Synthesis, *In Vitro* Antioxidant and Antimicrobial Activities of Some New 2-(3-Alkyl/Aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl-azomethine)phenyl Benzenesulfonate Derivatives

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Summary: Eight new 2-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonates (**3**) were obtained by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with 2-benzenesulfonyloxybenzaldehyde (**2**). Moreover, eight *N*-acetyl derivatives (**4**) of compounds **3** were obtained. Then, seven new 2-[1-(morpholine-4-yl-methyl)-3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonates (**5**) were obtained by the reactions of compounds **3** with formaldehyde and morpholine. The structures of twenty-three newly synthesized compounds were established from infrared, ^{1}H-NMR, ^{13}C-NMR, MS and UV spectral data. In addition, **3**, **4** and **5** type compounds were examined for their *in vitro* potential antioxidant and antibacterial activities. Three different methods were used for the antioxidant assay and the compounds **5** showed a significant activity for iron-binding. Antimicrobial activity was determined on six bacteria using the agar well diffusion method

Keywords: 4,5-Dihydro-1*H*-1,2,4-triazole; Schiff base; Mannich base; Antioxidant activity; Antimicrobial activity

Introduction

The balance between pro-oxidants and antioxidants reflects the morphological consequences. Oxidative stress can occur with an imbalance in favor of pro-oxidants and/or against antioxidants. This situation may cause cellular dysfunction or death [1]. The naturally occurring and synthetically derived antioxidants, which can provide the active ingredients to reduce or prevent the effect of oxidative stress, have attracted the attention of scientists [2].

Moreover, emerging antibiotic resistance is accepted as one of the most significant public health problems of the last few decades. The rapid emergence and prevalence of antibiotic-resistant pathogens require a serious effort to identify, develop and design new antibiotics [3]. Design and synthesis of novel heterocycles can play an important role because of their importance in medicinal chemistry.

Triazoles containing three nitrogen atoms are heterocyclic compounds. Some of the modern drugs which containing a triazole moiety are alprazolam, triazolam, estazolam (hypnotic, sedative, tranquilizer), trazodone (antidepressant, anxiolytic), trapidil (hypotensive), terconazole (antifungal), hexaconazole (antifungal), etizolam (amnesic, anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant), rilmazafon (hypnotic, anxiolytic) and rizatriptan (antimigraine agent) [4]. 1,2,4-Triazoles and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have been exhibited a wide range of biological activities [5-12].

Experimental

Materials and Methods

Chemical reagents were purchased from Merck AG, Aldrich and Fluka. Melting points were determined in open capillary tubes on an Electrothermal Melting-point Apparatus and were uncorrected. The infrared spectra were collected on a Perkin Elmer Instruments Spectrum One FT-IR spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded in DMSO-d_6 (deuterated dimethyl sulfoxide) with TMS as internal standard using a Bruker Ultrashield spectrophotometer at 400 MHz and 100 MHz, respectively. LC-MS/MS was performed on a Thermo Scientific Q Exactive Mass Spectrometer. UV absorption spectra were evaluated in 10 mm quartz cells between 200 and 400 nm using a PG Instruments Ltd T80 UV/Vis spectrometer.

Synthesis

General procedure for the synthesis of 2-(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)-phenyl benzenesulfonates (**3**)

2-Hydroxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (30 mL) was reacted with benzene-

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sulfonyl chloride (0.01 mol). Triethylamine (0.01 mol) in 10 mL ethyl acetate was added to this solution slowly by stirring at 0-5 °C for 2 hours. After that the mixture was refluxed for 3 hours and filtered. Then the filtrate was evaporated *in vacuo*, the crude product was washed with water and recrystallized from ethanol to afford novel compound **2** [13]. The corresponding compound **1** (0.01 mol) was dissolved in ethanoic acid (20 mL) and treated with 2-benzenesulfonyloxybenzaldehyde (**2**) (0.01 mol). After that the mixture was refluxed for 1.5 hours and evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from ethanol gave pure compounds **3a-h** as colorless crystals.

2-(3-Methyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonate **(3a)**

White solid; yield: 98 %; m.p. 213 °C; IR (cm^{-1}) υ_{max}: 3175 (NH), 1702 (C=O), 1618, 1603 (C=N), 1350 and 1193 (SO_2), 736 (1,2-disubstituted benzenoid ring), 761 and 682 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.23 (s, 3H, CH_3), 7.28-7.30 (m, 1H, Ar-H), 7.48 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.58-7.63 (m, 3H, Ar-H), 7.75-7.79 (m, 1H, Ar-H), 7.85-7.88 (m, 2H, Ar-H), 7.93-7.95 (m, 1H, Ar-H), 9.78 (s, 1H, N=CH), 11.88 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 10.96 (CH_3), [123.38, 126.54, 127.21, 128.07, 128.42 (2C), 129.74 (2C), 132.67, 133.58, 135.24, 146.27] (Ar-C), 144.19 (N=CH), 147.81 (Triazol-C_3), 151.06 (Triazol-C_5); UV [Ethanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 294 (11.300), 254 (13.870), 220 (19.790). LC-MS/MS: m/z = 359.08008 $[M+H]^+$ calculated: 359.08085.

2-(3-Ethyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonate **(3b)**

White solid; yield: 98 %; m.p. 202 °C; IR (cm^{-1}) υ_{max}: 3167 (NH), 1690 (C=O), 1595 (C=N), 1356 and 1182 (SO_2), 736 (1,2-disubstituted benzenoid ring), 760 and 680 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 1.21 (t, 3H, CH_2CH_3; *J* = 7.60 Hz), 2.62 (q, 2H, $\underline{CH_2}CH_3$; *J* = 7.60 Hz), 7.29-7.31 (m, 1H, Ar-H), 7.48-7.50 (m, 1H, Ar-H), 7.58-7.63 (m, 3H, Ar-H), 7.75-7.77 (m, 1H, Ar-H), 7.85-7.87 (m, 2H, Ar-H), 7.91-7.94 (m, 1H, Ar-H), 9.78 (s, 1H, N=CH), 11.91 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 10.05 ($CH_2\underline{CH_3}$), 18.38 ($\underline{CH_2}CH_3$), [123.40, 126.42, 127.25, 128.10, 128.43 (2C), 129.71 (2C), 132.65, 133.56, 135.25, 147.81] (Ar-C), 146.19 (N=CH), 148.35 (Triazol-C_3), 151.21 (Triazol-C_5); UV [Ethanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 294 (13.470), 256 (14.330), 220 (20.220). LC-MS/MS: m/z = 373.09650 $[M+H]^+$ calculated: 373.09650.

