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Synthesis, biological evaluation and molecular docking studies of new 2-ethoxy-4-{[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate derivatives on human aldose reductase enzyme

Gül Özdemir[1] · Namık Kılınç[2] · Sevda Manap[1] Murat Beytur*[1] Muzaffer Alkan[3] Haydar Yüksek[1]

Abstract

A series of 2-ethoxy-4-{[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonates **(3)** were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with 2-ethoxy-4-formyl-phenyl benzenesulfonate (**2**). *N*-acetyl derivatives (**4**) of compounds **3** were also obtained. Then, the compounds **3** have been treated with morpholine and 2,6-dimethylmorpholine in the presence of formaldehyde to synthesize 2-ethoxy-4-{[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)** and 2-ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonates **(6)**, respectively. The structures of twenty-six new compounds were identified by using elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and MS spectral data. In addition, *in vitro* antibacterial activities of the new compounds were evaluated against six bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Klepsiella pneumonia* according to agar well diffusion method. Furthermore, in order to determine the possible antidiabetic properties of the synthesized 1,2,4-triazole derivatives, inhibition effects on the AR enzyme were investigated and molecular docking studies were carried out to determine the receptor-ligand interactions of these compounds. IC_{50} values of triazole-derived compounds (6a, 6b, 6d-g) against AR enzyme were determined as 0.95 µM, 0.75 µM, 1.83 µM, 0.62 µM, 1.05 µM, 1.06 µM, respectively. Considering the docking scores and binding energies obtained docking studies, it has been shown that molecules fit very well to the active site of the AR enzyme.

Key words 4,5-Dihydro-1*H*-1,2,4-triazole · Schiff base · Mannich base · Antibacterial activity · Molecular docking · Aldose reductase

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Introduction

Triazoles are heterocyclic compounds that contain three nitrogen atoms. 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) (Sahu et al. 2013). 1,2,4-Triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have been found to have a broad spectrum of biological activities (Kaczor et al. 2013; Aktaş-Yokuş et al. 2015; Thakkar et al. 2017; Yüksek et al. 1997; Abuelhassan et al. 2018; Khalid et al. 2018).

The classical Mannich reaction, a three-component condensation between structurally diverse substrates containing at least one active hydrogen atom, an aldehyde component and an amine reagent leads to a class of compounds known as Mannich bases (Roman 2015). Mannich bases have applications in the pharmaceutical field and in other industries, such as the petroleum, the cosmetics, the dyes and the food industries, etc. The principal advantage of the Mannich reaction is that it enables two different molecules to be bonded together in one step (Tramontini and Angiolini 1994). Mannich bases acquired from 1,2,4-triazole derivatives are reported to possess biological activities including; antifungal, antioxidant, antilipase and antibacterial properties (Manap et al. 2020; Wang et al. 2017; Ceylan 2016).

Diabetes mellitus (DM), the most common chronic disease known to date, is a disorder affecting approximately 463 million people worldwide, and by 2045 the number of diabetic patients is expected to increase to 700 million (Saeedi et al. 2019). Numerous studies have shown that diabetes mellitus can cause various complications such as cardiovascular complications, diabetic nephropathy, liver complications, neuropathy, and cataract, which are the main causes of morbidity and mortality (Meng et al. 2019). Hyperglycemia is a condition characterized by diabetes and plays an important role in the development and progression of these complications resulting from irreversible long-term damage to biological macromolecules as well as acute and reversible changes in cellular metabolism (Ottana et al. 2011). Under hyperglycemic conditions, autooxidation reactions of glucose promoting intermolecular crosslinking and lipid oxidation produce reactive oxygen species (ROS) that can damage tissues, cellular membranes, and biomolecules (Brownlee 2005). In addition, highly reactive dicarbonyl compounds produced during glucose auto-oxidation reactions lead to the glycation of macromolecules resulting in the synthesis of advanced glycation end products (AGEs) (Jay et al. 2006).

In the case of hyperglycemia, a significant increase in polyol pathway activity increases both cellular osmotic stress and oxidative stress, thus contributing significantly to diabetes-related tissue damage, particularly in insulin-independent tissues of glucose uptake (Giacco and Brownlee 2010). Increased glucose flux along the polyol pathway triggers a range of metabolic events that can result in tissue and vascular damage, decrease in blood flow and decrease nerve conduction rate, thereby critically contributing to the etiology of

DM-related chronic complications (Maccari and Ottanà 2015). Especially in the lens, the accumulation of sorbitol in the cell leads to swelling, membrane permeability changes and osmotic stress, thereby promoting cataractogenesis (Brownlee 2001). In addition, NADPH depletion due to activation of the polyol pathway in hyperglycemic conditions disrupt the activity of NADPH-dependent enzymes such as glutathione reductase and indirectly contributes to the production of intracellular reactive oxygen species (Evans et al. 2002). However, the changing $NADH/NAD^+$ ratio, during the second step of the polyol pathway (conversion of sorbitol to fructose by SDH enzyme), increases the redox imbalance, causing many metabolic and signal pathways to be affected (Williamson et al. 1993).

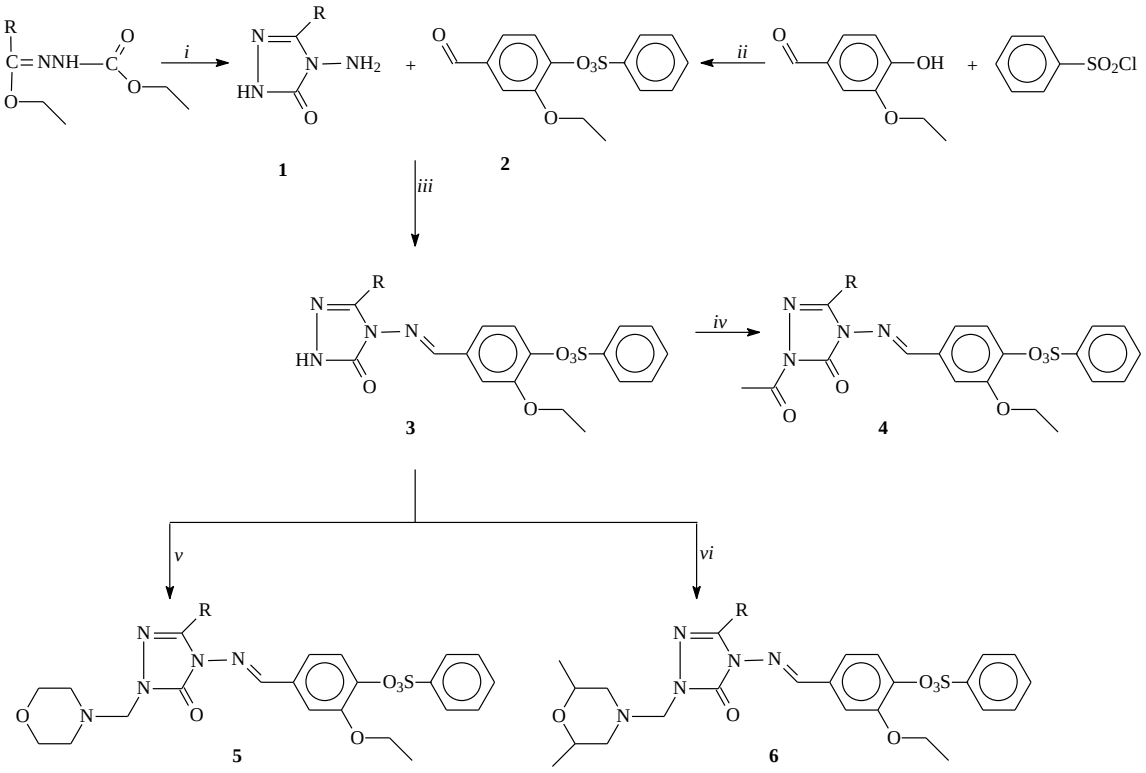
Aldose reductase (EC1.1.1.21) (ALR2), an enzyme detected in the lens, brain, skeletal muscle, liver, and kidney tissue, is an aldo-keto reductase that catalyzes the reduction of glucose to sorbitol using the NADPH cofactor (Maccari et al. 2008). The AR enzyme has been extensively investigated as an enzyme that has been critically involved in the emergence and progression of many pathologies associated with diabetes mellitus, especially in the past four decades. It is clear that the way to onset and delay diabetic complications is to block the activity of the polyol pathway through the inhibition of aldose reductase enzyme. However, there is no worldwide commercially available antidiabetic drug that inhibits the aldose reductase enzyme, yet (except for some eastern countries) (Balestri et al. 2017). So, in our study, the inhibition effects of newly-synthesized triazole compounds on the recombinant human aldose reductase enzyme were investigated and enzyme-inhibitor interactions are computed by molecular docking studies.

