ACTA CHEMICA IASI, **34_1**, 186-203 (2026)
DOI: 10.47743/achi-2026-1-0008

Article

CuAAC SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF 4-CARBOXYMETHYL-SUBSTITUTED 1,2,3-TRIAZOLES

Codruța Frîncul[a], Alina Ghinet[a,b,c], Samir Jawhara[d,e,f], Dylan Lambert[d,e,f], Dalila Belei[a], Elena Bîcu[a*]

[a]*Faculty of Chemistry, 'Al. I. Cuza' University of Iasi, B-dul Carol I, Nr. 11, Corp A, Ro-700506, Iasi, Romania*

[b]*Laboratoire de Chimie Durable et Santé, JUNIA, 16 rue Colson, F-59000, Lille Cedex, France*

[c]*Univ. Lille, Inserm, CHU Lille, JUNIA, U1352 - BioPrev - Better aging: from inflammaging to prevention, 59000, Lille, France*

[d]*CNRS, UMR 8576-UGSF—Unité de Glycobiologie Structurale et Fonctionnelle, INSERM U1285*

[e]*Medicine Faculty, University of Lille, F-59000 Lille, France*

[f]*CHU Lille, Service de Parasitologie Mycologie, Pôle de Biologie Pathologie Génétique, F-59000 Lille, France*

Abstract: A series of 1,4-disubstituted 1,2,3-triazole derivatives was synthesized via copper(I)-catalyzed azide–alkyne cycloaddition between substituted aryl azides and methyl propiolate. The reactions were performed using a $CuSO_4$/sodium ascorbate catalytic system in a mixed solvent medium, leading to the formation of the desired compounds in good yields. The structures of the obtained derivatives were confirmed by spectroscopic methods, including NMR analysis. Furthermore, the synthesized compounds were subjected to biological evaluation to investigate their potential antifungal activity against *Candida albicans*.

***Keywords*:** 1,2,3-Triazoles; Click Chemistry, Antifungal activity, Candida albicans

* Elena, Bîcu, *e-mail:* elena@uaic.ro

Introduction

Click chemistry has become a widely used approach in contemporary organic synthesis, as it allows molecular fragments to be connected through simple, robust, and highly selective transformations. Among the reactions included in this concept, the copper(I)-catalyzed azide-alkyne cycloaddition is of particular interest, providing direct access to 1,4-disubstituted 1,2,3-triazoles under mild reaction conditions. The importance of this transformation is closely related to the properties of the 1,2,3-triazole ring, which combines chemical stability with the ability to establish hydrogen bonding, dipole-dipole interactions, and π-stacking contacts with biological targets.[1,2,3] Due to these features, triazole derivatives have been extensively explored in medicinal chemistry and have been reported for antimicrobial, antifungal, anticancer, and antidiabetic activities. Therefore, the incorporation of a triazole moiety into bioactive molecular frameworks offers a useful strategy for the design of new heterocyclic compounds, including structures investigated as enzyme inhibitors in antidiabetic drug discovery.[4] Triazole compounds substituted at the 4th position of the 1,2,3-triazole core, particularly those bearing hydroxymethyl or carboxyethyl groups, have provided a suitable framework for assessing how the nature of the substituents influences the chemical properties and biological behavior of these molecules.[5,6] In this context, grafting ester groups onto the triazole skeleton is of particular interest, given the possibility of their subsequent transformation into carboxyl group and their use as linking fragments for coupling with macromolecular skeletons, such as dextran, leading to the production of hybrid molecules with antibacterial and antifungal properties that are more effective than fluconazole or even voriconazole.[6] Furthermore, modifications to the methylene group adjacent to the nitrogen atom in 1st position generated

stereogenic centers, opening up the possibility of separating enantiomers and evaluating the differences in behavior associated with them.[7,8] This highlights the importance of fine adjustments to the molecular skeleton, both from a chemical and stereochemical perspective, contributing to a deeper understanding of the relationship between the molecular structure and biological activity of 1,2,3-triazole derivatives.[7]

