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Mediterranean Journal of Chemistry

Mediterranean Journal of Chemistry **2018**, 7(4), 267-271

Synthesis, characterization and antibacterial activity of Methyl (*2R*)-2-benzamido-2-{[(*1R*)-2-methoxy-2-oxo-1-phenylethyl]amino}acetate

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Abstract: The present work covers the recent synthetic of methyl (*2R*)-2-benzamido-2-{[(*1R*)-2-methoxy-2-oxo-1-phenylethyl]amino}acetate, via *N*-alkylation of methyl α-azido glycinate *N*-benzoylated with methyl 2-amino-2-phenylacetate in methylene chloride and presence of triethylamine as a basic catalyst. The structure of the prepared compound was determined by spectroscopic methods: ^{1}H-NMR, ^{13}C-NMR, MS data, elemental analysis and confirmed by X-Ray diffraction. This compound was screened *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 confirmed that the title compound had a bactericidal effect against the strains tested.

Keywords: Antibacterial activity; aminoesters carboxylic; *N*-alkylation.

Introduction

Amino acid derivatives are an important group of peptidomimetics [1]. They exhibit several applications in medicinal chemistry. The amino acids were used as starting keys for synthesis peptides, are known to contribute to various chemotherapeutic effects, as antileukemic [2], antitumor [3], antimicrobial [4], and antiviral agents [5].
Heterocyclic α-amino acids frameworks constitute an essential pharmacophore in many naturally occurring and biologically active agents [6].

In continuation of our ongoing research [7,8], this work deals to describe the synthesis and design of the methyl (2R)-2-benzamido-2-{[(1R)-2-methoxy-2-oxo-1-phenylethyl]amino}acetate **2**. This latter was obtained through N-alkylation of methyl α-azido glycinate N-benzoylated 1 by methyl 2-amino-2-phenylacetate. The structure of the title compound was determined by usual spectroscopic techniques, such as NMR, MS, elemental analysis and corroborated by X-ray crystallography.

Results and Discussion

Chemistry

The methyl α-azido glycinate *N*-benzoylated **1** was prepared using Steglich method [9] and Achamlale's procedure [10,11] by the reaction of sodium azide with the methyl α-bromo glycinate.

Azide **1** is obtained with a good yield (92% yield), as a white solid. After that, it was substituted with methyl 2-amino-2-phenylacetate (derived from L-phenylglycine) through *N*-alkylation reaction in the presence of methylene chloride at room temperature (Scheme 1). After chromatography on a column of silica gel, we isolated only one regisomer in the form of a single crystal [12] whose configuration is well defined (Figure 2).

The structure of the compound 2 was established by ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum reveals in particular, a singlet at 4,65ppm attributed to the proton of the secondary amino group (**NH**-CH-Ph). The proton of the second amino group (Bz-**NH**) appears at 6,75 ppm. A singlet at 3,3 ppm corresponds to methylenic proton (NH-**CH**-Ph).

A doublet centred at 5,52ppm due to the resonance of the other methylenic proton (NH-**CH**-NH). The same for ^{13}C NMR spectrum

taken in $CDCl_3$ as solvent we note the presence of the main signals, at 61.97 ppm attributed to the

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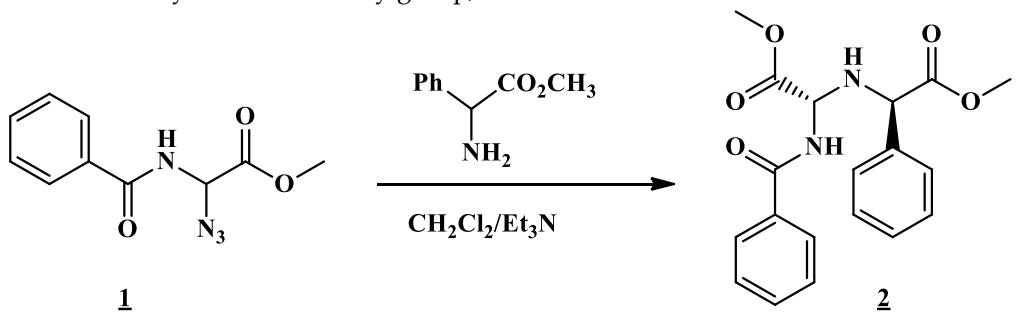
DOI: http://dx.doi.org/10.13171/mjc74181110-alami

Received August 11, 2018
Accepted October 10, 2018
Published November 6, 2018

carbon (-CH-phenyl), at 52.44 and 52.88 ppm attributed to two methyls of the methoxy group, 63.62 ppm attributed to carbon (NH-CH-NH).



