Synthesis of some 1,2,3-triazoles derivatives and evaluation of their antimicrobial activity

Ismail Fichtali[1], Marwa Chraibi[2], Fatima El Aroussi[1], Abdeslem Ben-Tama[1], El Mestafa El Hadrami[1*], Kawtar Fikri Benbrahim[2] and Salah-Eddine Stiriba[3]

[1]*Laboratoire de Chimie Organique Appliquée, Université Sidi Mohammed Ben Abdallah, Faculté des Sciences et Techniques, Route Immouzer, BP 2202 Fès, Morocco*
[2]*Laboratoire de biotechnologie microbienne, Université Sidi Mohammed Ben Abdallah, Faculté des Sciences et Techniques, Route Immouzer, BP 2202 Fès, Morocco*
[3]*Instituto de Ciencia Molecular (ICMol), Universidad de Valencia, C/ Catedrático José Beltrán 2, 46980, Valencia, Spain*

_____

ABSTRACT

*All 1,2,3-triazoles derivatives were synthesized via 1,3-dipolar CuAAC, while the derivative **14** was synthesized by Huisgen 1,3-dipolar cycloaddition reaction between dimethylacetylene dicarboxylate and N-protected methyl azido glycinate. All synthesized compounds structures were confirmed by spectral techniques ^1H NMR, ^{13}C NMR, and mass spectrometry (MS). The antimicrobial activity of these compounds was tested against two Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), four Gram-positive bacteria (Micrococcus luteus, Bacillus cereus, Bacillus subtilis and Staphylococcus aureus) and one fungal strain (Candida albicans). The compounds **14** showed an interesting inhibitory effect against all Gram-positive bacteria studied compared to the other derivatives.*

Keywords*: Antimicrobial activity, cycloaddition, 1,2,3-triazole.*
_____

INTRODUCTION

During the last few years, an increased interest in antimicrobial activity of synthetic molecules, due to the emergence of the multi-drug resistant bacteria which represents a major problem in the human health worldwide [1]. Hence, nowadays the major challenge is to fight multi-drug resistance, by the discovery and the development of new potent antimicrobial compounds [2,3].

The click chemistry approach was extensively used for the bioactive molecules synthesis, which can solve and reduce the multi-drug resistance using synthesized molecules such as 1,2,3-triazoles. The 1,2,3-triazole nucleus, is one of the most pharmacologically active compound that exhibits various activities [4] such as, antiviral, anticonvulsant, anti-inflammatory, anticancer, antioxidant, antimicrobial, and antifungal [5–11]. In addition, some 1,2,3-triazoles are used in medicinal chemistry and form the basic structure of some drugs available in the market, like tazobactam [12], cefatrizine [13], and carboxyamidotriazole [14] (Figure 1). Moreover, the stability of 1,2,3-triazole derivatives in various extreme conditions, acidic and basic media, oxidative and reductive conditions, indicates their aromatic character [15]. They also contribute in the formation of the hydrogen bonds which lead to bind the biomolecular targets and increase the solubility [10].
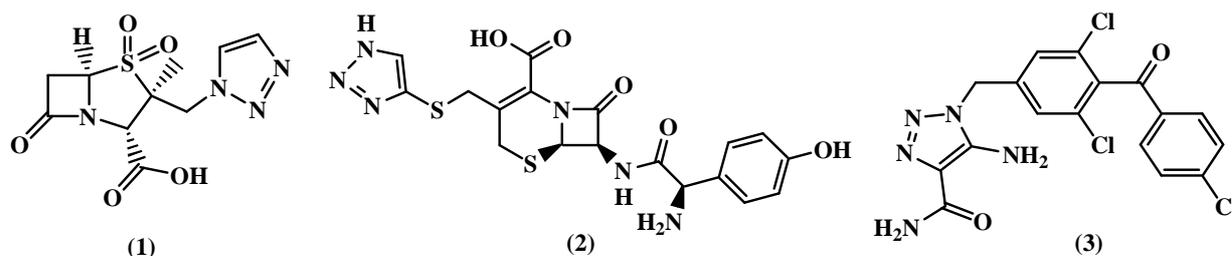
_____

Figure 1: Structures of some drugs containing 1,2,3-triazole nucleus. (1) tazobactam, (2) cefatrizine and (3) carboxyamidotriazole

The 1,4-disubstituted 1,2,3-triazoles are synthesized by Meldal [16] and Sharpless [17], using the copper(I)-catalyzed cycloaddition reaction between azides and terminal alkynes (CuAAC), which modified the original cycloaddition of Huisgen [18]. High efficiency and selectivity are observed when this reaction is carried out in aqueous media [19]. In addition, CuAAC reaction introduces various substituents allowing the obtainment of triazoles library that can be important in the construction of bioactive and functional molecules.

