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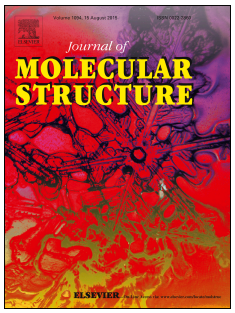

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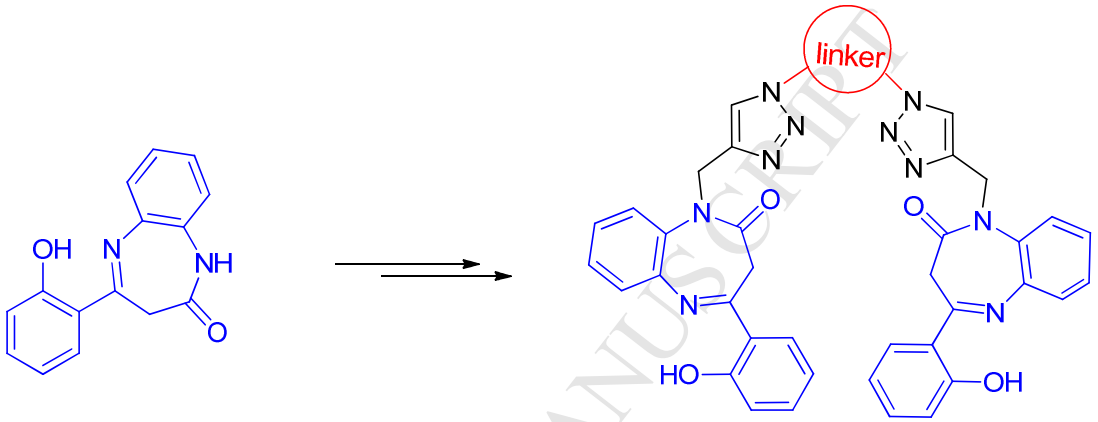
Graphical abstract



Design and Microwave-assisted Synthesis of Dimers of 1,5-benzodiazepine-1,2,3-triazole hybrids bearing alkyl/aryl spacers and Their Biological Assessment

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Abstract

Large series of novel *N*-bis-1,2,3-triazolo-linked-1,5-benzodiazepin-2-ones (BZD) have been synthesized under microwave irradiation through a Cu(I)-catalyzed double 1,3-dipolar alkyne-azide coupling reaction. This process is of considerable synthetic advantages in terms of time saving and remarkable yields. The chemical structures of the isolated compounds have been elucidated on the basis of extensive spectroscopic methods including 1D & 2D NMR, IR and HRMS. All the synthesized compounds have been evaluated for their antimicrobial and antioxidant activities. In *vitro* antimicrobial environment, almost all of compounds have shown an interesting activity. Among the dimers of 1,5-benzodiazepine-1,2,3-triazole compounds tested, the results revealed that the potent antibacterial was recorded to compounds **3g**,**l** for Gram-positive (within MIC = 31.25 and 125 µg/ml), and **3h**,**k** for Gram-negative (within MIC = 31.25 and 125 µg/ml). Besides, the results demonstrate that compounds **3h**,**k** have demonstrated the strongest potential against all the tested fungus (within MIC = 62.5 and 125 µg/ml). The antioxidant evaluation indicated that most compounds exhibited a moderate to good biological activity.

Keywords: 1,5-benzodiazepin-2-one, 1,2,3-triazole, microwave irradiation, click chemistry, antimicrobial activity, antioxidant activity.

1. Introduction

Among the large varieties of heterocycles, benzodiazepines have found their place in medicinal chemistry thanks to their clinical success as the most prescribed medicines [1]. Therefore, a considerable interest has been focused on the synthesis of new benzodiazepines. The use of this class of compounds with therapeutic purposes is not only limited to treatment of depression [2], epilepsy [3], but also psychomotor agitation [4], seizures and muscle spasms [5]. Furthermore, many examples of benzodiazepines are described as excellent samples of anticancer [6], antiviral [7] and antimicrobial agents [8]. Conformational constraint in their structures plays a key role in the determination of their bioactivities [9]. Interestingly, since *N*-functionalization of benzodiazepines (BZD) leads to the rigid analogues, the development of such scaffolds is highly desired [10]. In recent years, alkylating agents have been extensively studied and leading to the development of many biologically active compounds, particularly molecules that have been based on a triazole moiety [11]. Moreover, triazoles are an important class of *N*-heterocycles [12]. Their applications have been extended to widespread pathologies such as epilepsy [13], inflammation [14] and viral diseases [15]. In addition, they are considered to be excellent antibiotic agents [16]. A recent research in drug discovery has aimed at introducing the 1,2,3-triazole moiety as a connecting unit to link together two or more pharmacophores. The 1,5-benzodiazepine scaffold containing triazole moiety has gained popularity thanks to their pharmacological proprieties [17]. Further, it has been reported that many biologically active 1,2,3-bistriazole derivatives, which have interesting molecular symmetry, have shown a broad range of clinical applications [18]. In addition, amide-linked 1,4-disubstituted 1,2,3-bistriazoles present an interesting cytotoxicity [19]. Phenanthroline-2,9-bistriazoles reveal good anti-selective G-quadruplex intercalators [20] and the deoxystreptamine dimers (sugar-based bistriazole) serve also as human m-RNA ligands [21]. On the other hand, microbial infections are the most prevailing and spread infectious diseases worldwide [22]. Furthermore, their serious medical problem is resistance and the rapid rate of their development has led to increasing levels of resistance to classical antibiotics [23]. Therefore, the discovery and the development of effective antibacterial and antifungal drugs with novel mechanisms of action have become priority tasks for infectious-disease-research programs. On the other hand, it has been established that a free phenolic hydroxyl group connected to a benzodiazepine ring leads to new hydroxybenzodiazepine systems with a better reducing ability towards the most recommended AAPH and DPPH peroxyl radicals for measuring radical-scavenging activity in *vitro* [24, 25]. Since it looked encouraging, we aimed to

examine the possibility of our new dimers of 1,5-benzodiazepines-1,2,3-triazoles hybrids bearing two important phenolic moieties to act as radical scavengers. As part of our ongoing program directed towards the development of 1,5-benzodiazepine derivatives bearing a triazole moiety, we have focused our effort on the design of novel symmetric hybrid conjugates linking the benzodiazepine system to two 1,2,3-triazole units via a copper(I)-catalyzed 1,3-dipolar cyclisation. The synthesized compounds prompted us to explore the antimicrobial and the antioxidant effects of these new 1,2,3-triazolo-1,5-benzodiazepin-2-one derivatives. Their activities have been evaluated and discussed.

2. Experimental section

2.1. Material and instruments

Reagents were commercial products of analytical purity (Merck, Fluka, and Aldrich) and used without prior purification. ^{1}H, ^{13}C and two-dimensional NMR spectra were recorded on a Bruker AM-400 spectrometer at room temperature in $CDCl_3$ and DMSO at 300-400 MHz and at 75-100 MHz with all chemical shifts, reported in ppm, using residual non-deuterated solvent peaks as internal reference. Coupling constants are given in Hz and the abbreviations used for signal multiplicity are the following: s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet) and (br) broad signal. The ^{13}C NMR spectra are proton decoupled. IR spectra (In KBr pellets) were recorded on a Perkine Elmer Spectrum two FT-IR instrument with the Universal ATR Sampling Accessory, only structurally significant bands are reported. HRMS spectra were acquired with an electrospray ionisation (ESI) mass spectroscopy data were recorded on an UPLC Waters device (in positive mode); for the voltages of the mass spectroscopies, the following abbreviations are used: C Capillary (kV), SC Sampling Cone, EC Extraction Cone. Calibration was performed with sodium formate (range from 100 to 1000 $g.mol^{-1}$) and the lockspray (lockmass on the leucine encephaline 556.2771 $g.mol^{-1}$) was used without collision energy, the relative intensity of peaks is given in brackets. Microwave experiments were conducted with a Biotage AB Initiator EXP EU device with a maximum power of 800 W. Reactions were performed in open-air vessel with a magnetical stirrer. The temperature was measured by IR detection, after calibration using an optical fiber inside the reaction mixture. The power was fixed at 400 W and the temperature evolved in restreint-free conditions. Irradiation was carried out according to the conditions of times and temperatures indicated in the tables or in the experimental part. All the reactions were followed by TLC using aluminium sheets of Merck silica gel 60 F254, 0.2 mm. Melting

points of all the synthesized compounds were determined on BUCHI-510 capillary melting point apparatus.

2.2. Chemistry

2.2.1. Synthesis of *N*-(prop-1-yn)-4-(2-hydroxyphenyl)-1,5-benzodiazepin-2-one (2)

In a rounded bottom flask of 25 mL, an excess of propargyl bromide (0.45 mL, 1.5 equiv., 5.94 mmol) was added to a solution of the compound **1** (1 g, 1 equiv., 3.96 mmol) and NaH (0.123 g, 1.3 equiv., 5.14 mmol, 60 % dispersion in mineral oil) in *N*,*N*-dimethylformamide (15 mL). The resulting mixture was stirred for 12 h (monitored by TLC) and then organic materials were extracted with dichloromethane (3 × 30 mL). The combined organic layer was dried over $MgSO_4$, then filtered. The filtrate was concentrated in vacuum to give a residue, purified over silica gel by flash column chromatography (Cyclohexane/EtOAc 8:2) to afford the corresponding compound **2**.

Yellow solid; m.p.: 115-117 °C, 0.78 g, yield 78 %. IR (KBr, cm^{-1}): 3376 (O-H), 3320 (≡C-H), 1670 (C=O), 1560 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δH 2.25 (t, 1H, H_{22}, J= 3 Hz), 2.94 (d, 1H, H_{6a}, J= 12 Hz), 4.22 (t, 2H, H_{20}, J= 12 Hz), 4.68 (d, 1H, H_{6b}, J= 18 Hz), 6.85-6.95 (m, 2H, Ar-H), 7.23-7.36 (m, 4H, Ar-H), 7.66 (d, 1H, Ar-H, J= 6 Hz), 7.77 (d, 1H, Ar-H, J= 6 Hz), 13.79 (s, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δC 37.18 (C_{20}), 37.65 (C_6), 72.24 (C_{21}), 78.44 (C_{22}), 117.56 (C_{Ar}), 117.73 (C_{Ar}), 117.98 (C_{Ar}), 118.65 (C_{Ar}), 121.27 (C_{Ar}), 125.55 (C_{Ar}), 126.73 (C_{Ar}), 128.95 (C_{Ar}), 133.65 (C_{Ar}), 134.16 (C_{Ar}), 137.97 (C_{Ar}), 161.65 (C_5), 163.78 (C_{14}), 164.59 (C_7). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 291 (100, $M+H^+$), 329 (30, $M+K^+$) HRMS ES^+ for $C_{18}H_{15}N_2O_2$ m/z: $[M+H]^+$ Calc. 291.1134, found: 291.1124. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 289 (30, $[M-H]^-$), 181 (40), 1116 (100), HRMS ES^- for $C_{18}H_{13}N_2O_2$ m/z: $[M-H]^-$ Calc. 289.1079, found: 289.1076.

2.2.2. Synthesis of *N*-bis-1,2,3-triazole linked 1,5-benzodiazepines (3a-l)

In a rounded bottom flask of 25 mL, a mixture of compound **2** (500 mg, 1 equiv., 1.72 mmol) and DIPEA (0.89 mL, 3 equiv., 5.16 mmol) was stirred in dry DMF (10 mL), followed by addition of CuI (4.1 mg, 10 mol %, 0.172 mmol) and the appropriate diazide derivative $\mathbf{DA_{1\text{-}12}}$ (1 equiv., 1 mmol). The reaction mixture was stirred under microwave irradiation (400 W) for 6-12 min. After completion of the reaction as determined by TLC, the reaction was quenched with crushed ice (50 g) to avoid the apparition of side products. Organic materials were extracted with EtOAc (3 × 30 mL), then combined and dried over anhydrous $MgSO_4$. The crude reaction was filtered and the filtrate was concentrated under reduced pressure. The

crude material was purified by flash column chromatography on silica gel and concentrated under vacuum to afford a crude material, purified by column chromatography on silica gel (cyclohexane/EtOAc from 8:2 to 5:5) as eluent, affording the pure cycloadducts **3a-l** in 67-92% yields.