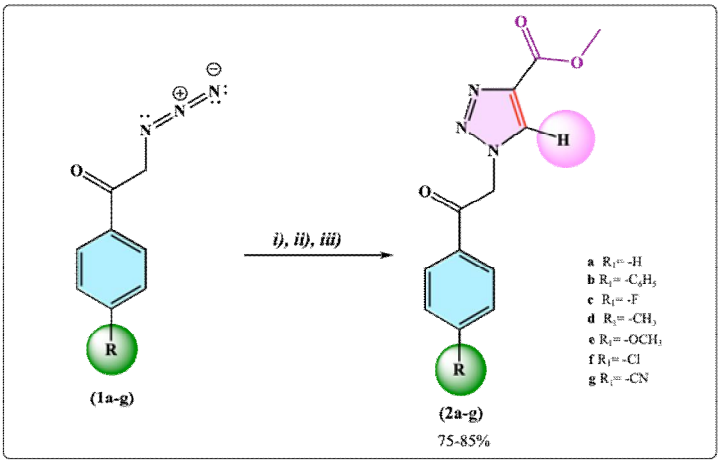
The current work aims to investigate a limited series of seven triazole derivatives (Scheme 1, **2a-g**), six of which are not reported in the literature (Scheme 1, **2b-g**), which have a carboxymethyl group in 4th position of the triazole skeleton. The choice of methyl propiolate as the dipolarophile in the click chemistry strategy described herein (Scheme 1) was motivated by the aim of assessing the impact of the carboxymethyl group on the biological profile of the resulting compounds, with particular focus on antifungal activity.

Comparison of the newly synthesized compounds with related analogues reported in the literature provided insights into how this subtle structural variation affects interactions with fungal targets and, consequently, antifungal efficacy. The aim of this study was therefore to evaluate their antifungal potential against the SC5314 wild-type strain of *Candida albicans*, with literature data for reference antifungal agents, such as fluconazole, being considered only as contextual support for interpreting the reported MIC values.

Results and Discussion

For the synthesis of the targeted 1,2,3-triazole derivatives, a cycloaddition reaction between an azide and a terminal alkyne was employed, using a catalytic system based on copper (II) salts and sodium ascorbate to generate *in situ* the monovalent copper species responsible for catalysis, as shown in Scheme 1.[6]

The reactions were performed in a monophasic *tert*-butanol (*t*-BuOH) medium, with acetonitrile (MeCN) added to achieve complete solubilization of the azide substrates. Once azides **1a-g** was fully dissolved in the reaction medium, methyl propiolate was added, followed by the addition of aqueous copper sulfate and sodium ascorbate solutions. The reaction mixture was maintained under vigorous stirring for 24 hours at room temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC) until the azides **1a-g** were completely consumed, using ethyl acetate : cyclohexane (v : v) as the eluent. After the reaction was complete, the solution was treated with cold water and stirred for 30 minutes, resulting in the formation of a cream-green precipitate. Subsequently, a diluted ammonia solution was added to remove residual copper species from the reactant system. The precipitate was vacuum filtered and dried at room temperature.



Scheme 1. Synthesis of 1,4-disubstituted 1,2,3-triazole derivatives.[6]
Reagents and conditions: i) methyl propiolate, ii) $CuSO_4 \cdot 5H_2O$, iii) sodium ascorbate, t-BuOH-CH_3CN (4 : 1, v : v), r.t., 24h.

After complete drying, the obtained solid still exhibited a greenish coloration, and the NMR analysis of the crude product indicated the presence of small traces of copper-containing species in addition to the

desired triazole derivative, most likely in the form of unidentified complexes. As a result, an additional purification step was necessary before proceeding with the biological evaluation. Compounds **2b-g** were further purified by column chromatography, employing an ethyl acetate: cyclohexane mixture (*2v : v*) as the eluent. After this step, the 1,2,3-triazole derivatives were obtained as white, crystalline solids with melting points ranging from 179 to 277 °C. Tables 1 and 2 summarize the most representative ^{1}H and ^{13}C NMR signals for compounds **2b-g**.

Table 1. ^{1}H-NMR chemical shifts representative for compounds **2b-g**.

^{1}H-NMR (DMSO-d_6, 500 Hz), δ, ppm			
Compound	OCH_3	CH_2	$H_{triazole}$
2b	3.86	6.31	8.75
2c	3.86	6.27	8.72
2d	3.86	6.23	8.72
2e	3.88	6.21	8.72
2f	3.84	6.26	8.70
2g	3.86	6.33	8.71

In the ^{1}H-NMR proton spectra recorded in DMSO-d_6, the signal attributed to the methoxy (-OCH_3) group is observed constantly in the range 3.84-3.88 ppm. The methylene (-CH_2-) protons adjacent to nitrogen in position one of the triazole skeleton appears as characteristic signals located between 6.21 and 6.33 ppm, slightly shifted depending on the nature of the aromatic substituent. The heterocyclic proton of the triazole nucleus is detected around 8.70-8.72 ppm, a typical range for this proton, suggesting the formation and closure of the 1,2,3-triazole heterocycles.

Table 2. ^{13}C-NMR chemical shifts representative for compounds **2b-g**.

