



Synthesis, characterization and antimicrobial activity of novel benzophenone derived 1,2,3-triazoles

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Abstract

A series of new benzophenone derived from 1,2,3-triazoles **3a-j** was synthesized, in good yields, using Copper-Catalyzed Alkyne-Azide Cycloaddition (CuAAC) reaction between a variety of commercial and synthesized alkynes and 4-(azidomethyl)benzophenone. The structure of synthesized compounds were confirmed by spectral techniques ¹H NMR, ¹³C NMR, and high-resolution mass spectrometry (HRMS). The prepared compounds were also tested in vitro for their antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and fungi (*Candida albicans*). Among the synthesized compounds **3a** and **3b** showed an interesting antimicrobial activity against all the tested strains except *E. coli* which showed a slightly resistance.

Keywords: antimicrobial activity, benzophenone, carbohydrates, cycloaddition, 1,2,3-triazole.

Introduction

Recently, the rates of microbial threats associated with the increasing emergence of antimicrobial resistance in hospitals are major concerns for public health and scientific communities around the world. Especially numerous multi-drug resistant Gram-positive bacteria pathogens, which are methicillin-resistant like *Staphylococcus aureus* (MRSA), are growing threat to human health [1,2]. Similarly, the fungal infections caused by various species, such as, *Candida* and *Aspergillus*, have been rising in prevalence all over the world [3,4]. This has required new efforts for the development of new powerful antimicrobial agents with broad spectrum of activity, that have an important role to control the emerging multi-drug resistance strains of bacteria and fungi [5,6].

The benzophenones (still called diphenyl ketones) are a class of molecules obtained by two pathways, natural and synthetic, and are pharmacologically active compounds [7,8]. Some derivatives of benzophenones display substantial activities including antifungal [9], anti-viral [10], antibacterial [11], antioxidant [12], and cytotoxic [13]. Numerous glycosyls derivative and N-heterocyclic compounds (such as, imidazole, benzimidazole, isatin, 1,2,4-triazole) exhibit good biological activities and are essentials intermediates in the synthesis of biologically active molecules [14–17]. Amongst the various drugs responsible of the antimicrobial activity there are the nitrogenous compounds, especially the 1,2,3-triazoles and their derivatives which have become a class of very active compounds with a broad spectrum of chemotherapeutic activities [18,19].

Many 1,2,3-triazoles, are proved potent and having several biological properties, such as, antiviral [20], antiepileptic [21], antifungal [22], antibacterial [2], antimicrobial [23], antiprotozoal [24], antioxidant [25], anti-inflammatory [26], and anticancer activities [27]. These 1,2,3-triazoles are incorporated in medicinal chemistry and form some drugs available in markets. Among the most well known structures (figure 1), we cite Tazobactam (**A**) [28], Cefatrizine (**B**) [29], Rufinamide (**C**) [30], and Carboxyamidotriazole (**D**) [31].

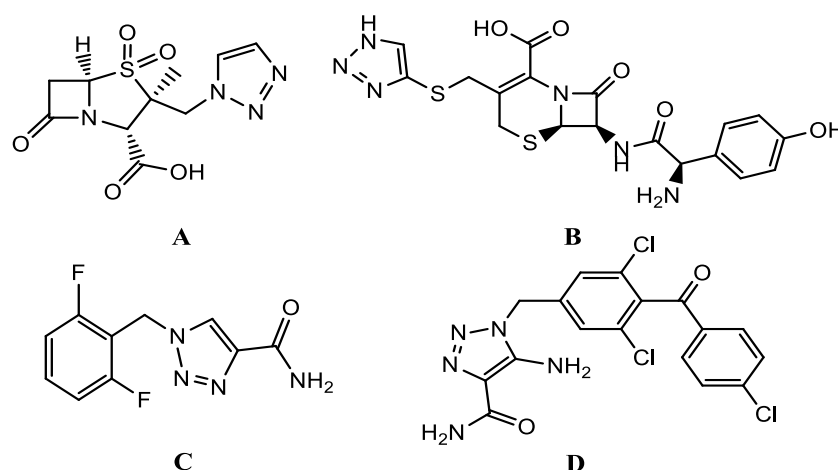


Figure 1: Structures of some drugs containing 1,2,3-triazole nucleus

Different methods are available for the synthesis of 1,2,3-triazoles [32]. The Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) is one of the best click reactions to date [33]. This method was discovered by Meldal and Sharpless in 2002 [34,35], which modified the classical Huisgen 1,3-dipolar cycloaddition [36] allowing the regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles.

