

Short Note

# Methyl *N*-[1-(Benzoylamino)-2-methoxy-2-oxoethyl]-tryptophanate

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**Abstract:** The title compound, methyl *N*-[1-(benzoylamino)-2-methoxy-2-oxoethyl]tryptophanate **2**, was synthesized in high yield, via *N*-alkylation reaction of methyl 2-azido-2-benzamidoacetate with methyl 2-amino-3-(1*H*-indol-3-yl)propanoate in acetone, with the presence of diisopropylethylamine as a base. The structure of the prepared compound was characterized by <sup>1</sup>H, <sup>13</sup>C NMR in addition to MS, X-Ray diffraction data, and elemental analysis. This compound was tested in vitro for its antibacterial activity against Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enteric*. The MIC values showed that the synthesized compound had a bactericidal effect against the strains tested.

**Keywords:** antibacterial activity; carboxylic amino esters; *N*-alkylation

## 1. Introduction

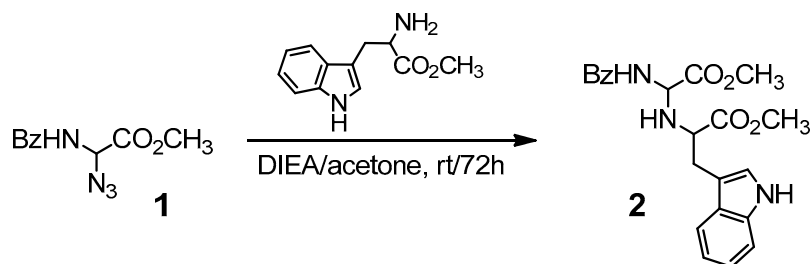
Heterocyclic  $\alpha$ -amino acids play a predominant role in medicinal chemistry because of the large activity spectrum they present. The rapid assembly of molecular diversity is an important goal of synthetic organic chemistry and is one of the key paradigms of modern drug discovery. The synthesis of new  $\alpha$ -carboxylic amino esters containing heterocyclic systems occupies an important place in the realm of synthetic organic chemistry [1–3] and they are the fundamental units of life because of their wide utility of such compounds as components of proteins, peptides, and as starting materials for the synthesis of naturally occurring biologically active compounds [4–6].

Recently, heterocyclic  $\alpha$ -amino acids have shown biological properties including antibacterial activity [7]. In view of these observations and in continuation of our previous work in heterocyclic chemistry, we synthesized the methyl *N*-[1-(benzoylamino)-2-methoxy-2-oxoethyl]tryptophanate **2** through *N*-alkylation between methyl 2-azido-2-benzamidoacetate and methyl 2-amino-3-(1*H*-indol-3-yl)propanoate [8,9]. Compound **2** was obtained with good yield (86% yield) and characterized by spectroscopic techniques, such as 1D and 2D NMR spectroscopy, mass spectrometry (MS), X-ray crystallography, MS data, and elemental analysis.

## 2. Results

### 2.1. Chemistry

The starting methyl 2-azido-2-benzamidoacetate **1** was prepared from glycine derivative by reaction with sodium azide in room temperature using acetone as solvent. This intermediate azide compound **1** was obtained pure with a good yield (92% yield), as a white solid after chromatography on silica gel column (ether/hexane: 1/4). After that, the compound **1** was substituted by 2-amino-3-(1*H*-indol-3-yl)propanoate via *N*-alkylation reaction in the presence of diisopropylethylamine as base in acetone at room temperature (Scheme 1).



Scheme 1. Synthesis strategy of compound 2.

A single crystal of the title compound was obtained by recrystallization from Acetone and its structure was established on the basis of NMR spectroscopy ( $^1\text{H}$ ,  $^{13}\text{C}$ ) (Figures 1 and 2), MS data and elemental analysis, and X-ray crystallography (Figure 3). The definite assignment the chemical shifts of protons and carbons are shown in Table 1.