^{13}C-NMR (DMSO-d_6, 125 Hz), δ, ppm							
Compound	O**C**H$_3$	**C**H$_2$	**C**H	**C**OOCH$_3$	**C**O	**C**-F	**C**≡N
2b	51.9	56.29	130.8	160.8	191.1	-	-
2c	51.9	56.2	130.8	160.8	190.3	165.7	-
2d	51.7	55.9	130.6	160.6	190.8	-	-
2e	51.6	55.7	130.7	160.6	189.6	-	-
2f	51.9	56.3	130.9	160.8	190.9	-	-
2g	51.7	56.4	130.6	160.5	191.1	-	117.9

The ^{13}C-NMR spectra also support the structures attributed to compounds **2b-g**. Thus, the carbon atoms of the methoxy group are identified at values between 51.6-51.9 ppm, and the methylene carbon at approximately 55.7-56.4 ppm. The tertiary carbon of the 1,2,3-triazole rings observed in the range 130.6-130.9 ppm, while the quaternary carbon of the carboxylate (-COOCH$_3$) group appears at values of 160.5-160.8 ppm. The signals corresponding to the carbonyl group linked to the phenyl unit are located around 189.6-191.1 ppm. In the case of derivative **2c**, the presence of the fluorine atom is confirmed by the characteristic C-F signal observed in the range 166.6-164.7 ppm, as well as by the specific signal of the nitrile group of compound **2g**, recorded at approximately 117.9 ppm.

Biological evaluation

We evaluated compounds **2a-g** for their minimum inhibitory concentrations (MICs) against the opportunistic yeast *Candida albicans*, a major etiological agent of candidiasis.

The MIC values were successfully determined for compounds **2b** and **2d**, which were found to be 16.07 μg/mL and 12.96 μg/mL, respectively (Table 3). In contrast, accurate MIC determination for compounds **2a, 2c**,

2e, 2f, and **2g** proved challenging. This difficulty appears to be associated with adaptive resistance mechanisms exhibited by *Candida albicans*.

Of note, these compounds **2a, 2c, 2e, 2f**, and **2g** showed antifungal activity at high concentrations. However, increasing the concentration did not result in a proportional inhibitory effect. Instead, *Candida albicans* appeared to activate defense mechanisms that reduced susceptibility to these molecules, suggesting the involvement of inducible resistance pathways.

Table 3. MIC values of the tested compounds.

Compound	R	M (g/mol)	MIC (µg/mL)
2a	H	245.23	245.23
2b	$-C_6H_5$	321.34	**16.07**
2c	-F	263.22	263.22
2d	$-CH_3$	259.27	**12.96**
2e	$-OCH_3$	275.26	275.26
2f	-Cl	279.68	279.68
2g	-CN	270.24	**27.02**
Fluconazole[a]			**0.125** [9]

a= The MIC value reported for fluconazole was taken from the literature and is included only as a reference value for comparison.

A comparative analysis of the antifungal data obtained for compounds **2a-g** highlights a clear dependence of the biological response on the nature of the aromatic substituent attached to the triazole core. From an electronic standpoint, a first structure-activity relationship can be outlined by comparing substituents with different inductive and resonance effects. The derivative bearing a *para*-methyl group (**2d**), characterized by a weak electron-donating effect, exhibits the most pronounced antifungal response within the series. A distinct behavior is observed for compound **2g**, which contains a cyano substituent and displays a MIC value of 27.02 µg/mL,

being markedly more active than the **2c** and **2f** substituted analogues. This result indicates that the presence of an electron-withdrawing group does not necessarily lead to a decrease in antifungal activity. In the case of the cyano derivative, the stronger dipolar character of the substituent, together with its ability to participate as a hydrogen-bond acceptor, may contribute to additional interactions within the biological environment.

Steric and hydrophobic factors further contribute to defining this structure-activity relationship. Although compound **2b** features an extended aromatic system, its biological response does not exceed that of the more structurally compact *para*-tolyl analogue (**2d**), suggesting that increased steric bulk may restrict optimal accommodation within the biological target site. In contrast, a moderate hydrophobic character, as observed for compound **2d**, appears to support both membrane permeation and the establishment of molecular-level interactions. Such a balance between lipophilicity and steric accessibility is frequently discussed in the context of antifungal agents involved in membrane-associated processes.