2-[3-(n-Propyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(3c)**

White solid; yield: 98 %; m.p. 190 °C; IR (cm^{-1}) υ_{max}: 3163 (NH), 1693 (C=O), 1594 (C=N), 1354 and 1190 (SO_2), 740 (1,2-disubstituted benzenoid ring), 755 and 690 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 0.95 (t, 3H, $CH_2CH_2CH_3$; *J* = 7.20 Hz), 1.65 (sext, 2H, $CH_2\underline{CH_2}CH_3$; *J* = 7.20 Hz), 2.58 (t, 2H, $\underline{CH_2}CH_2CH_3$; *J* = 7.20 Hz), 7.30 (dd, 1H, Ar-H; *J* = 8.40, 1.20 Hz), 7.49 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.58-7.63 (m, 3H, Ar-H), 7.74-7.76 (m, 1H, Ar-H), 7.84-7.87 (m, 2H, Ar-H), 7.90-7.93 (m, 1H, Ar-H), 9.78 (s, 1H, N=CH), 11.91 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 13.44 ($CH_2CH_2\underline{CH_3}$), 18.87 ($CH_2\underline{CH_2}CH_3$), 26.55 ($\underline{CH_2}CH_2CH_3$), [123.42, 126.38, 127.23, 128.13, 128.44 (2C), 129.71 (2C), 132.67, 133.56, 135.22, 146.82] (Ar-C), 146.17 (N=CH), 147.82 (Triazol-C_3), 151.15 (Triazol-C_5), 164.64 (COO); UV [Ethanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 294 (11.830), 256 (15.140), 220 (20.490). LC-MS/MS: m/z = 387.11179 $[M+H]^+$ calculated: 387.11215.

2-(3-Benzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonate **(3d)**

White solid; yield: 98 %; m.p. 195 °C; IR (cm^{-1}) υ_{max}: 3180 (NH), 1704 (C=O), 1590 (C=N), 1354 and 1190 (SO_2), 736 (1,2-disubstituted benzenoid ring), 764 and 683 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 4.00 (s, 2H, CH_2Ph), 7.25-7.35 (m, 6H, Ar-H), 7.45-7.66 (m, 5H, Ar-H), 7.81-7.88 (m, 3H, Ar-H), 9.73 (s, 1H, N=CH), 11.95 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 30.89 (CH_2Ph), [123.43, 126.34, 126.72, 128.10, 128.44 (2C), 129.67 (2C), 132.71, 133.47, 135.19, 146.11] (Ar-C), [127.17, 128.39 (2C), 128.77 (2C), 135.67] (Ar-C linked C-3), 145.92 (N=CH), 147.85 (Triazol-C_3), 151.05 (Triazol-C_5); UV [Ethanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 292 (10.520), 258 (12.570), 214 (25.070). LC-MS/MS: m/z = 435.11176 $[M+H]^+$ calculated: 435.11215.

2-[3-(p-Methylbenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(3e)**

White solid; yield: 97 %; m.p. 193 °C; IR (cm^{-1}) υ_{max}: 3181 (NH), 1705 (C=O), 1590 (C=N), 1354 and 1190 (SO_2), 829 (1,4-disubstituted

benzenoid ring), 737 (1,2-disubstituted benzenoid ring), 763 and 683 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.26 (s, 3H, $PhCH_3$), 3.95 (s, 2H, CH_2Ph), 7.13 (d, 2H, Ar-H; *J* = 8.00 Hz), 7.18 (d, 2H, Ar-H; *J* = 8.00 Hz), 7.28 (dd, 1H, Ar-H; *J* = 8.00, 1.20 Hz), 7.48-7.51 (m, 1H, Ar-H), 7.53-7.68 (m, 4H, Ar-H), 7.81-7.84 (m, 2H, Ar-H), 7.87-7.89 (m, 1H, Ar-H), 9.73 (s, 1H, N=CH), 12.00 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 20.58 ($PhCH_3$), 30.49 (CH_2Ph), [123.42, 126.34, 127.18, 128.10, 128.39 (2C), 129.67 (2C), 132.70, 133.49, 135.19, 146.26] (Ar-C), [128.64 (2C), 128.99 (2C), 132.55, 135.80] (Ar-C linked C-3), 145.90 (N=CH), 147.84 (Triazol-C_3), 151.05 (Triazol-C_5); UV [Ethanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 294 (11.050), 258 (13.860), 220 (25.230). LC-MS/MS: m/z = 449.12781 $[M+H]^+$ calculated: 449.12780.

2-[3-(p-Methoxybenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(3f)**

White solid; yield: 95 %; m.p. 199 °C; IR (cm^{-1}) υ_{max}: 3192 (NH), 1705 (C=O), 1594 (C=N), 1353 and 1189 (SO_2), 824 (1,4-disubstituted benzenoid ring), 740 (1,2-disubstituted benzenoid ring), 764 and 685 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 3.72 (s, 3H, OCH_3), 3.93 (s, 2H, CH_2Ph), 6.89 (d, 2H, Ar-H; *J* = 8.80 Hz), 7.21 (d, 2H, Ar-H; *J* = 8.80 Hz), 7.27-7.29 (m, 1H, Ar-H), 7.49 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.52-7.62 (m, 3H, Ar-H), 7.67 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.82-7.84 (m, 2H, Ar-H), 7.89-7.91 (m, 1H, Ar-H), 9.74 (s, 1H, N=CH), 12.00 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 30.03 (CH_2Ph), 55.05 (OCH_3), [123.42, 126.37, 127.20, 128.12, 128.40 (2C), 129.67 (2C), 132.70, 133.50, 135.19, 146.42] (Ar-C), [113.98 (2C), 127.43, 129.84 (2C), 158.14] (Ar-C linked C-3), 145.93 (N=CH), 147.84 (Triazol-C_3), 151.07 (Triazol-C_5); UV [Ethanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 294 (14.850), 258 (18.210), 228 (24.630), 216 (22.740). LC-MS/MS: m/z = 487.10461 $[M+Na]^+$ calculated: 487.10466.

