Research Article

Synthesis, Characterization, and Antibacterial Activity of Diethyl 1-((4-Methyl-2-phenyl-4,5-dihydrooxazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate

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The compound, diethyl 1-((4-methyl-2-phenyl-4,5-dihydrooxazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate 2, was synthesized in high yield, through 1,3-dipolar cycloaddition reaction of 4-(azidomethyl)-4-methyl-2-phenyl-4,5-dihydrooxazole and diethyl but-2-yne-dioate in the absence of a solvent. The structure of the synthesized compound was established on the basis of NMR spectroscopy (1H, 13C), X-ray crystallography, and MS data. The prepared compound was also tested in vitro for its antibacterial activity against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli). The calculation of MBC/MIC ratio showed that this triazole derivative 2 had a bactericidal effect on the two strains tested.

1. Introduction

Heterocycles having five-membered rings such as those containing the 1,2,3-triazole moiety play an important role in various biochemical processes. 1,2,3-Triazoles are an important class of heterocyclic compounds due to their wide usage as synthetic intermediates and pharmaceuticals [1–3]. Many triazole derivatives are found to exhibit various pharmacological properties such as antimicrobial [4], antiepileptic [5], antitubercular [6], and antibacterial [7], and a large number of 1,2,3-triazoles have also been reported with significant anticancer activities [8–10]. Since they are nontoxic, highly stable compounds and mostly water soluble, the 1,2,3-triazole derivatives could be ideal drug candidate and they could participate actively in molecular interactions by hydrogen bond formation [11] which implies their facility to binding with the biological targets [12].

In continuation of our research interest in heterocyclic amino acids and their precursors [13–18], we report in the present study our results concerning the synthesis and antibacterial activity of a new 1,2,3-triazole compound, as diethyl 1-((4-methyl-2-phenyl-4,5-dihydrooxazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate, an oxazolinic precursor of heterocyclic amino acids via 1,3-dipolar cycloaddition reaction between 2-phenyl-4-methyl-4-(azidomethyl)oxazoline and diethyl but-2-yne-dioate. The synthesized triazole derivative was characterized by spectroscopic techniques, such as 1D and 2D NMR spectroscopy, mass spectrometry (MS), and X-ray crystallography. In addition it was evaluated for its antibacterial activity in vitro against Escherichia coli ATCC 25922 (E. coli) and Staphylococcus aureus ATCC 29213 (S. aureus).

2. Results and Discussions

2.1. Chemistry: The starting 2-phenyl-4-methyl-4-(azidomethyl)oxazoline 1 was prepared from oxazoline derivative by reaction with sodium azide in reflux of DMF, using El Hajji’s method [19]. This intermediate azide compound was obtained pure with a 92% yield, as colorless oil after
Scheme 1: Synthesis of compound 2 with good yield using the 1,3-dipolar cycloaddition reaction of 4-(azidomethyl)-4-methyl-2-phenyl-4,5-dihydrooxazole and diethyl but-2-yne diolate in the absence of a solvent.

2.2. Biological Activity. The synthesized compound was tested for its in vitro antibacterial activity against the Gram-positive and the Gram-negative bacteria: Staphylococcus aureus ATCC 29213 (S. aureus) and Escherichia coli ATCC 25922 (E. coli) using the liquid serial dilutions method [20] for determination of MIC. The latter is defined according to the Antibiogram Committee of the French Society for Microbiology (CA-SFM) as being the lowest concentration that results in the inhibition of visible bacterial growth [21].

The determination of the minimum inhibitory concentration (MIC) was realized by the preparation of a series of dilutions of 1/2 of the synthetic product to test on liquid medium (microdilution).
Figure 2: $^1$H-$^13$C 2D correlation spectroscopy identifies coupling between protons and carbons in compound 2.

Table 1: $^1$H (300.13 MHz) and $^{13}$C (75.47 MHz) NMR spectral data for compound 2 in CDCl$_3$, including results obtained by homonuclear 2D shift-correlated and heteronuclear 2D shift-correlated HSQC ($^1$J$_{CH}$). Chemical shifts (δ in ppm) and coupling constants (J in Hz). The definite assignment of the chemical shifts of protons and carbons of compound 2.

