass spectrum from **3** showed a molecular ion (m/z) 414.01.

3.2. Preparation of a [1,5]Dioxonane-6,9-dione analog (4).

Several dioxanone derivatives have been synthesized from some reagents such as *o*-phthalaldehyde [34], 2,3-trimethylene-5,6-dihydro-4H-pyran [35], fullerene/$FeCl_3$ [36]. In this study, a [1,5]Dioxonane-6,9-dione derivative was synthesized via reaction of chloramphenicol with succinic acid using *N,N´*-dicyclohexylcarbodiimide as catalyst (Figure 2). The ^{1}H NMR spectrum from **4** showed several bands at 2.22-6.22 ppm for 2[1,5]Dioxonane-6,9-dione fragment; at 6.26 ppm for methylene bound to amide group; at 7.74-7.80 ppm for phenyl group bound to the nitro group; at 7.96 ppm from amide group. ^{13}C NMR spectra display chemical shifts at 29.70-49.16 and 69.50-79.73 ppm for [1,5]Dioxonane-6,9-dione fragment; at 64.40 ppm for methylene bound to amide group; at 122.85-146.90 ppm for phenyl bound to the nitro group; at 170.12-171.22 ppm for ester groups. Additionally, the mass spectrum from **4** showed a molecular ion (m/z) 404.01.

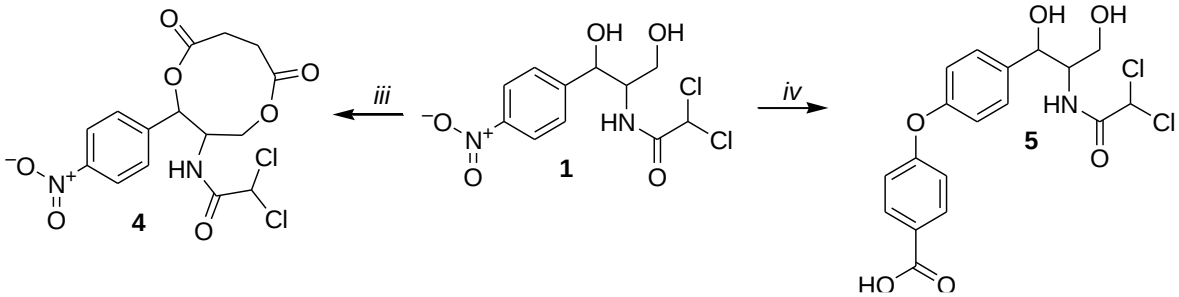

Figure 2. Synthesis of a [1,5]Dioxonane-6,9-dione analog (**4**) and a phenoxy-benzoic acid derivative (**5**). Reagents and Conditions: *iii* = succinic acid, *N,N´*-dicyclohexylcarbodiimide, EtOH, 72 h, room temperature; *iv* = 4-hydroxibenzoic acid, potassium carbonate anhydrous, dimethyl sulfoxide, 72 h, 90 ºC.

3.3. Synthesis of an ether derivative (5).

Several protocols for synthesis of some ether derivatives have been reported using different reagents such as Ta/Al_2O_3 [37], palladium [38], tert-butyl nitrite [39], ceric ammonium nitrate [40], dimethyl sulfoxide/potassium carbonate [41]. In this way, compound **5** was synthesized from chloramphenicol, dimethyl sulfoxide, and potassium carbonate anhydrous (Figure 2). The ^{1}H NMR spectrum from **5** showed several bands at 4.06-5.02 ppm for methylene groups bound to hydroxyl groups; at 6.20 ppm for methylene bound to amide group; at 6.35 ppm for both hydroxyl and amide groups; at 6.66-8.10 ppm for phenyl groups. ^{13}C NMR spectra display chemical shifts at 57.70-63.04 ppm for methylene groups linked to hydroxyl groups; at 64.44 ppm for methylene bound to amide group; at 114.82-158.30 ppm for phenyl groups; at 163.22 for amide group; at 168.40 ppm for carboxyl group. Besides, the mass spectrum from **5** showed a molecular ion (m/z) 413.04.