2.2.2.1. 1,1'-((1,1'-(ethane-1,2-diyl) bis(1*H*-1,2,3-triazole-4,1-iyl)) bis(methylene)) bis(1,5-benzodiazepin-2-one) (3a)

Yellow solid; m.p.: 217-219 °C, 460 mg, yield 92%. IR (KBr, cm^{-1}): 3390 (O-H), 1691 (C=O), 1599 (C=N).^{1}H NMR (300 MHz, $CDCl_3$): δH 2.97 (d, 2H, H_{20a}, J= 12 Hz), 4.17 (dd, 2H, H_{20b}, J= 20 Hz, J= 12 Hz), 4.77 (d, 2H, H_{6a}, J= 16 Hz,), 4.86 (s, 4H, $H_{26,26'}$), 5.13 (d, 2H, H_{6b}, J= 15 Hz), 6.87-6.94 (m, 2H, Ar-H), 7.02 (d, 2H, Ar-H, J= 6 Hz), 7.28-7.40 (m, 6H, Ar-H), 7.48 (s, 2H, $H_{25,25'}$), 7.83 (d, 2H, Ar-H, J= 6 Hz), 8.03 (s, 2H, H_{triaz}), 13.93 (broad singlet, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δC 38.17 ($C_{6,6'}$), 44.68 ($C_{26,26'}$), 49.58 ($C_{20,20'}$), 117.96 (C_{Ar}), 118.25 (C_{Ar}), 119.09 (C_{Ar}), 123.20 (C_{Ar}), 125.21 (C_{triaz}), 126.05 (C_{Ar}), 126.97 (C_{Ar}), 127.56 (C_{Ar}), 129.28 (C_{Ar}), 134.13 (C_{Ar}), 135.31 (C_{Ar}), 138.29 (C_{Ar}), 144.24 (C_{Ar}), 162.16 ($C_{5,5'}$), 164.16 ($C_{14,14'}$), 164.96 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 731 (20, $M+K^+$), 715 (40, $M+Na^+$), 693 (100, $M+H^+$), 347 (80, 0.5 $M+H^+$), HRMS ES^+ for $C_{38}H_{33}N_{10}O_4$ $[M+H]^+$ calc. 693.2686, found: 693.2696. ESI (-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 791 (50), 713 (10), 691 (100, $[M-H]^-$), 297 (50), 126 (90), HRMS ES^- for $C_{38}H_{31}N_{10}O_4$ $[M-H]^-$ calc. 691.2530, found: 691.2516.

2.2.2.2. 1,1'-((1,1'-(hexane-1,6-diyl)bis(1*H*-1,2,3-triazole-4,1-diyl))bis(methylene))bis(1,5-benzodiazepin-2-one) (3b)

Yellow solid; m.p.: 231-233 °C, 430 mg, yield 86%. IR (KBr, cm^{-1}): 3338 (O-H), 1691 (C=O), 1566 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): δH 1.24 (m, 4H, $H_{28,28'}$), 1.81 (m, 4H, $H_{27,27'}$), 2.92 (d, 2H, $H_{6a,6a'}$, J= 12 Hz,), 4.16-4.23 (m, 6H, $H_{6b,6b'-26,26'}$), 4.82 (d, 2H, $H_{20a,20a'}$, J= 15 Hz), 5.10 (d, 2H, $H_{20b,20b'}$, J= 15 Hz), 6.87-6.92 (m, 2H, Ar-H), 6.96 (d, 2H, Ar-H, J=9 Hz), 7.20-7.35 (m, 8H, Ar-H), 7.54 (s, 2H, H_{triaz}), 7.78 (d, 2H, Ar-H, J= 9 Hz), 8.00 (d, 2H, Ar-H, J= 6 Hz), 13.90 (s, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 25.77 ($C_{28,28'}$), 29.82 ($C_{27,27'}$), 38.24 ($C_{6,6'}$), 44.73 ($C_{20,20'}$), 50.09 ($C_{26,26'}$), 117.74 (C_{Ar}), 118.01 (C_{Ar}), 118.27 (C_{Ar}), 119.12 (C_{Ar}), 123.35 (C_{Ar}), 124.08 (C_{triaz}), 126.05 (C_{Ar}), 126.89 (C_{Ar}), 127.57 (C_{Ar}), 129.34 (C_{Ar}), 134.12 (C_{Ar}), 135.21 (C_{Ar}), 138.36 (C_{Ar}), 162.19 ($C_{5,5'}$), 164.13 ($C_{14,14'}$), 164.99 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 772 (20), 771 (100, $M+Na^+$), 749 (65, $M+H^+$), HRMS ES^+ for $C_{42}H_{41}N_{10}O_4$ $[M+H]^+$ calc. 749.3312, found: 749.3309. ESI (-)-

MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 810 (50), 783 (20), 747 (90, $[M-H]^-$), 213 (100), HRMS ES^- for $C_{42}H_{39}N_{10}O_4$ $[M-H]^-$ calc. 747.2316, found: 747.2318.

2.2.2.3. 1-((1-(6-azidohexyl)-1*H*-1,2,3-triazol-4-*yl*)methyl)-(1,5-benzodiazepin-2-one) (3b')

It was obtained as described in the last procedure and was purified as minor product after purification as yellow solid; m.p.: 117-119 °C, 135 mg, yield 27%. IR (KBr, cm^{-1}): 3386 (O-H), 1690 (C=O), 1598 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δH 1.24-1.42 (m, 4H, $H_{28,29}$), 1.52-1.60 (m, 2H, H_{30}), 1.84-1.93 (m, 2H, H_{27}), 3.00 (d, 1H, H_{6b}, J= 12 Hz), 3.25 (t, 2H, H_{31}, J= 9 Hz), 4.25 (d, 1H, $H_{6b,}$ J= 12 Hz), 4.28-4.34 (td, 2H, $H_{26,}$ J= 6Hz), 4.90 (d, 1H, H_{20b}, J= 15 Hz), 5.17 (d, 2H, H_{20a}, J= 15 Hz), 6.98 (t, 2H, Ar-H, J= 9 Hz), 7.05 (d, 2H, Ar-H, J=9 Hz), 7.25 (s, 1H, Ar-H), 7.28-7.46 (m, 5H, Ar-H), 7.59 (s, 1H, Ar-H), 7.80 (d, 1H, Ar-H, J= 6 Hz), 8.07 (d, 1H, Ar-H, J= 6 Hz).^{13}C NMR (75 MHz, $CDCl_3$): δC 26.09 (C_{28}), 28.60 (C_{29}), 29.95 (C_{30}), 30.90 (C_{27}), 38.24 (C_6), 44.64 (C_{20}), 50.28 (C_{26}), 51.23 (C_{31}), 117.94 (C_{Ar}), 118.31 (C_{Ar}), 119.18 (C_{Ar}), 123.38 (C_{Ar}), 124.08 (C_{Ar}), 126.11 (C_{Ar}), 126.87 (C_{Ar}), 127.67 (C_{Ar}), 129.37 (C_{Ar}), 134.24 (C_{Ar}), 135.18 (C_{Ar}), 138.22 (C_{Ar}), 143.70 (C_{Ar}), 162.21 (C_5), 164.19 (C_{14}), 164.98 (C_7). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 497 (30, $M+K^+$), 427 (60), 459 (80, $M+H^+$), HRMS ES^+ for $C_{24}H_{27}N_8O_2$ m/z: $[M+H]^+$ Calc. 459.2348, found: 459.2342. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 457 (60, $[M-H]^-$), 416 (20), 252 (40), HRMS ES^- for $C_{24}H_{25}N_8O_2$ m/z: $[M-H]^-$ Calc. 457.2179, found: 457.2173.

2.2.2.4. 1,1'-((1,1'-(1,4-phenylenebis(methylene)) bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene)) bis(1,5-benzodiazepin-2-one) (3c)

Yellow solid; m.p.: 170-172 °C, 410 mg, yield 82%. IR (KBr, cm^{-1}): 3386 (O-H), 1676 (C=O), 1576 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δH 2.99 (d, 2H, H_{6a}, J= 12 Hz), 4.24 (d, 2H, H_{6b}, J= 12 Hz), 4.89 (d, 2H, H_{20a}, J= 15 Hz), 5.17 (d, 2H ,H_{20b}, J= 15 Hz), 5.45 (d, 2H, $H_{26,26'}$, J= 15 Hz), 5.54 (d, 2H, $H_{26,26'}$, J= 15 Hz), 6.95-7.07 (m, 4H, Ar-H), 7.25 (s, 2H, Ar-H), 7.28 (s, 2H, Ar-H), 7.32-7.47 (m, 8H, Ar-H), 7.59 (s, 2H, H_{triaz}), 7.84 (d, 2H, Ar-H, J= 9 Hz), 8.07 (d, 2H, Ar-H, J= 9 Hz), 13.96 (s, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 37.15 ($C_{6,6'}$), 43.68 ($C_{20,20'}$), 52.67 ($C_{26,26'}$), 116.93 (C_{Ar}), 117.24 (C_{Ar}), 118.09 (C_{Ar}), 122.29 (C_{Ar}), 123.26 (C_{Ar}), 125.05 (C_{Ar}), 125.89 (C_{Ar}), 126.55 (C_{Ar}), 127.79 (C_{Ar}), 128.28 (C_{triaz}), 130.48 (C_{Ar}), 133.11 (C_{Ar}), 134.11 (C_{Ar}), 137.28 (C_{Ar}), 143.28 (C_{Ar}), 161.12 ($C_{5,5'}$), 163.05 ($C_{14,14'}$),

163.94 ($C_{7,7'}$). ESI-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 807 (10, $M+K^+$), 791 (20, $M+Na^+$), 769 (40, $M+H^+$), 385 (100, 0.5 $M+H^+$), HRMS ES^+ for $C_{44}H_{37}N_{10}O_4$ m/z: $[M+H]^+$ calc. 769.3068, found: 769.2028. ESI-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 767 (40), $[M-H]^-$, HRMS ES^- for $C_{44}H_{35}N_{10}O_4$ m/z: $[M-H]^-$ calc. 767.2843, found: 767.2838.

2.2.2.5. 1,1'-((1,1'-(methylenebis(4,1-phenylene))bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene))bis(1,5-benzodiazepin-2-one) (3d)

Yellow solid; m.p.: 175-177 °C, 390 mg, yield 78%. IR (KBr, cm^{-1}): 3355 (O-H), 1676 (C=O), 1584 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δ_H 2.90 (d, 2H, H_{6a}, J= 12 Hz), 4.14 (d, 2H, H_{6b}, J=12 Hz), 4.81 (d, 2H, H_{20a}, J= 15 Hz), 5.07 (d, 2H, H_{20b}, J= 15 Hz), 5.39 (s, 2H, $H_{30,30'}$), 6.88 (t, 2H, Ar-H, J= 9 Hz), 6.96 (d, 2H, Ar-H, J= 9 Hz), 7.15-7.38 (m, 16H, Ar-H), 7.49 (s, 2H, H_{triaz}), 7.75 (d, 2H, Ar-H, J= 9 Hz), 7.97 (d, 2H, Ar-H, J= 9 Hz), 13.85 (s, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δC 38.20 ($C_{6,6'}$), 43.31 ($C_{20,20'}$), 44.67 (C_{30}), 117.99 (C_{Ar}), 118.26 (C_{Ar}), 119.09 (C_{Ar}), 121.44 (C_{Ar}), 123.32 (C_{Ar}), 124.24 (C_{Ar}), 126.05 (C_{Ar}), 126.91 (C_{Ar}), 127.54 (C_{triaz}), 128.67 ($C_{Ar,}$), 128.69 (C_{Ar}), 128.77 (C_{Ar}), 129.33 (C_{Ar}), 134.11 (C_{Ar}), 135.14 (C_{Ar}), 138.37 (C_{Ar}), 144.27 (C_{Ar}), 162.21 ($C_{5,5'}$), 164.19 ($C_{14,14'}$), 164.98 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 853 (40, M+Na+), 831 (70, $M+H^+$), 416 (50, 0.5 $M+H^+$), HRMS ES^+ for $C_{49}H_{39}N_{10}O_4$ m/z: $[M+H]^+$ Calc. 831.3217, found: 831.3213. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 843 (60), 829 (50, $[M-H]^-$), 826 (30), 305 (10), HRMS ES^- for $C_{49}H_{37}N_{10}O_4$ m/z: $[M-H]^-$ Calc. 829.3023, found: 829.3017.