<table>
<thead>
<tr>
<th>Position</th>
<th>δ$_H$</th>
<th>δ$_C$</th>
<th>Correlation H-H</th>
<th>Correlation C-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td>139.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>127.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>159.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.27–4.42 (q, J = 7.15)</td>
<td>62.76</td>
<td>2H$^7$–2H$^7$ and 2H$^7$–3H$^8$</td>
<td>C$^7$–2H$^7$</td>
</tr>
<tr>
<td>8</td>
<td>1.38 (t, J = 7.15)</td>
<td>14.14</td>
<td>3H$^8$–3H$^8$ and 3H$^8$–2H$^7$</td>
<td>C$^8$–3H$^8$ and C$^8$–2H$^7$</td>
</tr>
<tr>
<td>6’</td>
<td></td>
<td>158.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7’</td>
<td>4.24–4.38 (q, J = 7.15)</td>
<td>61.71</td>
<td>2H$^7$–2H$^7$ and 2H$^7$–3H$^8$</td>
<td>C$^7$–2H$^7$</td>
</tr>
<tr>
<td>8’</td>
<td>1.30 (t, J = 7.15)</td>
<td>13.66</td>
<td>3H$^8$–3H$^8$ and 3H$^8$–2H$^7$</td>
<td>C$^8$–3H$^8$ and C$^8$–2H$^7$</td>
</tr>
<tr>
<td>9</td>
<td>4.76–4.93 (AB, J = 13.76)</td>
<td>56.38</td>
<td>2H$^9$–2H$^9$</td>
<td>C$^9$–2H$^9$</td>
</tr>
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<td>10</td>
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<td></td>
</tr>
<tr>
<td>11</td>
<td>4.13–4.53 (AB, J = 9.05)</td>
<td>75.13</td>
<td>2H$^{11}$–2H$^{11}$</td>
<td>C$^{11}$–2H$^{11}$</td>
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<td>12</td>
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<td>164.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1.41 (s)</td>
<td>25.19</td>
<td>3H$^{13}$–3H$^{13}$</td>
<td>C$^{13}$–3H$^{13}$</td>
</tr>
<tr>
<td>14–19</td>
<td>7.35–7.84 (m)</td>
<td>128.21–132.06</td>
<td>5H$<em>{arom}$–5H$</em>{arom}$</td>
<td>5C$<em>{arom}$–5H$</em>{arom}$</td>
</tr>
</tbody>
</table>
The minimum bactericidal concentration (MBC) was regarded as being the lowest concentration, in product tested, having shown an absence of growth.

According to our study, compound 2 has an inhibitory activity on the \textit{S. aureus} and \textit{E. coli} strains (MIC = 1.25 mg/mL). The results of the minimum bactericidal concentration, having shown a maximum number of 5 colonies on can, are MBC = 2.5 mg/mL for ATCC strains of \textit{S. aureus}, \textit{E. coli}, and \textit{B. subtilis}.

The calculation of ratio MBC/MIC showed that this triazole derivative has a bactericidal effect on the 2 strains tested.

### 3. Experimental Protocols

3.1. Chemistry. Melting point was determined with an electrothermal melting point apparatus and was uncorrected. NMR spectra ($^1$H and $^{13}$C) were recorded on a Bruker AM 300 (operating at 300.13 MHz for $^1$H, at 75.47 MHz for $^{13}$C) spectrometer (City of Innovation, USMBA-Fez). NMR data are listed in ppm and are reported relative to tetramethylsilane ($^1$H, $^{13}$C); residual solvent peaks are used as internal standard. All reactions were followed by TLC. TLC analyses were carried out on 0.25 mm thick precoated silica gel plates (Merck Fertigplatten Kieselgel 60F254) and spots were visualized under UV light or by exposure to vaporized iodine. Mass spectra were recorded on a PolarisQ Ion Trap GC/MSn Mass Spectrometer (City of Innovation, USMBA-Fez). ORTEP of compound 2 was obtained on a Bruker APEXIICCD detector diffractometer (CNRST-Rabat).

