Chapter 9
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Characterization and Antibacterial Activity of New α-Aminoester Derivative, Synthesized via *N*-Alkyaltion of Methyl α-Azido Glycinate

Oumaima Karai[1], Younas Aouine[1,2], Anouar Alami[1*], Hassane Faraj[1] and Abdelilah El Hallaoui[1]

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ABSTRACT

The amino ester derivative was synthesized via *N*-alkylation of methyl α-azido glycinate *N*-benzoylated **1** with methyl 2-amino-2-phenylacetate in methylene chloride and in presence of triethylamine as 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 synthesized compound showed an interesting inhibitory effect against all the strains tested.

Keywords: Antibacterial activity; carboxylic amino esters; N-alkylation.

1. INTRODUCTION

Amino acid derivatives are an important group of peptidomimetics [1]. They exhibit a 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 to 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 determinated by usual spectroscopic techniques, such NMR, MS, elemental analysis and corroborated by X-ray crystallography.

2. EXPERIMENT

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 tetra-methylsilane (^{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

[1]*Organic Chemistry Laboratory (LCO), Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, P.B. 2626, Fez 30000, Morocco.*
[2]*Department of Chemistry, Faculty of Sciences, Ibn Zohr University, P.B. 8106, Cité Dakhla, Agadir 80060, Morocco.*
**Corresponding author: E-mail: anouar.alami@usmba.ac.ma;*

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).

The ORTEP of compound **2** was obtained on a Bruker APEXII CCD detector diffractometer (CNRST-Rabat, NMR 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, C=O). 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.

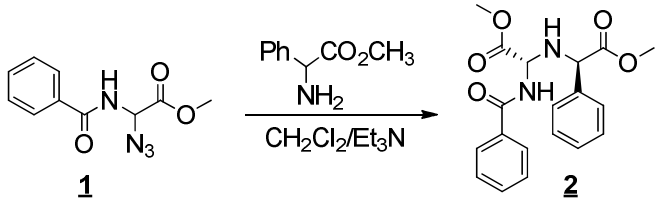
Concerning the disc diffusion method and resazurin microtiter-plate assay, the operative protocol adopted has already been validated in our previous paper [8].

3. RESULTS AND DISCUSSION

3.1 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 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 (Fig. 2).



Scheme 1. Synthesis strategy of compound 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 proton of secondary amino group (**N<u>H</u>**-CH-Ph). The proton of the second amino group (Bz-**N<u>H</u>**) appears at 6,75 ppm. A singlet at 3,3 ppm corresponds to methylenic proton (NH-**CH**-Ph). A doublet centered at 5,52ppm due to the resonance of the other methylenic proton (NH- **C<u>H</u>**-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 carbon (-**<u>C</u>**H-phenyl), at 52.44 and 52.88 ppm attributed to two methyl of the methoxy group, 63.62 ppm attributed to carbon (NH-**<u>C</u>**H-NH).

This attribution has been carried out in basis of HSQC (Fig. 1) 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 is showing in Table 1.