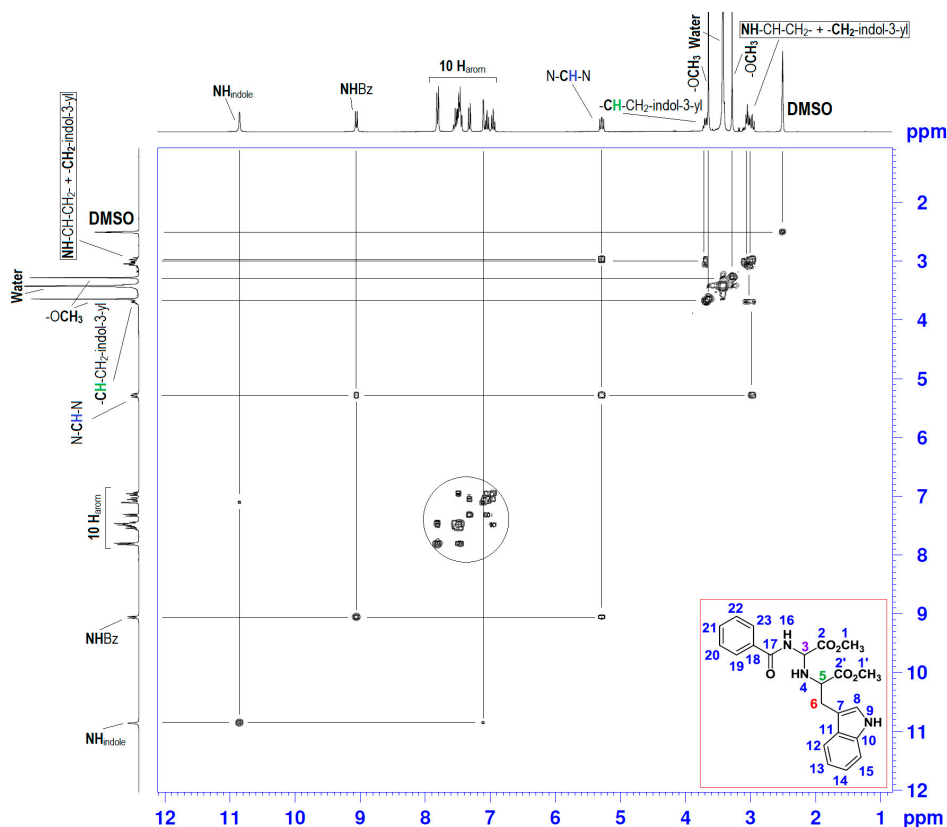


Figure 1. Homonuclear  $^1\text{H}$ - $^1\text{H}$  2D spectrum of compound 2.