In the light of the previous informations and in the continuity of our research [20], we report in this paper the synthesis of a variety of 1,2,3-triazoles. The synthesized triazoles were characterized by spectroscopic techniques, such as, ^{1}H NMR, ^{13}C NMR, and mass spectra (MS). In addition, they were evaluated for their *in vitro* antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus* and *Candida albicans.*

MATERIALS AND METHODS

1. Chemistry
Melting points (°C) were recorded on Kofler bench, and were uncorrected. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Bruker 300 spectrometer with chemical shift values (δ) given in part per million (ppm) relative to TMS (0.00 ppm) and using $CDCl_3$, or DMSO-d6 as solvents, the coupling constants *J* values are given in hertz. All columns chromatographic separations were carried using silica gel 60 (Merck 230-400 mesch). The progress of all reactions was monitored by the thin layer chromatography (TLC) using Silica gel 60-F254 F254 plat with visualization under UV light and iodine. The mass spectra (MS) were recorded in the ESI mode at the mass Spectrometry Service of the Universidad de Valencia and the data reported in m/e (intensity to 100%). All reagents were purchased from commercial sources and used without further purification. All solvents were dried and distilled prior to their use.

1.1. General procedure for the synthesis of 1,2,3-triazole *9-14*
The 1,2,3-triazoles derivatives (**8-13**) were synthesized by the method outlined in scheme 1. To the equimolar solution (1.28mmol) of azides **1-3** and alkynes **4-7** in an EtOH/H_2O mixture (8 mL, 1/1, V/V) we added $CuSO_4.5H_2O$ (0.05 equiv) and sodium ascorbate (0.1 equiv) successively. The reaction mixture was stirred at room temperature for 4 to 5 hours, and the progress of the reaction was monitored by TLC. After completion of the reaction, the solvents were evaporated under reduced pressure. The water (20mL) was added and extracted with ethyl acetate (2 × 20mL), the organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure to afford the crude product. The obtained crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (4:1 v/v) as eluent.

However, the 1,3-dipolar cycloaddition between the azide 1 (0.2g, 0.85mmol) and the dimethyl acetylenedicarboxylate (0.12g, 0.85mmol) in dichloromethane (15mL), leads to the synthesis of 1,2,3-triazole **14** outlined in scheme 2. the reaction mixture was stirred for 24h at room temperature (monitored by TLC, eluent, hexane/ethyl acetate, 1/1: V/V). After completion of the reaction, 30 mL of water was added and extracted. The organic layer was dried over anhydrous Na_2SO_4. After evaporation of the solvent, the resulting crude product was purified by column chromatography (hexane/ethyl acetate 2:1 v/v) as eluent to give the pure desired product.

1.2. Spectral data
The analytical data of all the isolated 1,2,3-triazoles is given as under.
Methyl 2-(4-((1H-1,2,4-triazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-2-benzamidoacetate (8)

_____
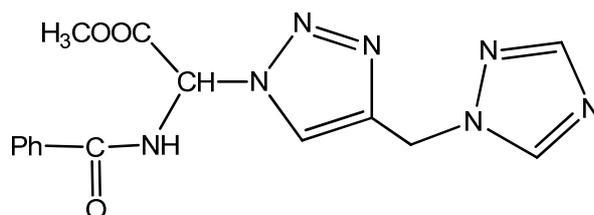
White solid product, m.p. 172°C, Yield: 69%, R_f : 0.2 (ethyl acetate). ^{1}H NMR (DMSO-d6, 300MHz): 4.20 (s, 3H, CH_3); 6.02 (s, 2H, CH_2); 7.63 (d, 1H, CH, *J*= 8.1Hz); 7.95-8.10 (m, 3H, CH_{arom}); 8.35-8.39 (m, 2H, CH_{arom}); 8.46 (s, 1H, $CH_{1,2,4-triaz}$); 8.70 (s, 1H, CH_{triaz}); 9.14 (s, 1H, $CH_{1,2,4-triaz}$); 10.70 (d, 1H, NH, *J=8.1Hz*). ^{13}C NMR (DMSO-d6, 75MHz): 44.24 (CH_2); 53.76 (CH_3); 65.19 (CH); 124.25 (CH_{triaz}); 128.05 ($2CH_{Ar}$); 128.76 (C_{Ar}); 129.00 ($2CH_{Ar}$); 132.36 (C_{triaz}); 132 .90 (CH_{Ar}); 142.51 ($CH_{1,2,4-triaz}$); 152.36 ($CH_{1,2,4-triaz}$); 166.57 (C=O); 166.87 (C=O). MS, m/z: 364.10 [M+Na]$^+$.