2.1.2.6. 1,1'-((1,1'-(1,4-phenylene) bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene)) bis(1,5-benzodiazepin-2-one) (3e)

Yellow solid; m.p.: 192-194 °C, 420 mg, yield 84%. IR (KBr, cm^{-1}): 3401 (O-H), 1706 (C=O), 1591 (C=N). ^1H NMR (300 MHz, $CDCl_3$): δH 3.19 (br, 2H, H_{6a}), 4.33 (br, 2H, H_{6b}), 5.13 (s, 4H, $H_{20,20'}$) 6.70 (d, 2H, Ar-H, J= 9 Hz), 7.00 (d , 2H, Ar-H, J= 9 Hz), 7.33-7.48 (m, 10H, Ar-H, $H_{27,27'}$), 7.51 (s, 2H, H_{triaz}), 7.88-7.96 (m, 4H, Ar-H) , 8.18 (s, 2H, Ar-H), 13.78 (s, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δC 38.24 ($C_{6,6'}$), 43.39 ($C_{20,20'}$), 114.00 ($C_{Ar,}$), 117.62 (C_{Ar}), 118.07 (C_{Ar}), 118.93 (C_{Ar}), 121.45 ($C_{27,27'}$), 122.99 ($C_{7,7'}$), 125.64 (C_{triaz}), 126.65 (C_{Ar}), 127.18 (C_{Ar}), 129.85 (C_{Ar}) , 133.94 (C_{Ar}), 134.70 (C_{Ar}), 138.29 (C_{Ar}), 143.66 (C_{Ar}), 149.19 (C_{Ar}), 161.35 ($C_{5,5'}$), 164.58 ($C_{14,14'}$), 164.74 ($C_{7,7'}$) . ESI-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 763 (40, $M+Na^+$), 741 (100, $M+H^+$), 385 (20), HRMS ES^+ for $C_{42}H_{33}N_{10}O_4$ m/z: $[M+H]^+$ calc. 741.2999, found: 741.3018. ESI-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z*

(rel. int.): 739 (100, $[M-H]^-$), 334 (50), 217 (20), HRMS ES^- for $C_{42}H_{31}N_{10}O_4$ m/z: $[M-H]^-$ calc. 739.2466, found: 739.2467.

2.2.2.7. 1,1'-((1,1'-(1,3-phenylene) bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene)) bis(1,5-benzodiazepin-2-one) (3f)

Yellow solid; m.p.: 210-212 °C, 400 mg, yield 80%. IR (KBr, cm^{-1}): 3391 (O-H), 1673 (C=O), 1566 (C=N). ^1H NMR (400 MHz, $CDCl_3$): δH 2.94 (d, 2H, H_{6a}, J= 12 Hz), 4.22 (d, 2H, H_{6b}, J= 12 Hz), 4.93 (d, 2H, H_{20a}, J= 16 Hz), 5.22 (d, 2H, H_{20b}, J= 16 Hz), 6.92 (t, 2H, H-Ar, J= 8 Hz), 6.99 (d, 2H, H-Ar, J= 8 Hz), 7.10-7.57 (m, 8H, H-Ar), 7.68 (d, 2H, J= 8 Hz), 7.91 (d, 2H, J= 8 Hz), 8.01 (s, 2H, H_{triaz}), 8.05 (d, 2H, H_{Ar}, J= 8 Hz), 13.93 (s, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δC 37.41 ($C_{6,6'}$), 43.70 ($C_{20,20'}$), 117.24 (C_{Ar}), 117.53 (C_{Ar}), 118.31 (C_{Ar}), 122.14 (C_{Ar}), 125.20 (C_{Ar}), 126.26 (C_{Ar}), 126.63 (C_{Ar}), 126.70 (C_{Ar}), 126.91 (C_{Ar}), 127.26 (C_{triaz}), 128.54 (C_{Ar}), 129.25 (C_{Ar}), 133.41 (C_{Ar}), 133.46 (C_{Ar}), 134.35 (C_{Ar}), 134.65 (C_{Ar}), 137.51 (C_{Ar}), 161.45 ($C_{5,5'}$), 163.31 ($C_{14,14'}$), 164.20 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 779 (70, $M+K^+$), 763 (40, $M+Na^+$), 741 (40, $M+H^+$), 371 (30, 0.5 $M+H^+$), HRMS ES^+ for $C_{42}H_{33}N_{10}O_4$ m/z: $[M+H]^+$ Calc. 741.2778, found: 741.2771. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 845 (60), 836 (20), 823 (80, $[M-H]^-$), 325 (40), 311 (20), HRMS ES^- for $C_{42}H_{31}N_{10}O_4$ m/z: $[M-H]^-$ Calc. 739.2567, found: 739.2559.

2.2.2.8. 1,1'-((1,1'-(oxybis(4,1-phenylene)) bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene)) bis(1,5-benzodiazepin-2-one) (3g)

Yellow solid; m.p.: 167-169 °C, 435 mg, yield 87% IR (KBr, cm^{-1}): 3379 (O-H), 1671 (C=O), 1588 (C=N). ^1H NMR (400 MHz, $CDCl_3$): δH 2.95 (d, 2H, H_{6a}, J= 16 Hz), 4.19 (d, 2H, H_{6b}, J= 12 Hz), 4.91 (d, 2H, H_{20a}, J= 16 Hz), 5.18 (d, 2H, H_{20b}, J= 16 Hz), 6.92 (t, 2H, Ar-H, J= 8 Hz), 6.98 (d, 2H, Ar-H, J= 8 Hz), 7.27 (t, 2 H, Ar-H, J= 4 Hz), 7.33-7.37 (m, 8 H, Ar-H), 7.79 (d, 2H, Ar-H, J= 8 Hz), 7.86 (d, 2H, Ar-H, J= 8 Hz), 7.98 (d, Ar-H, J= 8 Hz), 8.04 (s, 2H, H_{triaz}), 8.07 (d, 2H, Ar-H, J= 4 Hz), 13.88 (s, 2H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δC 37.42 ($C_{6,6'}$), 43.85 ($C_{20,20'}$), 117.16 (C_{Ar}), 117.58 (C_{Ar}), 118.46 (C_{Ar}), 120.04 (C_{Ar}), 121.54 (C_{triaz}), 122.40 (C_{Ar}), 125.56 (C_{Ar}), 126.27 (C_{Ar}), 126.89 (C_{Ar}), 128.56 (C_{Ar}), 128.95 (C_{Ar}), 133.53 (C_{Ar}), 134.19 (C_{Ar}), 137.69 (C_{Ar}), 139.60 (C_{Ar}), 140.24 (C_{Ar}), 144.43 (C_{Ar}), 161.43 ($C_{5,5'}$), 163.38 ($C_{14,14'}$), 164.43 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 855 (10, $M+Na^+$), 833 (50, $M+H^+$), 417 (100, 0.5 $M+H^+$), HRMS ES^+ for $C_{48}H_{37}N_{10}O_5$ m/z: $[M+H]^+$ calc. 833.2948, found: 833.2963. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z*

(rel. int.): 831 (100, $[M-H]^-$), 325 (40), 311 (30), 116 (80), HRMS ES^- for $C_{48}H_{35}N_{10}O_5$ m/z: $[M-H]^-$ calc. 831.2792, found: 831.2790.

2.2.2.9. 1,1'-((1,1'-(sulfonylbis(4,1-phenylene))bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene))bis(1,5-benzodiazepin-2-one) (3h)

Yellow solid; m.p.: 185-187 °C, 415 mg, yield 83%. IR (KBr, cm^{-1}): 3402 (O-H), 1668 (C=O), 1578 (C=N). ^1H NMR (400 MHz, $CDCl_3$): δH 2.92 (d, 2H, H_{6a}, J= 12 Hz), 4.18 (d, 2H, H_{6b}, J= 12 Hz), 4.86 (d, 2H, H_{20a}, J= 16 Hz), 5.18 (d, 2H, H_{20b}, J= 16 Hz), 6.88 (t, 2H, Ar-H, J= 8 Hz), 6.95 (d, 2H, Ar-H, J= 8 Hz), 7.01-7.17 (m, 4H, Ar-H), 7.23 (t, 2H, Ar-H, J= 8 Hz), 7.29-7.36 (m, 6H, Ar-H), 7.61 (t, 4H, Ar-H, J= 8 Hz), 7.78 (d, 2H, Ar-H, J= 8 Hz), 7.99 (s, 2H, H_{triaz}), 8.04 (t, 2H, Ar-H, J= 8 Hz), 13.85 (s, 2H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δC 37.22 ($C_{6,6'}$), 43.77 ($C_{20,20'}$), 116.98 (C_{Ar}), 117.30 (C_{Ar}), 118.15 (C_{Ar}), 118.93 (C_{Ar}), 121.37 (C_{Ar}), 122.27 (C_{Ar}), 123.96 (C_{Ar}), 125.17 (C_{Ar}), 125.98 (C_{Ar}), 126.62 (C_{Ar}), 128.32 (C_{Ar}), 131.86 (C_{triaz}), 133.17 (C_{Ar}), 134.17 (C_{Ar}), 137.37 (C_{Ar}), 143.56 (C_{Ar}), 155.90 (C_{Ar}), 161.21 ($C_{5,5'}$), 163.10 ($C_{14,14'}$), 164.12 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 903 (10, $M+Na^+$), 881 (100, $M+H^+$), 441 (50, 0.5 $M+H^+$), HRMS ES^+ for $C_{48}H_{37}N_{10}O_6S$ m/z: $[M+H]^+$ calc. 881.2618, found: 881.2602. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 879 (100, $[M-H]^-$), 100 (100), 116 (80), 126 (20), HRMS ES^- for $C_{48}H_{35}N_{10}O_6S$ m/z: $[M-H]^-$ calc. 879.2462, found: 879.2421.