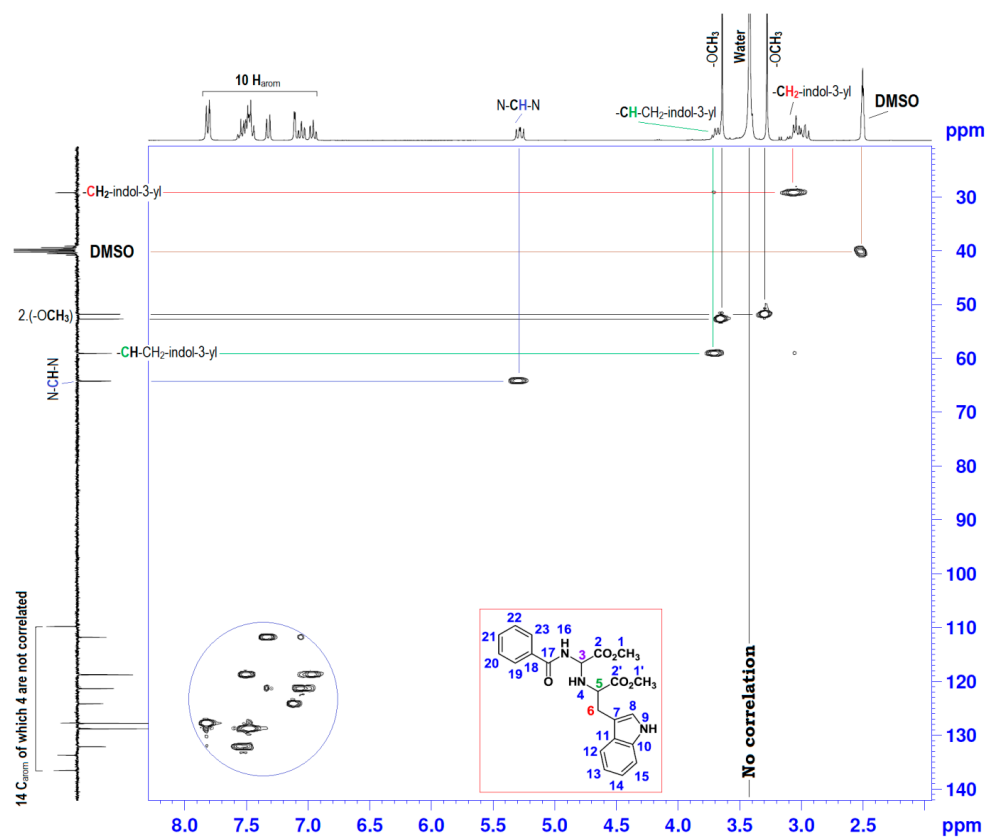


Figure 2. Heteronuclear  $^1\text{H}$ - $^{13}\text{C}$  2D spectrum of compound 2.

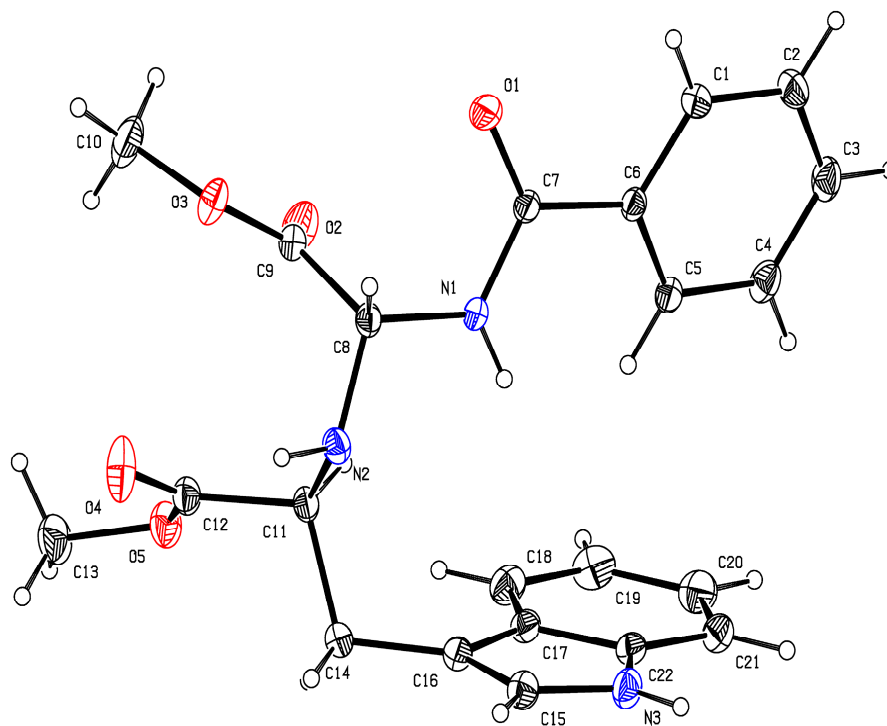


Figure 3. ORTEP view of compound 2 showing the atom-numbering scheme.

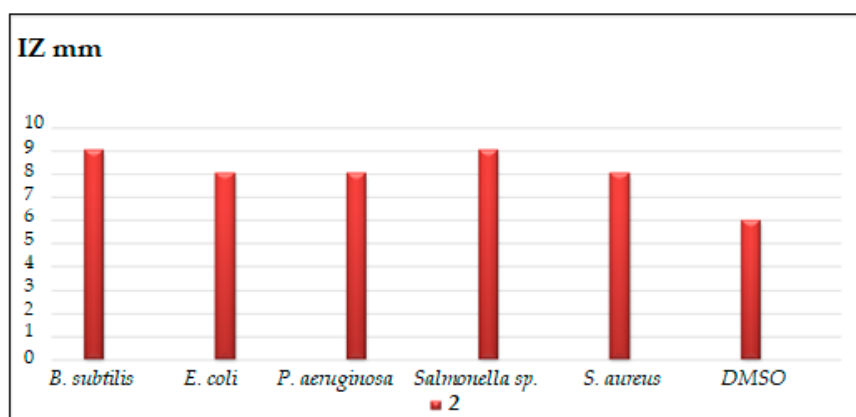
**Table 1.**  $^1\text{H}$  (300.13 MHz) and  $^{13}\text{C}$  (75.47 MHz) NMR spectral data for compound **2** in  $\text{DMSO-}d_6$ , including results obtained by homonuclear 2D shift-correlated and heteronuclear 2D shift-correlated HMBC. Chemical shifts ( $\delta$  in ppm) and coupling constants ( $J$  in Hz).