Based on all the previous informations, and in the interest of synthesis of new antimicrobial agents, our research aims the incorporation of the benzophenone with triazole nucleus, because both moieties are known for their antimicrobial properties [37]. In order to improve the antimicrobial effect, different benzophenone derived 1,2,3-triazoles were synthesized using CuAAC of compounds resulting from N-alkylation [38], O-alkylation [39,40], and substitution of 4-bromomethyl-benzophenone. Combining the activities of the benzophenone group, the 1,2,3-triazole, the carbohydrates derivative, and the N-heterocyclic compounds, we synthesized a new series of benzophenone derived 1,2,3-triazoles scaffold. The synthesized triazoles were characterized by spectroscopic techniques, such as, ^1H NMR, ^{13}C NMR, and mass spectra (MS). In addition they were evaluated for their antibacterial and antifungal activities in vitro against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*.

Material and methods

1. Chemistry

Melting points were recorded on Kofler bench, and were uncorrected. ^1H NMR (300MHz) and ^{13}C NMR (75MHz) spectra were recorded on Bruker spectrometers with chemical shift values (δ) given in part per million (ppm) relative to TMS (0.00 ppm) and using CDCl_3 as solvent, the coupling constants (J) are expressed in hertz (Hz) and singlet (s), doublet (d), doublet of a doublet (dd), and triplet (t) as well as multiplet (m). Flash chromatography was conducted using flash silica gel 60 (Merck 230-400 mesh). The reaction progress was monitored by TLC using Silica gel 60-F254 plat with visualization under UV light. 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

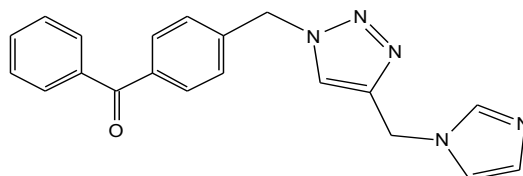
The synthetic route of benzophenone derived 1,2,3-triazoles **1-10** was outlined in Scheme 1. In a round-bottom flask 100 ml equipped with a magnetic stirrer bar, 4-(azidomethyl)benzophenone (1.26 mmol), alkyne (1.26 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.063 mmol), sodium ascorbate (0.126 mmol), Et-OH (5mL), H_2O (5mL) were added. 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 distilled water was added and extracted with diethyl ether, 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 (3:1 v/v) as eluent.

1.2. Spectral data

The analytical data of all the isolated benzophenone derived 1,2,3-triazoles is given as under.

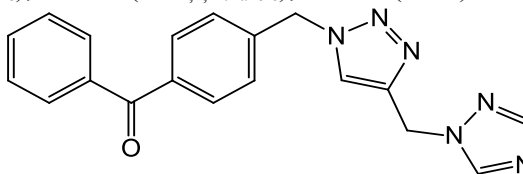
4-[4-(1H-imidazol-1-yl)methyl]-1H-1,2,3-triazol-1-yl]methylbenzophenone (3a)

White solid product, m.p. 132°C (dichloromethane/hexane:1/10), Yield: 77%, R_f : 0.1 (ethyl acetate). ^1H NMR (CDCl_3 , 300MHz): 5.24 (s, 2H, $\text{CH}_2\text{-N}_{\text{imidazole}}$); 5.57 (s, 2H, $\text{CH}_2\text{-N}_{\text{triazole}}$); 7.31 (s, 1H, $\text{H}_{\text{imidazole}}$); 7.34 (s, 1H, $\text{H}_{\text{imidazole}}$); 7.39 (s, 1H, $\text{H}_{\text{imidazole}}$); 7.44-7.49 (m, 3H, H_{Ar}); 7.56-7.62 (m, 2H, H_{Ar}); 7.73-7.79 (m+s, 5H, H_{Ar} , $\text{H}_{\text{triazole}}$). ^{13}C NMR (CDCl_3 , 75MHz): 42.80 ($\text{CH}_2\text{-N}_{\text{imidazole}}$); 54.14 ($\text{CH}_2\text{-N}_{\text{triazole}}$); 120.57 ($\text{CH}_{\text{imidazole}}$); 122.39 ($\text{CH}_{\text{triazole}}$); 128.12 (2CH_{Ar}); 128.71 (2CH_{Ar}); 130.28 (2CH_{Ar}); 131.09 (2CH_{Ar}); 133.07 (CH_{Ar}); 137.34 ($\text{CH}_{\text{imidazole}}$); 138.39 ($\text{C}_{\text{triazole}}$); 138.69 (C_{Ar} , $\text{CH}_{\text{imidazole}}$); 142.37 (C_{Ar}); 144.51 (C_{Ar}); 196.11 (C=O). MS, m/z: 344.10 [$\text{M}+\text{H}$] $^+$.