Results and Discussion

Chemistry

In the present study, 2-ethoxy-4-{[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonates **(3)** were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with 2-ethoxy-4-formyl-phenyl benzenesulfonate (**2**), which was synthesized by the reaction of 3-ethoxy-4-hydroxybenzaldehyde with benzenesulfonyl chloride by using triethylamine. Then, the reactions of compounds **3a**, **3b**, **3d**, **3e** and **3g** with acetic anhydride were investigated and 2-ethoxy-4-{[1-acetyl-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonates (**4)** were prepared. Finally, the compounds **3a**, **3b**, **3d**, **3e**, **3f** and **3g** were treated morpholine and 2,6-dimethylmorpholine in the presence of formaldehyde to synthesize 2-ethoxy-4-{[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)** and 2-ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonates **(6)** (Özdemir et al. 2018), respectively (Scheeme **1**). The starting compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(1)** were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones with an aqueous solution of hydrazine hydrate as described in the literature (Ikızler and Un 1979; Ikızler and Yüksek 1993).

The structures of newly synthesized compounds were identified by using elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and MS spectral data.



i) $N_2H_4.H_2O$, reflux, 6 h; *ii*) Et_3N; *iii*) AcOH, reflux, 1 h; *iv*) Ac_2O, reflux; *v*) morpholine, EtOH, reflux; *vi*) 2,6-dimethylmorpholine, EtOH, reflux
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 = $CH_2C_6H_4Cl$ (*m*-), i) R = C_6H_5

Scheme **1**. Synthetic route of compounds **1-6**

Antimicrobial Activity

The microbiological results are given in Table 1. Compounds **3** and **6** against to *Bacillus subtilis* and compounds **5** against to *Bacillus cereus* and *Escherichia coli* did not display any antimicrobial activity. The active compounds are emphasized by using bold italic characters in the table. The highest zone diameter was obtained from the compound **3f** against *Bacillus cereus* and compounds **6a** and **6f** against *Escherichia coli*. The compounds **4** did not display any antimicrobial activity against to all tested microorganisms.

Table 1 Antimicrobial activity of the new compounds (**3-6**).

Compound	Microorganisms and inhibition zone (mm)					
	Bs	**Bc**	**Pa**	**Kp**	**Sa**	**Ec**
3a	-	8	11	7	12	9
3b	-	10	13	7	7	11
3c	-	14	16	10	8	14
3d	-	7	8	7	8	12
3e	-	16	14	8	7	16
3f	-	***21***	16	8	7	16
3g	-	15	***17***	9	7	11
3h	-	***17***	16	10	10	***18***
3i	-	8	9	9	7	11
5a	-	-	9	14	9	-
5b	10	-	-	15	10	-
5d	-	-	11	11	11	-
5e	-	-	-	10	13	-
5f	11	-	-	13	10	-
5g	7	-	-	12	12	-
6a	-	14	***18***	7	-	***21***
6b	-	***17***	9	8	9	14
6d	-	10	12	7	8	***17***
6e	-	12	14	7	11	***18***
6f	-	14	**17**	9	7	***20***
6g	-	***18***	13	8	8	13
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).

In Vitro Inhibition and Molecular Docking Studies

In the present study, the inhibition effects of newly synthesized compounds **6a**, **6b**, **6d-g** on recombinant human aldose reductase enzyme were investigated. IC_{50} and K_i values of newly synthesized triazole compounds were calculated, and inhibition types were determined from

K_i plots. IC_{50} values of compounds **6a**, **6b**, **6d-g** were determined as 0.95 µM, 0.75 µM, 1.83 µM, 0.62 µM, 1.05 µM, 1.06 µM, respectively. K_i values were calculated as 0,42±0,09 µM, 0,74±0,49 µM, 1,43±0,41 µM, 0,27±0,02 µM, 0,68±0,33 µM, 2,04±0,35 µM, respectively. The inhibition type of the compounds **6a** and **6f** were competitive, the inhibition type of the compound **6e** was uncompetitive, and the inhibition type of the other compounds was determined as non-competitive (Table 2). Molecular docking studies were also conducted to support and verify experimental inhibition studies. Molecular docking studies showed that compounds **6a**, **6e** and **6f** were the compounds that best interacted with the active site of the AR enzyme, according to docking scores and binding energies. It has been shown that the molecular docking results are highly correlated with the experimental results and the inhibition mechanism is valid.

Table 2 Cumulative in vitro inhibition results of newly synthesized compounds **6a**, **6b**, **6d-g** on recombinant human AR enzyme

Compound	IC_{50} (µM)	Ki (µM)	Inhibition Type
6a	0,95	0,42±0,09	Competitive
6b	0,75	0,74±0,49	Noncompetitive
6d	1,83	1,43±0,41	Noncompetitive
6e	0,62	0,27±0,02	Uncompetitive
6f	1,05	0,68±0,33	Competitive
6g	1,06	2,04±0,35	Noncompetitive

One of the most widely used docking validations is the re-docking method (Yuriev et al. 2011). In this method, the ligand is extracted from the receptor and re-dock to the respective protein. In this study, co-crystallized ligand tolrestat was extracted from the receptor and re-docked into the active site of the AR enzyme. After docking, the best pose of the tolrestat was aligned with the co-crystalized ligand and RMSD (Root Mean Square Deviation) of the ligand was calculated. RMSD value less than 2 Å proves the accuracy of the docking protocol (Wang et al. 2003). When the best pose of the tolrestat conformation superimposed with the co-crystallized ligand, RMSD value determined as 0,9812 Å (Fig. 3).