2.2.2.10. 1,1'-((1,1'-(ethane-1,2-diylbis(4,1-phenylene))bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene))bis(1,5-benzodiazepin-2-one) (3i)

Yellow solid; m.p.: 178-180 °C, 335 mg, yield 67%. IR (KBr, cm^{-1}): 3391 (O-H), 1675 (C=O), 1568 (C=N). ^1H NMR (400 MHz, $CDCl_3$): δH 2.88 (d, 2H, H_{6a}, J= 8 Hz), 4.12 (d, 2H, H_{6b}, J= 8 Hz), 4.78 (d, 2H, H_{20a}, J= 12 Hz), 5.06 (d, 2H, H_{20b}, J= 16 Hz), 5.33-5.45 (2d, 4H, $H_{29,29'}$, J= 16 Hz), 6.86 (t, 2H, J= 8 Hz), 6.94 (d, 2H, Ar-H, J= 8 Hz), 7.08-7.23 (m, 8H, Ar-H), 7.26-7.31 (m, 8H, Ar-H), 7.50 (s, 2H, H_{triaz}), 7.73 (d, 2H, Ar-H, J= 8 Hz), 7.96 (d, 2H, Ar-H, J= 4 Hz), 13.83 (s, 2H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δC 37.23 ($C_{29,29'}$), 43.79 ($C_{6,6'}$), 48.73 ($C_{20,20'}$), 116.99 (C_{Ar}), 117.32 (C_{Ar}), 118.16 (C_{Ar}), 118.95 (C_{Ar}), 121.40 (C_{Ar}), 121.64 (C_{Ar}), 122.28 (C_{Ar}), 125.18 (C_{Ar}), 125.98 (C_{Ar}), 126.64 (C_{Ar}), 128.32 (C_{Ar}), 131.88 (C_{triaz}), 133.19 (C_{Ar}), 134.19 (C_{Ar}), 137.37 (C_{Ar}), 143.57 (C_{Ar}), 145.92 (C_{Ar}), 161.21 ($C_{5,5'}$), 163.09 ($C_{14,14'}$), 164.12 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 867 (50, $M+Na^+$), 845 (100, $M+H^+$), HRMS ES^+ for $C_{50}H_{41}N_{10}O_4$ m/z: $[M+H]^+$ calc. 845.2962, found:

845.2950. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 867 (30), 843 (100, $[M-H]^-$), 827 (10), HRMS ES^- for $C_{50}H_{39}N_{10}O_4$ m/z: $[M-H]^-$ calc. 843.2252, found: 843.2516.

2.2.2.11. 1,1'-((1,1'-(1,3-phenylenebis(methylene))bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene))bis(1,5 -benzodiazepin-2-one) (3j)

Yellow solid; m.p.: 143-145 °C, 445 mg, yield 89%. IR (KBr, cm^{-1}): 3402 (O-H), 1660 (C=O), 1578 (C=N). ^1H NMR (400 MHz, $CDCl_3$): δH 3.00 (d, 2H, H_{6a}, J= 12 Hz), 4.24 (d, 2H, H_{6b}, J= 12 Hz), 4.87 (d, 2H, H_{20a}, J= 12 Hz), 5.17 (d, 2H, H_{20b}, J= 16 Hz), 5.45 (d, 2H, H_{26a}, J= 16 Hz), 5.54 (d, 2H, H_{26b}, J= 16 Hz), 6.96 (t, 2H, Ar-H, J= 8 Hz), 7.06 (d, 2H, Ar-H, J= 4 Hz), 7.20-7.23 (m, 2H, Ar-H), 7.31-7.46 (m, 10H, Ar-H), 7.61 (s, 2H, H_{triaz}), 7.84 (d, 2H, Ar-H, J= 4 Hz), 8.09 (d, 2H, Ar-H, J= 8Hz), 13.99 (s, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δC 37. 42 ($C_{6,6'}$), 43.96 ($C_{20,20'}$), 53.04 ($C_{26,26'}$), 117.22 (C_{Ar}), 117.50 (C_{Ar}), 118.33 (C_{Ar}), 122.54 (C_{Ar}), 125.28 (C_{Ar}), 126.15 (C_{Ar}), 126.26 (C_{triaz}), 126.79 (C_{Ar}), 126.91 (C_{Ar}), 127.63 (C_{Ar}), 128.54 (C_{Ar}), 129.25 (C_{Ar}), 133.35 (C_{Ar}), 133.40 (C_{Ar}), 134.41 (C_{Ar}), 134.75 (C_{Ar}), 137.57 (C_{Ar}), 161.40 ($C_{5,5'}$), 163.35 ($C_{14,14'}$), 164.21 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 791 (30, $M+Na^+$), 769 (50, $M+H^+$), 385 (100, 0.5 $M+H^+$), HRMS ES^+ for $C_{44}H_{37}N_{10}O_4$ m/z: $[M+H]^+$ calc. 769.2999, found: 769.2990. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 769 (30, $[M-H]^-$), 250 (80), 297 (50), 325 (20), 116 (100), HRMS ES^- for $C_{44}H_{35}N_{10}O_4$ m/z: $[M-H]^-$ calc. 767.2843, found: 767.2835.

2.2.2.12. 1,1'-((1,1'-(pyridine-2,6-diyl)bis(1H-1,2,3-triazole-4,1-diyl))bis(methylene)) bis(1,5-benzodiazepin-2-one) (3k)

Yellow solid; m.p.: 185-187 °C, 355 g, yield 71%. IR (KBr, cm^{-1}): 3391 (O-H), 1675 (C=O), 1568 (C=N). ^1H NMR (400 MHz, $CDCl_3$): δH 2.93 (d, 2H, H_{6a}, J= 12 Hz), 4.21 (d, 2H, H_{6b}, J= 12 Hz), 4.92 (d, 2H, H_{20a}, J= 16 Hz), 5.20 (d, 2H, H_{20b}, J= 16 Hz), 6.94 (t, 2H, H-Ar, J= 8 Hz), 6.99 (d, 2H, H-Ar, J= 8 Hz), 7.10-7.52 (m, 9H, H-Ar,), 7.68 (d, 2H, H-Ar, J= 8 Hz), 7.91 (d, 2H, J= 9 Hz), 8.01 (s, 2H, H_{triaz}), 8.09 (d, 2H, J= 8 Hz), 13.38 (s, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δC 37.63 ($C_{6,6'}$), 43.56 ($C_{20,20'}$), 117.31 (C_{Ar}), 118.31 (C_{Ar}), 122.91 (C_{Ar}), 125.20 (C_{Ar}), 126.26 (C_{Ar}), 126.63 (C_{Ar}), 126.70 (C_{Ar}), 126.83 (C_{triaz}), 127.14 (C_{Ar}), 128.54 (C_{Ar}), 129.25 (C_{Ar}), 133.42 (C_{Ar}), 133.56 (C_{Ar}), 134.61 (C_{Ar}), 134.64 (C_{Ar}), 137.56 (C_{Ar}), 161.71 ($C_{5,5'}$), 163.40 ($C_{14,14'}$), 164.45 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 742 (60, $M+H^+$), 423 (60, 0.5 $M+H^+$), 112 (20), HRMS ES^+ for $C_{41}H_{32}N_{11}O_4$ m/z: $[M+H]^+$ Calc. 742.2648, found: 742.2656. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel.

int.): 844 (60), 845 (20), 843 (100, [M-H]$^-$), 311 (40), HRMS ES$^-$ for $C_{41}H_{30}N_{11}O_4$ m/z: [M-H]$^-$ Calc. 740.2674, found: 740.2679.

2.2.2.13. 1,1'-((1,1'-(3,3'-dimethyl-[1,1'-biphenyl]-4,4'-diyl)bis(1H-1,2,3-triazole-4,1-diyl)) bis(methylene))bis(1,5-benzodiazepin-2-one) (3l)

Yellow solid; m.p.: 167-169 °C, 405 mg, yield 81%. IR (KBr, cm^{-1}): 3391 (O-H), 1660 (C=O), 1582 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δH 2.29 (s, 6H, CH_3, $H_{32,32'}$), 3.07 (d, 2H, H_{6b}, J= 12 Hz), 4.30 (d, 2H, H_{6a}, J= 12 Hz), 5.06 (d, 2H, H_{20b}, J= 16 Hz), 5.31 (d, 2H, H_{20a}, J= 16 Hz), 7.01 (t, 2H, Ar-H, J=8 Hz), 7.07 (d, 2H, Ar-H, J= 12 Hz), 7.37 (t, 2H, J= 8 Hz), 7.45-7.48 (m, 10H, Ar-H), 7.58 (d, 2H, J= 8 Hz), 7.61 (s, 2H, H_{triaz}), 7.90 (d, 2H, Ar-H, J= 8 Hz), 8.19 (d, 2H, Ar-H, J= 8 Hz), 14.01 (s, 2H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δC 17.39 ($C_{32,32'}$), 37.51 ($C_{6,6'}$), 43.96 ($C_{20,20'}$), 117.25 (C_{Ar}), 117.54 (C_{Ar}), 118.38 (C_{Ar}), 122.68 (C_{Ar}), 124.88 (C_{Ar}), 125.41 (C_{Ar}), 125.60 (C_{Ar}), 125.67 (C_{Ar}), 126.22 (C_{Ar}), 126.26 (C_{triaz}), 126.87 (C_{Ar}), 128.58 (C_{Ar}), 129.56 (C_{Ar}), 133.23 (C_{Ar}), 133.39 (C_{Ar}), 134.39 (C_{Ar}), 135.30 (C_{Ar}), 137.74 (C_{Ar}), 140.59 (C_{Ar}), 161.45 ($C_{5,5'}$), 163.43 ($C_{14,14'}$), 164.34 ($C_{7,7'}$). ESI(+)-MS CH_3CN [C= 1, SC= 30, EC= 3] *m/z* (rel. int.): 867 (10, M+Na$^+$), 845 (100, M+H$^+$), 423 (70, 0.5 M+H$^+$), HRMS ES$^+$ for $C_{50}H_{41}N_{10}O_4$ m/z: [M+H]$^+$ calc. 845.3312, found: 845.3312. ESI(-)-MS CH_3CN [C= 1, SC= 30, EC= 2] *m/z* (rel. int.): 844 (60), 845 (20), 831 (30), 843 (100), [M-H]$^-$, 325 (70), 311 (40), 100 (100), HRMS ES$^-$ for $C_{50}H_{39}N_{10}O_4$ m/z: [M-H]$^-$ calc. 843.3156, found: 843.3102.

2.3. Biological evaluation of the synthesized compounds

2.3.1. Antimicrobial activity

2.3.1.1. Microorganisms

The antibacterial activity of the former compounds has been tested against nine microorganisms, including reference strains consisting of Gram-positive: *B. subtilus* (ATCC 6633), *S. epidermis* (CI), *S. aureus* (ATCC 25923) and *S. aureus* (ATCC 29213) and Gram-negative rods: *E. coli* (ATCC 25922), *K. pneumoniae* (CI), *E. fecalis* (ATCC 29212), *S. enterica* (CIP 5262) and *E. faecium* (CI). On the other side, *C. albicans* (ATCC 90028), *C. glabrata* (ATCC 90030) and *C. keusei* (ATCC 6258) have been used for the determination of antifungal activity. Antibacterial and antifungal activities were compared with the activities of the standard drugs Amoxicillin (AMX), Ampiclline (AMP) and Amphotericin (AB). The microorganism strains used in the biological assays are listed in Figure 3-5. Different

American-Type Cell Culture (ATCC) reference bacteria and fungi as well as clinical isolates strains have been used.

2.3.2. Antioxidant activity

All synthesized hydroxybenzodiazepines **3a-l** were evaluated for their in *vitro* antioxidant activity including three tests DPPH, ABTS and FRAP at several concentrations (0.62-1.25-2.50-5.0 mg/mL). The antioxidant capacity of the test has been expressed as IC_{50} values, which indicates the concentration of sample (mg/mL), required to scavenge 50% of DPPH, ABTS and EC_{50} values, that present the effective concentration providing 0.5 of absorbance in the reducing power assay (FRAP). Trolox has been chosen as the standard. All the tests have been carried out in triplicate.

3. Results and discussion

3.1. Chemistry

We have initiated our work by the design and the preparation of the dipolarophilic system starting from 4-(2-hydroxyphenyl)-3*H*-1,5-benzodiazepin-2-one **2** which has been prepared according to the method described by Eiden. *et al* [26] using the 4-hydroxycoumarin and orthophenylenediamine as starting materials (scheme 1). The propargylation of **1** in dry DMF using NaH as a catalyst at room temperature has provided the dipolarophile **2** in excellent yield (72%). The ^{1}H NMR spectrum, recorded at 300 MHz in $CDCl_3$ spectrometer revealed new signals in the aliphatic region: a doublet at δ_H= 2.96 ppm and a singlet at δ_H= 2.25 ppm corresponding to the alkyne protons of the propargyl moiety. In addition, the ^{1}H NMR spectrum has shown two doublets at δ_H= 4.24 ppm and 4.71 ppm, assigned to the methylene protons H_{3a} and H_{3b} corresponding to the diazepine ring with a coupling constant of 18 Hz for each other. We notice the disappearance of the NH proton signal and the presence of the phenolic hydrogen singlet deshielded at 13.90 ppm [27].

