Research Article

Synthesis, Characterization, and Antibacterial Activity of Diethyl 1-((4-Methyl-2-phenyl-4,5-dihydrooxazol-4-yl)methyl)-1*H*-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)-1*H*-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-ynedioate 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)-1*H*-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-ynedioate. 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-ynedioate in the absence of a solvent.

FIGURE 1: ^1H - ^1H correlation spectroscopy identifies coupling between protons in compound **2**.

chromatography on silica gel column. In the second time, compound **1** was submitted to 1,3-dipolar cycloaddition reaction at room temperature with diethyl but-2-ynedioate in the absence of a solvent.

The reaction was monitored by thin-layer chromatography and after consumption of the starting material, stirring was stopped. The recrystallization of the crude mixture in ether/hexane (v/v) led to the cycloadduct **2** with a 75% yield (Scheme 1).

The structure of compound **2** was established on the basis of NMR spectroscopy (^1H and ^{13}C) (Figures 1 and 2), X-ray crystallography (Figure 3), and MS data. The definite assignment of the chemical shifts of protons and carbons is shown in Table 1.

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).

Heteronuclear ^1H - ^{13}C 2D
spectrum for compound 2 FIGURE 2: ^1H - ^{13}C 2D correlation spectroscopy identifies coupling between protons and carbons in compound 2.TABLE 1: ^1H (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 ($^1J_{\text{CH}}$). 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.

Position	δ_{H}	δ_{C}	Correlation H-H	Correlation C-H
4	—	139.60	—	—
5	—	127.00	—	—
6	—	159.93	—	—
7	4.27–4.42 (q, $J = 7.15$)	62.76	$2\text{H}^7\text{-}2\text{H}^7$ and $2\text{H}^7\text{-}3\text{H}^8$	$\text{C}^7\text{-}2\text{H}^7$
8	1.38 (t, $J = 7.15$)	14.14	$3\text{H}^8\text{-}3\text{H}^8$ and $3\text{H}^8\text{-}2\text{H}^7$	$\text{C}^8\text{-}3\text{H}^8$ and $\text{C}^8\text{-}2\text{H}^7$
6'	—	158.73	—	—
7'	4.24–4.38 (q, $J = 7.15$)	61.71	$2\text{H}^{7'}\text{-}2\text{H}^{7'}$ and $2\text{H}^{7'}\text{-}3\text{H}^{8'}$	$\text{C}^{7'}\text{-}2\text{H}^{7'}$
8'	1.30 (t, $J = 7.15$)	13.66	$3\text{H}^{8'}\text{-}3\text{H}^{8'}$ and $3\text{H}^{8'}\text{-}2\text{H}^{7'}$	$\text{C}^{8'}\text{-}3\text{H}^{8'}$ and $\text{C}^{8'}\text{-}2\text{H}^{7'}$
9	4.76–4.93 (AB, $J = 13.76$)	56.38	$2\text{H}^9\text{-}2\text{H}^9$	$\text{C}^9\text{-}2\text{H}^9$
10	—	70.77	—	—
11	4.13–4.53 (AB, $J = 9.05$)	75.13	$2\text{H}^{11}\text{-}2\text{H}^{11}$	$\text{C}^{11}\text{-}2\text{H}^{11}$
12	—	164.46	—	—
13	1.41 (s)	25.19	$3\text{H}^{13}\text{-}3\text{H}^{13}$	$\text{C}^{13}\text{-}3\text{H}^{13}$
14–19	7.35–7.84 (m)	128.21–132.06	$5\text{H}_{\text{arom}}\text{-}5\text{H}_{\text{arom}}$	$5\text{C}_{\text{arom}}\text{-}5\text{H}_{\text{arom}}$

FIGURE 3: Molecular structure of compound showing the atom numbering scheme. H atoms are represented as small spheres of arbitrary radii.