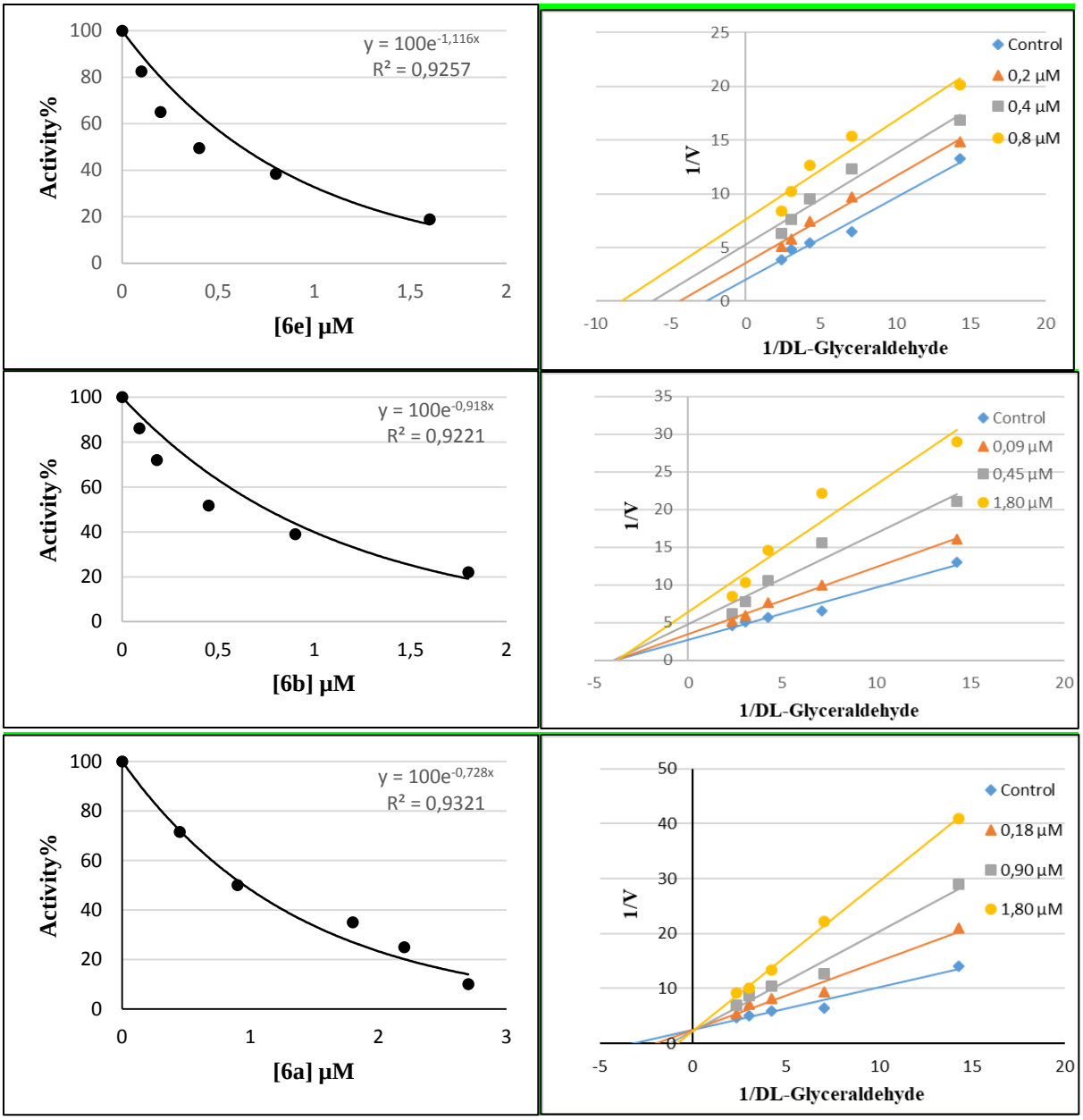


Fig. 1 IC_{50} (left) and K_i plots (right) of compounds **6e**, **6b** and **6a** showing the best in vitro inhibition effect against the aldose reductase enzyme

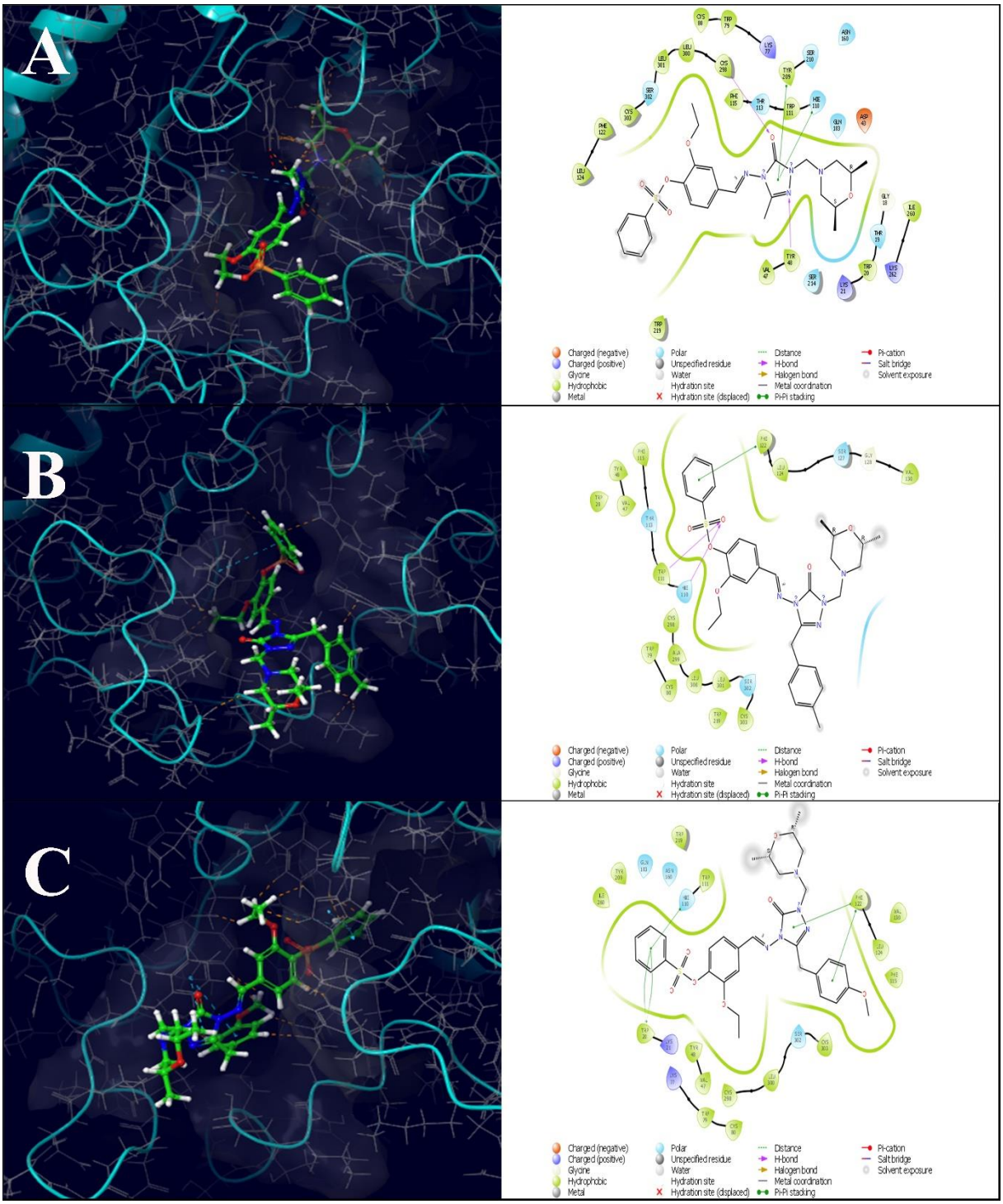


Fig. 2 Docking pose (left) and 2D ligand-receptor interaction diagram (right) of best-scored compounds **6a** (A), **6e** (B) and **6f** (C). Ligand binding sites are represented as solid surfaces