Fungal culture conditions and antifungal susceptibility testing

The minimum inhibitory concentration (MIC) assay was performed using the SC5314 wild-type strain of *Candida albicans*.[10] Strains were maintained on Sabouraud dextrose agar and incubated at 37 °C for 24 h prior to use. For liquid cultures, a single well-isolated colony was inoculated into Sabouraud dextrose broth (Sigma-Aldrich, Saint-Quentin Fallavier, France) and grown at 37 °C for 24 h with constant shaking. Yeast cells were subsequently recovered by centrifugation (2500 rpm, 5 min.), thoroughly rinsed with sterile phosphate-buffered saline (PBS), and resuspended in PBS to obtain the appropriate cell density for downstream assays. Antifungal susceptibility testing was carried out by determining minimum inhibitory

concentrations (MICs) using the broth microdilution method, performed in strict accordance with the reference protocol issued by the Clinical and Laboratory Standards Institute. The experimental design was consistent with procedures previously detailed by Pfaller *et al.*[11]

Experimental

Chemistry

Starting materials are commercially available and were used without further purification (suppliers: Carlo Erba Reagents S.A.S., Tokyo Chemical Industry Co. Ltd. and Acros Organics). Melting points were measured on an MPA 100 OptiMelt® apparatus and are uncorrected. Nuclear Magnetic Resonance (NMR) spectra were acquired at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR, on a Bruker Avance III 500 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) with tetramethylsilane (TMS) as internal standard, at 25 °C. Chemical shifts (δ) are expressed in part per million (ppm) relative to the residual solvent signal (2.50 ppm for DMSO-d_6) in ^{1}H NMR spectra and (39.52 ppm for DMSO-d_6) in ^{13}C NMR spectra, respectively. Splitting patterns are designed as: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; q, quintuplet; br s, broaden singlet; br t, broaden triplet. Coupling constants (*J*) are reported in Hertz (Hz). Thin layer chromatography (TLC) was realized on Macherey Nagel silica gel plates with fluorescent indicator and were visualized under a UV-lamp at 254 nm and 365 nm. IR spectra were recorded on a FTIR Bruker Tensor 27 spectrometer. MALDI-TOF MS spectra were acquired on a Bruker Rapiflex MALDI-TOF mass spectrometer equipped with a Smartbeam 3D laser FlexControl (Bruker Daltonics, Version 4.0) operated in positive reflectron ion mode. Samples (20 µg/mL in 0.1 % TFA) were mixed 1:1 with a freshly prepared sDHB matrix solution (50 mg/mL in ACN/0.1 % TFA in H_2O, *3v : 2v*), and 1 µL of the mixture was deposited on a stainless-

steel target plate and air dried at room temperature. External calibration was performed using the Bruker Peptide Calibration Standard Kit.

General procedure for the synthesis of triazoles 2a-g by classical click chemistry

Synthesis of azides

The 1,2,3-triazoles were obtained from *ω*-bromoacetophenones. The first step consisted of the reaction between sodium azide (1.2 eq.) and different *ω*-bromoacetophenones (1.0 eq.), in a biphasic equivolumic system of chloroform and water, using tetra-*n*-butylammonium bromide (0.13 eq.) as phase transfer agent. The medium was stirred vigorously for 36 hours at room temperature. Next, the organic phase was washed three times with water, followed by drying over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude was a yellow liquid. Ethanol was added over this liquid, and the mixture was cooled to 5 °C, when a white precipitate was obtained in the flask. Then, this was filtered and dried to obtain the pure intermediate azide **1a-g**. The azides synthesized in this study have been previously reported in the literature. Therefore, their structural confirmation was carried out by correlating the experimentally recorded spectral data and physicochemical properties with those reported in earlier studies. The comparative assessment showed good agreement between the obtained results and the literature data, supporting the correct structural assignment of the prepared azide precursors.[12,13,14]

Synthesis of 1,2,3-triazoles

The second step was aimed at closing the substituted 1,2,3-triazole ring from the reaction between the azide derivative **1a-g** and a marginal alkyne. The 1,2,3-triazole ring closure reaction was performed in *tert*-butanol system, to which acetonitrile was added to ensure complete

dissolution of the azide (1.0 eq.). After the complete dissolution of the compounds **1a-g**, methyl propiolate (1.2 eq.), variously substituted, was introduced into the reaction system, followed by the addition of $CuSO_4 \cdot 5H_2O$ (0.1 eq.) and sodium ascorbate (0.2 eq.), previously dissolved in water, to generate Cu(I) in the reaction system, acting as a catalyst. The mixture was vigorously stirred for 24h at room temperature. After the complete consumption of azide **1a-g**, monitored by TLC, cold water was added to the final solution until a suspension was formed. A diluted ammonia solution was added to the medium to complex the copper in the reaction system. The mixture was then cooled on ice-bath and stirred for 30 minutes. The solid obtained was filtered and dried. Purification was performed by column chromatography on silica gel stationary phase using a mixture of ethyl acetate and cyclohexane as the mobile phase to obtain pure 1,2,3-triazole **2a-g**. Triazole **2a**[15] presented the same physico-chemical properties as previously described.