Scheme 1. Synthesis strategy of compound **2**.

This attribution has been carried out by the HSQC (Figure1) NMR spectrum. Which, show correlations between the protons and the neighboring carbons. As it shows an interaction between amide proton and the adjacent asymmetric carbon. In contrast, the proton of the amine showed no correlation.

The definite assignment the chemical shifts of protons and carbons are shown in Table 1.

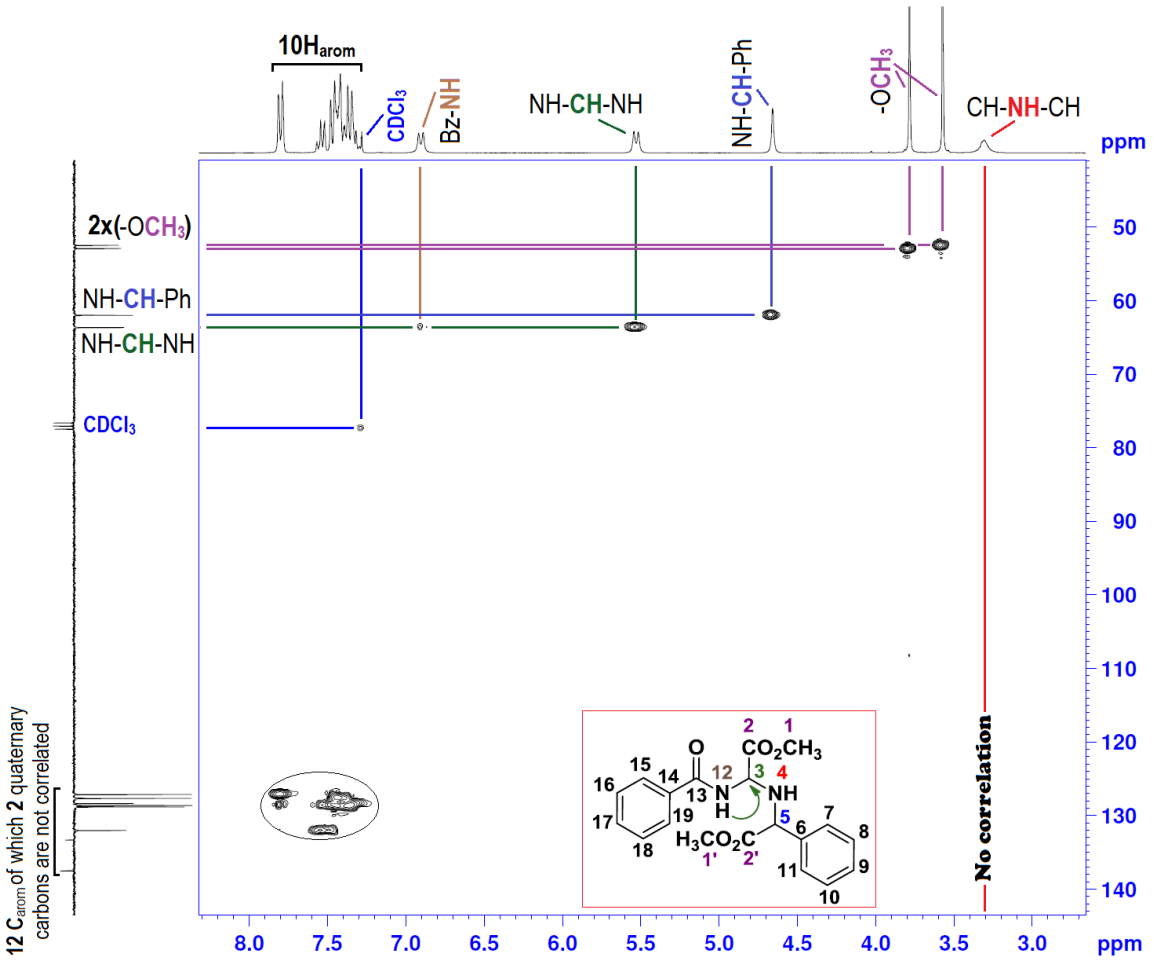


Figure 1. Heteronuclear ^{1}H-^{13}C 2D spectrum of compound **2**.