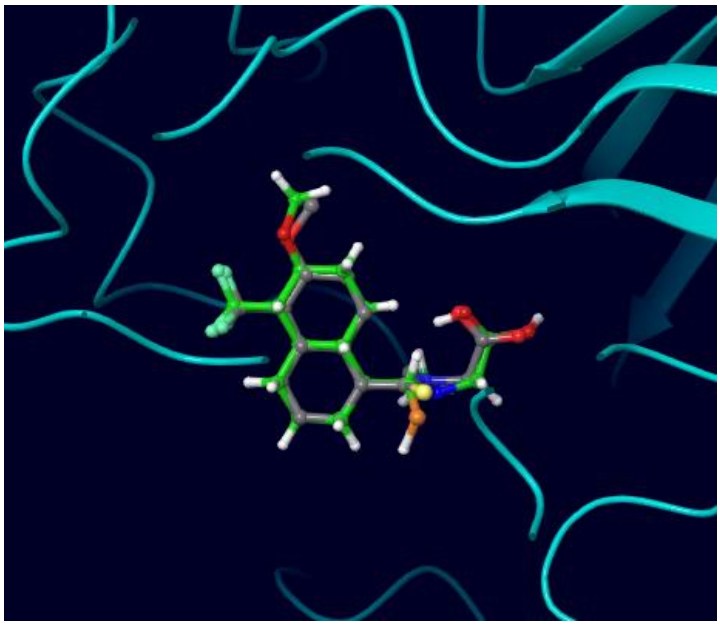


Fig. 3 Docking validation of tolrestat adduct AR enzyme. AR receptor is depicted in the ribbon model. The co-crystallized ligand is represented in gray ball and stick modeling, while the re-docked ligand is shown in green ball and stick modeling.

In recent years, triazoles and their heterocyclic derivatives have attracted great attention due to their potent pharmacological properties. Numerous triazole compounds have often been used as clinical drugs or candidates for the treatment of various types of diseases, showing wide potential as medicinal agents (Zhou and Wang 2012). It has been shown in many studies that triazole derivative compounds have antioxidant (Barbuceanu et al. 2014), anticancer (Tyagi et al. 2015), antibacterial (Almajan et al. 2009), antitubercular (Shaikh et al. 2016), analgesic (Vijesh et al. 2013), antifungal (Wu et al. 2018), anti-inflammatory (Khan et al. 2020), anticonvulsant (Sari et al. 2018), and antiviral (Chen et al. 2019) properties. Although the antidiabetic properties of triazole derivatives are known, there is no experimental study on the synthesis of triazole compounds targeting the aldose reductase enzyme. Therefore, in this study, we synthesized six new triazole-derived compounds (**6a**, **6b**, **6d-g**) and examined the inhibition effects of these compounds on the diabetes mellitus related enzyme aldose reductase. IC_{50} values of compounds **6a**, **6b**, **6d-g** determined as 0.95 µM, 0.75 µM, 1.83 µM, 0.62 µM, 1.05 µM, 1.06 µM, respectively. The inhibition results show that all compounds synthesized inhibited the aldose reductase enzyme at micromolar and sub-micromolar concentrations. According to the inhibition results, the best potential inhibitor for AR enzyme was compound **6e** (IC_{50}= 0,62 µM), while compound **6d** (IC_{50}= 1,83 µM) showed the lowest inhibition effect. In addition to determining the IC_{50} values of the synthesized compounds, K_i values, which are an expression of the inhibitors' affinity for the enzyme, were also determined. K_i values of the synthesized triazole derivative compounds **6a**, **6b**, **6d-g** were determined as 0,42±0,09 µM, 0,74±0,49 µM, 1,43±0,41 µM, 0,27±0,02 µM, 0,68±0,33 µM, 2,04±0,35 µM, respectively. According to these results, it is clear that the inhibitor with the highest affinity for the AR enzyme is compound **6e**. It is seen that the determined K_i constants are correlated with the inhibition results.

Molecular docking studies were also conducted to theoretically verify the experimentally obtained inhibition results and to elucidate the inhibition mechanisms by determining the inhibitor-receptor interactions. Therefore, the newly synthesized triazole derivatives were docked against aldose reductase enzyme (PDB code: 2FZD) (AR-tolrestat adduct). With docking studies, docking score, XP GScore (Extra precision glide score), glide evdw (Van der Waals energy), glide ecoul (coulomb energy), glide energy (Model energy) and glide emodel (Modified Coulomb-van der Waals interaction energy) values of the compounds were determined (Table 3). Considering the docking scores and binding energies, it is observed that the compound that interacts best with the active site of the AR enzyme is **6a**, which has the lowest IC_{50} value with competitive inhibition type. The compound **6a** made hydrogen bonding with the amino acid residues TYR48 and CYS298 and pi-pi stacking with the amino acid residues HIS110 and TYR209 in the active pocket of the AR enzyme (Fig. 2A). It has been confirmed that compound **6e**, which shows the best inhibitory effect experimentally, is one of the best inhibitor candidates, considering the docking score and binding energies.

In order to emphasize how important the results obtained from docking studies for the synthesized triazole derivative compounds, docking studies were also performed for well-known AR enzyme inhibitors tolrestat, sorbinil, and zopolrestat. It shows that the compounds **6a**, **6b**, **6e** and **6f**, which have very good inhibitory effects against the AR, interact better with the catalytic active site of the AR enzyme than both sorbinil and zopolrestat (Table 3).

Table 3 Molecular docking scores and binding energies of compounds **6a**, **6b**, **6d-g** with AR enzyme and comparisons with reference inhibitors tolrestat, sorbinil and zopolrestat

Compound	Docking Score	XP GScore	Glide Δvdw	Glide Δcoulomb	Glide Energy	Glide Emodel
6a	-8,85	-8,85	-41,68	-1,12	-42,81	-81,73
6b	-7,97	-7,97	-48,85	-0,14	-48,99	-61,17
6d	-6,79	-6,79	-41,11	-4,14	-45,23	-64,26
6e	-8,31	-8,31	-52,91	-3,99	-56,91	-92,35
6f	-8,11	-8,11	-53,23	-1,48	-54,71	-86,28
6g	-7,38	-7,38	-46,52	-0,21	-46,72	-89,76
Tolrestat	-12,03	-12,16	-20,69	-12,87	-33,57	-37,88
Sorbinil	-7,81	-7,86	-28,89	-2,15	-31,05	-43,59
Zopolrestat	-7,46	-7,46	-36,75	-10,01	-46,77	-68,31

Conclusion

In the present study, new 1,2,4-triazole derivatives (**3-6**) were designed and synthesized. Their structures were identified using elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. The target compounds were also investigated for their antimicrobial potential. In addition, the inhibition effects of the six 1,2,4-triazole compounds on the AR enzyme were

examined and docking studies were carried out to determine the inhibitor-receptor interactions. Inhibition studies have shown that all of the compounds investigated, especially compound **6e**, are strong potential AR enzyme inhibitors. Docking studies of the investigated compounds against the AR enzyme show that the compounds fitted in the active site of the enzyme very well, compared to some well-known standard AR inhibitors and confirmed the experimental inhibition results and mechanism of inhibition.