Position	$\delta_{\text{H}}$	$\delta_{\text{C}}$	Correlation H-H	Correlation C-H
1	3.28 (s)	51.78	$3\text{H}^1\text{-}3\text{H}^1$	$\text{C}^1\text{-}3\text{H}^1$
2	-	166.61	-	-
1'	3.64 (s)	52.61	$3\text{H}^{1'}\text{-}3\text{H}^{1'}$	$\text{C}^1\text{-}3\text{H}^{1'}$
2'	-	174.89	-	-
3	5.25–5.31 (dd, $J_1 = 10.17$ ; $J_2 = 7.73$ )	64.18	$1\text{H}^3\text{-}1\text{H}^3$ ; $1\text{H}^3\text{-}1\text{H}^4$ ; $1\text{H}^3\text{-}1\text{H}^{16}$	$\text{C}^3\text{-}1\text{H}^3$
4	2.94–3.02 (m)	-	$1\text{H}^4\text{-}1\text{H}^4$ ; $1\text{H}^4\text{-}1\text{H}^3$ ; $1\text{H}^4\text{-}1\text{H}^5$	-
5	3.67–3.72 (t, $J = 6.90$ )	59.06	$1\text{H}^5\text{-}1\text{H}^5$ ; $1\text{H}^5\text{-}1\text{H}^4$ ; $1\text{H}^5\text{-}2\text{H}^6$	-
6	2.94–3.02 (m)	29.18	$2\text{H}^6\text{-}2\text{H}^6$ ; $2\text{H}^6\text{-}1\text{H}^5$	$\text{C}^6\text{-}2\text{H}^6$
9	10.9 (s)	-	$1\text{H}^9\text{-}1\text{H}^9$ ; $1\text{H}^9\text{-}1\text{H}^8$	-
16	9.1 (s)	-	$1\text{H}^{16}\text{-}1\text{H}^{16}$ ; $1\text{H}^{16}\text{-}1\text{H}^3$	-
17	-	170.38	-	-
7, 8; 10–15 and 18–23	6.93–7.82 (m)	109.80–136.48	$10\text{H}^{\text{ar}}\text{-}10\text{H}^{\text{ar}}$ ; $1\text{H}^8\text{-}1\text{H}^9$	$10\text{C}^{\text{ar}}\text{-}10\text{C}^{\text{ar}}$

## 2.2. Biological Activity

### 2.2.1. Disc Diffusion Method

The synthesized product exhibited varying antibacterial activity as is shown by the inhibition zones (IZ) in Figure 4. The results from the disc diffusion assay indicated that the tested compound showed higher antibacterial activity against Gram-positive bacteria (IZ 08–09) than against Gram-negative bacteria (IZ 07–10).



**Figure 4.** Antibacterial activity (inhibition zone (IZ) measured in mm) of compound **2** against pathogenic bacteria.

### 2.2.2. Resazurin Microtiter Plate Assay

In the present study, we used the modified resazurin microtiter plate assay to evaluate the antimicrobial activity of synthesized products. This method provided reproducible and accurate results and allowed direct comparison of the antibacterial activity of the tested compound.

The average of MIC of this compound varied between 2.5–5 mg/mL against the strains tested. As is shown in Table 2, the compound **2** showed similar MIC value of 2.5 mg/mL against Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enteric*, the same value against Gram-positive bacteria *Bacillus subtilis* except *Staphylococcus aureus* which present a MIC of 5 mg/mL.

**Table 2.** Antibacterial activity minimum inhibitory concentration (MIC) in mg/mL of compound 2 against pathogenic bacteria presented.