2-[3-(p-Chlorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(3g)**

White solid; yield: 96 %; m.p. 234 °C; IR (cm^{-1}) υ_{max}: 3181 (NH), 1705 (C=O), 1604, 1589 (C=N), 1354 and 1190 (SO_2), 823 (1,4-disubstituted benzenoid ring), 734 (1,2-disubstituted benzenoid ring), 762 and 684 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 4.01 (s, 2H, CH_2Ph), 7.27-7.29 (m, 1H, Ar-H), 7.31-7.33 (m, 2H, Ar-H), 7.38-7.41 (m, 2H, Ar-H), 7.47-7.52 (m, 1H, Ar-H), 7.54-7.60 (m, 3H, Ar-H), 7.67-7.69 (m, 1H, Ar-H), 7.82-7.88 (m, 3H, Ar-H), 9.74 (s, 1H, N=CH), 11.95 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 30.22 (CH_2Ph), [123.41, 126.38, 127.11, 128.11, 128.42 (2C), 129.67 (2C), 132.76, 133.49, 135.20, 147.85] (Ar-C), [128.37 (2C), 130.71 (2C), 131.45, 134.63] (Ar-C linked C-3), 145.78 (Triazol-C_3), 146.05 (N=CH), 151.03 (Triazol-C_5); UV [Ethanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 292 (13.100), 258 (16.020), 220 (31.000). LC-MS/MS: m/z = 491.05505 $[M+Na]^+$ calculated: 491.05512; 492.05856 $[(M+2)+Na]^+$ calculated: 493.05157.

2-(3-Phenyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonate **(3h)**

White solid; yield: 96 %; m.p. 208 °C; IR (cm^{-1}) υ_{max}: 3184 (NH), 1701 (C=O), 1603, 1586 (C=N), 1352 and 1193 (SO_2), 733 (1,2-disubstituted benzenoid ring), 764 and 703 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 7.33 (dd, 1H, Ar-H; *J* = 8.40, 1.20 Hz), 7.46-7.49 (m, 1H, Ar-H), 7.53-7.64 (m, 7H, Ar-H), 7.71-7.75 (m, 1H, Ar-H), 7.82-7.90 (m, 5H, Ar-H), 9.79 (s, 1H, N=CH), 12.45 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: [123.44, 126.39, 127.02, 128.21, 128.46 (2C), 129.75 (2C), 132.91, 133.59, 135.27, 147.99] (Ar-C), [126.46, 128.09 (2C), 128.48 (2C), 130.13] (Ar-C linked C-3), 144.54 (Triazol-C_3), 148.15 (N=CH), 151.21 (Triazol-C_5); UV [Ethanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 262 (19.800), 250 (22.140), 232 (20.130), 218 (20.680). LC-MS/MS: m/z = 421.09705 $[M+H]^+$ calculated: 421.09650.

General method for the synthesis of 2-(1-acetyl-3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonates **(4)**

The corresponding compound **3** (0.01 mol) was refluxed with acetic anhydride (15 mL) for half an hour. Then absolute ethyl alcohol (50 mL) were added and the mixture was refluxed for one hour. The solution was evaporated *in vacuo* at 40-45 °C and the pure compounds **4** were obtained by several recrystallizations of the residue from ethanol.

2-(1-Acetyl-3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonate **(4a)**

White solid; yield: 84 %; m.p. 184 °C; IR (cm^{-1}) υ_{max}: 1766, 1700 (C=O), 1616, 1599 (C=N), 1343 and 1192 (SO_2), 729 (1,2-disubstituted

benzenoid ring), 762 and 696 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.31 (s, 3H, CH_3), 2.53 (s, 3H, $COCH_3$), 7.25 (dd, 1H, Ar-H; *J* = 8.40, 0.80 Hz), 7.49 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.60-7.67 (m, 3H, Ar-H), 7.78-7.82 (m, 1H, Ar-H), 7.86-7.88 (m, 2H, Ar-H), 7.96-7.98 (m, 1H, Ar-H), 9.67 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 11.10 (CH_3), 23.49 ($COCH_3$), [123.32, 126.68, 126.78, 128.14, 128.42 (2C), 129.75 (2C), 133.24, 133.1, 135.36, 147.75] (Ar-C), 144.56 (Triazol-C_3), 148.06 (N=CH), 148.09 (Triazol-C_5), 165.94 ($\underline{C}OCH_3$); UV [Ethanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 290 (12.085), 248 (16.080), 218 (20.330). LC-MS/MS: m/z = 423.07312 $[M+Na]^+$ calculated: 423.07336.

2-(1-Acetyl-3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonate **(4b)**

White solid; yield: 75%; m.p. 154 °C; IR (cm^{-1}) υ_{max}: 1734, 1720 (C=O), 1613, 1598 (C=N), 1373 and 1190 (SO_2), 757 (1,2-disubstituted benzenoid ring), 757 and 702 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 1.22 (t, 3H, CH_2CH_3; *J* = 7.20 Hz), 2.54 (s, 3H, $COCH_3$), 2.70 (q, 2H, $\underline{CH_2}CH_3$; *J* = 7.20 Hz), 7.26 (dd, 1H, Ar-H; *J* = 8.40, 1.20 Hz), 7.50 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.60-7.66 (m, 3H, Ar-H), 7.77-7.79 (m, 1H, Ar-H), 7.85-7.88 (m, 2H, Ar-H), 7.94-7.97 (m, 1H, Ar-H), 9.66 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 9.51 ($CH_2\underline{CH_3}$), 18.48 $\underline{CH_2}CH_3$), 23.52 ($COCH_3$), [123.35, 126.67, 126.71, 128.18, 128.43 (2C), 129.89 (2C), 133.24, 133.58, 135.36, 147.97] (Ar-C), 148.02 (N=CH), 148.10 (Triazol-C_3), 150.04 (Triazol-C_5), 165.92 ($\underline{C}OCH_3$); UV [Ethanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 290 (12.970), 248 (17.120), 218 (21.170). LC-MS/MS: m/z = 437.08920 $[M+Na]^+$ calculated: 437.08901.