Methyl 2-(4-((1H-benzo[d]imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-2-benzamidoacetate (9)
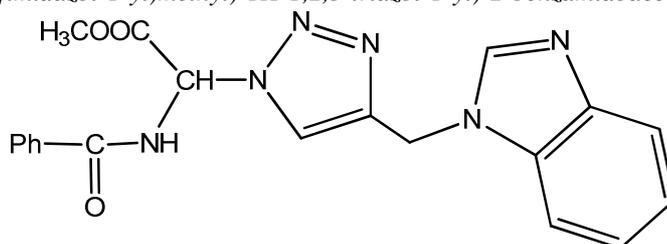
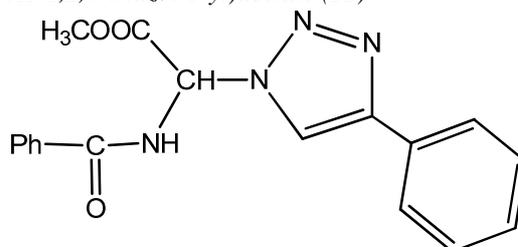White solid product, m.p. 180°C, Yield: 73,3%, R_f : 0.1 (ethyl acetate). ^{1}H NMR (DMSO-d6, 300MHz): 3.71 (s, 3H, CH_3); 5.61(s, 2H, CH_2); 7.10 (d, 1H, CH, *J*=7.8Hz); 7.19 (s, 1H, CH_{triaz}); 7.47-7.62 (m, 5H, CH_{arom}); 7.87-7.89 (m, 4H, CH_{arom}), 8.28 (s, 1H, $CH_{benzimidazole}$); 10.18 (d, 1H, NH, *J*=7.8Hz). ^{13}C NMR (DMSO-d6, 75MHz): 40.55 (CH_2); 53.78 (CH_3); 65.31 (CH); 122.97 (CH_{triaz}); 124.09 ($CH_{benzimidazole}$); 128.11 ($4CH_{Ar}$); 129.01 ($4CH_{Ar}$); 132.51 ($2C_{Ar}$), 132.90 (CH_{arom}); 143.02 (C_{Ar}+ C_{triaz}); 166.65 (C=O); 167.04 (C=O). MS, m/z: 391.1 [M+H]$^+$.

Methyl 2-benzamido-2-(4-(p-tolyl)-1H-1,2,3-triazol-1-yl)acetate (10)
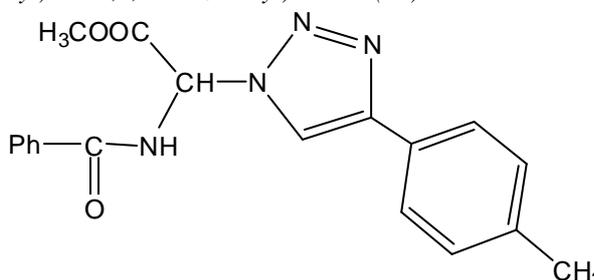
White solid product, m.p. 132°C, Yield: 73%, R_f : 0.25 (2/1 : hexane / ethyl acetate). ^{1}H NMR (CDCl$_3$, 300MHz): 2.37 (s, 3H, CH_3); 3.89 (s, 3H, CH_3); 7.01 (d, 1H, CH, *J*=7.8Hz); 7.22 (d, 2H, CH_{arom}, J=8.1Hz), 7.42-7.58 (m, 3H, CH_{arom}); 7.73 (d, 2H, CH_{arom}, *J*=8.1Hz); 7.84-7.87 (m, 2H, CH_{arom}); 8.04 (d, 1H, NH, *J*=7.8Hz); 8.20 (s, 1H, CH_{triaz}). ^{13}C NMR (CDCl$_3$, 75MHz): 21.54 (CH_3); 54.35 (CH_3); 64.13 (CH); 121.14 (C_{Ar}); 126.00 ($2CH_{Ar}$); 127.45 (C_{Ar}); 127.72 ($2CH_{Ar}$); 129.07 ($2CH_{Ar}$); 129.76 ($2CH_{Ar}$); 132.18 (CH_{triaz}); 133.07 (CH_{Ar}); 138.51 (C_{Ar}); 148.06 (C_{triaz}); 165.73 (C=O); 167.36 (C=O). MS, m/z: 373.10 [M+Na]$^+$.