Spectral data and physico-chemical properties of newly synthesized compounds

*Methyl 1-(2-([1,1'-biphenyl]-4-yl)-2-oxoethyl)-1H-1,2,3-triazole-4-carboxylate **(2b):*** The general procedure was used with 0.4 g 1-([1,1'-biphenyl]-4-yl)-2-azidoethan-1-one **1b** (1.68 mmol, 1 eq.), 0.17 g methyl propiolate (2.14 mmol, 1.2 eq.), 0.042 g $CuSO_4 \cdot 5H_2O$ (0.16 mmol, 0.1 eq.), 0.066 g sodium ascorbate (0.33 mmol, 0.2 eq.) and 5 mL of *tert*-butanol. Purification by column chromatography was performed using a mixture of ethyl acetate (EtOAc) and cyclohexane (*2v : v*). White solid, 0.65 g, 80 % yield, m.p. (ethanol) = 224 °C, R_f (EtOAc : cyclohexane 1 : 1, *v : v*) = 0.2; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.75 (s, 1H, Ar*H*), 8.16 (d, *J* = 8.0 Hz,

2H, Ar*H*), 7.93 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.80 (d, *J* = 7.0 Hz, 2H, Ar*H*), 7.53 (t, *J* = 7.0 Hz, 2H, Ar*H*), 7.45 (t, *J* = 7.0 Hz, 1H, Ar*H*), 6.31 (s, 2H, -C*H*$_2$), 3.86 (s, 3H, COOC*H*$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ: 191.1 (CO), 160.8 ($COOCH_3$), 145.6 (C), 138.7 (C), 138.6 (C), 132.7 (C), 130.8 (CH), 129.2 (2CH), 129.0 (2CH), 128.7 (CH), 127.2 (2CH), 127.1 (2CH), 56.2 (CH_2), 51.9 (CH_3) ppm; IR *v*: 1725 (-$COOCH_3$), 1685 (>C=O), 1237 (C-O), 900-500 (C-H) cm^{-1}; MS (*m/z*): MS (MALDI-TOF/TOF): *m/z* = 322 $[M+H]^+$.

Methyl 1-(2-(4-fluorophenyl)-2-oxoethyl)-1H-1,2,3-triazole-4-carboxylate ***(2c):*** The general procedure was used with 0.4 g 2-azido-1-(4-fluorophenyl)ethan-1-one **1c** (2.20 mmol, 1 eq.), 0.22 g methyl propiolate (2.65 mmol, 1.2 eq.), 0.055 g $CuSO_4 \cdot 5H_2O$ (0.22 mmol, 0.1 eq.), 0.087 g sodium ascorbate (0.44 mmol, 0.2 eq.) and 5 mL of *tert*-butanol. Purification by column chromatography was performed using a mixture of ethyl acetate (EtOAc) and cyclohexane (*2v : v*). White solid, 0.43 g, 75 % yield, m.p. (ethanol) = 200 °C, R_f (EtOAc : cyclohexane 1 : 1, *v : v*)= 0.2; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ: 8.72 (s, 1H, Ar*H*), 8.17 (q, *J* = 5.5 Hz, 2H, Ar*H*), 7.46 (t, *J* = 8.5 Hz, 2H, Ar*H*), 6.27 (s, 2H, -C*H*$_2$), 3.86 (s, 3H, COOC*H*$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ: 190.3 (CO), 165.7 (d, *J* = 251.2 Hz, C-F), 160.8 ($COOCH_3$), 138.7 (C), 131.4 (d, *J* = 10.0 Hz, 2CH), 130.8 (CH), 130.7 (d, *J* = 2.5 Hz, C), 116.2 (d, *J* = 22.0 Hz, 2CH), 56.2 (CH_2), 51.9 (CH_3) ppm; IR *v*: 1720 (-$COOCH_3$), 1686 (>C=O), 1251 (C-O), 1164 (C-F), 900-500 (C-H) cm^{-1}; MS (*m/z*): MS (MALDI-TOF/TOF): *m/z* = 264 $[M+H]^+$.

Methyl 1-(2-oxo-2-(p-tolyl)ethyl)-1H-1,2,3-triazole-4-carboxylate ***(2d):*** The general procedure was used with 0.4 g 2-azido-1-(*p*-tolyl)ethan-1-one **1d**