2-[1-Acetyl-3-(n-propyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(4c)**

White solid; yield: 96%; m.p. 160 °C; IR (cm^{-1}) υ_{max}: 1734, 1719 (C=O), 1614, 1600 (C=N), 1370 and 1191 (SO_2), 754 (1,2-disubstituted benzenoid ring), 754 and 700 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 0.98 (t, 3H, $CH_2CH_2CH_3$; *J* = 7.20 Hz), 1.69 (sext, 2H, $CH_2\underline{CH_2}CH_3$; *J* = 7.20 Hz), 2.54 (s, 3H, $COCH_3$), 2.66 (t, 2H, $\underline{CH_2}CH_2CH_3$; *J* = 7.20 Hz), 7.26 (dd, 1H, Ar-H; *J* = 8.40, 0.80 Hz), 7.50 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.60-7.66 (m, 3H, Ar-H), 7.77-7.79 (m, 1H, Ar-H), 7.85-7.88 (m, 2H, Ar-H), 7.94-7.96 (m, 1H, Ar-H), 9.66 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 13.41 ($CH_2CH_2\underline{CH_3}$), 18.46 ($CH_2\underline{CH_2}CH_3$), 23.53 ($COCH_3$), 26.54 ($\underline{CH_2}CH_2CH_3$), [123.36, 126.64, 126.70, 128.18, 128.41 (2C), 129.86 (2C), 133.24, 133.58, 135.35, 148.01] (Ar-C), 147.92 (Triazol-C_3), 148.11 (N=CH), 148.90 (Triazol-C_5), 165.93 ($\underline{C}OCH_3$); UV [Ethanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 290 (14.560), 248 (19.610), 220 (25.350). LC-MS/MS: m/z = 451.10464 $[M+Na]^+$ calculated: 451.10466.

2-(1-Acetyl-3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonate **(4d)**

White solid; yield: 79%; m.p. 117 °C; IR (cm^{-1}) υ_{max}: 1735, 1701 (C=O), 1588 (C=N), 1352 and 1187 (SO_2), 763 (1,2-disubstituted benzenoid ring), 763 and 700 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.55 (s, 3H, $COCH_3$), 4.00 (s, 2H, CH_2Ph), 7.25-7.36 (m, 6H, Ar-H), 7.47-7.66 (m, 5H, Ar-H), 7.81-7.88 (m, 3H, Ar-H), 9.73 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 23.58 ($CO\underline{C}H_3$), 30.89 (CH_2Ph), [123.43, 126.34, 126.72, 128.10, 128.44 (2C), 129.67 (2C), 132.72, 133.47, 135.30, 146.11] (Ar-C), [127.16, 128.39 (2C), 128.77 (2C), 135.67] (Ar-C linked C-3), 145.92 (N=CH), 147.58 (Triazol-C_3), 151.05 (Triazol-C_5), 165.90 ($\underline{C}OCH_3$); UV [Ethanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 290 (10.160), 250 (13.050), 214 (23.790). LC-MS/MS: m/z = 499.10434 $[M+Na]^+$ calculated: 499.10466.

2-[1-Acetyl-3-(p-methylbenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(4e)**

White solid; yield: 82%; m.p. 128 °C; IR (cm^{-1}) υ_{max}: 1739, 1720 (C=O), 1615, 1601 (C=N), 1353 and 1199 (SO_2), 839 (1,4-disubstituted benzenoid ring), 739 (1,2-disubstituted benzenoid ring), 762 and 703 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.27 (s, 3H, $PhCH_3$), 2.54 (s, 3H, $COCH_3$), 4.05 (s, 2H, CH_2Ph), 7.15 (d, 2H, Ar-H; *J* = 8.00 Hz), 7.23 (d, 2H, Ar-H; *J* = 8.00 Hz), 7.22-7.25 (m, 1H, Ar-H), 7.51 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.56-7.63 (m, 3H, Ar-H), 7.68-7.70 (m, 1H, Ar-H), 7.82-7.84 (m, 2H, Ar-H), 7.89-7.91 (m, 1H, Ar-H), 9.63 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 20.60 ($PhCH_3$), 23.58 ($CO\underline{C}H_3$), 30.47 (CH_2Ph), [123.36, 126.58, 126.67, 128.16, 128.38 (2C), 128.84 (2C), 131.43, 133.26, 133.51, 135.30, 147.89] (Ar-C), [128.64 (2C), 128.99 (2C), 131.43, 136.12] (Ar-C

linked C-3), 147.57 (Triazol-C_3), 148.13 (N=CH), 148.25 (Triazol-C_5), 165.90 ($\underline{C}OCH_3$); UV [Ethanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 290 (14.770), 248 (19.900), 218 (32.100). LC-MS/MS: m/z = 513.12018 $[M+Na]^+$ calculated: 513.12031.

2-[1-Acetyl-3-(p-methoxybenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzene-sulfonate **(4f)**

White solid; yield: 83%; m.p. 162 °C; IR (cm^{-1}) υ_{max}: 1736, 1718 (C=O), 1612, 1599 (C=N), 1353 and 1193 (SO_2), 820 (1,4-disubstituted benzenoid ring), 758 (1,2-disubstituted benzenoid ring), 758 and 684 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.54 (s, 3H, $COCH_3$), 3.73 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2Ph), 6.90 (d, 2H, Ar-H; *J* = 8.80 Hz), 7.23-7.28 (m, 3H, Ar-H), 7.52 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.56-7.64 (m, 3H, Ar-H), 7.68-7.70 (m, 1H, Ar-H), 7.83-7.85 (m, 2H, Ar-H), 7.92-7.94 (m, 1H, Ar-H), 9.63 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 23.57 ($CO\underline{C}H_3$), 30.02 (CH_2Ph), 55.07 (OCH_3), [123.36, 126.60, 128.17, 128.39 (2C), 129.67 (2C), 133.25, 133.53, 135.29, 147.90] (Ar-C), [113.88 (2C), 126.68, 129.83 (2C), 158.33] (Ar-C linked C-3), 147.62 (Triazol-C_3), 148.13 (N=CH), 148.40 (Triazol-C_5), 165.89 ($\underline{C}OCH_3$); UV [Ethanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 284 (14.010), 220 (27.880), 212 (26.190). LC-MS/MS: m/z = 529.11523 $[M+Na]^+$ calculated: 529.11523.