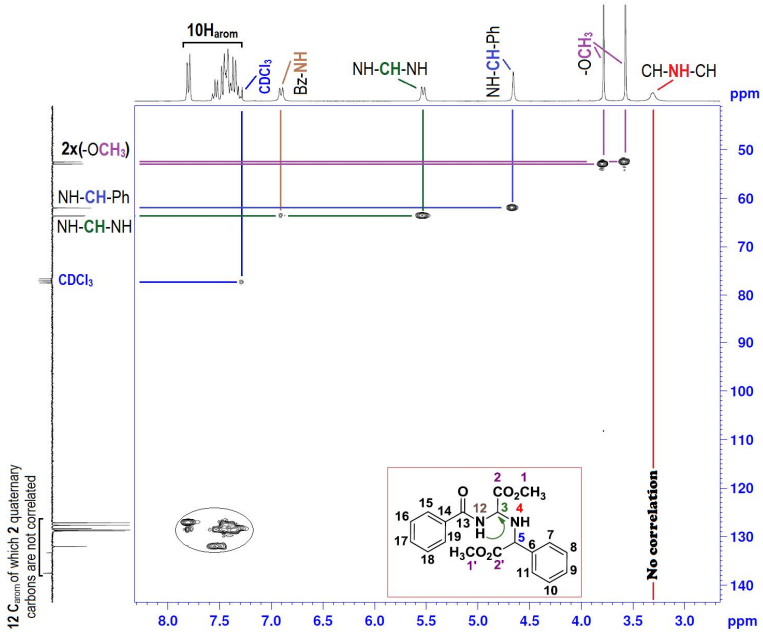


Fig. 1. Heteronuclear ^{1}H-^{13}C 2D spectrum of compound 2

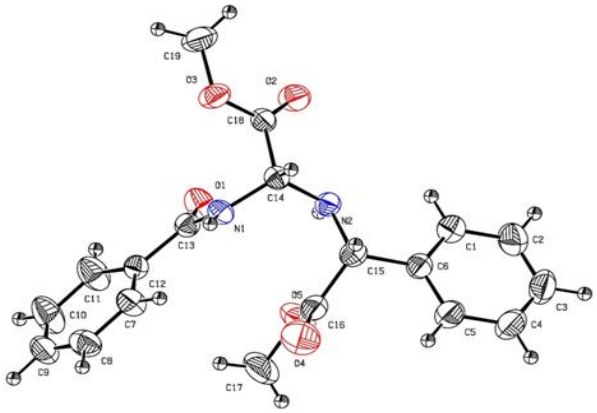


Fig. 2. ORTEP diagram of compound 2

3.2 Biological Activity

3.2.1 Disc diffusion method

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

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	$10C_{arom}$-$10H_{arom}$

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

	Compound 2	Chloramphenicol
E. coli CIP 53126	1.25	0.05
S. 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

3.2.2 Resazurin microtiter plate assay

In this study, we used the modified resazurin microtiter plate assay it's 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.

As can be seen in this Table 2, the 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.

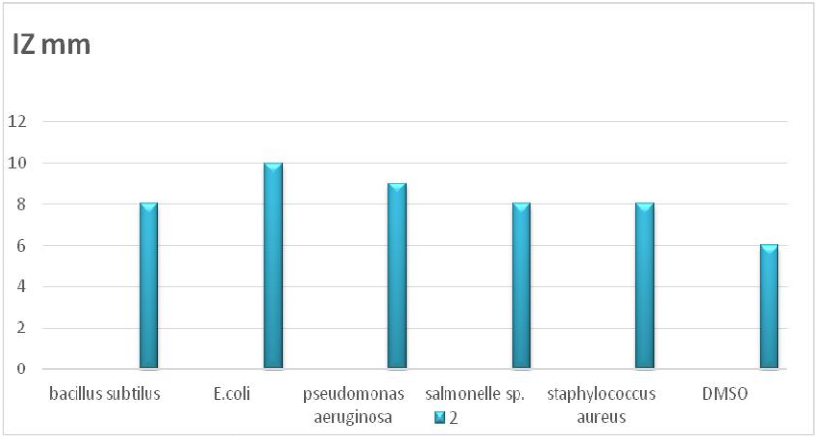


Fig. 3. Antibacterial activity (inhibition zone (IZ) measured in mm) of compound against pathogenic bacteria

4. 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Oumaima Karai
Organic Chemistry Laboratory (LCO), Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, P.B. 2626, Fez 30000, Morocco.

She was born in Fez, Morocco, in 1990. She obtained her Bachelor's and Master's degrees in chemistry from the Sidi Mohammed Ben Abdellah University Faculty of Sciences and Technologies, Fez, Morocco, in 2011 and 2013. She is pursuing for her Ph.D. degree at Sidi Mohammed Ben Abdellah University Faculty of Sciences Dhar El Mahraz, Fez, Morocco. Her research interests include synthesis and spectroscopic study of new compounds derived from heterocyclic and non-heterocyclic carboxylic α,α-diamino-diesters, and on the study of their biological and antioxydant activities.

Younas Aouine
Organic Chemistry Laboratory (LCO), Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, P.B. 2626, Fez 30000, Morocco and Department of Chemistry, Faculty of Sciences, Ibn Zohr University, P.B. 8106, Cité Dakhla, Agadir 80060, Morocco.

He was born in Aït-Seghrouchen of Taza, Morocco, in 1979. He received his higher education diploma in Chemistry from the Sidi Mohamed Ben Abdellah University of Fez, Morocco, in 2003. He obtained his advanced degree (D.E.S.A) and Ph.D. degree in Organic and Heterocyclic Chemistry from the same university, in 2005 and 2015, respectively. Since 2006, he has been working as a researcher in organic chemistry laboratory (LCO) with Professors Alami A. and El Hallaoui A., at Faculty of Sciences DM, University of Fez. In 2008, he joined the Ministry of Education as a professor of Physical Sciences and Chemistry in Imzouren High School, Al Hoceima, Northern Morocco. Since 2018, he joined the Department of Chemistry of the Ibn Zohr University, Agadir, as an assistant professor. His teaching has been devoted to organic and heterocyclic chemistry courses. His current research is focalized firstly on synthesis and characterization of new heterocyclic α-amino acids and their precursors and on the other hand on the study of their biological and electrochemical activities. https://orcid.org/0000-0001-7691-5598.