Methyl 2-benzamido-2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetate (11)

White solid product, m.p. 148°C, Yield: 75%, Rf : 0.25 (2/1 : hexane / ethyl acetate). 1H NMR (CDCl3, 300MHz): 3.90 (s, 3H, CH_3); 7.02 (d, 1H, CH, *J*=7.8Hz); 7.31-7.59 (m, 6H, CH_{arom}); 7.83-7.87 (m, 4H, CH_{arom}); 8.00(d, 1H, NH, *J*=7.8Hz); 8.24 (s, 1H, CH_{triaz}). ^{13}C NMR (CDCl$_3$, 75MHz): 54.56 (CH_3); 64.28 (CH); 121.68 (CH_{Ar}); 126.26

_____

$(2CH_{Ar})$; 127.87 $(2CH_{Ar})$; 128.81 (CH_{triaz}); 129.25 $(4CH_{Ar})$; 130.43 (C_{Ar}); 132.32 (C_{Ar}); 133.27 (C_{Ar}); 148.26 (C_{triaz}); 165.84 (C=O); 167.52 (C=O). MS, m/z: 359.10 $[M+Na]^+$.

*1-octyl-4-phenyl-1H-1,2,3-triazole (**12**)*
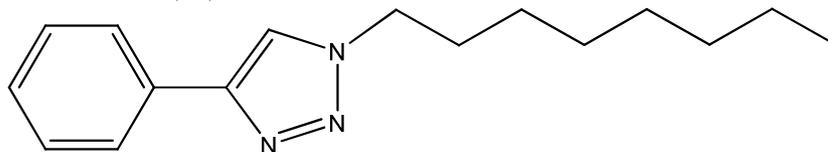
White solid product, m.p. 84°C, Yield: 72%, R_f : 0.4 (2/1: hexane / ethyl acetate). ^{1}H NMR (CDCl$_3$, 300MHz): 0.85 (t, 3H, CH$_3$, J_3=6.7Hz); 1.26-1.34 (m, 10H, 5CH$_2$); 1.89-1.97 (m, 2H, CH$_2$); 4.39 (t, 2H, CH$_2$-N, J= 7.2Hz); 7.29-7.45 (m, 3H, CH$_{Ar}$); 7.74 (s, 1H, CH$_{triaz}$); 7.81-7.85 (m, 2H, CH$_{Ar}$). ^{13}C NMR (CDCl$_3$, 75MHz): 14.55 (CH$_3$); 23.09 (CH$_2$); 27 (CH$_2$); 29.47 (CH$_2$); 29.54 (CH$_2$); 30.86 (CH$_2$); 32.20 (CH$_2$); 50.94 (CH$_2$-N); 119.80 (C$_{Ar}$); 126.19 (2CH$_{Ar}$); 128.56 (CH$_{Ar}$); 129.32 (2CH$_{Ar}$); 131.27 (CH$_{triaz}$); 146.94 (C$_{triaz}$). MS, m/z: 358.10 $[M+H]^+$.

*4-phenyl-1-tetradecyl-1H-1,2,3-triazole (**13**)*
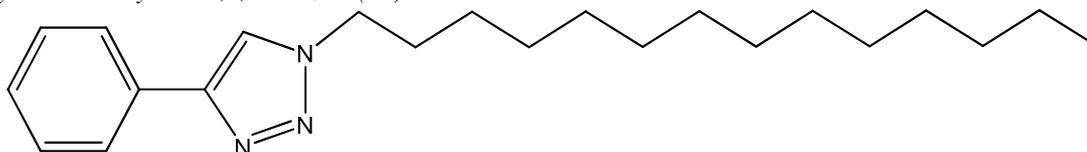
White solid product, m.p. 100°C, Yield: 81.7%, R_f : 0.4 (2/1 : hexane / ethyl acetate). ^{1}H NMR (CDCl$_3$, 300MHz): 0.86 (t, 3H, CH$_3$, J= 6Hz); 1.25-1.33 (m, 22H, 11CH$_2$); 1.88-198 (m, 2H, CH$_2$); 4.38 (t, 2H, CH$_2$-N, J=7.5Hz); 7.30-7.44 (m, 3H, CH$_{Ar}$); 7.74 (s, 1H, CH$_{triaz}$); 7.82-7.84 (m, 2H, CH$_{Ar}$). ^{13}C NMR (CDCl$_3$, 75MHz): 14.51 (CH$_3$); 23.08 (CH$_2$); 26.90 (CH$_2$); 29.41 (CH$_2$); 29.75-30.07 (7CH$_2$); 30.75 (CH$_2$); 32.32 (CH$_2$); 50.83 (CH$_2$-N); 119.78 (C$_{Ar}$); 126.08 (2CH$_{Ar}$); 128.45 (CH$_{Ar}$); 129.21 (2CH$_{Ar}$); 131.17 (CH$_{triaz}$); 148.12 (C$_{triaz}$). MS, m/z: 342.20 $[M+H]^+$.