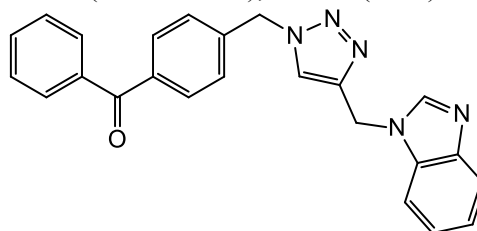
4-[4-(1H-1,2,4-triazol-1-yl)methyl]-1H-1,2,3-triazol-1-yl]methylbenzophenone (3b)

Yellow oil product, Yield: 77%, R_f : 0.4 (hexane/ethyl acetate: 1:1 v/v). ^1H NMR (CDCl_3 , 300MHz): 5.46 (s, 2H, $\text{CH}_2\text{-N}_{1,2,4\text{-triazole}}$); 5.58 (s, 2H, $\text{CH}_2\text{-N}_{\text{triazole}}$); 7.32-7.35 (m, 2H, H_{Ar}); 7.43-7.58 (m, 3H, H_{Ar}); 7.60 (s, 1H, $\text{H}_{1,2,3\text{-triazole}}$); 7.73-7.79 (m, 4H, H_{Ar}); 7.90 (s, 1H, $\text{H}_{1,2,4\text{-triazole}}$); 8.21 (s, 1H, $\text{H}_{1,2,4\text{-triazole}}$). ^{13}C NMR (CDCl_3 , 75MHz): 45.30 ($\text{CH}_2\text{-N}_{1,2,4\text{-triazole}}$); 54.27 ($\text{CH}_2\text{-N}_{1,2,3\text{-triazole}}$); 123.49 ($\text{CH}_{1,2,3\text{-triazole}}$); 128.27 (2CH_{Ar}); 128.81 (2CH_{Ar}); 130.39 (2CH_{Ar}); 131.20 (2CH_{Ar}); 133.18 (CH_{Ar}); 137.46 ($\text{C}_{1,2,3\text{-triazole}}$); 138.49 (C_{Ar}); 138.75 (C_{Ar}); 142.82 (C_{Ar}); 152.67 ($\text{CH}_{1,2,4\text{-triazole}}$); 156.10 ($\text{CH}_{1,2,4\text{-triazole}}$); 196.24 (C=O). MS, m/z: 345.10 [$\text{M}+\text{H}$] $^+$.



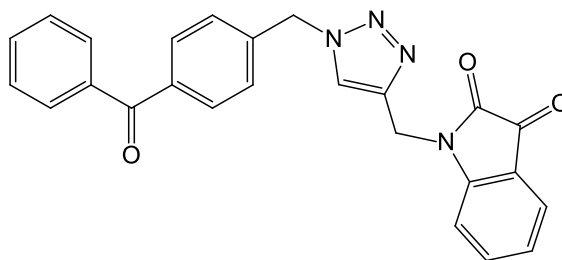
4-[4-(1H-benzo[d]imidazol-1-yl)methyl]-1H-1,2,3-triazol-1-yl]methylbenzophenone (3c)

Brown oil product. Yield: 79%, R_f : 0.2 (ethyl acetate). ^1H NMR (CDCl_3 , 300MHz): 5.30 (s, 2H, $\text{CH}_2\text{-N}_{\text{benzimidazole}}$); 5.36 (s, 2H, $\text{CH}_2\text{-N}_{\text{triazole}}$); 7.11-7.14 (m, 4H, H_{Ar}); 7.29-7.47 (m, 5H, H_{Ar}); 7.57-7.60 (m+1, 5H, H_{Ar} + $\text{H}_{\text{triazole}}$); 7.85 (s, 1H, $\text{H}_{\text{benzimidazole}}$). ^{13}C NMR (CDCl_3 , 75MHz): 40.93 ($\text{CH}_2\text{-N}_{\text{benzimidazole}}$); 54.13 ($\text{CH}_2\text{-N}_{\text{triazole}}$); 110.39 (CH_{Ar}); 120.70 (CH_{Ar}); 122.78 (CH_{Ar}); 122.91 (CH_{Ar}); 123.69 ($\text{CH}_{\text{triazole}}$); 128.15 (2CH_{Ar}); 182.81 (2CH_{Ar}); 130.37 (2CH_{Ar}); 131.11 (2CH_{Ar}); 133.18 (CH_{Ar}); 133.89 ($\text{C}_{\text{benzimidazole}}$); 137.44 (C_{Ar}); 138.34 ($\text{C}_{\text{triazole}}$); 138.92 (2C_{Ar}); 143.18 ($\text{C}_{\text{benzimidazole}}$); 143.88 ($\text{CH}_{\text{benzimidazole}}$); 196.27 (C=O). MS, m/z: 394.10 [$\text{M}+\text{H}$] $^+$.