Experimental

Chemistry

Chemical reagents used in this paper were bought from Merck AG, Aldrich and Fluka. Recombinant human aldose reductase enzyme, β-nicotinamide adenin dinucleotide phosphate (β-NADPH), DL-Glyceraldehyde, and sodium phosphate were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Melting points were taken using an Electrothermal Melting-point Apparatus in an open capillary tube and were not corrected. The infrared spectra were recorded on a Perkin Elmer Instruments Spectrum One FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined in deuterated dimethyl sulfoxide with TMS as internal standard using a Bruker Ultrashield spectrophotometer at 400 MHz and 100 MHz, respectively. Elemental analyses were carried out on a LECO, CHNS-932 for C, H, and N. Electrospray ionisation mass spectrometry (ESI-MS) was performed on a TSQ Quantum Access Max Triple Stage Quadrupole Mass Spectrometer.

General procedure for the synthesis of 2-ethoxy-4-{[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonates (3) 3-Ethoxy-4-hydroxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (30 mL) was reacted with benzenesulfonyl chloride (0.01 mol), and to this solution was added triethylamine (0.01 mol) in 10 mL ethyl acetate slowly by stirring at 0-5 °C. Stirring was continued for 2 hours; then the mixture was refluxed for 3 hours and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from ethanol to afford novel compound **2**. White solid; yield 88%; m.p. 73-75 °C. IR (cm^{-1}): 2848 and 2749 (CHO), 1692 (C=O), 1371 and 1195 (SO_2), 746 and 696 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.96 (s, 1H, CHO), 7.87-7.81 (m, 3H, Ar-H), 7.70-7.66 (m, 2H, Ar-H), 7.57 (dd, 1H, Ar-H, J = 8.40, 2.00 Hz), 7.51 (m, 1H, Ar-H), 7.44 (d, 1H, Ar-H, J = 8.40 Hz), 3.87 (q, 2H, OCH_2CH_3, J = 7.20 Hz), 1.11 (t, 3H, OCH_2CH_3, J = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.93 (CHO), [151.15, 141.81, 135.91, 135.07, 134.94, 129.52 (2C), 128.10 (2C), 124.51, 122.76, 113.41] (ar-C), 64.20 ($O\underline{C}H_2CH_3$), 13.97 ($OCH_2\underline{C}H_3$). The corresponding compound **1** (0.01 mol) was dissolved in ethanoic acid (20 mL) and by treated 2-ethoxy-4-formyl-phenyl benzenesulfonate (**2**) (0.01 mol). The mixture was refluxed for 1.5 hours and then evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from ethanol gave pure compounds **3a-i** as uncolored crystals.

2-Ethoxy-4-[(3-methyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethine]-phenyl benzenesulfonate (3a) White solid; yield: 98 %; m.p. 180-182 °C. IR (cm^{-1}): 3176 (NH), 1707 (C=O), 1597 (C=N), 1371 and 1183 (SO_2), 746 and 692 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.84 (s, 1H, NH), 9.68 (s, 1H, N=CH), 7.86-7.82 (m, 3H, Ar-H), 7.69-7.66 (m, 2H, Ar-H), 7.47 (s, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 7.30 (d, 1H, Ar-H, *J* = 8.40 Hz), 3.83 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), 2.27 (s, 3H, CH_3), 1.10 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.35 (Triazole C_5), 150.86 (N=CH), 144.28 (Triazole C_3), [151.15, 139.48, 135.13, 134.84, 133.77, 129.47 (2C), 128.14 (2C), 124.26, 119.97, 112.76] (ar-C), 64.04 ($O\underline{C}H_2CH_3$), 14.04 ($OCH_2\underline{C}H_3$), 11.03 (CH_3); MS (70 eV): *m/z* (%) 115.09 (100), 143.11 (20), 146.10 (8), 288.23 (8), 403.07 (M+1, 9), 444.11 (24), 805.16 (2M+1, 20); Anal. Calcd. for $C_{18}H_{18}N_4O_5S$: C, 53.72; H, 4.51; N, 13.92. Found: C, 53.55; H, 4.44; N, 13.59.

2-Ethoxy-4-[(3-ethyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethine]-phenyl benzenesulfonate (3b) White solid; yield: 97 %; m.p. 179-181 °C. IR (cm^{-1}): 3197 (NH), 1698 (C=O), 1597 (C=N), 1359 and 1177 (SO_2), 751 and 701 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.87 (s, 1H, NH), 9.68 (s, 1H, N=CH), 7.86-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.46 (s, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 7.30 (d, 1H, Ar-H, *J* = 8.08 Hz), 3.83 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 2.68 (q, 2H, CH_2CH_3, *J* = 7.60 Hz), 1.20 (t, 3H, CH_2CH_3, *J* = 7.20 Hz), 1.10 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.36 (Triazole C_5), 150.87 (N=CH), 148.04 (Triazole C_3), [151.28, 139.48, 135.16, 134.83, 133.80, 129.47 (2C), 128.13 (2C), 124.28, 119.85, 112.80] (ar-C), 64.01 ($O\underline{C}H_2CH_3$), 18.43 ($\underline{C}H_2CH_3$), 14.03 ($OCH_2\underline{C}H_3$), 9.94 ($CH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.12 (100), 143.14 (35), 146.10 (7), 288.19 (8), 416.97 (M+1, 5), 458.10 (12), 855.19 (2M+1, 7); Anal. Calcd. for $C_{19}H_{20}N_4O_5S$: C, 54.80; H, 4.84; N, 13.45. Found: C, 54.79; H, 4.83; N, 12.98.

2-Ethoxy-4-{[3-(*n*-propyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (3c) White solid; yield: 96 %; m.p. 144-146 °C. IR (cm^{-1}): 3169 (NH), 1701 (C=O), 1577 (C=N), 1366 and 1199 (SO_2), 750 and 686 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.87 (s, 1H, NH), 9.69 (s, 1H, N=CH), 7.87-7.82 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.46 (s, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 7.31 (d, 1H, Ar-H, *J* = 8.80 Hz), 3.84 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 2.64 (t, 2H, $\underline{CH}_2CH_2CH_3$; *J* = 7.20 Hz), 1.68 (sext, 2H, $CH_2\underline{CH}_2CH_3$; *J* = 7.20 Hz), 1.10 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz), 0.95 (t, 3H, $CH_2CH_2CH_3$; *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.35 (Triazole C_5), 150.86 (N=CH), 146.93 (Triazole C_3), [151.28, 139.48, 135.16, 134.84, 133.80, 129.48 (2C), 128.13 (2C), 124.32, 119.77, 112.87] (ar-C), 64.00 ($O\underline{C}H_2CH_3$), 26.64 ($\underline{C}H_2CH_2CH_3$), 18.92 ($CH_2\underline{C}H_2CH_3$), 14.03 ($OCH_2\underline{C}H_3$), 13.49 ($CH_2CH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.12 (100), 143.04 (23), 146.10 (7), 288.26 (7), 431.05 (M+1, 27), 472.10 (70); Anal. Calcd. for $C_{20}H_{22}N_4O_5S$: C, 55.80; H, 5.15; N, 13.02. Found: C, 55.67; H, 5.10; N. 13.04.