*Dimethyl 1-(1-benzamido-2-methoxy-2-oxoethyl)-1H-1,2,3-triazole-4,5-dicarboxylate (**14**)*
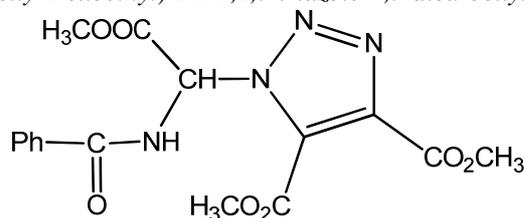
White solid product, m.p. 126°C, Yield: 72%, R_f : 0.35 (1/1 : hexane / ethyl acetate). ^{1}H NMR (CDCl$_3$, 300MHz): 3.85, 3.97, 4.05 (3s, 9H, 3O-CH$_3$); 7.43-7.56 (m, 3H, CH$_{Ar}$); 7.73 (d, 1H, CH, J=8.4 Hz); 7.79-7.83 (m, 2H, CH$_{Ar}$); 7.96 (d, 1H, NH, J=8.4Hz). ^{13}C NMR (CDCl$_3$, 75MHz): 53.20, 54.21, 54.63 (3CH$_3$); 64.90 (CH); 127.78 (2CH$_{Ar}$); 129.16 (2CH$_{Ar}$); 130.11(C$_{Ar}$); 132.22 (C$_{triaz}$); 133.16 (CH$_{Ar}$); 143.43 (C$_{triaz}$); 159.70 (C=O); 160.49 (C=O); 165.70 (C=O); 166.90 (C=O). MS, m/z: 399.00 $[M+Na]^+$.

2.Biology

2.1.Minimum inhibitory concentration determination (MIC) against bacterial strains

The MIC value, which represents the lowest concentration that completely inhibits the growth of microorganisms were performed in 96 well-microplate using the microdilution assay according to the protocol previously described by Bouhdid et al. [20] with slight modifications. Briefly, a stock solution of each product was prepared in (DMSO). Then, a serial dilutions of all tested 1,2,3-triazoles were prepared in Mueller Hinton Broth medium (MHB) at final concentrations ranged between 5 mg/mL and 0.004 mg/mL. The 12th well was considered as growth control (free-drug control). Afterwards, 50 μL of bacterial inoculum was added to each well at a final concentration of 10^6 CFU/mL. After incubation at 37 °C for 24 h, 10 μL of rezasurin were added to each well as bacterial growth indicator. After further incubation at 37 °C for 2 h, the bacterial growth was revealed by the change of coloration from purple to pink [21]. Experiments were carried out in triplicates.

### 2.2. Determination of minimum inhibitory concentration against Candida albicans

The determination of MIC against *Candida albicans* was performed in 96 well-microplate using the microdilution assay according to the protocol previously described by CLSI [22], with slight modifications. Firstly, from the stock

_____

solution of synthesized products serial dilutions were prepared in malt extract broth. The 12th well was considered as growth control. Then, 50 µL of the inoculum were added to each well at a final concentration of 10^3 CFU/mL. Afterwards, the microplate was incubated at 30° C for 48 h, Experiments were carried out in triplicate.

RESULTS AND DISCUSSION

The synthetic routes for the preparation of 1,2,3-triazoles **8-13** and **14** are outlined in Scheme 1 and 2. To study the synthesis of 1,2,3-triaozles, we have proceeded a set of reactions. Firstly we protected both acid and amine functions of the amino acid glycine to give N-protected methyl glycinate, the bromination, and azidation reactions, were then carried out to achieve the synthesis of compound **1**[23]. While azides **2** and **3** were prepared by a nucleophilic substitution with sodium azide [24]. The alkynes **4** and **5** were synthesized according the N-alkylation [25] with propargyl bromide in DMF in the presence of anhydrous K_2CO_3 and a phase-transfer catalyst (TBAB). The 1,3-dipolar cycloaddition of the dipoles azides **1-3** with the alkynes **4-7** using a catalytic quantity of $CuSO_4.5H_2O$ and sodium ascorbate in $EtOH/H_2O$ at room temperature give's a variety of 1,4-disubstituted 1,2,3-triazoles **8-13** (Scheme 1) in good yields 69-81.7% (Table 1). Whereas, the cycloaddition between the azide **1** and the dimethyl acetylenedicarboxylate in dichloromethane at room temperature, leads to the synthesis of 1,2,3-triazole **14** outlined in scheme 2. with a good yield of 72%