2-[1-Acetyl-3-(p-chlorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(4g)**

White solid; yield: 73%; m.p. 153 °C; IR (cm^{-1}) υ_{max}: 1735, 1706 (C=O), 1619, 1602 (C=N), 1353 and 1196 (SO_2), 836 (1,4-disubstituted benzenoid ring), 735 (1,2-disubstituted benzenoid ring), 755 and 683 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.54 (s, 3H, $COCH_3$), 4.12 (s, 2H, CH_2Ph), 7.23 (dd, 1H, Ar-H; *J* = 8.00, 1.20 Hz), 7.37-7.43 (m, 4H, Ar-H), 7.49 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.58-7.64 (m, 3H, Ar-H), 7.70-7.74 (m, 1H, Ar-H), 7.82-7.85 (m, 2H, Ar-H), 7.88-7.90 (m, 1H, Ar-H), 9.65 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 23.56 ($CO\underline{C}H_3$), 30.20 (CH_2Ph), [123.32, 126.59, 126.62, 128.16, 128.43 (2C), 129.84 (2C), 133.29, 133.57, 135.31, 147.81] (Ar-C), [128.40 (2C), 130.71 (2C), 131.73, 135.19] (Ar-C linked C-3), 147.68 (N=CH), 147.88 (Triazol-C_3), 148.14 (Triazol-C_5), 165.88 ($\underline{C}OCH_3$); UV [Ethanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 290 (8.170), 248 (10.160), 216 (20.760). LC-MS/MS: m/z = 533.06561 $[M+Na]^+$ calculated: 533.06569; 535.06213 $[(M+2)+Na]^+$ calculated: 535.06274.

2-(1-Acetyl-3-phenyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonate **(4h)**

White solid; yield: 77%; m.p. 160 °C; IR (cm^{-1}) υ_{max}: 1728, 1701 (C=O), 1603, 1586 (C=N), 1353 and 1189 (SO_2), 753 (1,2-disubstituted benzenoid ring), 753 and 688 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.52 (s, 3H, $COCH_3$), 7.28 (dd, 1H, Ar-H; *J* = 8.00, 0.80 Hz), 7.50 (t, 1H, Ar-H; *J* = 7.60 Hz), 7.57-7.66 (m, 6H, Ar-H), 7.74-7.76 (m, 1H, Ar-H), 7.82-7.89 (m, 4H, Ar-H), 7.91-7.92 (m, 1H, Ar-H), 9.67 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 23.62 ($CO\underline{C}H_3$), [123.44, 126.39, 126.64, 128.21, 128.65 (2C), 129.76 (2C), 132.91, 133.61, 135.28, 148.15] (Ar-C), [126.46, 128.08 (2C), 128.46 (2C), 130.13] (Ar-C linked C-3), 147.99 (Triazol-C_3), 148.25 (Triazol-C_5), 150.17 (N=CH), 166.17 ($\underline{C}OCH_3$); UV [Ethanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 260 (24.610), 222 (27.900), 216 (27.110). LC-MS/MS: m/z = 485.08902 $[M+Na]^+$ calculated: 485.08901.

General procedure for the synthesis of 2-[1-(morpholine-4-yl-methyl)-3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzene-sulfonates **(5)**

Compound **3** (5 mmol) was dissolved absolute ethanol and then formaldehyde (37%, 10 mmol) and morpholine (6 mmol) were added to this solution. The reaction mixture was refluxed for four hours and then, the mixture was left at room temperature for overnight. After cooling the mixture in the refrigerator, the solid formed was obtained by filtration, washed with cold ethanol and recrystallization gave pure compounds **5**.

2-[1-(Morpholine-4-yl-methyl)-3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(5a)**

Yield: 86%; m.p. 167 °C; IR (cm^{-1}): 1700 (C=O), 1594 (C=N), 1387 and 1195 (SO_2), 768 (1,2-disubstituted benzenoid ring), 768 and 686 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.32 (s, 3H, CH_3), 2.74 (t, *J* = 4.4 Hz, 4H, CH_2NCH_2), 3.74 (t, *J* = 4.4 Hz, 4H, CH_2OCH_2), 4.63 (s, 2H, NCH_2N), 7.26-7.31 (m, 1H, Ar-H), 7.35-7.37 (m, 1H, Ar-H), 7.44-7.51 (m, 3H, Ar-H), 7.60-7.61 (m, 1H, Ar-H), 7.93-7.95 (m, 3H, Ar-H), 9.86 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 11.36 (CH_3), 50.53

(CH_2NCH_2), 66.54 (NCH_2N), 66.86 (CH_2OCH_2), [123.73, 126.86, 127.43, 127.73, 128.91 (2C), 129.19 (2C), 132.22, 134.26, 135.07, 148.20] (Ar-C), 143.75 (Triazol-C_3), 148.65 (Triazol-C_5), 150.64 (N=CH). LC-MS/MS: m/z = 480.13135 $[M+Na]^+$ calculated: 480.13121.

2-[1-(Morpholine-4-yl-methyl)-3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(5b)**

Yield: 77%; m.p. 125 °C; IR (cm^{-1}): 1699 (C=O), 1593 (C=N), 1380 and 1192 (SO_2), 764 (1,2-disubstituted benzenoid ring), 764 and 685 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.29 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 2.71 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 2.75 (t, *J* = 4.4 Hz, 4H, CH_2NCH_2), 3.74 (t, *J* = 4.4 Hz, 4H, CH_2OCH_2), 4.65 (s, 2H, NCH_2N), 7.26-7.32 (m, 1H, Ar-H), 7.35-7.37 (m, 1H, Ar-H), 7.44-7.53 (m, 3H, Ar-H), 7.59-7.60 (m, 1H, Ar-H), 7.92-7.95 (m, 3H, Ar-H), 9.85 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 10.48 ($CH_2\underline{C}H_3$), 19.07 ($\underline{C}H_2CH_3$), 50.52 (CH_2NCH_2), 66.51 (NCH_2N), 66.89 (CH_2OCH_2), [123.73, 126.74, 127.43, 127.80, 128.92 (2C), 129.19 (2C), 132.18, 134.29, 135.05, 148.66] (Ar-C), 147.65 (Triazol-C_3), 149.07 (Triazol-C_5), 150.80 (N=CH). LC-MS/MS: m/z = 494.14686 $[M+Na]^+$ calculated: 494.14686.