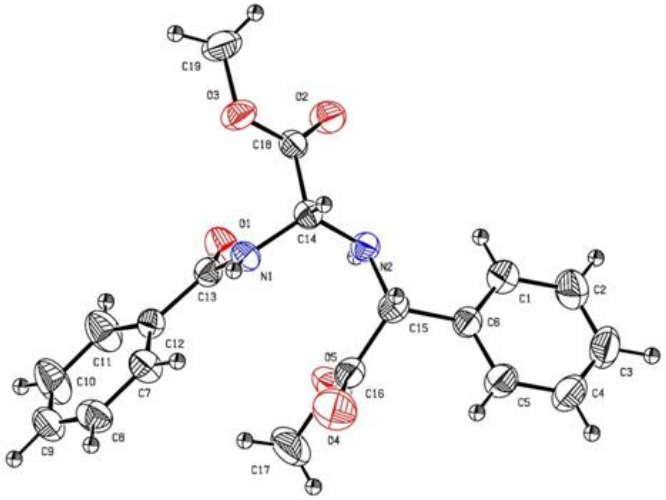


Figure 2. ORTEP diagram of compound **2**.

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 HMBC. Chemical shifts (**δ** in ppm) and coupling constants (*J* in Hz).

Position	δ_H	δ_C	Correlation C-H
1	3.51 (s)	52.44	C^1-$3H^1$
2	-	167.14	-
1’	3.78 (s)	52.88	$C^{1'}$-$3H^{1'}$
2’	-	173.63	-
3	5.52 (d, *J* = 8.4 Hz)	63.62	C^3-$1H^3$ and C^3-$1H^{12}$
4	3.3 (e)	-	-
5	4.65 (s)	61.67	C^5-$1H^5$
12	6.75 (d, *J* = 8.4 Hz)	-	-
13	-	170.14	-
6–11 and 14–19	7.28–7.82 (m)	127.13–137.48	$10C_{arom}$-$10H_{arom}$

Biology Activity

Disc Diffusion Method

The synthesized product exposed diversified antibacterial activity as is shown by the inhibition zones (IZ) in Figure 3. The results from the disc diffusion assay indicated that the tested compound showed important antibacterial activity against Gram-positive bacteria (IZ 08) and Gram-negative bacteria (IZ 08-10).

Resazurin microtiter-plate assay

In this study, we used the modified resazurin microtiter plate assay it is a dye used as an oxidation-reduction indicator in bacterial cell viability assays to evaluate the antimicrobial activity of synthesized products [8]. This method provided the reproducible and accurate results and allowed direct comparison of the antibacterial activity of the tested compounds.

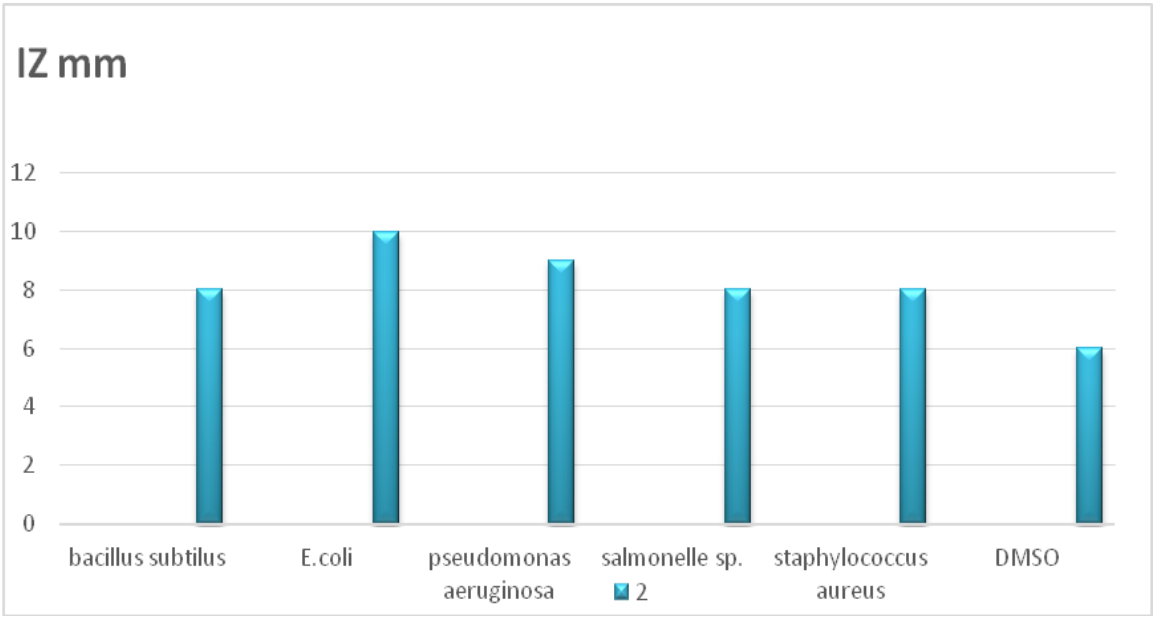


Figure 3. Antibacterial activity (inhibition zone (IZ) measured in mm) of compound **2** against pathogenic bacteria