	Compound 2	Chloramphenicol
<i>E. Coli</i> CIP 53126	2.5	0.05
<i>S. aureus</i> CIP 483	5	0.095
<i>S. enterica</i> CIP 8039	2.5	0.05
<i>B. subtilis</i> CIP 5262	2.5	0.095
<i>P. aeruginosa</i> CIP 82118	2.5	0.05

### 3. Materials and Methods

#### 3.1. Chemistry

All solvents were purified following the standard techniques and commercial reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Melting point was determined with an Electrothermal melting point apparatus and was uncorrected. NMR spectra ( $^1\text{H}$  and  $^{13}\text{C}$ ) were recorded on a Bruker AM 300 spectrometer (operating at 300.13 MHz for  $^1\text{H}$ , at 75.47 MHz for  $^{13}\text{C}$ ) (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany). NMR data are listed in ppm and are reported relative to tetra-methylsilane ( $^1\text{H}$ ,  $^{13}\text{C}$ ); residual solvent peaks being 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 60F<sub>254</sub>) 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 (CNRST-Rabat). Ortep of compound 2 was obtained on a Bruker APEXII CCD detector diffractometer (CNRST-Rabat).

To a solution of 2.6 mmol of *N*-benzoylated methyl  $\alpha$ -azidoglycinate 1 and 3.12 mmol of diisopropylethylamine (DIEA) in 10 mL of acetone, 2.86 mmol of 2-amino-3-(1*H*-indol-3-yl)propanoate was added. The reaction mixture was stirred for 72 h at room temperature. The solvent was evaporated under reduced pressure. The residue was quenched with saturated aqueous solution of ammonium chloride (20 mL) and extracted with methylene chloride (3  $\times$  20 mL). The organic layer was dried over sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and the solvent was removed under reduced pressure. The product obtained was recrystallized from acetone.

*N*-benzoylated methyl  $\alpha$ -azidoglycinate 1: Yield = 92% (white solid); m.p = 80–82 °C.  $^1\text{H}$ -NMR (DMSO,  $\delta_{\text{H}}$  ppm): 3.74 (s, 3H, -OCH<sub>3</sub>); 5.82 (d, 1H, N-CH-N<sub>3</sub>,  $J$  = 7.80Hz); 7.49–7.91 (m, 5H, 5H<sub>arom</sub>); 9.83 (d, 1H, NHBz,  $J$  = 7.80Hz).  $^{13}\text{C}$ -NMR (DMSO,  $\delta_{\text{C}}$  ppm): 53.41 (C, -OCH<sub>3</sub>); 65.62 (1C, N-CH-N<sub>3</sub>); 128.03, 129.04, 132.76 and 132.87 (6C, C<sub>arom</sub>); 167.18 and 167.67 (2C, CO). MS ESI  $m/z$  (%) = 235.

Methyl *N*-[1-(benzoylamino)-2-methoxy-2-oxoethyl]tryptophanate 2: Yield = 86% (white solid); m.p. = 174–176 °C.  $^1\text{H}$ -NMR (DMSO,  $\delta_{\text{H}}$  ppm): 2.94–3.02 (m, 3H, NH-CH-CH<sub>2</sub>- and -CH<sub>2</sub>-indol-3-yl); 3.28 (s, 3H, -OCH<sub>3</sub>); 3.64 (s, 3H, -OCH<sub>3</sub>); 3.67–3.72 (t, 1H, N-CH-CH<sub>2</sub>-,  $J$  = 6.90 Hz); 5.25–5.31 (dd, 1H, N-CH-N,  $J_1$  = 10.17 Hz and  $J_2$  = 7.73 Hz); 6.93–7.82 (m, 10H, 10H<sub>arom</sub>); 9.1 (s, 1H, NHBz); 10.9 (s, 1H, NH<sub>indole</sub>).  $^{13}\text{C}$ -NMR (DMSO,  $\delta_{\text{C}}$  ppm): 29.18 (1C, -CH<sub>2</sub>-indol-3-yl); 51.78 and 52.65 (2C, -OCH<sub>3</sub>); 59.06 (1C, -CH-CH<sub>2</sub>-indol-3-yl); 64.18 (1C, N-CH-N); 109.80, 111.79, 118.74, 121.33, 124.15, 127.74, 127.78, 128.78, 132.12, 133.71 and 136.48 (14C, C<sub>arom</sub>); 166.61, 170.38 and 174.89 (3C, CO). Calcd. for C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>5</sub> (%): C, 64.54; H, 5.66; N, 10.26; Found (%): C 64.28, H 5.71, N 10.16. MS ESI  $m/z$  (%) = 409.60.