2-[1-(Morpholine-4-yl-methyl)-3-(n-propyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(5c)**

Yield: 98%; m.p. 84 °C; IR (cm^{-1}): 1699 (C=O), 1586 (C=N), 1377 and 1190 (SO_2), 755 (1,2-disubstituted benzenoid ring), 755 and 692 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 1.01 (t, *J* = 7.2 Hz, 3H, $CH_2CH_2CH_3$), 1.74 (sext, *J* = 7.6 Hz, 2H, $CH_2\underline{CH_2}CH_3$), 2.66 (t, *J* = 7.2 Hz, 2H, $\underline{CH_2}CH_2CH_3$), 2.74 (t, *J* = 4.8 Hz, 4H, CH_2NCH_2), 3.73 (t, *J* = 4.8 Hz, 4H, CH_2OCH_2), 4.65 (s, 2H, NCH_2N), 7.27-7.30 (m, 1H, Ar-H), 7.32-7.37 (m, 1H, Ar-H), 7.44-7.50 (m, 3H, Ar-H), 7.58-7.60 (m, 1H, Ar-H), 7.91-7.95 (m, 3H, Ar-H), 9.86 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 13.67 ($CH_2CH_2\underline{C}H_3$), 19.65 ($CH_2\underline{C}H_2CH_3$), 27.24 ($\underline{C}H_2CH_2CH_3$), 50.51 (CH_2NCH_2), 66.50 (NCH_2N), 66.89 (CH_2OCH_2), [123.72, 126.69, 127.44, 127.79, 128.92 (2C), 129.18 (2C), 132.18, 134.29, 135.03, 147.92] (Ar-C), 146.55 (Triazol-C_3), 148.67 (Triazol-C_5), 150.76 (N=CH). LC-MS/MS: m/z = 508.16275 $[M+Na]^+$ calculated: 508.16251.

2-[1-(Morpholine-4-yl-methyl)-3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(5d)**

Yield: 72%; m.p. 105 °C; IR (cm^{-1}): 1700 (C=O), 1583 (C=N), 1387 and 1189 (SO_2), 762 (1,2-disubstituted benzenoid ring), 762 and 701 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.75 (t, *J* = 4.4 Hz, 4H, CH_2NCH_2), 3.74 (t, *J* = 4.4 Hz, 4H, CH_2OCH_2), 4.02 (s, 2H, CH_2Ph), 4.67 (s, 2H, NCH_2N), 7.26-7.37 (m, 9H, Ar-H), 7.41-7.47(m, 2H, Ar-H), 7.81 (dd, *J* = 8.0, 1.6 Hz, 1H, Ar-H), 7.85-7.87 (m, 2H, Ar-H), 9.75 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 31.70 (CH_2Ph), 50.53 (CH_2NCH_2), 66.62 (NCH_2N), 66.89 (CH_2OCH_2), [123.85, 126.63, 127.44, 127.66, 128.86 (2C), 129.11 (2C), 132.23, 134.23, 134.85, 147.77] (Ar-C), [127.16, 128.65 (2C), 128.84 (2C), 135.26] (Ar-C linked triazol-C_3), 145.46 (Triazol-C_3), 148.72 (Triazol-C_5), 150.63 (N=CH). LC-MS/MS: m/z = 556.16272 $[M+Na]^+$ calculated: 556.16251.

2-[1-(Morpholine-4-yl-methyl)-3-(p-methylbenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(5e)**

Yield: 72%; m.p. 110 °C; IR (cm^{-1}): 1702 (C=O), 1590 (C=N), 1381 and 1195 (SO_2), 839 (1,4-disubstituted benzenoid ring), 758 (1,2-disubstituted benzenoid ring), 756 and 685 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.32 (s, 3H, $PhCH_3$), 2.74 (t, *J* = 4.8 Hz, 4H, CH_2NCH_2), 3.74 (t, *J* = 4.8 Hz, 4H, CH_2OCH_2), 3.97 (s, 2H, CH_2Ph), 4.66 (s, 2H, NCH_2N), 7.12 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.19 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.26 (m, 1H, Ar-H), 7.31-7.38 (m, 3H, Ar-H), 7.43-7.47 (m, 2H, Ar-H), 7.83-7.88 (m, 3H, Ar-H), 9.73 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 21.03 ($PhCH_3$), 31.27 (CH_2Ph), 50.52 (CH_2NCH_2), 66.60 (NCH_2N), 66.89 (CH_2OCH_2), [123.83, 126.66, 127.43, 127.70, 128.84 (2C), 129.11 (2C), 132.20, 134.23, 134.89, 147.77] (Ar-C), [128.76 (2C), 129.33 (2C), 132.15, 136.75] (Ar-C linked triazol-C_3), 145.68 (Triazol-C_3), 148.72 (Triazol-C_5), 150.63 (N=CH). LC-MS/MS: m/z = 570.17841 $[M+Na]^+$ calculated: 570.17816.

2-[1-(Morpholine-4-yl-methyl)-3-(p-chlorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(5g)**

Yield: 82%; m.p. 94 °C; IR (cm^{-1}): 1705 (C=O), 1585 (C=N), 1357 and 1191 (SO_2), 845 (1,4-disubstituted benzenoid ring), 776 (1,2-disubstituted benzenoid ring), 776 and 686 (monosubstituted

benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.74 (t, J = 4.4 Hz, 4H, CH_2NCH_2), 3.74 (t, J = 4.4 Hz, 4H, CH_2OCH_2), 4.00 (s, 2H, CH_2Ph), 4.66 (s, 2H, NCH_2N), 7.24-7.52 (m, 10H, Ar-H), 7.79-7.81 (m, 1H, Ar-H), 7.92-7.95 (m, 2H, Ar-H), 9.80 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 31.10 (CH_2Ph), 50.52 (CH_2NCH_2), 66.66 (NCH_2N), 66.88 (CH_2OCH_2), [123.81, 126.63, 127.46, 127.58, 128.81 (2C), 129.12 (2C), 132.33, 134.26, 134.94, 148.18] (Ar-C), [128.86 (2C), 130.22 (2C), 133.09, 133.67] (Ar-C linked triazol-C_3), 145.01 (Triazol-C_3), 148.72 (Triazol-C_5), 150.60 (N=CH). LC-MS/MS: m/z = 590.12366 $[M+Na]^+$ calculated: 590.12354; 592.12018 $[(M+2)+Na]^+$ calculated: 592.12059.