The preparation of the diazides (12 diazides) required extensive experiments, following different methods. *i)* Starting from the halogenated compounds, SN_2 reaction with NaN_3 (sodium azide) proceeded for the appropriate time and solvent (in DMSO at room temperature starting from the dichlorinated derivatives [28], in DMF under reflux from the dibrominated compounds [29]), leading to desired diazides in good yields (91-98%). *ii)* For the second procedure, the diazide compounds have been prepared from the appropriate diamine, through a two-step process: diazotization with the sodium nitrite ($NaNO_2$) in acidic conditions

followed by the addition of the sodium azide [30]. The desired diazides (**DA_{1-12}**) have been obtained in good yields (74-97%). We have continued our work by the double 1,3-dipolar cyclization (CuAAC) starting from benzodiazepine **2** as a dipolarophilic system. The 1,6-diazidohexane (**DA_2**= N_3-$(CH_2)_6$-N_3) was chosen as a model (Scheme 2). We have explored the suitable conditions carrying out Cu (I)-catalysed Huisgen 1,3-dipolar cyclization with alkyne **2**. The use of 2 equiv. of alkyne was necessary to give access to the *N*-bis-1,2,3-triazole linked to the 1,5-benzodiazepine systems. Table 1 summarizes the most relevant result of this study.

The course of the reaction has been followed by TLC. We observed two products, the cycloadduct **3b**, the major compound and the minor cycloadduct **3b'**. The efficiency of the reaction is due to a weak steric hindrance and the good reactivity of *N*-propargylated benzodiazepine. The $^{1}H/^{13}C$ NMR, 2D experiments data have confirmed the structure of **3b** and **3b'**. Thus, the ^{1}H NMR spectrum of **3b'** shows five set of signals for the methylene protons of the hexylchain and two doublets at 3.00 and 4.25 ppm corresponding to the H_6 protons. The benzodiazepine core adopts a unique preferential conformation [31] where its equatorial proton is deshielded by the C_5 aryl ring π-electron current and the axial proton is shielded by the benzene ring of the benzodiazepine. While the bis-1,2,3-triazolo-benzodiazepine **3b** displays three signals for the methylene protons of the hexyl chain, one singlet for the triazolic proton and two doublets at 4.82 and 5.10 ppm ($J_{gem}\approx$ 15 Hz) represent the methylene attached to the amido nitrogen.

The macrocycle **3b** is highly symmetric as shown by its ^{1}H NMR spectrum which revealed a second set of two doublets at δ_H=2.91 and 4.18 ppm ($J_{gem}\approx$ 12 Hz) indicating the resonance of the methylene protons of the benzodiazepine cores. The AB system observed at ambient operating temperature, demonstrates the non-equivalence of the hydrogens of (**H_a-H_b**) due to the fact that the *3H*-1,5-benzodiazepine ring adopts a unique, non-planar and locked conformation. Based on these results, we have assumed the importance of the introduction of triazolyl groups at N1 resulting in perihydrogen interaction between the triazolyl groups and the other hydrogens of the benzene ring, which could lead to a constrained interesting conformation of N1-substituted benzodiazepine derivatives. This is further supported by the diastereotopic nature of the methylene protons of both methylene triazolyl group and the benzodiazepine core. (Figure 1, 2)

Attempts using 2 equiv. of triethylamine at room temperature, led to the mono and bis cycloadducts (**3b**, **3b')** after 24 hours with moderate yields (entry 1). This may be explained

by the poor solubility of copper iodide in such solvent [32]. In contrast, microwave assisted cycloaddition in DMF, has given cycloadducts with good conversions (72% for **3b,** and 20% for **3b'**) in just few minutes (15 min, entry 4). Optimal conditions have been obtained for this reaction in dry DMF with 2 equiv., of DIEPA under microwave radiation (400 W, 6 min) in the presence of a catalytic amount of CuI (entry 5). It is interesting to mention that the reaction yield has been improved with DIPEA from 72% (entry 4) to 87% (entry 5). These conditions with a bulkier basis have been found to be the adequate method giving only the bistriazole in excellent yield with few traces of mono-1,2,3-triazole **3b'**. Using these optimal conditions, the scope of this approach was subsequently explored under microwave irradiation completed within 6-12 min with various diazides (12 diazides). (Scheme 3)

The consumption of both the azido linker and the alkyne was monitored by a thin layer of chromatography and allowed the isolation of pure bis-triazoles **3a-l**. Structural assignments for the compounds **3a-l** were established by being based on their spectroscopic data mainly 1D, 2D NMR and ESI low and high resolution MS. The structures of **3a-l** were confirmed by ^{13}C and DEPT NMR spectra, which present the signals of the benzodiazepine and the bistriazole systems resonating in the range of 114.0-164.7 ppm, especially with the remarkable signals in the region of 122.2–124.1 ppm, which can easily be assigned to the C_{25} of the triazole moiety. It is important to show that, despite the complex topology of the targets, the ^{1}H and ^{13}C NMR spectra of **3a-l** present highly symmetric patterns. For example, the AB system has been observed in the ^{1}H NMR spectra of all compounds. The reaction took place regioselectively at the alkynes moiety to produce the corresponding 1,4-disubstituted-1,2,3-triazole. The reaction was found to be totally regioselective and the bis-1,4-triazole linked to the benzodiazepine adducts **3a-l** were isolated in good yields ranging from 67 to 92% in short reaction times. (Table 2)

3.2. Biological evaluation of the synthesized compounds

3.2.1. Antimicrobial activity

Here, we have investigated the antimicrobial activity of 1,5-benzodiazepines (BZD) and the series of the *N*-bis-1,2,3-triazole linked to the 1,5-benzodiazepines conjugates which have been evaluated through the determination of the minimal inhibitory concentration (MIC) by the microdilution method. Most of synthesized products have shown, either moderate or important antibacterial activity against Gram-positive and Gram-negative bacteria with MIC of 31.25-250 µg/mL, and a remarkable antifungal activity with MIC of 62.5-250 µg/mL.

In *vitro*, antibacterial and antifungal screening results are summarized in histograms represented in figure 3, 4 and 5.

In the first screening phase against *B. subtilus*, two compounds **3g** and **3l** have exhibited an excellent activity compared to standard antibiotic amoxicillin (AMX) with MIC= 31.25 µg/mL. Among triazoles derivatives, the compounds **3e**, **3g** and **3l** emerged as potential antibacterial agents comparable with the standard drugs AMP and AMX against bacterial pathogens: *S. aureus*. (Figure 3)

On the other hand, compounds **3b**, **3c**, **3f**, **3g**, **3h**, **3k** and **3l** have been significantly the potent derivatives against most of the used Gram-negative bacteria with a MIC ranging from 31.25 to 125 µg/mL as compared to the benzodiazepines **1** and **2** and to AMX. (Figure 4)

It is also very clear, that most of the tested compounds have displayed an excellent activity two times higher for **3g** and **3i** and three times higher for the rest of bis-1,2,3-triazolobenzodiazepines against *E. fecalis* compared to the positive control (AMX). It is worthwhile to notice that compounds **3a**, **3b**, **3d**, **3f**, **3g**, **3h**, **3k** and **3l** are four times as active towards *E. faecium* (CI) than Amoxacilline (AMX). (Figure 4)

The data revealed also that most of bis-1,2,3-triazolobenzodiazepines are very potent compared to benzodiazepines **1** and **2** without the triazoles moieties. This effect may be explained by the importance of the triazole unit for the antibacterial activity of the bis-triazolo benzodiazepine analogues.

Additionally, the results of antifungal screening indicate that all bis triazolobenzodiazepines showed a very important activity against all used fungal with an MIC = 62.5-250 µg/mL. Indeed, the compounds **3h** and **3k** are the most potent antifungal agents showing a MIC of 62.5 µg/mL against *C. keusi* and *C.glabrata*. (Figure 5)

Structure-activity relationships (SAR)

Our studies were devoted to the analysis of qualitative and quantitative structure-activity relationships (SAR) of benzodiazepine derivatives. As described in the literature, benzodiazepine derivatives revealed to be excellent antimicrobial agents [33]. For the first screening of the in *vivo* antimicrobial results, we noticed the presence of activity in our synthesized compounds. In addition, independently of the nature of the substituent attached to the benzodiazepine borne by the triazole, compounds **3a-l** were found to be more active than compounds **1** and **2**. These results conclude the importance of the introduction of the triazole moiety via the methylene linker to improve the activity of benzodiazepine **1**. Particularly, for Gram-positive, compounds **3g** and **3l** can be optimized for the broad spectrum of activity against bacteria compared to Amoxicillin (AMX). In our case, the compound having ether

group attached to two phenyl rings **3g** favors the potency. In fact, the ether group appeared to be much more important for antibacterial activity [34]. Also, it is interesting to note that the methyl substitution on the phenyl rings **3l** has shown a promising activity. As shown, compounds possessing electron-donating groups in the links found to enhance the bacterial inhibition effects. On the other hand, for Gram-negative, compounds with sulfonyl group at the triazole ring **3h** as well as **3k** with pyridine group are highly active against *E. fecalis*, *S. enterica* and *E. faecium*. These strong electron-withdrawing groups induce the potency of benzodiazepine derivatives. This result is supported by the studies of Konda et *al.* [35]. For antifungal activity and according to the minimum inhibitory concentration (MIC) values, compounds **3h** and **3k** are highly active against *C. keusi* and *C. glabrata* compared to the standard antifungal agent Amphotericin (AB). In our case, the presence of electron withdrawing link group increases the antifungal activity of the synthesized benzodiazepine derivatives.

3.2.2. Antioxidant activity

The DPPH method is known as a convenient method, which is rapid and simple used for the screening of many samples for radical scavenging activity [36]. These advantages made the DPPH an interesting method for the evaluation of our compounds. On the other hand, the assay with the ABTS, as a moderately stable nitrogen center radical known for its high solubility as compared to the DPPH radical [37], can be used to test the ability of compounds to donate $\mathbf{H}^{\cdot}$ able to neutralize $ABTS^{+}$ free radicals.

The investigation of antioxidant screening revealed that all the newly of 1,5-benzodiazepines-1,2,3-triazoles hybrids showed moderate radical scavenging capacity compared to the standard Trolox, described as a potent antioxidant agent with IC_{50}= 0,126±0,013 mg/ml in DPPH test and IC_{50}= 0,163±0.021 mg/ml in ABTS test.

As depicted in figure 6, the results showed that compound **1** exhibited an important DPPH and ABTS scavenging ability with IC_{50}= 3,05±0,05 mg/ml and IC_{50}= 2,43±0,06 mg/ml, respectively, when compared to compound **2** (IC_{50}= 3,14±0.02 mg/ml and IC_{50}= 2,78±0.12 mg/ml). Such results support the crucial role of the labile hydrogens in the interaction of the examined compounds with the stable free radicals DPPH and $ABTS^{+}$.[24]

Furthermore, the lowest capacity of neutralization of DPPH radical was observed for compound **3b** (IC_{50}= 3,01±0.06 mg/mL), while compounds **3h** and **3g** exhibited the highest scavenging abilities with IC_{50}= 1,88±0.02 mg/ml and IC_{50}= 2.07±0.03 mg/ml, respectively, when compared to other derivatives. The compounds **3h** and **3g** displayed the strongest

capacity for the neutralization of $ABTS^{+}$ free radicals with IC_{50}= 1.63±0.04 and 1.91±0.06 mg/mL respectively. These findings may be explained by the presence of sulfonyl group and oxygen atom in the linker moiety of both structures, which are able to contribute to the neutralization of free radicals, as well as the presence of phenolic hydroxyl groups connected to a benzodiazepine ring.