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 *S. aureus* and *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 *S. aureus*, *E. coli*, and *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 (^1H and ^{13}C) were recorded on a Bruker AM 300 (operating at 300.13 MHz for ^1H , 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 (^1H , ^{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 APEXII CCD 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

(Na_2SO_4), 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; R_f = 0.23 (ether/hexane). δ_H ppm (300.13 MHz; CDCl_3): 1.30 (3H, $\text{CH}_3\text{-CH}_2$, t, J = 7.15 Hz); 1.38 (3H, $\text{CH}_3\text{-CH}_2$, t, J = 7.15 Hz); 1.41 (3H, $\text{CH}_3\text{-Oxaz}$, s); 4.13–4.53 (2H, $\text{CH}_2\text{-Oxaz}$, AB, J = 9.05 Hz); 4.24–4.38 (2H, $\text{-CH}_2\text{-CH}_3$, q, J = 7.15 Hz); 4.27–4.42 (2H, $\text{-CH}_2\text{-CH}_3$, q, J = 7.15 Hz); 4.76–4.93 (2H, $\text{-CH}_2\text{-triazole}$, AB, J = 13.76 Hz); 7.35–7.84 (5H_{arom}, m). δ_C ppm (75.47 MHz; CDCl_3): 13.66 (1C, $\text{CH}_3\text{-CH}_2$); 14.14 (1C, $\text{CH}_3\text{-CH}_2$); 25.19 (1C, $\text{CH}_3\text{-Oxaz}$); 56.38 (1C, $\text{CH}_2\text{-triazole}$); 61.71 (1C, $\text{CH}_3\text{-CH}_2$); 62.76 (1C, $\text{CH}_3\text{-CH}_2$); 70.77 (1C, $\text{C}_q\text{-Oxaz}$); 75.13 (1C, $\text{CH}_2\text{-Oxaz}$); 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, CO); 164.46 (1C, 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 polypropylene microplate. The determination of the MIC 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)methyl)-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-ynedioate in the absence of a solvent.

The structure of the obtained compound was confirmed by NMR spectroscopy (^1H , ^{13}C), 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

- [1] N.-N. Su, Y. Li, S.-J. Yu, X. Zhang, X.-H. Liu, and W.-G. Zhao, "Microwave-assisted synthesis of some novel 1,2,3-triazoles by click chemistry, and their biological activity," *Research on Chemical Intermediates*, vol. 39, no. 2, pp. 759–766, 2013.
- [2] N.-N. Su, L.-X. Xiong, S.-J. Yu et al., "Larvicidal activity and click synthesis of 2-alkoxyl-2-(1,2,3-triazole-1-yl)acetamide library," *Combinatorial Chemistry and High Throughput Screening*, vol. 16, no. 6, pp. 484–493, 2013.
- [3] B. Schulze and U. S. Schubert, "Beyond click chemistry-supramolecular interactions of 1,2,3-triazoles," *Chemical Society Reviews*, vol. 43, no. 8, pp. 2522–2571, 2014.
- [4] Z.-J. Wang, Y. Gao, Y.-L. Hou et al., "Design, synthesis, and fungicidal evaluation of a series of novel 5-methyl-1H-1,2,3-triazole-4-carboxyl amide and ester analogues," *European Journal of Medicinal Chemistry*, vol. 86, pp. 87–94, 2014.
- [5] S. Ulloora, R. Shabaraya, and A. V. Adhikari, "Facile synthesis of new imidazo[1,2-a]pyridines carrying 1,2,3-triazoles via click chemistry and their antiepileptic studies," *Bioorganic and Medicinal Chemistry Letters*, vol. 23, no. 11, pp. 3368–3372, 2013.
- [6] K. Walczak, A. Gondela, and J. Suwiński, "Synthesis and anti-tuberculosis activity of N-aryl-C-nitroazoles," *European Journal of Medicinal Chemistry*, vol. 39, no. 10, pp. 849–853, 2004.
- [7] A. Kamal, S. M. A. Hussaini, S. Faazil et al., "Anti-tubercular agents. Part 8: Synthesis, antibacterial and antitubercular activity of 5-nitrofur based 1,2,3-triazoles," *Bioorganic and Medicinal Chemistry Letters*, vol. 23, no. 24, pp. 6842–6846, 2013.
- [8] W. Zhang, Z. Li, M. Zhou et al., "Synthesis and biological evaluation of 4-(1,2,3-triazol-1-yl)coumarin derivatives as potential antitumor agents," *Bioorganic and Medicinal Chemistry Letters*, vol. 24, no. 3, pp. 799–807, 2014.
- [9] R. Bollu, J. D. Palem, R. Bantu et al., "Rational design, synthesis and anti-proliferative evaluation of novel 1,4-benzoxazine-[1,2,3]triazole hybrids," *European Journal of Medicinal Chemistry*, vol. 89, pp. 138–146, 2014.
- [10] T. A. Dang Thi, N. T. Kim Tuyet, C. Pham The et al., "Synthesis and cytotoxic evaluation of novel amide-triazole-linked triterpenoid-AZT conjugates," *Tetrahedron Letters*, vol. 56, no. 1, pp. 218–224, 2015.
- [11] M. Whiting, J. Muldoon, Y.-C. Lin et al., "Inhibitors of HIV-1 protease by using in situ click chemistry," *Angewandte Chemie - International Edition*, vol. 45, no. 9, pp. 1435–1439, 2006.
- [12] W. S. Horne, M. K. Yadav, C. D. Stout, and M. R. Ghadiri, "Heterocyclic peptide backbone modifications in an α -helical coiled coil," *Journal of the American Chemical Society*, vol. 126, no. 47, pp. 15366–15367, 2004.
- [13] F. Zaid, S. El Hajji, A. El Hallaoui et al., "Synthesis of heterocyclic β -aminoalcohol precursors of heterocyclic α -amino acids," *Preparative Biochemistry and Biotechnology*, vol. 28, no. 2, pp. 137–153, 1998.
- [14] S. Achamlale, A. Elachqar, A. El Hallaoui et al., "Synthesis of biheterocyclic α -amino acids," *Amino Acids*, vol. 17, no. 2, pp. 149–163, 1999.
- [15] Y. Aouine, H. Faraj, A. Alami et al., "Synthesis of new triheterocyclic compounds, precursors of biheterocyclic amino acids,"