2-Ethoxy-4-[(3-benzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethine]-phenyl benzenesulfonate (3d) White solid; yield: 95 %; m.p. 181-183 °C. IR (cm^{-1}): 3169 (NH),

1704 (C=O), 1576 (C=N), 1347 and 1195 (SO_2), 755 and 704 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.02 (s, 1H, NH), 9.64 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.39 (m, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.31-7.27 (m, 5H, Ar-H), 7.25-7.21 (m, 1H, Ar-H), 4.06 (s, 2H, CH_2Ph), 3.80 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 1.12 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.53 (Triazole C_5), 150.81 (N=CH), 146.16 (Triazole C_3), [151.14, 139.50, 135.13, 134.83, 133.73, 129.47 (2C), 128.14 (2C), 124.25, 120.53, 111.94] (ar-C), [135.82, 128.65 (2C), 128.42 (2C), 126.68] (Ar-C linked C-3), 63.98 ($O\underline{C}H_2CH_3$), 31.16 (CH_2Ph), 14.03 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.11 (100), 143.14 (64), 146.04 (14), 288.18 (5), 479.14 (M+1, 9).

2-Ethoxy-4-{[3-(*p*-methylbenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (3e) White solid; yield: 98 %; m.p. 146-148 °C. IR (cm^{-1}): 3163 (NH), 1701 (C=O), 1573 (C=N), 1348 and 1194 (SO_2), 825 (1,4-disubstituted benzenoid ring), 760 and 701 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.99 (s, 1H, NH), 9.63 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.39 (m, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.29 (d, 1H, Ar-H, *J* = 8.40 Hz), 7.19 (d, 2H, ArH; *J* = 8.00 Hz), 7.09 (d, 2H, ArH; *J* = 7.60 Hz), 4.00 (s, 2H, CH_2Ph), 3.81 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 2.24 (s, 3H, PhCH_3), 1.12 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.77 (Triazole C_5), 150.81 (N=CH), 146.30 (Triazole C_3), [151.16, 139.50, 135.13, 134.83, 133.75, 129.47 (2C), 128.14 (2C), 124.25, 120.54, 111.92] (ar-C), [135.73, 132.71, 128.94 (2C), 128.51 (2C)] (Ar-C linked C-3), 63.97 ($O\underline{C}H_2CH_3$), 30.77 (CH_2Ph), 20.57 (PhCH_3), 14.03 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.12 (100), 143.10 (38), 146.04 (5), 288.18 (5), 493.13 (M+1, 7).

2-Ethoxy-4-{[3-(*p*-methoxylbenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (3f) White solid; yield: 95 %; m.p. 172-174 °C. IR (cm^{-1}): 3209 (NH), 1696 (C=O), 1595 (C=N), 1366 and 1174 (SO_2), 856 (1,4-disubstituted benzenoid ring), 755 and 685 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.98 (s, 1H, NH), 9.64 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.66-7.64 (m, 2H, Ar-H), 7.42 (m, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.30 (d, 1H, Ar-H, *J* = 8.00 Hz), 7.22 (d, 2H, ArH; *J* = 8.80 Hz), 6.85 (d, 2H, ArH; *J* = 8.80 Hz), 3.98 (s, 2H, CH_2Ph), 3.82 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 3.71 (s, 3H, OCH_3), 1.12 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.87 (Triazole C_5), 150.83 (N=CH), 146.46 (Triazole C_3), [151.15, 139.50, 135.14, 134.83, 133.76, 129.47 (2C), 128.14 (2C), 124.27, 120.47, 112.05] (ar-C), [158.06, 129.72 (2C), 127.57, 113.85 (2C)] (Ar-C linked C-3), 63.99 ($O\underline{C}H_2CH_3$), 55.01 (PhCH_3), 30.29 (CH_2Ph), 14.03 ($OCH_2\underline{C}H_3$ MS (70 eV): *m/z* (%) 115.10 (100), 143.08 (18), 146.02 (5), 509.10 (M+1, 7).

2-Ethoxy-4-{[3-(*p*-chlorobenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (3g) White solid; yield: 98 %; m.p. 170-172 °C. IR (cm^{-1}): 3169 (NH), 1704 (C=O), 1587 (C=N), 1370 and 1174 (SO_2), 845 (1,4-disubstituted benzenoid ring), 752 and 687 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.03 (s, 1H, NH), 9.64 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H),

7.40-7.34 (m, 6H, Ar-H), 7.29 (d, 1H, Ar-H, *J* = 8.40 Hz), 4.07 (s, 2H, CH_2Ph), 3.79 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 1.12 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.97 (Triazole C_5), 150.82 (N=CH), 145.80 (Triazole C_3), [151.12, 139.53, 135.12, 134.82, 133.68, 129.46 (2C), 128.14 (2C), 124.26, 120.53, 111.99] (ar-C), [134.82, 131.37, 130.56 (2C), 128.36 (2C)] (Ar-C linked C-3), 63.98 ($O\underline{C}H_2CH_3$), 30.48 (CH_2Ph), 14.04 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.09 (100), 143.07 (42), 146.03 (14), 288.18 (4), 513.03 (M, 2); Anal. Calcd. for $C_{24}H_{21}N_4O_5SCl$: C, 56.20; H, 4.13; N, 10.92. Found: C, 56.05; H, 4.24; N. 10.29.

2-Ethoxy-4-{[3-(*m*-chlorobenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (3h) White solid; yield: 97 %; m.p. 177-179 °C. IR (cm^{-1}): 3166 (NH), 1705 (C=O), 1576 (C=N), 1350 and 1194 (SO_2), 817 and 701 (1,3-disubstituted benzenoid ring), 754 and 691 (monosubstituted benzenoid ring). ^1H NMR (400 MHz, DMSO-d_6): δ 12.04 (s, 1H, NH), 9.64 (s, 1H, N=CH), 7.84-7.80 (m, 3H, Ar-H), 7.68-7.64 (m, 2H, Ar-H), 7.39-7.37 (m, 2H, Ar-H), 7.33-7.25 (m, 5H, Ar-H), 4.09 (s, 2H, CH_2Ph), 3.81 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), 1.11 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 151.97 (Triazole C_5), 150.85 (N=CH), 145.65 (Triazole C_3), [151.09, 139.55, 135.10, 134.85, 133.67, 129.47 (2C), 128.14 (2C), 124.23, 120.68, 111.84] (ar-C), [138.26, 132.94, 130.26, 128.73, 127.41, 126.74] (Ar-C linked C-3), 64.00 ($O\underline{C}H_2CH_3$), 30.70 (CH_2Ph), 14.04 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.09 (100), 122.50 (32), 143.05 (98), 146.03 (14), 221.96 (17), 262.99 (35), 304.04 (12), 339.16 (13), 379.15 (13), 514.31 (M+1, 3); Anal. Calcd. for $C_{24}H_{21}N_4O_5SCl$: C, 56.20; H, 4.13; N, 10.92. Found: C, 56.18; H, 4.07; N. 10.82.