(2.28 mmol, 1 eq.), 0.23 g methyl propiolate (1.74 mmol, 1.2 eq.), 0.057 g $CuSO_4 \cdot 5H_2O$ (0.22 mmol, 0.1 eq.), 0.090 g sodium ascorbate (0.45 mmol, 0.2 eq.) and 5 mL of *tert*-butanol. Purification by column chromatography was performed using a mixture of ethyl acetate (EtOAc) and cyclohexane (*2v : v*). White solid, 0.50 g, 85 % yield, m.p. (ethanol) = 213 °C, R_f (EtOAc : cyclohexane 1 : 1, *v : v*)= 0.26; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ: 8.72 (s, 1H, Ar*H*), 7.97 (d, *J* = 8.5 Hz, 2H, Ar*H*), 7.42 (d, *J* = 8.0 Hz, 2H, Ar*H*), 6.23 (s, 2H, -C*H*$_2$), 3.85 (s, 3H, COOC*H*$_3$), 2.41 (s, 3H, -C*H*$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ: 190.8 (CO), 160.6 ($COOCH_3$), 144.9 (C), 138.4 (C), 131.2 (C), 130.6 (CH), 129.4 (2CH), 128.1 (2CH), 55.9 (CH_2), 51.7 (CH_3), 21.1 (CH_3) ppm; IR *v*: 1716 (-$COOCH_3$), 1693 (>C=O), 1229 (C-O), 900-500 (C-H) cm^{-1}; MS (*m/z*): MS (MALDI-TOF/TOF): *m/z* = 260 $[M+H]^+$.

Methyl 1-(2-(4-methoxyphenyl)-2-oxoethyl)-1H-1,2,3-triazole-4-carboxylate ***(2e):*** The general procedure was used with 0.4 g 2-azido-1-(4-methoxyphenyl)ethan-1-one **1e** (1.45 mmol, 1 eq.), 0.21 g methyl propiolate (2.51 mmol, 1.2 eq.), 0.052 g $CuSO_4 \cdot 5H_2O$ (0.20 mmol, 0.1 eq.), 0.082 g sodium ascorbate (0.41 mmol, 0.2 eq.) and 5 mL of *tert*-butanol. Purification by column chromatography was performed using a mixture of ethyl acetate (EtOAc) and cyclohexane (*2v : v*). White solid, 0.49 g, 85 % yield, m.p. (ethanol) = 179 °C, R_f (EtOAc : cyclohexane 1 : 1, *v : v*) = 0.14; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ: 8.72 (s, 1H, Ar*H*), 8.06 (d, *J* = 9.0 Hz, 2H, Ar*H*), 7.13 (d, *J* = 9.0 Hz, 2H, Ar*H*), 6.21 (s, 2H, -C*H*$_2$), 3.88 (s, 3H, COOC*H*$_3$), 3.86 (s, 3H, OC*H*$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ: 189.6 (CO), 164.8 (C), 160.6 ($COOCH_3$), 138.4 (C), 126.5 (C), 130.7 (CH), 130.5 (2CH), 114.1 (2CH), 55.7 (CH_2), 55.5 (CH_3), 51.6 (CH_3) ppm; IR *v*:

1749 (-$COOCH_3$), 1677 (>C=O), 1235 (C-O), 900-500 (C-H) cm^{-1}; MS (MALDI-TOF/TOF): *m/z* = 276 $[M+H]^+$.

Methyl 1-(2-(4-chlorophenyl)-2-oxoethyl)-1H-1,2,3-triazole-4-carboxylate ***(2f):*** The general procedure was used with 0.4 g 2-azido-1-(4-chlorophenyl)ethan-1-one **1f** (2.04 mmol, 1 eq.), 0.20 g methyl propiolate (2.45 mmol, 1.2 eq.), 0.051 g $CuSO_4 \cdot 5H_2O$ (0.20 mmol, 0.1 eq.), 0.081 g sodium ascorbate (0.40 mmol, 0.2 eq.) and 5 mL of *tert*-butanol. Purification by column chromatography was performed using a mixture of ethyl acetate and cyclohexane (*2v : v*). White solid, 0.45 g, 80 % yield, m.p. (ethanol) = 236.4 °C, R_f (EtOAc : cyclohexane 1 : 1, *v : v*) = 0.26; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.70 (s, 1H, Ar*H*), 8.07 (d, *J* = 8.5 Hz, 2H, Ar*H*), 7.68 (d, *J* = 8.5 Hz, 2H, Ar*H*), 6.26 (s, 2H, -CH_2), 3.84 (s, 3H, COOCH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ: 190.9 (CO), 160.8 ($COOCH_3$), 139.4 (C), 138.7 (C), 132.7 (C), 130.9 (CH), 130.2 (2CH), 129.3 (2CH), 56.3 (CH_2) 51.9 (CH_3) ppm; IR *v*: 1720 (-$COOCH_3$), 1688 (>C=O), 1226 (C-O), 900-500 (C-H), 779 (C-Cl) cm^{-1}; MS (*m/z*): MS (MALDI-TOF/TOF): *m/z* = 280 $[M+H]^+$.