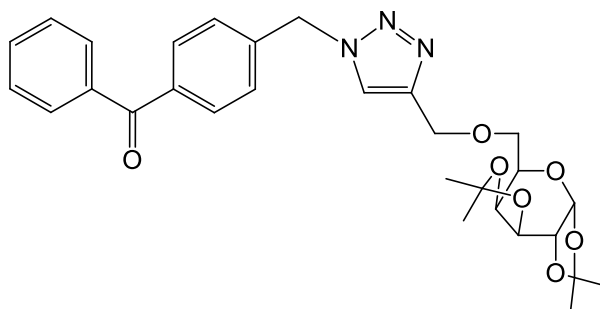


1-[1-(4-benzoylbenzyl)-1H-1,2,3-triazol-4-yl]methylisatin (3d)

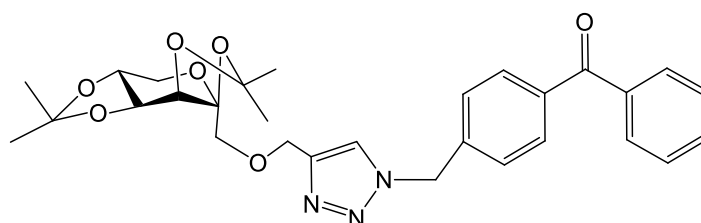
Yellow oil product, Yield: 81%, R_f : 0.4 (hexane/ethyl acetate: 1:1 v/v). ^1H NMR (CDCl_3 , 300MHz): 5.02 (s, 2H, $\text{CH}_2\text{-N}_{\text{isatin}}$); 5.59 (s, 2H, $\text{CH}_2\text{-N}_{\text{triazole}}$); 7.07-7.12 (m, 1H, CH_{Ar}); 7.28-7.36 (m, 3H, CH_{Ar}); 7.44-7.61 (m, 5H, CH_{Ar}); 7.69-7.78 (m, 5H, CH_{Ar} + $\text{H}_{\text{triazole}}$). ^{13}C NMR (CDCl_3 , 75MHz): 35.82 ($\text{CH}_2\text{-N}_{\text{isatin}}$); 54.32 ($\text{CH}_2\text{-N}_{\text{triazole}}$); 111.87 (CH_{Ar}); 117.93 (C_{Ar}); 124.48 ($\text{CH}_{\text{triazole}}$); 125.75 (CH_{Ar}); 128.36 (2CH_{Ar}); 128.80 (3CH_{Ar}); 130.41 (3CH_{Ar}); 131.19 (2CH_{Ar}); 133.15 (CH_{Ar}); 137.49 ($\text{C}_{\text{triazole}}$); 138.48 (C_{Ar}); 138.76 (C_{Ar}); 139.04 (C_{Ar}); 150.57 (C_{Ar}); 158.39 ($\text{C=O}_{\text{isatin}}$); 183.42 ($\text{C=O}_{\text{isatin}}$); 196.26 ($\text{C=O}_{\text{benzophenone}}$). MS, m/z: 445.10 [$\text{M}+\text{Na}$] $^+$.



4-[4-(1,2:3,4-di-O-isopropylidene- α -D-6-galacopyranosylmethyl)-1H-1,2,3-triazol-1-yl]methylbezophenone (**3e**)
White oil product, Yield: 76%, R_f : 0.2 (hexane/ethyl acetate: 1:1 v/v). ^1H NMR (CDCl_3 , 300MHz): 1.30, 1.40, 1.49 (3s, 12H, 4CH₃); 3.63-3.75 (m, 2H, CH₂-O); 3.98 (dd, 1H, CHO, $J_1=7.0$, $J_2=1.5$); 4.23 (dd, 1H, CHO, $J_1=7.9$, $J_2=1.8$); 4.29 (dd, 1H, CHO, $J_1=5.0$, $J_2=2.3$); 4.58 (dd, 1H, CHO, $J_1=7.9$, $J_2=2.3$); 4.74 (s, 2H, CH₂-O); 5.50 (d, 1H, CHO, $J=5.0$); 5.59 (s, 2H, CH₂-N); 7.34-7.49 (m, 5H, H_{Ar}); 7.58 (m, 1H, H_{triazole}); 7.75-7.77 (m, 4H, H_{Ar}). ^{13}C NMR (CDCl_3 , 75MHz): 24.46 (CH₃); 24.88 (CH₃); 25.96 (CH₃); 26.04 (CH₃); 53.60 (CH₂-N); 64.87 (CH₂-O); 66.77 (CHO); 69.47 (CH₂-O); 70.53 (CHO); 70.67 (CHO); 71.18 (CHO); 96.34 (CHO); 108.52-109.25 (2C); 122.69 (CH_{triazole}); 127.76 (2CH_{Ar}); 128.35 (2CH_{Ar}); 129.95 (2CH_{Ar}); 130.67 (2CH_{Ar}); 132.63 (CH_{Ar}); 137.22 (C_{Ar}); 137.89 (C_{Ar}); 138.99 (C_{Ar}); 145.97 (C_{triazole}); 195.80 (C=O). MS, m/z: 536.23 [M+H]⁺.