**Synthesis of Diethyl 1-((4-Methyl-2-phenyl-4,5-dihydrooxazol-4-yl)methyl)-1H-1,2,3-triazole-4,5-dicarboxylate 2.** A mixture of 0.65 mmol of 4-(azidomethyl)-4-methyl-2-phenyl-4,5-dihydrooxazole and 0.65 mmol of diethyl acetylenedicarboxylate was constantly stirred for 12 hours. After reaction, the reaction crude was treated with ethyl acetate, the organic layer was washed with water and dried with sodium sulfate.
(Na₂SO₄), and the solvent was removed. The product was purified by recrystallization in ether-hexane to afford the pure product. Yield = 75% (white solid); m.p. = 92–94 °C; Rₜ = 0.23 (ether/hexane). δH ppm (300.13 MHz; CDCl₃): 1.30 (3H, CH₃-CH₂-t, J = 7.15 Hz); 1.38 (3H, CH₃-CH₂-t, J = 7.15 Hz); 1.41 (3H, CH₃-Oxaz, s); 4.13–4.53 (2H, CH₂-Oxaz, AB, J = 9.05 Hz); 4.24–4.38 (2H, -CH₂-CH₃, q, J = 7.15 Hz); 4.27–4.42 (2H, -CH₂-CH₃, q, J = 7.15 Hz); 4.76–4.93 (2H, -CH₂-triazole, AB, J = 13.76 Hz); 7.35–7.84 (5H arom, m). δC ppm (75.47 MHz; CDCl₃): 13.66 (IC, CH₃-CH₂-); 14.14 (IC, CH₃-CH₂-); 25.19 (IC, CH₃-Oxaz); 56.38 (IC, CH₂-triazole); 61.71 (IC, CH₂-CH₃); 62.76 (IC, CH₂-CH₃); 70.77 (IC, C₈-Oxaz); 75.13 (IC, CH₂-Oxaz); 127.00 (C-5, of triazole ring); 128.21; 128.46; 131.74 and 132.06 (6C arom); 139.60 (C-4, of triazole ring); 158.73 and 159.93 (2C, C=O); 164.46 (IC, CN). MS-EI: [M+1]⁺ = 387.

3.2. Biological Activity. 40 mg of compound 2 was completely dissolved in 1 mL of DMSO. Then, 3 mL of BHI (Brain Heart Infusion) medium was added to give a final concentration of 10 mg/mL.

The MIC was determined by the method of dilution in liquid medium by microdilution. The method of microdilution consists in preparing of dilution series in a 96-well liquid medium by microdilution. The method of microdilution was carried out according to the protocol recommended by CLSI (Clinical and Standard Laboratories Institute) [20].

100 µL of the BHI medium (Brain Heart Infusion) was distributed in all the wells except for those of the first line. Then, 200 µL of the stock solution of product 2 was added in the first line. The realization of the dilution series was made by taking 100 µL first well of the first column and by adding it in the second well pertaining to the same column and so on until the penultimate well. The same stages were repeated for the other columns.

The wells were inoculated by 100 µL of the bacterial suspension with a concentration of 106 UFC/mL. The wells of the last line of the microplate contain only the inoculum (control). The microplate was incubated at 37°C for 24 hours. The MIC corresponds to the well, containing the lowest concentration of the product tested, which showed no visible bacterial growth.

4. Conclusion

The synthesis of diethyl 1-((4-methyl-2-phenyl-4,5-dihydrooxazol-4-yl)ethyl)-1H-1,2,3-triazole-4,5-dicarboxylate has been realized with good yield using the 1,3-dipolar cycloaddition reaction of 4-(azidomethyl)-4-methyl-2-phenyl-4,5-dihydrooxazole and diethyl but-2-yne dioate in the absence of a solvent.

The structure of the obtained compound was confirmed by NMR spectroscopy (¹H, ¹³C), X-ray crystallography, and MS data.

The biological tests, carried out on the synthesized compound, showed that this triazole derivative has a bactericidal effect against the Gram-positive and the Gram-negative bacteria: Staphylococcus aureus ATCC 29213 (S. aureus) and Escherichia coli ATCC 25922 (E. coli).

Conflicts of Interest

The authors declared that they have no conflicts of interest as regards this work.

References