2-[1-(Morpholine-4-yl-methyl)-3-phenyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl-azomethine]phenyl benzenesulfonate **(5h)**

Yield: 84%; m.p. 154 °C; IR (cm^{-1}): 1694 (C=O), 1585 (C=N), 1378 and 1192 (SO_2), 750 (1,2-disubstituted benzenoid ring), 777 and 699 (monosubstituted benzenoid ring); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.82 (t, J = 4.4 Hz, 4H, CH_2NCH_2), 3.75 (t, J = 4.4 Hz, 4H, CH_2OCH_2), 4.79 (s, 2H, NCH_2N), 7.26 (m, 1H, Ar-H), 7.31-7.38 (m, 1H, Ar-H), 7.44-7.49 (m, 6H, Ar-H), 7.54-7.58 (m, 1H, Ar-H), 7.87-7.90 (m, 5H, Ar-H), 9.85 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 50.54 (CH_2NCH_2), 66.91 (CH_2OCH_2), 66.96 (NCH_2N), [123.77, 126.92, 127.53, 127.63, 128.89 (2C), 129.21 (2C), 132.39, 134.40, 134.99, 148.83] (Ar-C), [126.34, 128.40 (2C), 128.54 (2C), 130.32] (Ar-C linked triazol-C_3), 144.13 (Triazol-C_3), 149.61 (Triazol-C_5), 150.89 (N=CH). LC-MS/MS: m/z = 542.14697 $[M+Na]^+$ calculated: 542.14686.

Biological protocols

Antioxidant activity

1,1-Diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$), ferrous chloride, 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α-tocopherol and ethylenediaminetetraacetic acid (EDTA) were purchased from E. Merck and Sigma–Aldrich. The reducing power of the newly synthesized compounds was estimated by the method of Oyaizu [14]. Free radical scavenging activity of the newly synthesized compounds was determined by $DPPH^{\cdot}$, by the method of Blois [15]. The chelation of ferrous ions by the synthesized compounds and standards were measured according to the method of Dinis et al. [16] as explained precisely in the literature [6, 8, 10].

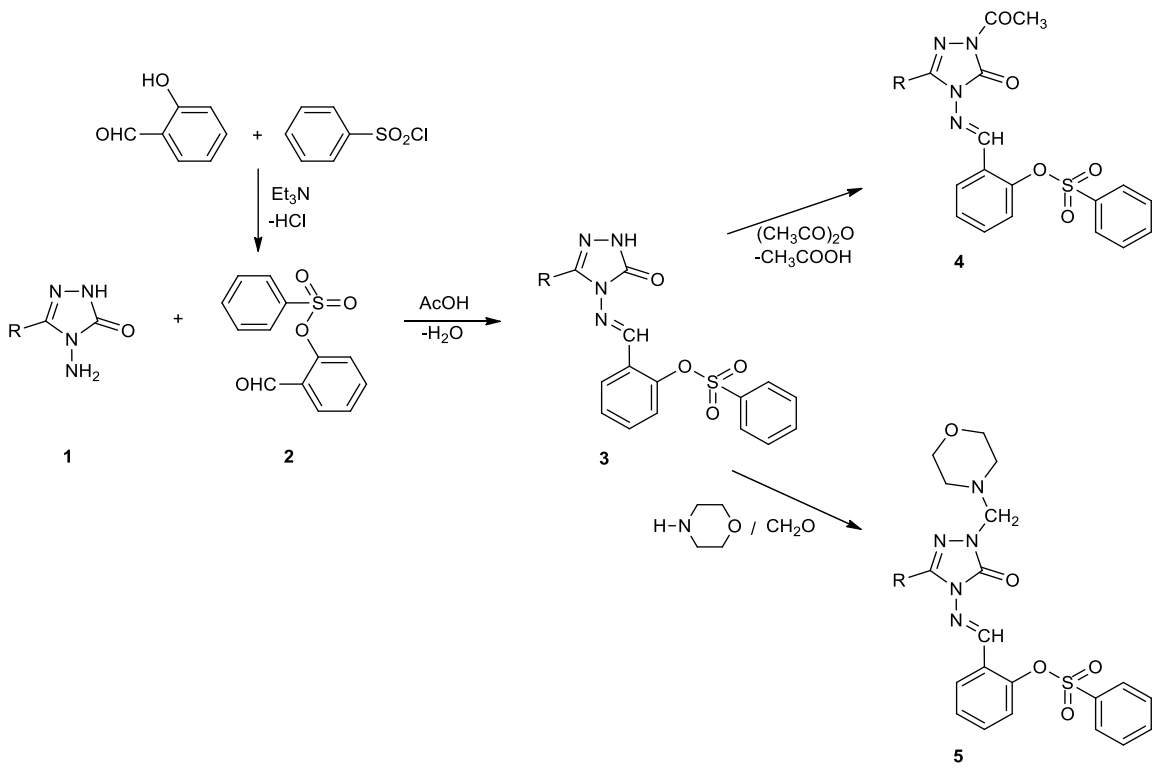
Antimicrobial activity

All bacterial and yeast strains were purchased from the company of Microbiological Environmental Protection Laboratories (France) and were as follows: *Bacillus substilis* (ATCC 11774), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 4352). Simple susceptibility screening test using agar well diffusion method was used [17,18] as explained precisely in the literature [8, 10]. All of the new compounds were weighed and dissolved in DMSO to prepare extract stock solution of 1 mg/ml.

Results and Discussion

Chemistry

In the present study, eight new 2-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl-azomethine)-phenyl benzenesulfonates (**3a-h**), eight new 2-(1-acetyl-3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl-azomethine)phenyl benzenesulfonates (**4a-h**) and seven new 2-[1-(morpholine-4-yl-methyl)-3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl-azomethine]-phenyl benzenesulfonates (**5a-e,g,h**) were synthesized (Scheme-1). The structures of twenty-three newly synthesized compounds were identified with data from infrared, ^{1}H-NMR, ^{13}C-NMR, MS and UV spectroscopy.



a) R = CH_3, b) R = CH_2CH_3, c) R = $CH_2CH_2CH_3$, d) R = $CH_2C_6H_5$, e) R = $CH_2C_6H_4CH_3$ (*p*-), f) R = $CH_2C_6H_4OCH_3$ (*p*-), g) R = $CH_2C_6H_4Cl$ (*p*-), h) R = C_6H_5

Scheme-1: Synthetic route of compounds **2-5.**

Antioxidant activity

The antioxidant activities of twenty-three novel compounds **3a-h**, **4a-h** and **5a-e,g,h** determined. The new compounds showed neither reductive activities nor scavenging effect. However, metal chelating activity was significant since it reduced the concentrations of the catalyzing transition metal. Chelating effects of the new compounds **3**, **4**, **5**, EDTA, α-tocopherol are respectively shown in Figs **1-3**. The data acquired from the figures disclose that the chelating activities of the compounds **5** were concentration-dependent, the other compounds were not. Thus, compounds **5** demonstrate a significant activity for iron-binding. Finally, Mannich bases were found to be most active when compared to Schiff bases for all concentrations.