Methyl 1-(2-(4-cyanophenyl)-2-oxoethyl)-1H-1,2,3-triazole-4-carboxylate ***(2g):*** The general procedure was used with 0.4 g 4-(2-azidoacetyl)benzonitrile **1g** (2.29 mmol, 1 eq.), 0.23 g methyl propiolate (2.73 mmol, 1.2 eq.), 0.057 g $CuSO_4 \cdot 5H_2O$ (0.22 mmol, 0.1 eq.), 0.091 g sodium ascorbate (0.45 mmol, 0.2 eq.) and 5 mL of *tert*-butanol. Purification by column chromatography was performed using a mixture of ethyl acetate and cyclohexane (*2v : v*). White solid, 0.46 g, 75 % yield, m.p. (ethanol) = 222 °C, R_f (EtOAc : cyclohexane 1 : 1, *v : v*) = 0.12; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.71 (s, 1H, Ar*H*), 8.22 (d, *J* = 8.5 Hz, 2H, Ar*H*),

8.11 (dd, *J* = 8.0, 1.5 Hz, 2H, Ar*H*), 6.33 (s, 2H, -C*H*$_2$), 3.86 (s, 3H, COOC*H*$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ: 191.1 (CO), 160.5 ($COOCH_3$), 138.5 (C), 136.9 (C), 132.9 (2CH), 130.6 (CH), 128.7 (2CH), 117.9 (C≡N), 116.0 (C), 56.4 (CH_2), 51.7 (CH_3) ppm; IR *ν*: 2232 (C≡N), 1721 (-$COOCH_3$), 1702 (>C=O), 1222 (C-O), 900-500 (C-H) cm^{-1}; MS (MALDI-TOF/TOF): *m/z* = 271 $[M+H]^+$.

Conclusions

In this study, a series of 1,4-disubstituted 1,2,3-triazoles derivatives (**2a-g**) bearing a carboxymethyl substituent at the 4th position were obtained through a Cu(I) mediated azide-alkyne cycloaddition protocol starting from appropriately substituted aryl azides and methyl propiolate. The synthetic approach proceeded under mild conditions using the $CuSO_4$/sodium ascorbate catalytic system in a *tert*-BuOH/MeCN/H_2O mixture, providing the target compounds in good yields. The structures of the synthesized molecules were confirmed by spectroscopic techniques, including NMR analysis.

Among the prepared molecules, derivatives **2a-g**, synthesized within the framework of the present work, were subjected to biological screening to assess their antifungal potential against *Candida albicans*. The minimum inhibitory concentration (MIC) values indicated that several members of this series exhibited measurable antifungal activity. Compounds **2b** and **2d** displayed the most pronounced antifungal response, with MIC values of 16.07 μg/mL and 12.96 μg/mL, respectively. These observations suggest that substitution on the aromatic fragment exerts a measurable influence on the biological profile of the triazole scaffold. Conversely, other derivatives from the series showed weaker inhibition, and increasing the concentration

of these molecules did not lead to a proportional increase in antifungal response. The atypical MIC behavior observed for some compounds may be associated with adaptive cellular responses in *Candida albicans*, which could reduce susceptibility to certain triazole derivatives. However, since no mechanistic experiments were performed in the present study, this interpretation should be regarded only as a possible hypothesis. Further investigations will be necessary to clarify whether inducible resistance pathways or adaptive stress responses are involved in the observed antifungal profile.

Overall, the obtained results underline the relevance of the 1,2,3-triazole framework as a versatile platform for the development of molecules with antifungal potential. The activity observed for derivatives **2b** and **2d** indicates that structural modulation of the aromatic substituent can influence biological response and may serve as a starting point for further structural refinement. Future investigations will focus on expanding this series of triazole derivatives and examining the relationship between electronic characteristics of the substituents and the antifungal profile of the resulting compounds.

Acknowledgements

The authors thank the CERNESIM Research Centre within the Institute for Interdisciplinary Research at the Alexandru Ioan Cuza University of Iasi for infrastructure used in NMR spectra recording and the support, the Erasmus+ KA131-HED program for providing the opportunity to carry out two internship mobilities at JUNIA (Lille, France) during the academic years 2023-2024 and 2024-2025 and to Assoc. Prof. Dr. Brîndușa-Alina Petre for performing the MALDI-TOF mass spectrometry analyses at the Proteomics Laboratory, TRANSCEND-IRO Center, Iasi.

Conflicts of interest

The authors declare no conflict of interest. This research was not funded by external agencies. Therefore, funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The authors have no personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results.