Journal Marocain de Chimie Hétérocyclique, vol. 7, no. 1, pp. 44–49, 2008.

- [16] Y. Aouine, H. Faraj, A. Alami, A. El-Hallaoui, A. Elachqar, and A. Kerbal, "Simple and efficient synthesis of racemic 2-(tert-Butoxycarbonylamino)-2-methyl-3-(1H-1,2,4-triazol-1-yl)propanoic acid, a new derivative of β -(1,2,4-Triazol-1-yl)alanine," *Molecules*, vol. 16, no. 4, pp. 3380–3390, 2011.
- [17] Y. Aouine, H. Faraj, A. Alami et al., "Triheterocyclic compounds, oxazolinic precursors of biheterocyclic amino acids, Part II: phenothiazine derivatives and structural study of regioisomers through 1H-15N 2D NMR HMBC," *Journal Marocain de Chimie Hétérocyclique*, vol. 133, no. 1, pp. 39–47, 2014.
- [18] A. Younas, E. H. Abdelilah, and A. Anouar, "N,N-dibenzyl-1-(1-[(4-methyl-2-phenyl-4,5-dihydrooxazol-4-yl)methyl])-1h-1,2,3-triazol-4-yl)methanamine," *MolBank*, vol. 2014, no. 1, article no. M819, 2014.
- [19] A. Atmani, A. E. Hallaoui, S. E. Hajji, M. L. Roumestant, and P. Viallefont, "From Oxazolines to Precursors of Aminoacids," *Synthetic Communications*, vol. 21, no. 22, pp. 2383–2390, 1991.
- [20] *Clinical and Laboratory Standards Institute CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, vol. 32, CLSI document, Approved Standard Ninth Edition edition, 2012.
- [21] "European Committee on Antimicrobial Susceptibility Testing EUCAST, Comité de l'antibiogramme de la société française de microbiologie, Recommandations 2015," V1.0 Janvier 2015.