Antimicrobial activity

Table-I summarizes promising microbiological results of the newly synthesized compounds. The compounds **3b** and **4f** showed the highest zone diameter against *Bacillus cereus*. The other active compounds emphasized by using bold italic characters in Table-I.

Table-I: Antimicrobial activity of the compounds **3**, **4** and **5**.

Compounds	Microorganisms and inhibition zone (mm)					
	Bs	**Bc**	**Pa**	**Kp**	**Sa**	**Ec**
3a	**11**	**14**	**15**	**15**	**12**	**10**
3b	***24***	**15**	***20***	**15**	***17***	***20***
3c	***22***	**15**	***18***	***17***	**12**	**15**
3d	***21***	**12**	**16**	**16**	**15**	**15**
3e	**13**	***17***	**12**	**10**	**8**	**13**
3f	**16**	**12**	**15**	***18***	**16**	**15**
3g	**15**	**14**	**10**	**12**	***17***	**13**
3h	***20***	**13**	**15**	**13**	**15**	**13**
4a	**15**	**10**	**13**	***17***	**12**	**16**
4b	***17***	**12**	**16**	***20***	**16**	***17***
4c	**15**	**10**	**14**	***17***	**13**	**15**
4d	***17***	**13**	**15**	**12**	***17***	**13**
4e	***20***	**12**	**14**	**10**	***18***	**13**
4f	***24***	**15**	***22***	***18***	***21***	***18***
4g	***21***	**14**	***20***	**12**	***23***	***17***
4h	***18***	**12**	***18***	**16**	**14**	**16**
5a	**14**	**14**	**14**	**10**	**9**	**-**
5b	***17***	**14**	***17***	***18***	***19***	**15**
5c	***17***	***21***	***18***	***19***	***17***	**14**
5d	***19***	***17***	**14**	***19***	***17***	**13**
5e	**16**	***20***	**15**	**12**	**13**	**12**
5g	***17***	***17***	**13**	**15**	***18***	**16**
5h	***17***	**13**	**10**	**13**	**14**	**-**
Amp.	**33**	**36**	**36**	**35**	**37**	**34**
Neo.	**17**	**17**	**17**	**16**	**13**	**16**
Str.	**12**	**12**	**12**	**11**	**21**	**10**

Bs: *Bacillus subtilis* (ATCC-11774), Bc: *Bacillus cereus* (ATCC-11778), Pa: *Pseudomonas aeruginosa* (ATCC-27853), Kp: *Klebsiella pneumoniae* (ATCC-4352) Sa: *Staphylococcus aureus* (ATCC-6538), Ec: *Escherichia coli* (ATCC-25922), Amp.: Ampicillin (X3261), Neo.: Neomycin (X3360), Str.: Streptomycin (X3385).

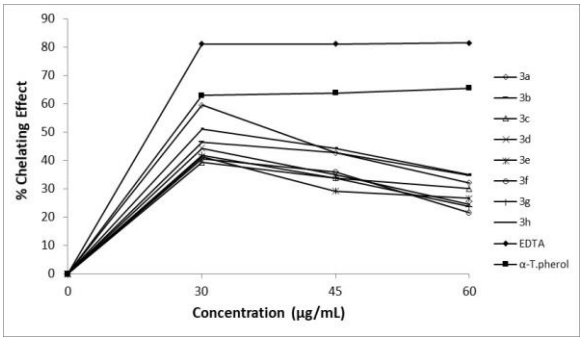


Fig. 1: Metal chelating effect of different amount of the compounds 3, EDTA and α-tocopherol on ferrous ions.

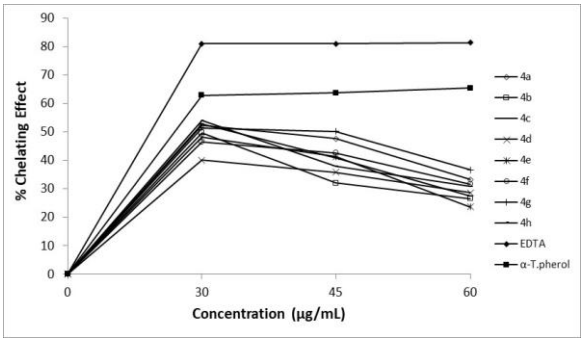


Fig. 2: Metal chelating effect of different amount of the compounds 4, EDTA and α-tocopherol on ferrous ions.

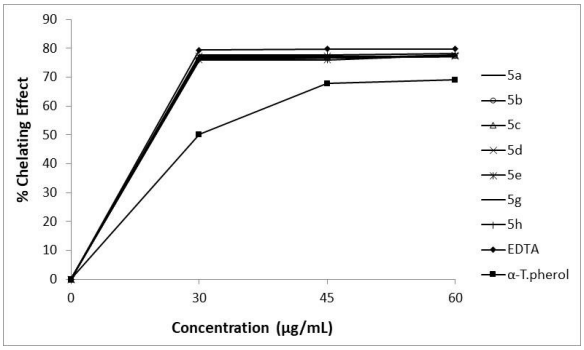


Fig. 3: Metal chelating effect of different amount of the compounds 5, EDTA and α-tocopherol on ferrous ions.

Conclusion

In the present study, new 1,2,4-triazole derivatives (**3a-h, 4a-h** and **5a-e,g,h**) were designed and synthesized. Their structures were identified with data from infrared, ^{1}H-NMR, ^{13}C-NMR, MS and UV spectroscopy. The target compounds were also investigated for their antioxidant and antimicrobial potential. The promising *in vitro* antioxidant and antimicrobial activity of the compound has encouraged us to further study of novel agents.

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