References

1. Usman, G. Alpha-glucosidase Inhibitors: Clinically promising candidates for anti-diabetic drug discovery; Elsevier: Olanda, **2019**; pp 119 – 136.
2. Finn, M. G.; Fokin, V. V. Click chemistry: function follows form. *Chem. Soc. Rev*. **2010**, *39(4)*, 1231 – 1232. https://doi.org/10.1039/C003740K
3. Matin, M. M.; Matin, P.; Rahman, M. R.; Ben Hadda, T.; Almalki, F. A.; Mahmud, S.; Ghoneim, M. M.; Alruwaily, M.; Alshehri, S. Triazoles and their derivatives: chemistry, synthesis, and therapeutic applications. *Front. Mol. Biosci*. **2022**, *9*, 864286. https://doi.org/10.3389/fmolb.2022.864286
4. Pan, R.; Pathak, T. Copper catalyzed azide–alkyne cycloaddition based synthesis and applications of oligo-1,2,3-triazoles. *Arkivoc* **2022**, *6*, 81 – 151. https://doi.org/10.24820/ark.5550190.p011.865
5. Lopez-Ruiz, A.; Ortega-Muñoz, M.; Santoyo-Gonzalez, F.; Megia-Fernandez, A. Click to learn, learn to click: undergraduate synthetic organic chemistry experiments. *Chem. Pap*. **2023**, *77*, 5227 – 5236. https://doi.org/10.1007/s11696-023-02858-5
6. Frîncul, C.; Ghinet, A.; Shova, S.; Ciobanu, C.-I.; Nica, A. S.; Petre, B. A.; Bîcu, E.; Belei, D. Regioselective C-alkylation in a series of functionalized 1,2,3-triazoles: an unexpected preference over O-alkylation. *J. Mol. Struct.* **2026**, *1355*, 144999. https://doi.org/10.1016/j.molstruc.2025.144999
7. Stanciu, M. C.; Belei, D.; Bicu, E.; Tuchilus, C. G.; Nichifor, M. Novel amphiphilic dextran esters with antimicrobial activity. *Int. J. Biol. Macromol*. **2020**, *150*, 746 – 755. https://doi.org/10.1016/j.ijbiomac.2020.02.021
8. Frîncul, C.; Ghinet, A.; Belei, D.; Chankvetadze, B.; Shova, S.; Lipka, E.; Dascălu, A.-E. Optimizing enantiomeric resolution of chiral triazoles in supercritical fluid chromatography. *Chirality* **2025**, *37*(2), e70016. https://doi.org/10.1002/chir.70016

9. Lo, W.-H.; Deng, F.-S.; Chang, C.-J.; Lin, C.-H. synergistic antifungal activity of chitosan with fluconazole against *Candida albicans*, *Candida tropicalis*, and fluconazole-resistant strains. *Molecules* **2020**, *25*(21), 5114. https://doi.org/10.3390/molecules25215114

10. Gillum, A. M.; Tsay, E. Y.; Kirsch, D. R. Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of S. cerevisiae ura3 and *E. coli* pyrF mutations. *Mol. Gen. Genet.* **1984**, *198*(2), 179 – 82. https://doi.org/10.1007/bf00328721

11. Pfaller, M. A.; Diekema, D. J.; Procop, G. W.; Rinaldi, M. G. Multicenter comparison of the VITEK 2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing amphotericin B, flucytosine, and voriconazole against *Candida spp. J. Clin. Microbiol*. **2007**, *45*(11), 3522 – 8. https://doi.org/10.1128/jcm.00403-07

12. Jagtap, R. A.; Jagdale, B. S.; Dhonnar, S. L.; Shaikh, S. A.; More, R. A.; Alavala, R. R.; Muteeb, G.; Fernandez-Conde, D.; Pawar, T. J.; Meshram, R. J.; Escobar-Cabrera, J.; Chobe, S. S. Efficient synthesis, dual anti-tubercular and antioxidant activity of triazole-acetophenone derivatives: enhanced efficacy via esterification and quantum mechanical validation of CYP121 binding. *RSC Adv*. **2026**, *16*(4), 3609 – 3622. https://doi.org/10.1039/D5RA09368F

13. Wu, X.; Guo, J.; Sun, M.-Y.; Du, T.; Hao, D.; Liu, D.; Wang, D.; Wen, S.; Yin, J.; Li, D.; He, J. Construction of heteroleptic copper complexes in perylene diimide-based COFs for heterogeneous metallaphotoredox catalysis. *J. Am. Chem. Soc*. **2026**, *148*(4), 4283 – 4294. https://doi.org/10.1021/jacs.5c17585

14. Abuhaie, C.-M.; Ghinet, A.; Farce, A.; Dubois, J.; Gautret, P.; Rigo, B.; Belei, D.; Bîcu, E., Synthesis and biological evaluation of a new series of phenothiazine-containing protein farnesyltransferase inhibitors. *Eur. J. Med. Chem.* **2013**, *59*, 101 – 110. https://doi.org/10.1016/j.ejmech.2012.11.008

15. Butler, C. R.; Bendesky, J.; Schoffstall, A. M., Regioselective reduction of 1 *H*-1,2,3-triazole diesters. *Molecules* **2021**, *26*(18), 5589. https://doi.org/10.3390/molecules26185589