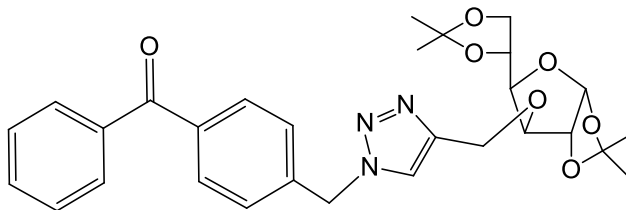


4-[4-(2,3:4,5-di-O-isopropylidene- β -D-fructopyranosylmethyl)-1H-1,2,3-triazol-1-yl]methylbezophenone (**3f**)
Yellow oil product, Yield: 73%, R_f : 0.2 (hexane/ethyl acetate: 1:1 v/v). ^1H NMR (CDCl_3 , 300MHz): 1.32, 1.35, 1.41, 1.52 (4s, 12H, 4CH₃); 3.62-3.92 (m, 4H, CH₂O + CH₂O); 4.22 (d, 1H, CHO, $J=7.9$); 4.33 (d, 1H, CHO, $J=3$); 4.58 (dd, 1H, CHO, $J=8$, $J=3$); 4.78 (2d, 2H, CH₂-O, $J=12$); 5.61 (s, 2H, CH₂-N); 7.34-7.51 (m, 4H, H_{Ar}); 7.58 (s, 1H, H_{triazole}); 7.76-7.81 (m, 5H, H_{Ar}). ^{13}C NMR (CDCl_3 , 75MHz): 24.05 (CH₃); 25.29 (CH₃); 25.83 (CH₃); 25.51 (CH₃); 53.65 (CH₂-N); 61.05 (CH₂-O); 65.53 (CH₂-O); 70.18 (CHO); 70.24 (CHO); 70.96 (CHO); 72.12 (CH₂-O); 102.57 (C-O); 108.52-108.93 (2C); 122.49 (CH_{triazole}); 127.70 (2CH_{Ar}); 128.37 (2CH_{Ar}); 129.97 (2CH_{Ar}); 130.71 (2CH_{Ar}); 132.66 (CH_{Ar}); 137.22 (C_{Ar}); 137.96 (C_{Ar}); 138.92 (C_{Ar}); 145.88 (C_{triazole}); 195.80 (C=O). MS, m/z: 536.24 [M+H]⁺.



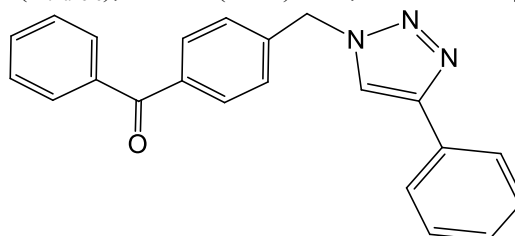
4-[4-(1,2:5,6-di-O-isopropylidene- α -D-glucofuranosylmethyl)-1H-1,2,3-triazol-1-yl]methylbezophenone (**3g**)
Yellow oil product, Yield: 72%, R_f : (hexane/ethyl acetate: 1:1 v/v). ^1H NMR (CDCl_3 , 300MHz): 1.30, 1.38, 1.49 (3s, 12H, 4CH₃); 3.96-4.12 (m, 4H, CH₂O + 2CHO); 4.29 (dd, 1H, CHO, $J=9.8$, $J=5.7$); 4.60 (d, 1H, CHO, $J=3.7$); 4.80 (2d, 2H, CH₂-O, $J=12.7$); 5.62 (s, 2H, CH₂-N); 5.86 (d, 1H, CHO, $J=3.9$); 7.35-7.61 (m, 5H, H_{Ar}); 7.63 (s, 1H, H_{triazole}); 7.77-7.82 (m, 4H, H_{Ar}). ^{13}C NMR (CDCl_3 , 75MHz): 26.78 (CH₃); 26.82 (CH₃); 27.36 (CH₃); 29.66 (CH₃); 53.92 (C-N); 64.14 (CH₂-O); 67.44 (CH₂-O); 72.37 (CHO); 81.89 (CHO); 82.24 (CHO); 82.54 (CHO); 105.24 (CHO); 109.04-111.88 (2C); 122.53 (CH_{triazole}); 127.66 (2CH_{Ar}); 128.38

(2CH_{Ar}); 129.96 (2CH_{Ar}); 130.74 (2CH_{Ar}); 132.70 (CH_{Ar}); 137.18 (C_{Ar}); 138.03 (C_{Ar}); 138.87 (C_{Ar}); 145.67 (C_{triazole}); 195.77 (C=O). MS, m/z: 536.23 [M+H]⁺.