3.4. Synthesis of two acetamides analogs (6 and 7).

There are several method for synthesis of acetamide derivatives: these protocol use some reagents such as iron(II)acetate [42], methyl methoxyacetate [43], triethylphosphine [44], cobalt(III)chloropentaamine chloride [45], hydrochloric acid [46], α-pyridinium acetamide [47] and others. In this research, two acetamide derivatives were synthesized from chloramphenicol and either phenylhydrazine or hydrazine using copper(II) chloride as a catalyst to form the compounds **6** or **7** (Figure 3). The ^{1}H NMR spectrum from **6** showed several bands at 3.62 ppm for methylene bound to both amide and imino groups; at 4.00-5.00 ppm for methylene groups linked to hydroxyl groups; at 6.00 ppm for amino, hydroxyl, and amide

groups; at 6.44 for imino group; at 6.80-7.26 ppm for phenyl group bound to the amino group; at 7.60-7.80 ppm for phenyl group linked to nitro group. ^{13}C NMR spectra display chemical shifts at 59.90-72.32 ppm for methylene bound to both hydroxyl groups; at 115.60-121.10, 128.44, and 149.12 ppm for phenyl group linked to the amino group; at 122.54-126.94, 146.68 and 150,30 ppm for phenyl bound to the nitro group; at 158.60 ppm for amide group; at 128.22 ppm for imino group. Additionally, the mass spectrum from **6** showed a molecular ion (m/z) 358.12.

Finally, the ^{1}H NMR spectrum from **7** showed several bands at 4.00-5.00 ppm for methylene groups linked to both hydroxyl groups; at 5.18 ppm for amide, hydroxyl, and amino groups; at 6.00 ppm for imino group; at 7.60-7.80 ppm for phenyl group. ^{13}C NMR spectra display chemical shifts at 59.90-72.32 ppm for methylene groups linked to both hydroxyl groups; at 122.54-126.94 and 146.72-150.36 ppm for phenyl group; at 125.70 ppm for imino group; at 158.24 ppm for amide group. Finally, the mass spectrum from **7** showed a molecular ion (m/z) 282.09.

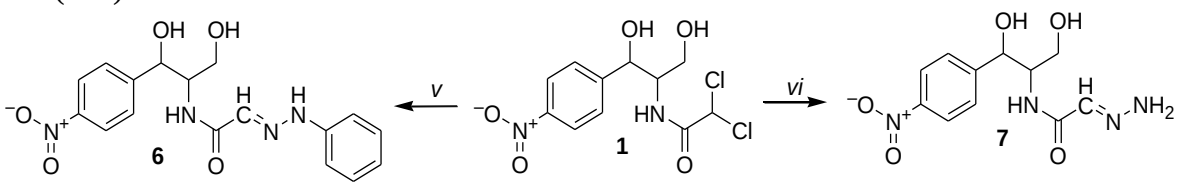

Figure 3. Synthesis of two acetamide derivatives (**6** and **7**). *Reagents and Conditions*: *v* = phenylhydrazine hydrochloride, Copper(II) chloride anhydrous, 72 h, room temperature; *vi* = hydrazine hydrochloride, Copper(II) chloride anhydrous, 72 h, room temperature.

3.5. Biological activity.

For several years, chloramphenicol has been used to treat some infectious diseases; however, this drug can produce some secondary effects such as aplastic anemia [48], anaphylaxis [49], bone marrow suppression [50], Gray syndrome [51], acidosis [52]. Besides, some studies indicate that chloramphenicol can induce bacterial resistance in some bacterial strains such as *Staphylococcus aureus* [53] and *Escherichia coli* [54, 55]. To evaluate the biological activity of chloramphenicol derivatives (compounds 2 to 7) on *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Klebsiella pneumoniae*, chloramphenicol was used as a control using the minimum inhibitory concentration (MIC) method. It is important to mention that dilutions of the different compounds studied (mg/mL) (Table 1) are expressed in the results as mmol/mL. The results (Figure 4) showed that bacterial growth of either *Staphylococcus aureus* or *Streptococcus pneumoniae* was inhibited by chloramphenicol (1.54×10^{-3} mmol/mL) and compound **6** (2.4×10^{-3} mmol/mL); however, this effect was higher in the presence of compound **4** (1.20×10^{-3} mmol/mL).

These data suggest that phenylhydrazine fragment bound to acetamide group could be the responsibility of the antibacterial effect of compound **6**. This hypothesis is supported by other studies which indicate that some acetamide derivatives exert antibacterial activity against *Staphylococcus aureus* [56-58] and *Streptococcus pneumoniae* [59-62]. However, the biological activity of **4** was different to **6**; these data suggest that [1,5]Dioxonane-6,9-dione fragment can exert higher antibacterial activity compared with acetamide groups. Here it is important to mention that some studies showed that some dioxo-dione derivatives decrease of bacterial growth of Gram-positive bacteria [63, 64].