The Ferric Reducing Antioxidant Power (FRAP) is another interesting way to evaluate antioxidant activity of drugs. It is a technique generally used for experimenting the reducing ability of antioxidant species by transferring a simple electron [38]. The results by reducing power assay presented in figure 7 have showed that the activity of all tested compounds increase compared to Trolox (EC_{50}= 0.072 ± 0.022 mg/mL) as follows:

3h> 3g> 3k> 3l> 3e> 1> 2> 3d> 3j> 3f> 3c> 3i> 3a> 3b.

The highest reducing activity is observed in compounds **3h** (EC_{50}= 1.12±0.08 mg/mL), **3g** (EC_{50}= 1.27±0.03 mg/mL**)**, **3k** (EC_{50}= 1.50±0.07 mg/mL), **3l** (EC_{50}= 1.82±0.13 mg/mL) and **3e** (EC_{50}= 2.06±0.05 mg/mL) which is probably due to the presence of different reducing groups on the linkers of such hybrid dimers.

On the other side, compounds **1** and **2** showed better activity than the rest of bis-1,2,3-triazole benzodiazepine with Half maximal effective concentration (EC_{50}) of (2.17±0.08), (2.53±0.06), respectively.

Structure-activity relationships (SAR)

The position and degree of hydroxylation, polarity, solubility and reducing potential are the main factors influencing the antioxidant activity of phenolic compounds [39]. As shown, the results of a triplicate assay can only give a suggestion on the potential of the tested compounds. For 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, the current research revealed the potential of 4-(2-hydroxyphenyl)-*1H*-1,5-benzodiazepin-2-one **1** as emerging free radical scavenger. The study established a structure-activity relationship (SAR) where the hydroxyl moiety of the 1,5-benzodiazepine was found to play a profound role and influence over antioxidant activity. The antioxidant potential of our compounds is moderate compared to Trolox. In fact, the potent antioxidant activity of the new purified compound could be attributed to the presence of two hydroxyl groups in the benzodiazepine core, which play an important role in the activation of free radicals. Introduction ether group into **3g** increases the scavenging activity and the presence of a sulfonyl group in **3h** has shown a promising activity. Generally, similar pattern of activity was also observed with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS). On the other hand, Ferric reducing

antioxidant power (FRAP) analysis of the synthesized compounds revealed that they are moderately to importantly active compared to Trolox.

4. Conclusion

In summary, we have successfully synthesized new benzodiazepine derivatives linked by 1,4-disubstitued 1,2,3-bistriazoles in a complete regioselective way using different aliphatic/aromatic moieties. The easy way and the high yields of the obtained products (67-92%) make this method attractive for the synthesis of potential biologically active molecules. Their evaluation as antimicrobial agents has revealed that all the screened compounds exhibit moderate to excellent activities. Only two products (**3h**,**k**) have given the highest antifungal activity. On the basis of current knowledge of SAR, biological evaluation results revealed that antimicrobial potency is considerably improved by the presence of triazole. Furthermore, all the synthesized compounds were evaluated for antioxidant activity. Amongst all the tested compounds, some bis-1,2,3-triazole benzodiazepines have shown an important antioxidant activity with regard to the starting compounds **1**,**2** in three tests. Further, it was noticed that **3g** and **3h** showed comparatively higher antioxidant activity than other bis-triazole benzodiazepines. These results illustrate the potential of this new class of molecules for diverse biologycal applications. Overall results suggested that nature and position of the substituents attached to the benzodiazepine skeleton had a considerable impact on the antioxidant effect of the tested molecules. These findings deserve to be studied more thoroughly in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at xxx

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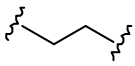
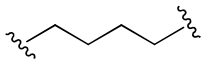
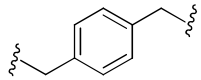
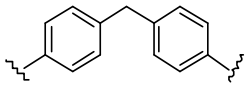
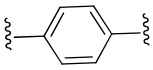
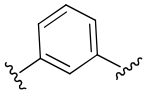
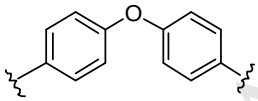
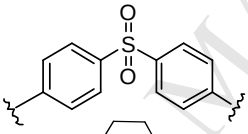
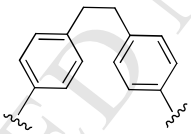
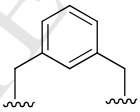
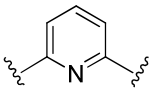
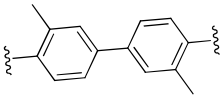
Table 1. Optimization of Cu(I)-catalyzed 1,3-dipolar cyclization for the synthesis of bis-1,2,3-triazoles **3b** and mono-1,2,3-triazole **3b'.**[a]

Entry	Solvent	Bases (2 equiv.)	Condition	Time	Yield %[c] (ratio 3b/3b')
1	DCM	Et_3N	rt	24 h	25/27
2	THF	Et_3N	rt	24 h	30/12
3	Toluene	Et_3N	reflux	24 h	45/10
4	DMF	Et_3N	MW[b]	15 min	72/20

Bold in entry highlights the optimal reaction conditions.
[a] Alkyne **2** (2 mmol), with diazide $\mathbf{D_{A2}}$ (1 mmol) and 0.2 mmol of CuI.
[b] The reaction was performed at *reflux* or *under MW* irradiation (400W).
[c] Ratios **(3b/3b')** were calculated on the crude product on the basis of integration signal intensities in ^{1}H NMR spectrum.

Table 2. 1,4-disubstituted bistriazoles **3a-l** prepared by the Cu(I)-catalyzed coupling under MW irradiation (400W).

Entry	Compd.	linkers	Yield (%)[a]	Time (min)
1	**3a**		92	6
2	**3b**		86	6
3	**3c**		82	8
4	**3d**		78	10
5	**3e**		84	8
6	**3f**		80	8
7	**3g**		87	10
8	**3h**		83	10
9	**3i**		67	12
10	**3j**		89	10
11	**3k**		71	12
12	**3l**		81	12

[a] Isolated yield for the bis-1,2,3-triazole after purification by column chromatography.

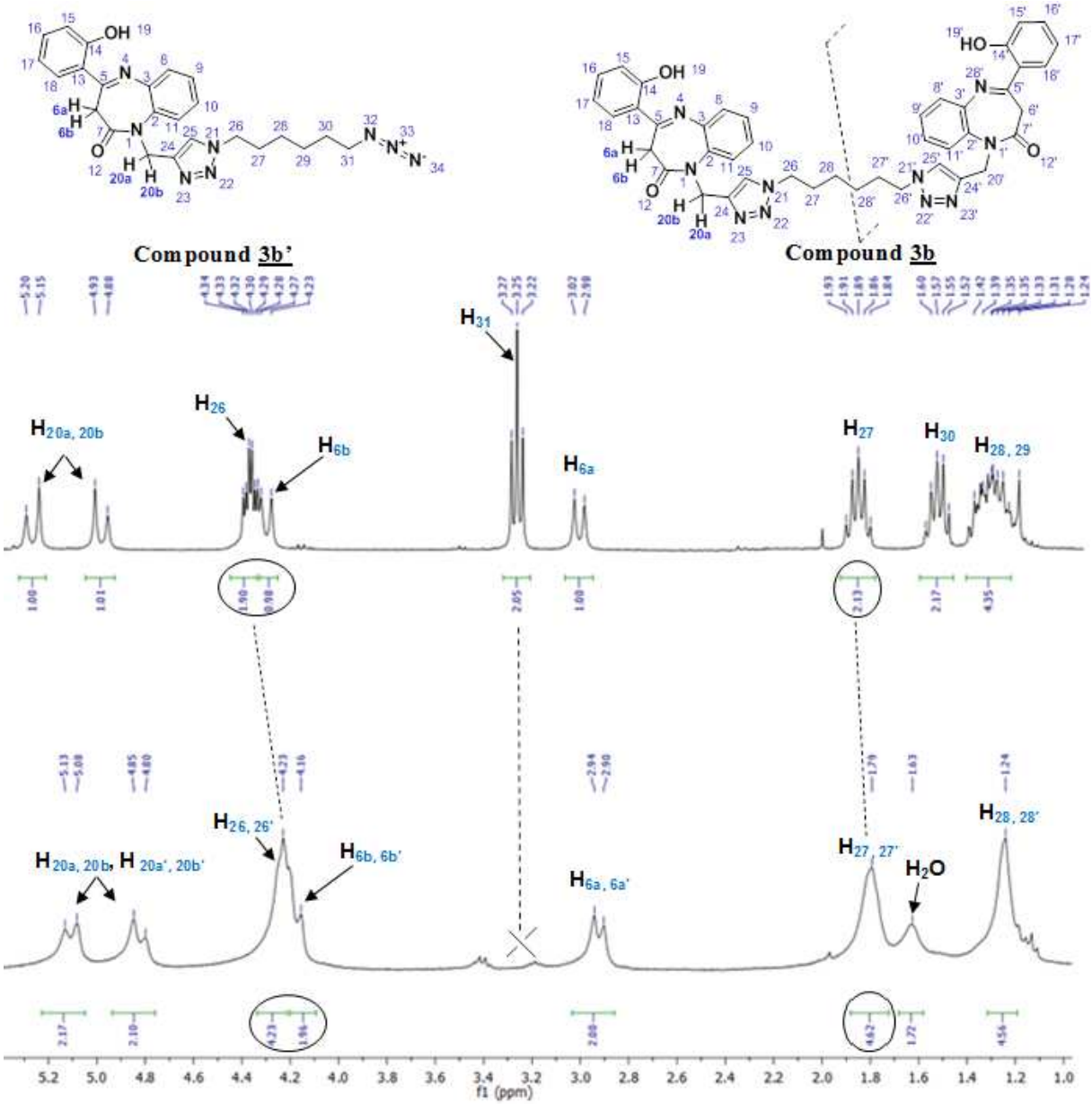


Figure 1. selected ^{1}H NMR chemical shifts of **3b'** (up) and **3b** (down) ($CDCl_3$, 300 MHz).