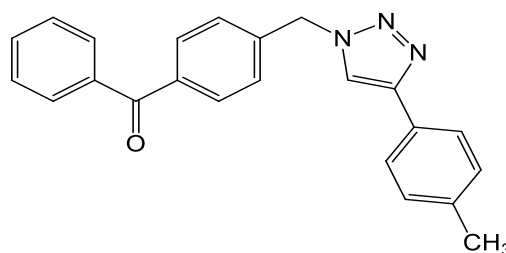
4-[4-(phenyl)-1H-1,2,3-triazol-1-yl]methylbenzophenone (3h)

White solid product, m.p. 137°C (dichloromethane/hexane:1/10), Yield: 87%, R_f: 0.5 (hexane/ethyl acetate: 1:1 v/v). ¹H NMR (CDCl₃, 300MHz): 5.67 (s, 2H, CH₂-N); 7.30-7.62 (m, 8H, H_{Ar}); 7.76-7.84 (m, 7H, H_{Ar}+H_{triazole}). ¹³C NMR (CDCl₃, 75MHz): 54.16 (CH₂-N); 120.10 (CH_{triazole}); 126.13 (2CH_{Ar}); 128.15 (2CH_{Ar}); 128.73 (CH_{Ar}); 128.80 (2CH_{Ar}); 129.27 (2CH_{Ar}); 130.42 (2CH_{Ar}); 130.75 (C_{Ar}); 131.18 (2CH_{Ar}); 133.14 (CH_{Ar}); 137.56 (C_{Ar}); 138.37 (C_{Ar}); 139.46 (C_{Ar}); 143.33 (C_{triazole}); 196.31 (C=O). MS, m/z: 362.12 [M+Na]⁺.



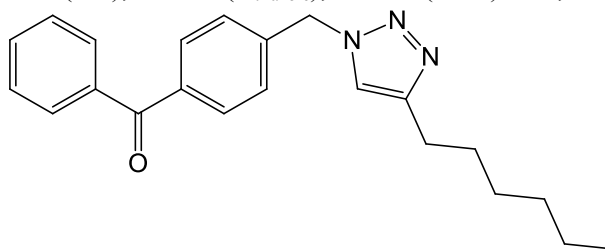
4-[4-(p-tolyl)-1H-1,2,3-triazol-1-yl]methylbenzophenone (3i)

White solid product, m.p. 170°C (dichloromethane/hexane:1/10), Yield: 82%, R_f: 0.5 (hexane/ethyl acetate: 1:1 v/v). ¹H NMR (CDCl₃, 300MHz): 2.37 (s, 3H, CH₃); 5.66 (s, 2H, CH₂-N); 7.23-7.62 (m, 8H, 7H_{Ar}+ 1H_{triazole}); 7.69-7.82 (m, 6H, H_{Ar}). ¹³C NMR (CDCl₃, 75MHz): 21.43 (CH₃); 53.50 (CH₂-N); 125.62 (CH_{triazole}); 127.92 (2CH_{Ar}); 128.52 (3CH_{Ar}); 129.72 (2CH_{Ar}); 130.15 (3CH_{Ar}); 130.89 (2CH_{Ar}); 132.84 (CH_{Ar}); 137.29 (C_{Ar}); 138.30 (C_{Ar}); 138.33 (C_{Ar}); 139.09 (C_{Ar}); 140.15 (C_{Ar}); 147.95 (C_{triazole}); 195.91 (C=O). MS, m/z: 354.15 [M+H]⁺.



4-[4-(hexyl)-1H-1,2,3-triazol-1-yl]methylbenzophenone (3j)

White solid product, M.p. 80°C (dichloromethane/hexane:1/10), Yield: 86%, R_f: 0.5 (hexane/ethyl acetate: 1:1 v/v). ¹H NMR (CDCl₃, 300MHz): 0.86 (t, 3H, CH₃, J=6.45); 1.24-1.36 (m, 6H, 3CH₂); 1.62-1.67 (m, 2H, CH₂); 2.70 (t, 2H, CH₂, J=7.65); 5.58 (s, 2H, CH₂-N); 7.32-7.61 (m, 6H, H_{Ar} + H_{triazole}); 7.75-7.80 (m, 4H, CH_{Ar}). ¹³C NMR (CDCl₃, 75MHz): 13.97 (CH₃); 22.47 (CH₂); 25.62 (CH₂); 28.84 (CH₂); 29.72 (CH₂); 31.46 (CH₂); 53.47 (CH₂-N); 120.68 (CH_{triazole}); 127.56 (2CH_{Ar}); 128.32 (2CH_{Ar}); 129.95 (2CH_{Ar}); 130.65 (2CH_{Ar}); 132.64 (CH_{Ar}); 137.08 (C_{Ar}); 137.74 (C_{Ar}); 139.28 (C_{Ar}); 148.72 (C_{triazole}); 196.11 (C=O). MS, m/z: 348.2 [M+H]⁺.