All the synthesized compounds were characterized by ^{1}H, ^{13}C NMR and by mass spectra. The ^{1}H NMR spectra of 1,2,3-triazoles **8-13** revealed a singlet between 7.19 and 8.71 relative to the CH of triazole nucleus (Scheme 1, Table 1). The ^{13}C NMR spectra of this compounds show's a signal from 122.97 to 132.77 ppm which correspond to the CH triazole (Table 1). The mass spectra recorded in the ESI mode confirmed the proposed structures.
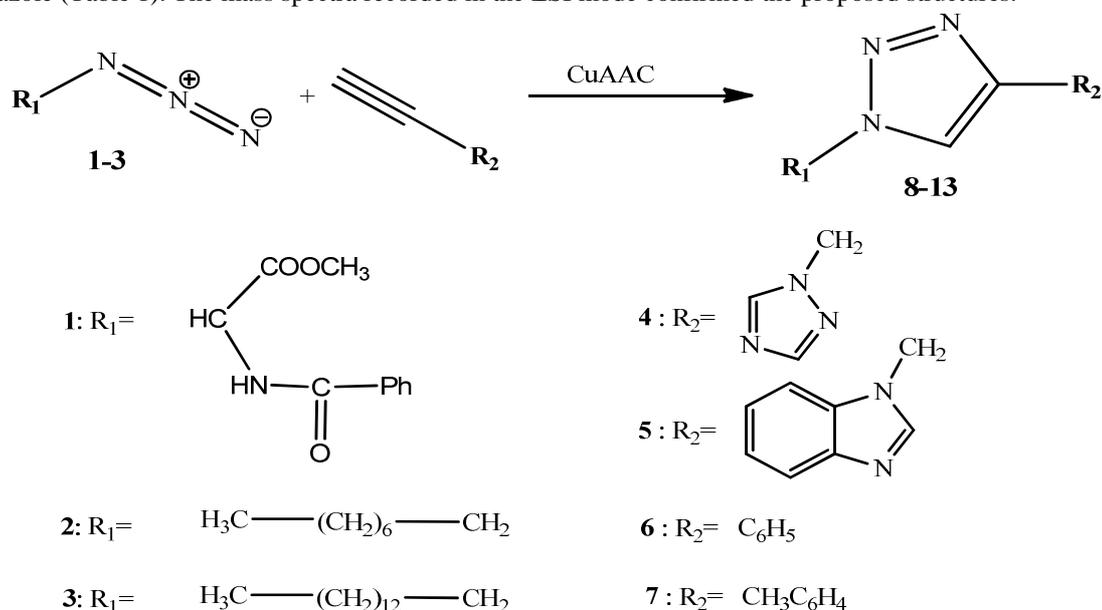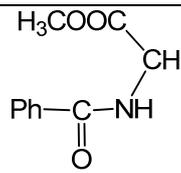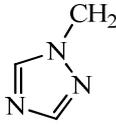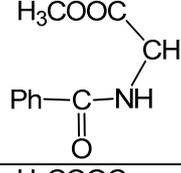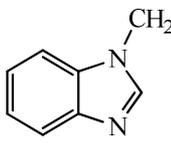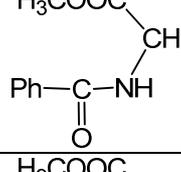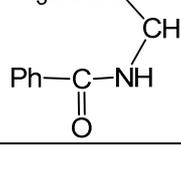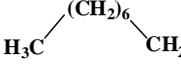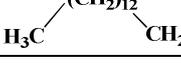