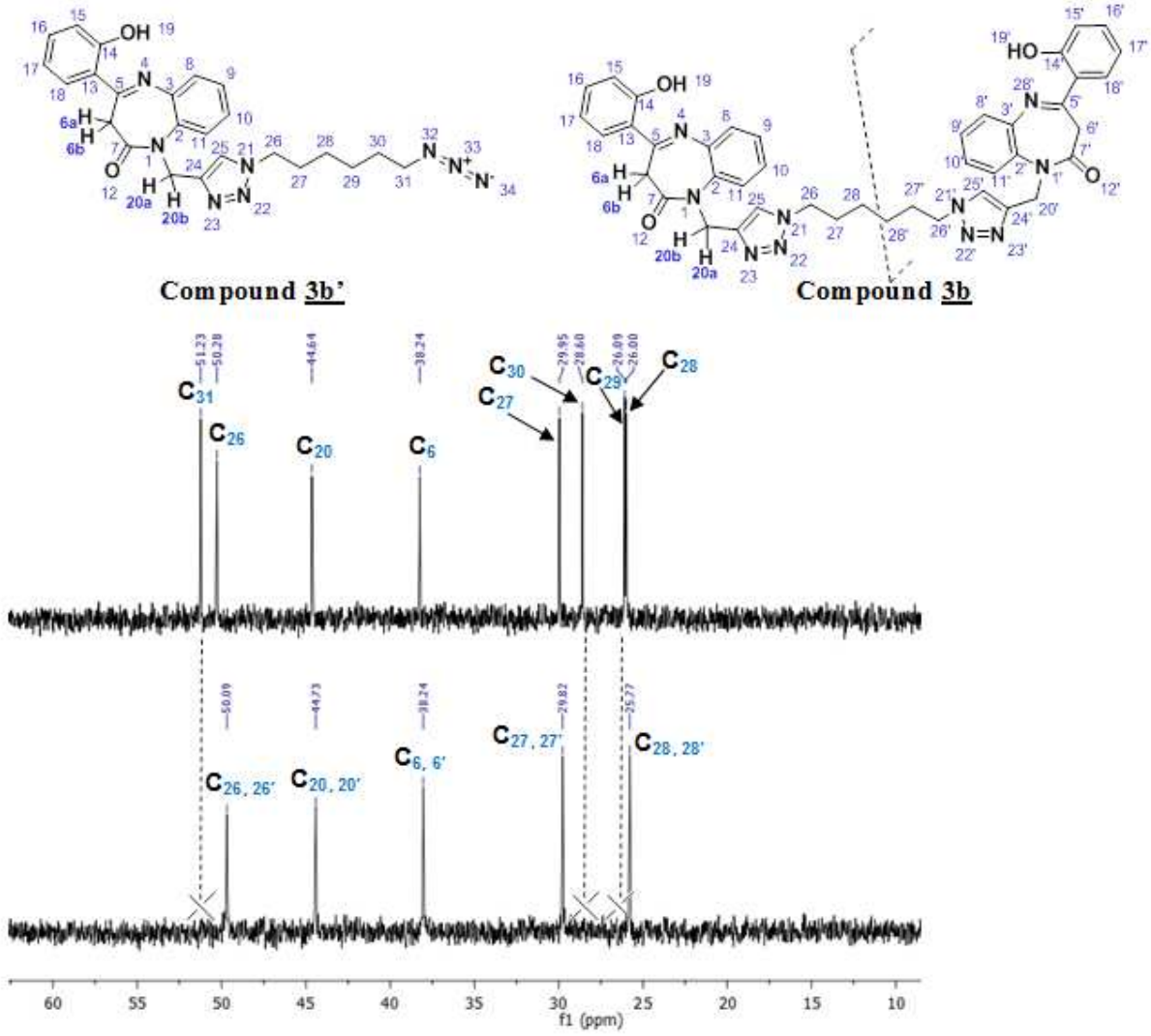


Figure 2. selected ^{13}C NMR chemical shifts of **3b'** (up) and **3b** (down) ($CDCl_3$, 75 MHz).

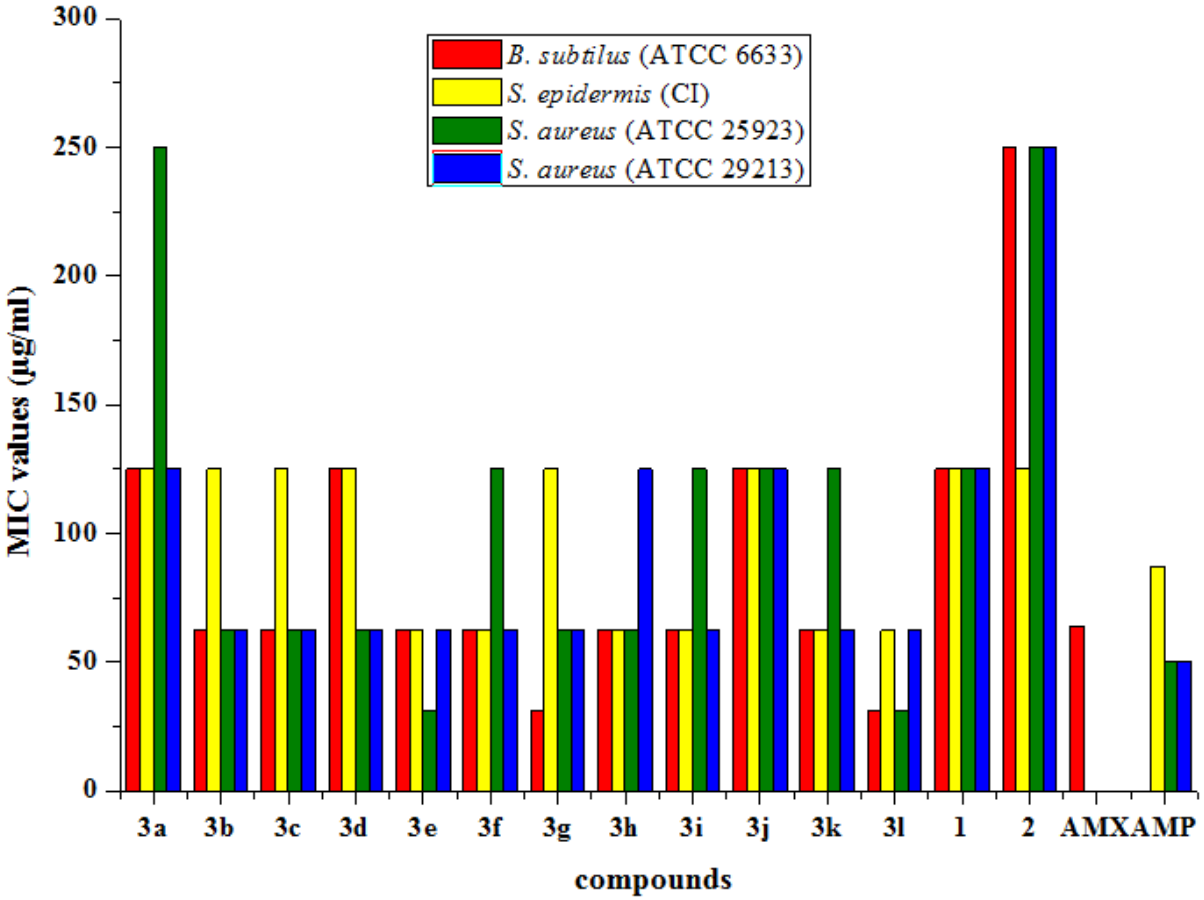


Figure 3. Antibacterial activity of synthesized compounds against Gram-positive bacteria.

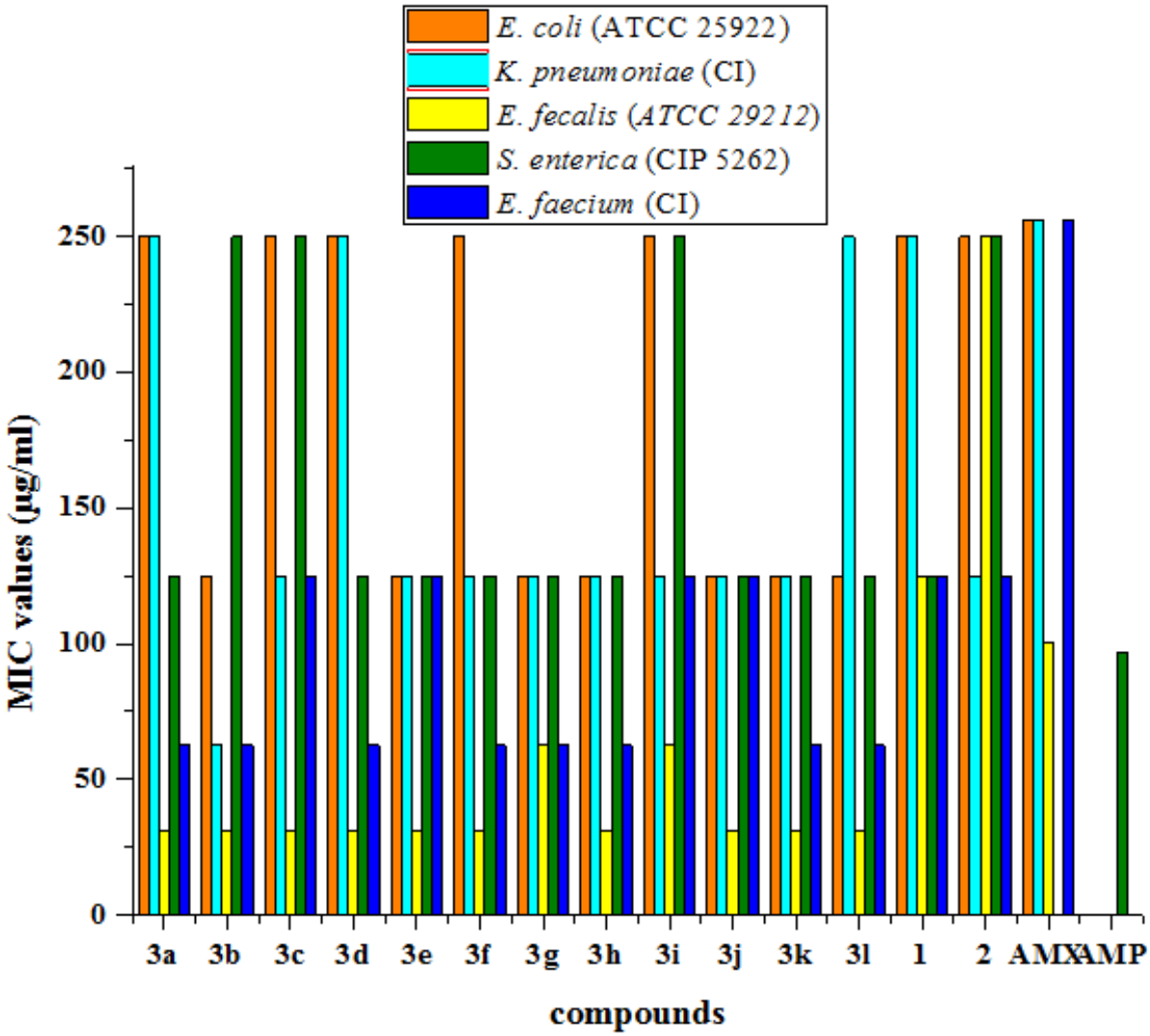


Figure 4. Antibacterial activity of synthesized compounds against Gram-negative bacteria.

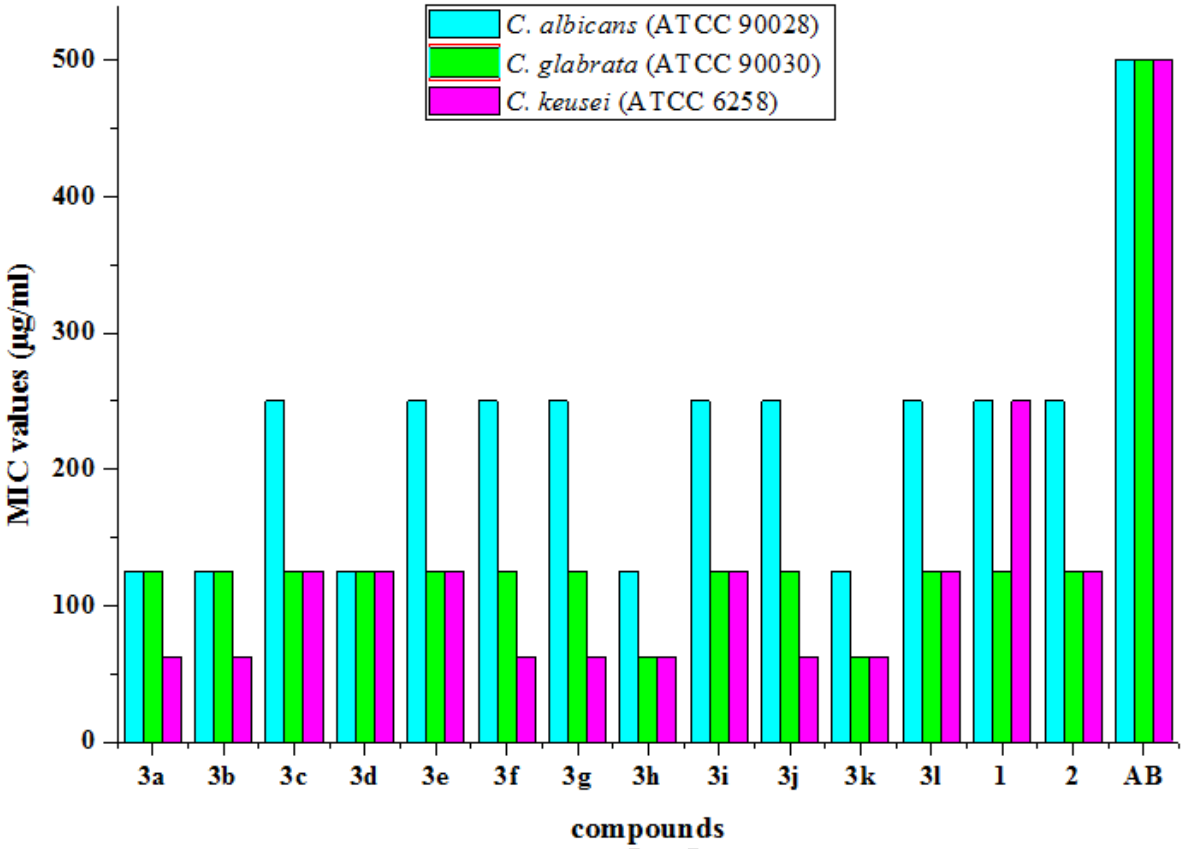


Figure 5. Antifungal activity of synthesized compounds.