2-Ethoxy-4-[(3-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethine]-phenyl benzenesulfonate (3i) White solid; yield: 95 %; m.p. 165-167 °C. IR (cm^{-1}): 3154 (NH), 1694 (C=O), 1581 (C=N), 1368 and 1179 (SO_2), 751 and 685 (monosubstituted benzenoid ring). ^1H NMR (400 MHz, DMSO-d_6): δ 12.41 (s, 1H, NH), 9.65 (s, 1H, N=CH), 7.91-7.89 (m, 2H, Ar-H), 7.86-7.80 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.54-7.52 (m, 3H, Ar-H), 7.46-7.42 (m, 2H, Ar-H), 7.32 (d, 1H, Ar-H, *J* = 8.40 Hz), 3.82 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 1.09 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.64 (Triazole C_5), 150.86 (N=CH), 146.56 (Triazole C_3), [151.25, 139.65, 135.18, 134.84, 133.62, 129.49 (2C), 128.12 (2C), 124.40, 120.24, 112.71] (ar-C), [130.14, 128.46 (2C), 128.00 (2C), 126.52] (Ar-C linked C-3), 63.95 ($O\underline{C}H_2CH_3$), 14.01 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.11 (100), 143.14 (72), 146.04 (24), 288.21 (65), 316.23 (26), 465.01 (M+1, 9), 506.03 (18), 554.52 (10).

General procedure for the Synthesis of 2-ethoxy-4-{[1-acetyl-3-alkyl(aryl)-4,5-dihydro-1*H*-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 0.5 h. After addition of absolute ethanol (50 mL), the mixture was refluxed for 1 h. Evaporation of the resulting solution at 40-45 °C *in vacuo* and several recrystallizations of the residue from an appropriate solvent gave pure compounds **4**.

2-Ethoxy-4-[(1-acetyl-3-methyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethine]-phenyl benzenesulfonate (4a) White solid; yield: 91 %; m.p. 204-206 °C. IR (cm^{-1}): 1761, 1692 (C=O), 1620, 1578 (C=N), 1379 and 1170 (SO_2), 753 and 689 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.56 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.52 (m, 1H, Ar-H), 7.49 (dd, 1H, ArH; *J* = 8.40, 2.00 Hz), 7.32 (d, 1H, Ar-H, *J* = 8.00 Hz), 3.84 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), 2.49 (s, 3H, $COCH_3$), 2.34 (s, 3H, CH_3), 1.10 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.97 ($\underline{C}OCH_3$), 154.47 (Triazole C_5), 147.77 (N=CH), 146.67 (Triazole C_3), [150.93, 139.85, 135.10, 134.88, 133.23, 129.50 (2C), 128.14 (2C), 124.36, 120.43, 113.00] (ar-C), 64.10 ($O\underline{C}H_2CH_3$), 23.39 ($CO\underline{C}H_3$), 14.03 ($OCH_2\underline{C}H_3$), 11.16 (CH_3); MS (70 eV): *m/z* (%) 115.10 (100), 143.06 (24), 146.06 (6), 445.05 (M+1, 3), 508.08 (12); Anal. Calcd. for $C_{20}H_{20}N_4O_6S$: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.16; H, 4.50; N. 12.27.

2-Ethoxy-4-[(1-acetyl-3-ethyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethine]-phenyl benzenesulfonate (4b) White solid; yield: 97 %; m.p. 154-156 °C. IR (cm^{-1}): 1763, 1690 (C=O), 1619, 1577 (C=N), 1367 and 1181 (SO_2), 751 and 691 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.55 (s, 1H, N=CH), 7.86-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 751-7.48 (m, 2H, Ar-H), 7.32 (d, 1H, Ar-H, *J* = 8.40 Hz), 3.84 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 2.75 (q, 2H, CH_2CH_3, *J* = 7.20 Hz), 2.50 (s, 3H, $COCH_3$), 1.24 (t, 3H, CH_2CH_3, *J* = 7.20 Hz), 1.10 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.94 ($\underline{C}OCH_3$), 154.49 (Triazole C_5), 150.16 (N=CH), 148.00 (Triazole C_3), [150.93, 139.85, 135.12, 134.87, 133.25, 129.50 (2C), 128.13 (2C), 124.39, 120.32, 113.03] (ar-C), 64.09 ($O\underline{C}H_2CH_3$), 23.41 ($CO\underline{C}H_3$), 18.52 ($\underline{C}H_2CH_3$), 14.02 ($OCH_2\underline{C}H_3$), 9.40 ($CH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.08 (100), 143.06 (57), 459.02 (M+1, 32), 522.03 (18); Anal. Calcd. for $C_{21}H_{22}N_4O_6S$: C, 55.01; H, 4.84; N, 12.22. Found: C, 54.94; H, 4.77; N. 11.91.

2-Ethoxy-4-[(1-acetyl-3-benzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethine]-phenyl benzenesulfonate (4d) White solid; yield: 90 %; m.p. 133-135 °C. IR (cm^{-1}): 1726, 1706 (C=O), 1606, 1577 (C=N), 1386 and 1196 (SO_2), 777 and 692 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.43-7.25 (m, 8H, Ar-H), 4.14 (s, 2H, CH_2Ph), 3.79 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 2.49 (s, 3H, $COCH_3$), 1.12 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.95 ($\underline{C}OCH_3$), 153.77 (Triazole C_5), 148.16 (N=CH), 147.93 (Triazole C_3), [150.87, 139.85, 135.10, 134.87, 133.21, 129.49 (2C), 128.13 (2C), 124.34, 121.01, 112.12] (ar-C), [134.72, 128.84 (2C), 128.48 (2C), 126.93] (Ar-C linked C-3), 64.05 ($O\underline{C}H_2CH_3$), 31.10 (CH_2Ph), 23.48 ($CO\underline{C}H_3$), 14.01 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.10 (100), 143.07 (98), 146.03 (8), 267.17 (8), 288.14 (6), 521.11 (M+1, 4), 584.07 (6).

2-Ethoxy-4-{[1-acetyl-3-(*p*-methylbenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (4e) White solid; yield: 83 %; m.p. 174-176 °C. IR (cm^{-1}): 1754, 1722 (C=O), 1602, 1577 (C=N), 1369 and 1196 (SO_2), 791 (1,4-disubstituted

benzenoid ring), 756 and 687 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.43-7.39 (m, 2H, Ar-H), 7.30 (d, 1H, Ar-H, J = 8.40 Hz), 7.23 (d, 2H, ArH; J = 8.00 Hz), 7.11 (d, 2H, ArH; J = 7.60 Hz), 4.08 (s, 2H, CH_2Ph), 3.80 (q, 2H, OCH_2CH_3, J = 6.80 Hz), 2.50 (s, 3H, $COCH_3$), 2.25 (s, 3H, $PhCH_3$), 1.12 (t, 3H, OCH_2CH_3, J = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.96 ($\underline{C}OCH_3$), 153.75 (Triazole C_5), 148.29 (N=CH), 147.92 (Triazole C_3), [150.87, 139.85, 135.12, 134.87, 133.22, 129.49 (2C), 128.14 (2C), 124.34, 121.03, 112.11] (ar-C), [136.03, 131.57, 128.71 (2C), 128.51 (2C)] (Ar-C linked C-3), 64.04 ($O\underline{C}H_2CH_3$), 30.72 (CH_2Ph), 23.47 ($CO\underline{C}H_3$), 20.58 ($PhCH_3$), 14.02 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.09 (100), 143.07 (70), 146.03 (18), 535.09 (M+1, 42), 552.10 (18), 598.09 (30).