Scheme 1: Synthetic route of 1,2,3-triazoles derivatives 8-13
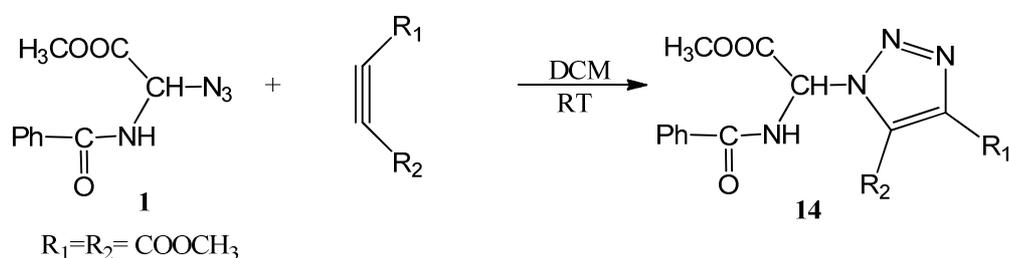
Scheme 2: Synthetic route of 1,2,3-triazoles derivative 14

_____

Table 1: Yields and characteristics of 1,2,3-triazoles 8-13

compounds	R_1	R_2	Yields	RMN ^{1}H δCH-triazole in ppm	RMN ^{13}C δCH-triazole in ppm
8	$H_3COOC-CH(-NH-C(=O)-Ph)$	$-CH_2-$(1,2,4-triazol-1-yl)	69%	8.70	124.25
9	$H_3COOC-CH(-NH-C(=O)-Ph)$	$-CH_2-$(benzimidazol-1-yl)	73,3%	7.19	122.97
10	$H_3COOC-CH(-NH-C(=O)-Ph)$	$CH_3C_6H_4$	73%	8.20	132.18
11	$H_3COOC-CH(-NH-C(=O)-Ph)$	C_6H_5	75%	8.24	128.81
12	$H_3C-(CH_2)_6-CH_2-$	C_6H_5	72%	7.74	131.27
13	$H_3C-(CH_2)_{12}-CH_2-$	C_6H_5	81.7%	7.74	131.17

The antibacterial activity against various bacteria was evaluated by observing the growth's inhibition of these strains tested in contact with different concentrations of each product's sample.

The results of the antibacterial activity of the synthesized products are represented in Table 2.

Table 2: The minimum inhibitory concentration (MIC mg/mL) exhibited by 1,2,3-triazole derivatives against the tested microbial strains.

Compounds	Bacterial strain						Fungal strain
	Gram -		Gram +				
	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Bacillus cereus*	*Staphylococcus aureus*	*Micrococcus luteus*	*Candida albicans*
8	-	-	-	5	-	5	-
9	-	-	-	1.25	-	1.25	-
10	-	-	5	5	5	5	-
11	-	-	5	5	5	5	-
12	-	-	5	5	-	5	-
13	-	-	-	-	-	-	-
14	-	2.5	1.25	1.25	1.25	1.25	-

As can be seen in this table, the compound **14** exercised an important inhibitory activity against Gram positive bacteria studied compared to rest of the compounds. Especially, *Staphylococcus aureus*, *Bacillus cereus* and *Micrococcus luteus* have shown a high sensitivity to this compound, with a MIC value of 1.25 mg/mL, while the growth inhibition of *Bacillus subtilis* was achieved at a MIC value of 2.5 mg/mL. Likewise, the compound **9** showed similar MIC value against *Bacillus cereus* and *Micrococcus luteus*. The compounds **8**, **10**, **11** and **12** exhibited the inhibitory effect at MIC value of 5 mg/mL. However, *S. aureus* was resistant to the compounds **8**, **9** and **12**, when *B. subtilis* was resistant to the compounds **8**, **9**. All Gram-negative bacteria (*E. coli, P. aeruginosa*) and *C. albicans* were resistant to all the tested compounds. Moreover, the compound **13** did not show any antimicrobial effect against all tested strains. In fact Gram-positive bacteria were generally found to be more sensitive than Gram negative bacteria, whose resistance is attributed to the structures of their cell wall. Outer membrane of the Gram-

_____

negative bacteria contains primarily lipopolysaccharides molecules and forms a hydrophilic barrier conferring protection against the effects of highly hydrophobic compounds [26].

CONCLUSION

The synthesis of a variety of 1,2,3-triazoles was performed via the 1,3-dipolar cycloaddition (compound 14) and 1,3-dipolar cycloaddition copper catalyzed reaction (compounds **8-13**). All compounds were obtained in good yields. The compounds structures obtained were confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry analysis.

All synthesized 1,2,3-triazoles were tested for their antimicrobial capacity, the compound **14** showed a promising inhibitory effect against all Gram-positive bacteria tested. However, no inhibition was observed against Gram-negative bacteria and *Candida albicans*.