2. Biology

2.1. Antimicrobial activity

Antimicrobial activity of benzophenone derived 1,2,3-triazoles **3a-j** was evaluated according to the disk-diffusion method [41] against different microorganisms, including three bacteria strains: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and one fungal strain *Candida albicans*. Mueller Hinton agar (MHA) medium was used for Bacteria, while MHA supplemented with 2% dextrose and 0.5 mg/L methylene blue was used for *C. albicans*. Plates were pre-incubated at 37 °C for 24 h. Then, 100 µL of microbial inoculum adjusted to 0.5 McFarland was spreaded on the plates surfaces using a sterile glass rod to prepare microbial lawns. A sterile paper disk (6 mm in diameter) was placed on the surface of each agar plate and impregnated with 10 µL of each triazoles solution (**3a-j**) at a final concentration of 100µg/disk. Then, Petri dishes were incubated at 37 °C during 24 h for bacteria, and 30 °C during 48 h for *C. albicans*. The diameters of the inhibition zones were measured in mm (including disk diameter) with caliper. A disk impregnated with dimethylsulfoxide at 2% as negative control was performed. Each experiment was carried out in triplicate.

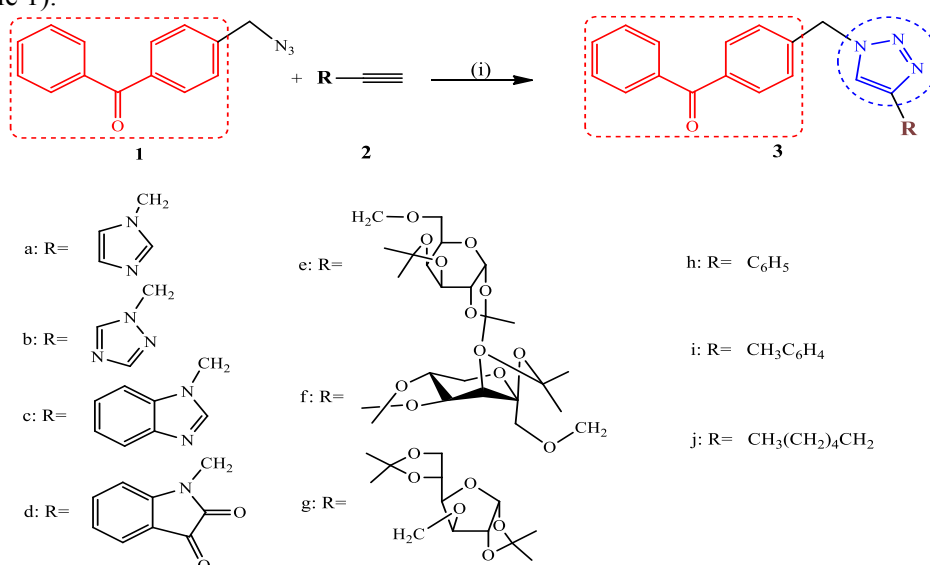
2.2. Minimum inhibitory concentration determination (MIC)

Minimum inhibitory concentration (MIC) of our triazoles were evaluated using the broth macro-dilution method with some modifications [1]. Briefly, the sterile nutrient broth or malt extract medium were used, for bacteria and *C. albicans* respectively, to perform the triazoles solutions (1 mL) using two-fold serial dilutions. Then, each tube was inoculated with a standardized microbial inoculum prepared in the same medium volume being 1 mL. Thus, triazoles solutions had the final concentrations of: 1000, 500, 250 ... 3.9 µg/mL. After vortexing, all tubes were incubated at 37°C for 24 h for bacteria and at 30 °C for *C. albicans*. The minimum inhibitory concentration (MIC) was determined as the lowest concentration showing no microbial growth compared to the positive control. DMSO (2 %) was used as a negative control.

3. Results and Discussion

We reported a synthesis of 1,4 and 1,5-disubstituted glucopyranosyl 1,2,3-triazole and the triazolyl analogue of the trityl cation by 1,3-dipolar cycloaddition [42,43]. We continued our experiments to apply this method for the preparation and the study of antimicrobial activity of benzophenone derived 1,2,3-triazoles. The 1,3-dipolar cycloaddition catalyzed by Cu(I) between benzophenonemethylazide (**1**) and various dipolarophiles (**2**) was realized leading to a variety of triazolylmethylbenzophenone (**3**) (Scheme 1). The cycloaddition products were purified by column chromatography and isolated with good yields (72-87%, see Table 1).