As can be seen in this Table 2, compound **2** exercised an important inhibitory activity against Gram-negative bacteria more than Gram-positive bacteria. Especially, *Escherichia coli* which have shown a high sensitivity to this compound, with a MIC value of 1.25 mg/mL. The compound **2** showed similar MIC value (2.5 mg/mL) against *salmonella enteric* and *Pseudomonas aeruginosa*, while the growth inhibition of *Bacillus subtilis and Staphylococcus aureus* were achieved at a MIC value 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
Escherichia coli CIP 53126	1,25	0,05
Staphylococcus aureus CIP 483	5	0,095
S. enterica CIP 8039	2,5	0,05
B. subtilus CIP 5262	5	0.095
P. aeruginosa CIP 82118	2,5	0,05

Experimental

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, Morocco). NMR data are listed in ppm and are reported relative to tetramethylsilane (^{1}H, ^{13}C); residual solvent peaks being used as an 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 $60F_{254}$), 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, Morocco). Ortep of compound **2** was obtained on a Bruker APEXII CCD detector diffractometer (CNRST-Rabat, Morocco). Elemental analysis was performed with Flash 2000 EA 1112, Thermo Fisher Scientific-Elemental Analyzer (CNRST-Rabat, Morocco).

To a stirred solution of 2 mmol of methyl 2-amino-2-phenylacetate and 4 mmol of triethylamine in 10 mL of dry methylene chloride, 2.6 mmol of *N*-benzoylated methyl α-azidoglycinate **1** were added. The mixture is stirred at 0°C for 1 hour then at room temperature for 16 hours. The resulting solution was washed with citric acid (15%), then with a saturated solution of sodium bicarbonate

($NaHCO_3$). A single crystal of the title compound is obtained by recrystallization from the ether.

Methyl(2R)-2-benzamido-2-{[(1R)-2-methoxy-2-oxo-1-phenylethyl]amino}acetate **2**: Yield = 86% (white solid); m.p = 126–128 °C.
^{1}H-NMR (300.13 MHz; $CDCl_3$, δ_H ppm): 3.3(e, 1H, NH-CH-Ph); 3.51(s, 3H, -OCH_3); 3.78(s, 3H, -OCH_3); 4.65(s, 1H, NH-CH-Ph); 5.52(d, 1H, N-CH-N, *J* = 8.4 Hz); 6.75(d, 1H, NHBz, *J* = 8.4 Hz); 7.28–7.82(m, 10H_{arom}).
^{13}C-NMR (75.47 MHz; $CDCl_3$, δ_C ppm): 52.44 (1C, OCH_3); 52.88 (1C, OCH_3); 61.97 (1C, NH-CH-Ph); 63.62 (1C, N-CH-N); 127.13–137.48 (10C, C_{arom}); 167.14, 170.14 and 173.63 (3C, CO).
Calcd. for $C_{19}H_{20}N_2O_5$ (%): C, 64.04; H, 5.66; N, 7.86; Found (%): C 63.84, H 5.67, N 7.89.
MS ESI *m/z* (%) = 356.49.

Concerning the disc diffusion method and resazurin microtiter assay plate testing, the operative protocol adopted has already been validated in our previous paper [8].

Conclusion

In summary, the synthesis of methyl (2R)-2-benzamido-2-{[(1R)-2-methoxy-2-oxo-1-phenylethyl]-amino}acetate **2** was performed via *N*-alkylation reaction. The spectroscopic and elemental data are in perfect agreement with the proposed structure of the obtained product. Indeed, the antibacterial screening of compound **2** showed good activity towards all bacterial strains when compared to standard drug Chloramphenicol.

Acknowledgements

This work was supported by Sidi Mohammed Ben Abdellah University (USMBA, Fez), Regional Center for Agronomic Research of Rabat (INRA, Rabat) and National Center for Scientific and Technical Research (CNRST, Rabat).

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