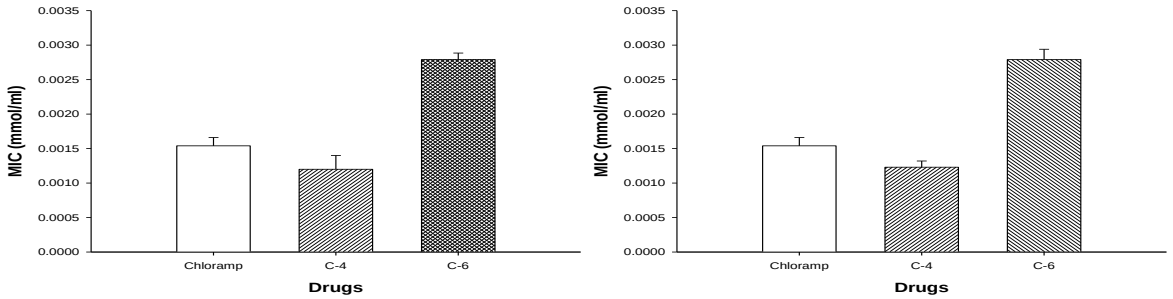

Figure 4. The biological activity produced by chloramphenicol (Chloramp) and compounds 4 and 6 against either *Staphyloccocus aureus* (Left) or *Streptococcus pneumoniae* (Right). The results showed that bacterial growth of this microorganism was significantly inhibited (p = 0.05) by compound 4 compared with chloramphenicol and compound 6. Each bar represents the mean ± S.E. of 9 experiments.

On the other hand, other experiments were carried out to evaluate the biological activity of chloramphenicol and compounds **2** to **7** against *Escherichia coli* and *Klebsiella pneumoniae*. The results (Figure 5) showed that compound **4** significantly decreased the bacterial growth of Gram-negative bacteria compared to compound **6** and chloramphenicol. All these data suggest that these chloramphenicol derivatives could interact with some biological molecule involved in the surface of Gram-positive or Gram-negative bacteria, which may decrease the bacterial growth of these microorganisms.

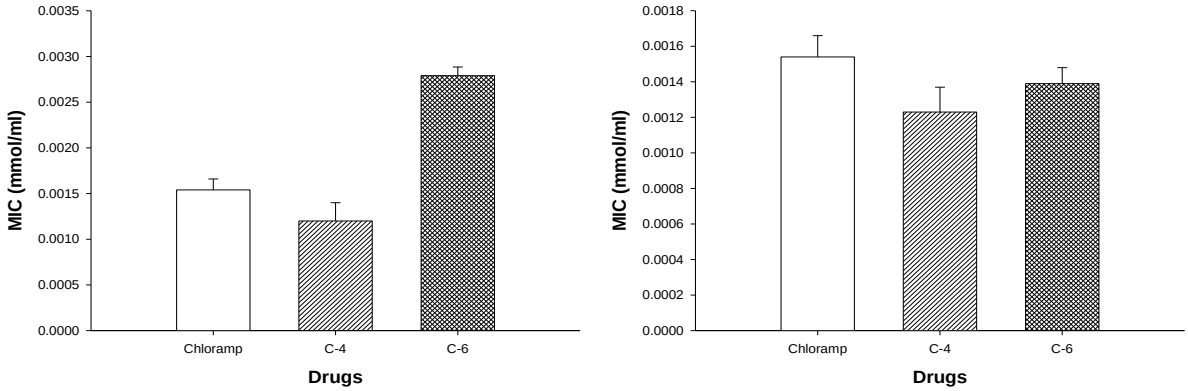

Figure 5. Antibacterial activity exerted by chloramphenicol (Chloramp) and compounds 4 and 6 against either *Klebsiella pneumoniae* (Left) or *Escherichia coli* (Right). The data showed that the bacterial growth of Gram-negative bacteria was significantly inhibited (p = 0.05) by compounds 4 and 6 compared with chloramphenicol. Each bar represents the mean ± S.E. of 9 experiments.

3.6. Ligand-protein interaction.

Various theoretical models are used to predict the interaction of different compounds with the surface of several proteins or enzymes; these models involve some parameters such as free binding energies and solvation energies [65-67]. For example, the Dockingserver software was used to analyze the interaction of some compounds such as coumarin-thiopropioinic acids [68], (1,2,3-triazole) [69], anthranilic acid derivatives [70], Pyrazolo[5, 1-c][1, 2, 4]triazoles [71] with both Gram-positive and Gram-negative bacteria. In this way, it was decided to use 5fpo and 3zbi proteins as theoretical models (Protein Data Bank) [25, 26] to evaluate the interaction of compounds **2** to **7** with either *Staphylococcus aureus* or *Escherichia coli* bacteria. The results (Tables 1 and 2) showed different amino acid residues involved in the interaction of compounds **4** and **6** with either 5fpo or 3zbi proteins surface compared with compounds **2**, **3**, **5**, **7,** and chloramphenicol; this phenomenon could be due to differences in their chemical

structure. Besides, other theoretical data showed differences in some thermodynamic parameters involved in the interaction of **4** and **6** with either 5fpo or 3zbi proteins compared with compounds **2**, **3**, **5**, **7,** and chloramphenicol (Tables 3 and 4).