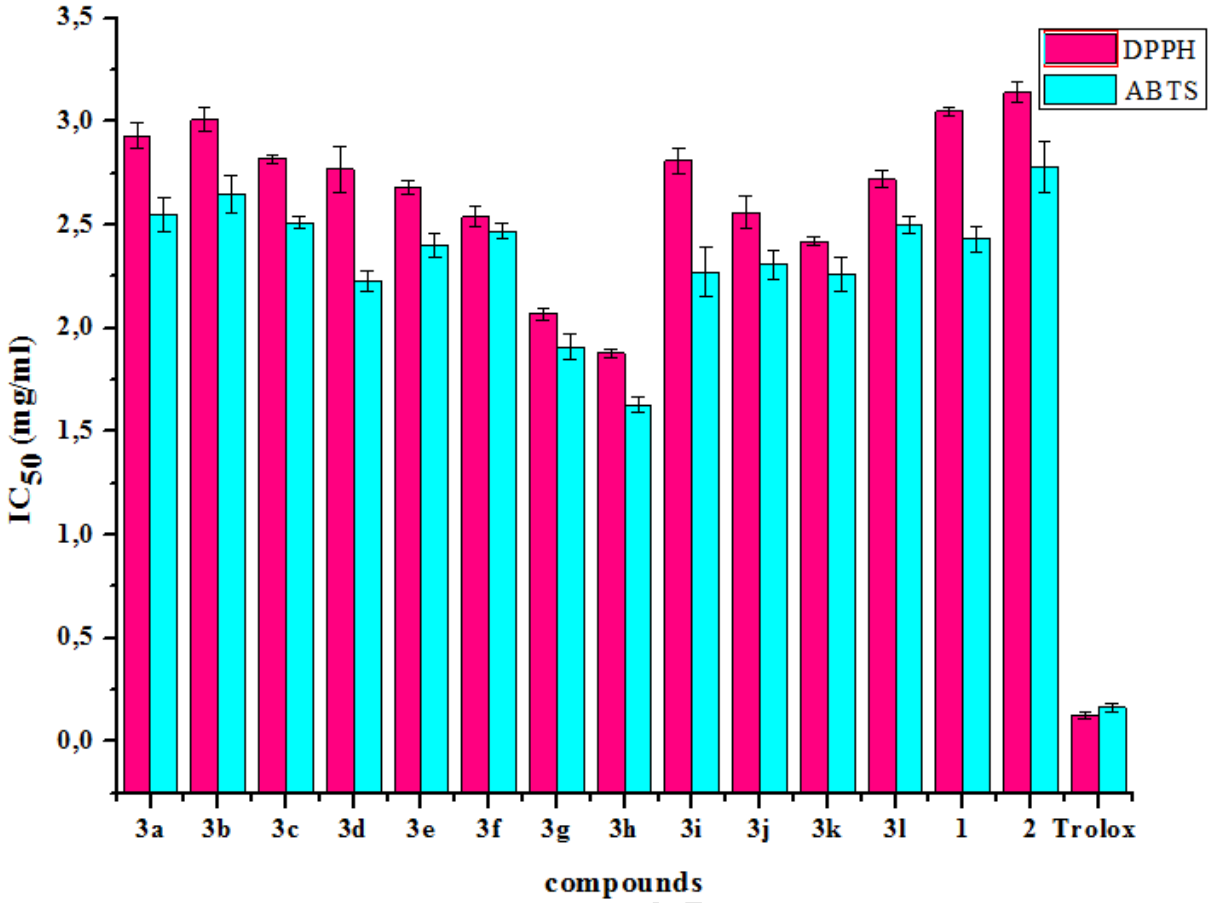


Figure 6. Antioxidant activity of synthesized compounds induced by IC_{50} values of the DPPH and ABTS free radical scavenging.

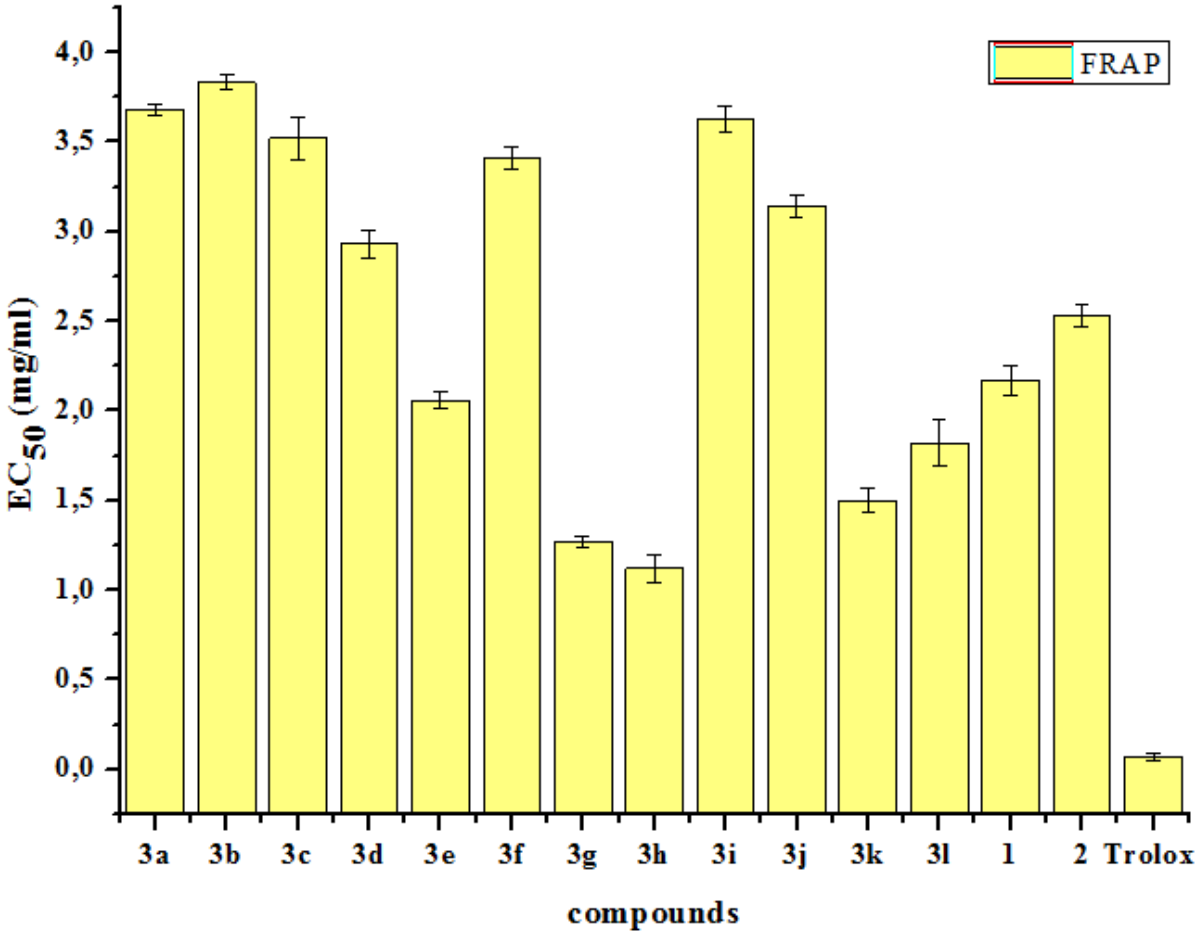
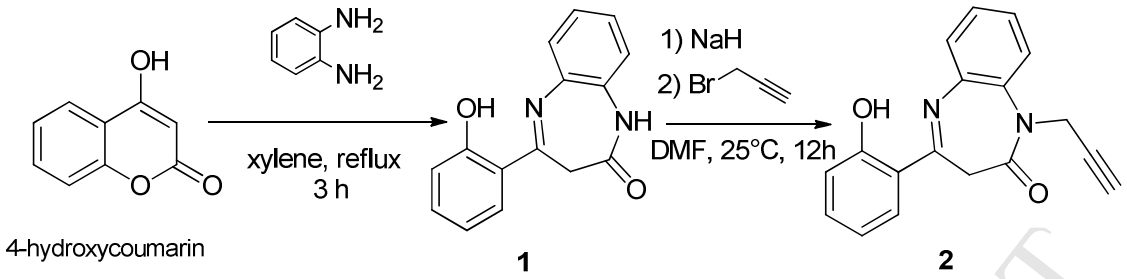
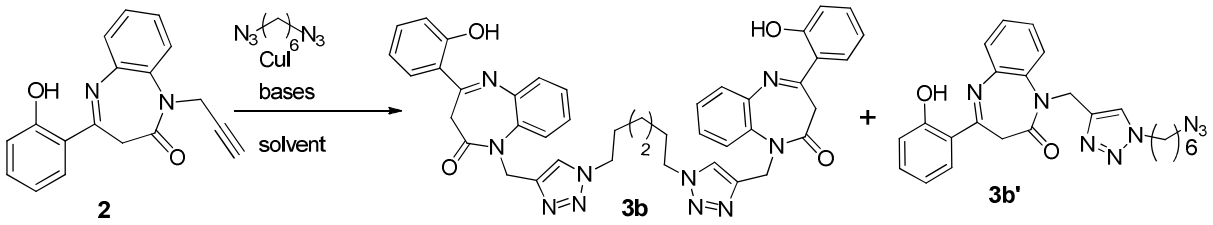


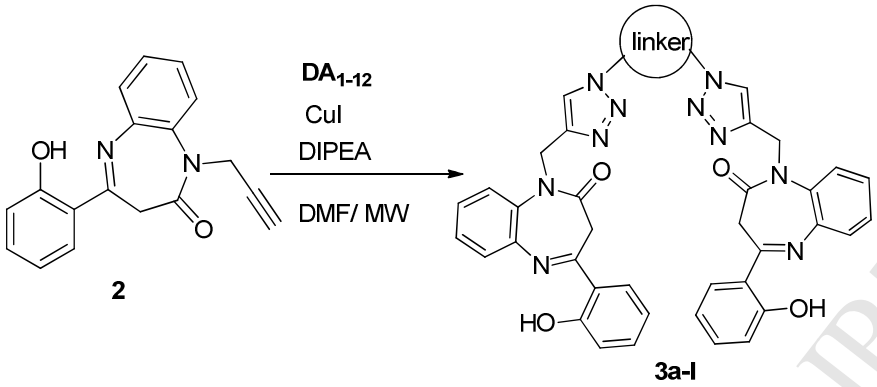
Figure 7. Antioxidant activity of synthesized compounds induced by EC_{50} values of the FRAP assay.



Scheme 1. General method for the synthesis of *N*-propargyl-1,5-benzodiazepinone **2**.



Scheme 2. General method for the synthesis of **3b** and **3b'**.



Scheme 3. General method for the synthesis of the bis-1,2,3-benzodiazepines **3a-l**.

Highlights

- A microwave assisted approach leading to new bis-triazole benzodiazepines
- Cycloadducts were confirmed using NMR, IR and HRMS.
- New bis-triazole benzodiazepines have shown an interesting antioxidant activity.
- These compounds exhibit a remarkable an in vitro antimicrobial activity.