The molecular structures of the new 1,2,3-triazolylmethylbenzophenone (**3a-3j**) were established on the basis of the ¹H and ¹³C NMR spectroscopic data and mass spectrometry. In fact, the ¹H NMR spectra of the compounds **3a-3j** shows a singlet between 5.36 and 5.67 ppm relative to the CH₂ between 1,2,3-triazole and benzophenone (Scheme 1, Table 1).



Scheme 1: Synthetic route of novel benzophenone derived 1,2,3-triazoles **3a-j** Reagent and condition: (i) CuSO₄•5H₂O (0.05 equiv), sodium ascorbate (0.1 equiv), Et-OH/H₂O = 1:1 (v/v).

¹³C NMR spectra of these compounds exhibit a signal from 195.77 to 196.31 ppm which correspond to the C=O of the benzophenone (Table 1). The mass spectra (MS) recorded in the ESI mode confirmed the proposed structures.

Table 1: Yields and characteristics of 1,2,3-triazolymethylbenzophenones **3a-3j**

Entry	Compounds	Yield%	RMN ¹ H δCH ₂ -triazole in ppm	RMN ¹³ C δCO benzophenone in ppm
1	3a	77	5.57	196.11
2	3b	77	5.58	196.24
3	3c	79	5.36	196.27
4	3d	81	5.59	196.26
5	3e	76	5.59	195.80
6	3f	73	5.67	195.80
7	3g	72	5.62	195.77
8	3h	87	5.67	196.31
9	3i	82	5.66	195.91
10	3j	86	5.58	196.11

The antimicrobial activity of tested compounds was qualitatively and quantitatively assessed by the disk-diffusion and broth dilution methods. The inhibition zone diameters (IZD) and MIC values are set in Table 2.

Table 2: *In vitro* antimicrobial activity of benzophenone derived 1,2,3-triazoles.

Compounds	Bacterial strains						Fungal strain	
	Gram -		Gram +				<i>Candida albicans</i>	
	<i>Escherichia coli</i>		<i>Bacillus subtilis</i>		<i>Staphylococcus aureus</i>			
	MIC (µg/mL)	Φ mm	MIC (µg/mL)	Φ mm	MIC (µg/mL)	Φ mm	MIC (µg/mL)	Φ mm
3a	250	8	31.25	14	31.25	16	15.63	19
3b	500	7	62.5	13	31.25	15	15.63	18
3c	500	7	125	11	250	9	15.63	17
3d	500	7	250	9	250	10	15.63	15
3e	1000	7	250	10	500	9.5	62.5	12
3f	1000	7	500	9	500	9	125	12
3g	1000	7	500	9	500	9	62.5	13.5
3h	1000	7	500	9	1000	7.5	250	9
3i	1000	7	1000	7	1000	7	250	9
3j	1000	7	1000	7	1000	7	250	9
DMSO	*	6	*	6	*	6	*	6

*: not done

The zones inhibition diameters and the MIC values indicate that all synthesized compounds **3a-j** exhibited an antimicrobial activity against Gram-, Gram+, and fungal strains. As can be seen from the Table 2, the ten compounds showed various degrees of antimicrobial activity depending upon the tested microbial strains.

Among all the tested microorganisms it was interesting to note that *Candida albicans* was more susceptible against all compounds studied especially **3a**, **3b**, **3c**, and **3d** which exercised the strongest inhibitory effect having the lowest MIC value (15.63 µg/mL) and the largest IZD 19, 18, 17 and 15 mm respectively. While **3h**, **3i** and **3j** exhibited the lowest activity with a higher MIC value (250µg/mL).

In contrast, *E. coli* was the most resistant strain to all synthesized compounds with the MIC values superior or equal to 250µg/mL. However, only **3a** was able to give a moderate inhibitory activity. Conversely, *Bacillus subtilis* and *Staphylococcus aureus* were the most sensitive strains particularly with **3a** and **3b** compounds. Moreover, a moderate inhibitory effect was observed for the molecules (**3c**, **3d**, **3e**, **3f**, **3g**) and (**3c**, **3d**, **3e**, **3f**, **3g**, **3h**) respectively against *Staphylococcus aureus* and *Bacillus subtilis*.

Conclusion

The synthesis of a series of 1,2,3-triazolylmethylbenzophenone has been realized with good yields using the CuAAC of a variety of alkynyl heterocycles, alkynyl carbohydrates or alkynes, and benzophenonemethylazide. The structures of the obtained compounds were confirmed by NMR spectroscopy (¹H and ¹³C) and mass spectrometry.

Overall, the molecules **3a**, and **3b** showed very promising antifungal and bacterial activity. They were more selective for the Gram-positive test pathogens than for the Gram-negative bacteria, which joined several studies who stipulate that Gram-positive bacteria were generally found to be more sensitive than Gram-negative [44,45].

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