REFERENCES

[1] Jones K.E., Patel N.G., Levy M.A., Storeygard A., Balk D., Gittleman J.L., Daszak P., *Nature,* **2008**, 451,990-993.
[2] Chua T., Moore C.L., Perri M.B., Donabedian S.M., Masch W., Vager D., Davis S.L., Lulek K., zimnicki B., Zervos M.J., *J. Clin. Microbiol.,* **2008**, 46, 2345-2352.
[3] Chu D.T.W., Plattner J.J., Katz L., *J. Med. Chem.,* **1996**, 3939 3853-3874.
[4] Kolb H.C., Sharpless K.B., *Drug Discovery Today*, **2003**, 8, 1128-1137
[5] He Y.-W., Dong C.-Z., Zhao J.-Y., Ma L.-L., Li Y.-H., Aisa H.A., *Eur. J. Med. Chem*., **2014**, 76, 245-255.
[6] Wilhelm E. a., Machado N.C., Pedroso A.B., Goldani B.S., Seus N., Moura S., Savegnago L., Jacob R.G., Alves D., *RSC Adv*., **2014**, 4, 41437-41445.
[7] Assis S.P.D.O., Silva M.T. D., Oliveira R.N.D., Lima V.L.D.M., *Sci. World J*., **2012**, 2012, 1-7.
[8] Kurumurthy C., Veeraswamy B., Rao P.S., Kumar G.S., Rao P.S., Reddy V.L., Rao J.V., Narsaiah B., *Bioorg. Med. Chem. Lett.*, **2014**, 24, 746-749.
[9] Mady M.F., Awad G.E.A., Jørgensen K.B., *Eur. J. Med. Chem*., **2014**, 84, 433-443.
[10] Zhang H.-Z., Wei J.-J., Kumar K. V., Rasheed S., Zhou C.-H., *Med. Chem. Res.,* **2015**, 24 182-196.
[11] Su N.-N., Li Y., Yu S.-J., Zhang X., Liu X.-H., Zhao W.-G., *Res. Chem. Intermed*., **2013**, 39, 759-766.
[12] Yang Y., Rasmussen B.A., Shlaes D.M., *Pharmacol. Ther.* 83 (**1999**) 141-151
[13] Weinstein A., *Drugs*., **1980**, 19, 137-154.
[14] Soltis M., Yeh H., Cole K., Whittaker N., Wersto R., Kohn E., *Drug Metab. Dispos.*, **1996**, 24, 799-806.
[15] Ramesh S., Ashok D., Goud G.L., Reddy V.P., *Russ. J. Gen. Chem.*, **2014**, 84, 1608-1614.
[16] Tornøe C.W., Christensen C., Meldal M., *J. Org. Chem.,* **2002**, 67, 3057–3064.
[17] Rostovtsev V. V,. Green L.G, Fokin V. V, Sharpless K.B., *Angew. Chem. Int. Ed. Engl*., **2002**, 41, 2596-2599.
[18] Huisgen R., In Synthetic applications of 1, 3-dipolar cycloaddition chemistry toward heterocycles and natural products, JOHN WILEY & SONS, INC., New York, **1984.**
[19] Sharpless K.B., Manetsch R., *Expert Opin. Drug Discov*., **2006**, 1, 525-538.
[20] Fichtali I., Laaboudi W., El Hadrami E. M., El Aroussi F., Ben-Tama A., Benlemlih M., Stiriba S.-E., *J. Mater. Environ. Sci.*, **2016**, 7, 1633-1641.
[21] Bouhdid S., Abrini J., Zhiri A., Espuny M.J., Manresa A., *J. Appl. Microbiol.*, **2009**, 106, 1558-1568.
[22] NCCLS. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Second Edition. NCCLS document M27-A2 (ISBN 1-56238-469-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, **2002**
[23] Bentama A., El Hadrami E.M., El Hallaoui A., Elachqar A., Lavergne J.-P., Roumestant M.-L., Viallefont Ph., *Amino Acids*, **2003**, 24, 423-426.
[24] Takasu K., Azuma T., Takemoto Y., S*Tetrahedron Lett.*, **2010**, 51, 2737-2740.
[25] Murty M.S.R., Ram K.R., Rao B.R., Rao R.V., Katiki M.R., Rao J.V., Pamanji R., Velatooru L. R., *Med. Chem. Res.*, **2014**, 23, 1661–1671
[26] Trombetta D., Castelli F., Sarpietro M.G., Venuti V., Cristani M., Daniele C., Antonella S., Gabriela M., Giuseppe B., Maria G., *J. Antimicrob. Agents Chemother*., **2005**, 49, 2474-2478.