Anouar Alami
Organic Chemistry Laboratory (LCO), Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, P.B. 2626, Fez 30000, Morocco.

He was born in Fez, Morocco, in 1966. He studied Chemistry at Montpellier II University, France and he obtained his Ph.D. degree in 1991. He then joined the Department of Chemistry at the FSDM, USMBA Fez, Morocco in 1992. He prepared his state doctorate thesis degree in Organic Chemistry in 1997 at USMBA. Among the responsibilities he assumed: Head of Department of Chemistry, elected for two terms 2013-2015 and 2016-2017 of the Council of FSDM, Chairman of the TRANSMEDITERRANEAN COLLOQUIUM ON HETEROCYCLIC CHEMISTRY, November 22-25, 2017, Fez, Morocco,

http://tramech9.raidghost.com/, Responsible for the doctoral training "Bioactive Molecules, Health and Biotechnologies", 2014-2018, Director of the Laboratory of organic chemistry, 2014-2018, Coordinator of the Bachelor's degree program "Chemistry Sciences", FSDM, 2005-2012, Project Manager "President of the Coordination Commission with higher education, from the Board of the Academy of Fez-Boulemane Region, 2009-2012, Elected Member of the Council of USMBA, Fez, 2009-2011, Elected member of the Management Board USMBA, Fez 2011, Chairman of the International Symposium on heterocyclic chemistry, October 26-29, 2011 http://ishc2011.6te.net/Sitefr/index.html, Elected member of the college of the chemistry department, 2000-2012. He strongly believes in the cognitive complementarity of science and in parallel to all his responsibilities, he had prepared a DEUG (2008) and a License (2010) in Private Law in French and a Master in Economics and Management (2013) at the Faculty of Economics, Law, and Social Sciences of Fez. Concerning the scientific research side. He had published more than 90 research articles indexed in several databases (Scopus, Web of science, Elsevier, Eric, IMIST…) till 2019. ORCID ID: https://orcid.org/0000-0002-3951-9382

Hassane Faraj
Organic Chemistry Laboratory (LCO), Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, P.B. 2626, Fez 30000, Morocco.

He was born in Fez, Morocco, in 1963. He studied Chemistry at Montpellier II University, France and he obtained his Ph.D. degree in 1991. He then joined the department of chemistry at the Faculty of Sciences Dhar El Marhaz, Sidi Mohamed Ben Abdellah University (Fez, Morocco) in 1993. His current research is focused on the synthesis and characterization of new heterocyclic α-amino acids and their precursors and on the other hand on the study of their biological and electrochemical activities. He has taken part in conferences and communications in national and international congresses and has published the results of research (+70 publications and communications) in several international journals.

Abdelilah El Hallaoui
Organic Chemistry Laboratory (LCO), Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, P.B. 2626, Fez 30000, Morocco.

He obtained a state doctorate thesis in chemistry in 1984 at the University of Montpellier (France), “Synthesis of amino acides enantiomerically pure”. The same year, he was recruited as an organic chemistry teacher at Sidi Mohamed Ben Abdellah University, Fez, Morocco, where he teaches advanced organic chemistry, asymmetric synthesis and strategy of synthesis. In 1986, he founded the Laboratory of Organic Chemistry (LCO) along with others, where he was responsible of amino acids team. This laboratory LCO developed new methodologies of synthesis of heterocyclic amino acids and their precursors (heterocyclic amino-aldehydes and amino alcohols) as well as the studies of the biological, electrochemical and structural properties of the synthesized products. He has taken part in conferences and communications in national and international congresses and published the results of research (+120 publications and communications) in several international journals. He was the founder member of the first TRAMECH in 2000. On the teaching and administrative level he had been the Head of the Department of Chemistry at the Faculty of Sciences Dhar El Mahraz, Fez, Morocco (1990-1992), Head of the Faculty of Sciences and Technologies, Fez, Morocco (1992- 1999), Member of several scientific commissions and Member of several review and expertise boards.

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