#### 3.2. Biological Activity

##### 3.2.1. Disc Diffusion Method

The antimicrobial studies was evaluated using the agar diffusion technic in petri dishes [10], this activity was carried out against five bacterial strains: *Staphylococcus aureus* CIP 483, *Bacillus subtilis* CIP 5262, *Escherichia coli* CIP 53126, *Pseudomonas aeruginosa* CIP 82118, and *salmonella enteric* CIP 8039.

Briefly, 100  $\mu\text{L}$  of suspension containing approximately  $5 \times 10^5$  colony-forming units (CFU)/mL of bacteria cells on Tryptic Soy Agar (TSA). The sterile filter discs (6 mm diameter) were separately impregnated with 15  $\mu\text{L}$  of the synthesized product and placed on the agar which had previously been inoculated with the tested microorganism. These petri dishes were incubated overnight at 37 °C [11]. After incubation, all plates were checked for inhibition zones and the diameters were measured in millimeters, chloramphenicol antibiotics (50 mg/mL) and DMSO were used as positive and negative control respectively.

### 3.2.2. Resazurin Microtiter Plate Assay

For the minimum inhibitory concentration (MIC) of the synthesized product, a modified resazurin microtiter plate assay was used as reported by Sarker [12]. Briefly, a volume of 100  $\mu\text{L}$  of each product solution (20 mg/mL, *v/v* in DMSO 90%), was pipetted into the first row of the 96 well plates, then to all other wells of microtiter plates, we added 100  $\mu\text{L}$  of tryptic soya broth. A serial dilution was achieved by starting transferring 100  $\mu\text{L}$  test material from first row to the subsequent wells in the next row of the same column and so that each well has 100  $\mu\text{L}$  of test material in serially descending concentrations. Then 10  $\mu\text{L}$  of bacterial suspension ( $10^8$  CFU/mL) was added to each well. The microplates were placed in an incubator set at 37 °C for 24 h. Each plate had a set of controls: A column with an antibiotic as positive control (chloramphenicol in serial dilution (50 mg/mL)), a column with all solutions except the test material, and a column with all solutions with the exception of the bacterial suspension. Finally, the resazurin solution was prepared by dissolving 270 mg of resazurin powder in 40 mL sterile distilled water [13]. A volume of 10  $\mu\text{L}$  of resazurin solution as indicator was added in each well after incubation. The microplates were placed again in an incubator at 37 °C for just 2 h. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value [14].

## 4. Conclusions

The synthesis of methyl *N*-[1-(benzoylamino)-2-methoxy-2-oxoethyl]tryptophanate **2** was performed via *N*-alkylation reaction and characterized by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, MS data, elemental analysis, and X-ray crystallography. The synthesized compound was screened for its antimicrobial activity. Antibacterial results indicated that the compound **2** had a bactericidal effect towards all bacterial strains when compared to standard drug Chloramphenicol.

**Supplementary Materials:** The following are available online at [www.mdpi.com/2017/3/M958](http://www.mdpi.com/2017/3/M958), Figure S1:  $^{13}\text{C}$ -NMR spectrum of compound **1**, Figure S2:  $^1\text{H}$ -NMR spectrum of compound **1**, Figure S3:  $^{13}\text{C}$ -NMR spectrum of compound **2**, Figure S4:  $^1\text{H}$ -NMR spectrum of compound **2**, Figure S5: Mass spectrum of compound **2**.

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**Author Contributions:** O.K. performed the experiments; H.F., A.A., and A.E.H. conceived and designed the experiments; M.E.H., M.R.K., and M.B. performed the antibacterial activity experiments; Y.A. analyzed the data and wrote the paper.

**Conflicts of Interest:** The authors declared that they have no conflicts of interest as regards this work.

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