Table 1. Interaction of compound 4 and indomethacin (control) with 5fpo-protein surface.

Chloramphenicol	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6	Compound 7
Met_{79}	Leu_{82}	Met_{79}	Leu_{82}	Leu_{80}	Leu_{82}	Leu_{80}
Leu_{80}	Asn_{84}	Leu_{80}	Glu_{110}	Leu_{82}	Glu_{110}	Leu_{82}
Leu_{82}	Lys_{112}	Ser_{81}	Lys_{112}	Leu_{111}	Leu_{111}	Lys_{112}
Glu_{110}	Ala_{117}	Leu_{82}	Ala_{117}	Lys_{112}	Lys_{112}	Glu_{167}
Lys_{112}	Arg_{133}	Lys_{112}	Arg_{133}	Arg_{133}	Ile_{113}	Tyr_{219}
Ala_{117}	Glu_{167}	Ala_{117}	Glu_{167}	Glu_{167}	Glu_{167}	Val_{281}
Arg_{133}	Arg_{194}	Arg_{133}	Arg_{194}	Tyr_{219}	Phe_{217}	Lys_{283}
Glu_{167}	Tyr_{219}	Glu_{167}	Tyr_{219}	Asp_{278}	Tyr_{219}	
Tyr_{219}	Lys_{307}	Tyr_{219}	Val_{281}	Val_{281}	Asn_{247}	
Val_{281}				Lys_{283}	Val_{281}	
				Lys_{307}	Lys_{283}	

Table 2. Interaction of chloramphenicol and compounds 2 to 7 with 3zbi -protein surface.

Chloramphenicol	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6	Compound 7
Ile_{791}	Ile_{791}	Thr_{792}	Phe_{803}	Ile_{791}	Ile_{791}	Ile_{791}
Thr_{792}	Thr_{792}	Phe_{803}	Leu_{805}	Thr_{792}	Thr_{792}	Thr_{792}
Phe_{803}	Phe_{803}	Leu_{805}	Ala_{823}	Ile_{795}	Gly_{793}	Gly_{793}
Val_{804}	Val_{804}	Ala_{823}	Ala_{831}	Lys_{796}	Ile_{795}	Val_{802}
Leu_{805}	Leu_{805}		Phe_{888}	Gln_{799}	Val_{802}	Phe_{803}
Ala_{823}	Ala_{823}			Arg_{801}	Phe_{803}	Ala_{823}
				Val_{802}	Leu_{805}	
				Phe_{803}	Ala_{823}	
				Ala_{823}		

Table 3. Thermodynamic parameters involved in the interaction of chloramphenicol and their derivatives with the 5fpo-protein surface.

Compound	Est. Free energy of Binding (kcal/mol)	Est. Inhibition Constant (Ki)	vdW + H-bond + desolv Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Interm. Energy	Interact Surface
Chloramphenicol	-7.84	1.78	-8.89	0.05	-8.84	702.91
2	-5.32	125.63	-6.76	-0.04	-6.81	853.63
3	-5.31	128.09	-6.94	0.03	-6.92	860.66
4	-7.07	6.62	-7.87	0.04	-7.83	803.93
5	-5.98	41.42	-6.36	-0.88	-7.24	851.29
6	-7.84	1.79	-7.71	-0.20	-7.91	759.21
7	-8.79	10.47	-7.94	-0.08	-8.02	692.93

Table 4. Thermodynamic parameters involved in the interaction of chloramphenicol and their derivatives with the 3zbi-protein surface.

Compound		Est. Free energy of Binding (kcal/mol)	Est. Inhibition Constant (Ki)	vdW + H-bond + desolv Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Interm. Energy	Interact Surface
Chloramphenicol		-5.82	54.16	-5.99	0.01	-5.98	423.32
2		-5.46	98.85	-7.00	0.04	-6.95	550.00
3		-5.49	94.60	-6.57	0.01	-6.57	518.97
4		-6.55	15.82	-7.64	-0.02	-7.49	591.75
5		-5.74	61.87	-7.79	-0.09	-7.88	649.74
6		-5.86	50.62	-7.66	-0.01	-7.68	603.96
7		-5.29	133.07	-5.74	-0.02	-5.76	479.98

4. Conclusions

In this study, an easy synthesis of some chloramphenicol derivatives (compounds **2** to **7**) is reported: it is noteworthy that these methods do not require special conditions or dangerous reagents. Besides, both compounds **4** and **6** showed antibacterial activity against either Gram-positive or Gram-negative bacteria; therefore, these chloramphenicol derivatives could be a good candidate as the bacterial agent.

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Conflict of Interest

We declare that this manuscript does not have any conflict of financial interests (political, personal, religious, ideological, academic, intellectual, commercial, or otherwise) for its publication.

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