2-Ethoxy-4-{[1-acetyl-3-(*p*-chlorobenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (4g) White solid; yield: 85 %; m.p. 143-145 °C. IR (cm^{-1}): 1726 (C=O), 1600, 1576 (C=N), 1369 and 1181 (SO_2), 849 (1,4-disubstituted benzenoid ring), 761 and 692 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.53 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.43-7.36 (m, 6H, Ar-H), 7.29 (d, 1H, Ar-H, J = 8.40 Hz), 4.16 (s, 2H, CH_2Ph), 3.79 (q, 2H, OCH_2CH_3, J = 6.80 Hz), 2.50 (s, 3H, $COCH_3$), 1.11 (t, 3H, OCH_2CH_3, J = 6.80 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.93 ($\underline{C}OCH_3$), 153.85 (Triazole C_5), 147.92 (N=CH), 147.84 (Triazole C_3), [150.88, 139.87, 135.09, 134.87, 133.17, 129.49 (2C), 128.14 (2C), 124.34, 121.01, 112.15] (ar-C), [133.75, 131.63, 130.76 (2C), 128.40 (2C)] (Ar-C linked C-3), 64.05 ($O\underline{C}H_2CH_3$), 30.43 (CH_2Ph), 23.46 ($CO\underline{C}H_3$), 14.02 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 115.09 (100), 143.08 (98), 146.03 (28), 288.17 (29), 554.60 (M, 9), 618.04 (15); Anal. Calcd. for $C_{26}H_{23}N_4O_6SCl$: C, 56.27; H, 4.18; N, 10.09. Found: C, 56.35; H, 4.15; N. 9.74.

General procedure for the synthesis of 2-ethoxy-4-{[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) Compound **3** (5 mmol) was dissolved absolute ethanol and to this solution were added formaldehyde (37%, 10 mmol) and morpholine (6 mmol). The reaction mixture was refluxed for 4 hours. 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 recrystallized from ethanol gave pure compounds **5** as colorless crystals.

2-Ethoxy-4-{[1-(morpholine-4-yl-methyl)-3-methyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (5a) White solid; yield: 75 %; m.p. 102-104 °C. IR (cm^{-1}): 1704 (C=O), 1610, 1576 (C=N), 1377 and 1197 (SO_2), 760 and 689 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.66 (s, 1H, N=CH), 7.85-7.80 (m, 3H, Ar-H), 7.69-7.66 (m, 2H, Ar-H), 7.49 (s, 1H, Ar-H), 7.47 (dd, 1H, ArH; J = 8.40, 2.00 Hz), 7.31 (d, 1H, Ar-H, J = 8.00 Hz), 4.53 (s, 2H, NCH_2N), 3.83 (q, 2H, OCH_2CH_3, J = 6.80 Hz), 3.55 (t, 4H, CH_2OCH_2; J = 4.40 Hz), 2.57 (t, 4H, CH_2NCH_2; J = 4.40 Hz), 2.31 (s, 3H, CH_3), 1.10 (t, 3H, OCH_2CH_3, J = 6.80 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.08 (Triazole C_5), 150.14 (N=CH), 143.12 (Triazole C_3), [150.88, 139.61,

135.13, 134.85, 133.57, 129.48 (2C), 128.14 (2C), 124.20, 120.15, 112.84] (ar-C),), 66.02 (CH_2OCH_2), 65.92 (NCH_2N), 64.05 ($O\underline{C}H_2CH_3$), 49.95 (CH_2NCH_2), 14.04 ($OCH_2\underline{C}H_3$), 10.91 (CH_3); MS (70 eV): *m/z* (%) 115.08 (20), 143.06 (3), 502.11 (M+1, 100); Anal. Calcd. for $C_{23}H_{27}N_5O_6S$: C, 55.08; H, 5.43; N, 13.96. Found: C, 54.87; H, 5.33; N. 13.60.

2-Ethoxy-4-{[1-(morpholine-4-yl-methyl)-3-ethyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (5b) White solid; yield: 77 %; m.p. 142-144 °C. IR (cm^{-1}): 1692 (C=O), 1610, 1577 (C=N), 1356 and 1179 (SO_2), 760 and 702 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.66 (s, 1H, N=CH), 7.86-7.82 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.49 (s, 1H, Ar-H), 7.47 (dd, 1H, ArH; *J* = 8.40, 2.00 Hz), 7.31 (d, 1H, Ar-H, *J* = 8.40 Hz), 4.54 (s, 2H, NCH_2N), 3.83 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 3.56 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 2.70 (q, 2H, CH_2CH_3, *J* = 7.20 Hz), 2.57 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 1.22 (t, 3H, CH_2CH_3, *J* = 7.20 Hz), 1.10 (t, 3H, OCH_2CH_3, *J* = 7.20 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.10 (Triazole C_5), 150.28 (N=CH), 146.83 (Triazole C_3), [150.88, 139.61, 135.15, 134.85, 133.59, 129.48 (2C), 128.13 (2C), 124.33, 120.05, 112.87] (ar-C), 66.02 (CH_2OCH_2), 65.96 (NCH_2N), 64.04 ($O\underline{C}H_2CH_3$), 49.97 (CH_2NCH_2), 18.33 ($\underline{C}H_2CH_3$), 14.03 ($OCH_2\underline{C}H_3$), 9.94 ($CH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 118.09 (27), 129.07 (38), 132.08 (27), 159.07 (10), 516.14 (M+1, 100); Anal. Calcd. for $C_{24}H_{29}N_5O_6S$: C, 55.91; H, 5.67; N, 13.58. Found: C, 54.95; H, 5.1; N. 13.25.

2-Ethoxy-4-{[1-(morpholine-4-yl-methyl)-3-benzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl Furan-2-carboxylate (5d) White solid; yield: 72 %; m.p. 151-153 °C. IR (cm^{-1}): 1712 (C=O), 1602, 1573 (C=N), 1375 and 1199 (SO_2), 753 and 696 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.61 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.68-7.64 (m, 2H, Ar-H), 7.40-7.37 (m, 2H, Ar-H), 7.32-7.28 (m, 5H, Ar-H), 7.23 (m, 1H, Ar-H), 4.58 (s, 2H, NCH_2N), 4.09 (s, 2H, CH_2Ph), 3.80 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), 3.56 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 2.59 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 1.12 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.50 (Triazole C_5), 150.16 (N=CH), 144.87 (Triazole C_3), [150.82, 139.63, 135.12, 134.85, 133.52, 129.47 (2C), 128.13 (2C), 124.28, 120.73, 111.97] (ar-C), [135.66, 128.59 (2C), 128.49 (2C), 126.77] (Ar-C linked C-3), 66.03 (CH_2OCH_2 + NCH_2N), 64.00 ($O\underline{C}H_2CH_3$), 49.98 (CH_2NCH_2), 30.98 (CH_2Ph), 14.02 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 118.09 (52), 132.06 (100), 159.07 (20), 173.06 (24), 578.16 (M+1, 14).

2-Ethoxy-4-{[1-(morpholine-4-yl-methyl)-3-(*p*-methylbenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (5e) White solid; yield: 71 %; m.p. 123-125 °C. IR (cm^{-1}): 1699 (C=O), 1591, 1575 (C=N), 1363 and 1197 (SO_2), 820 (1,4-disubstituted benzenoid ring), 753 and 689 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.61 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.40-7.38 (m, 2H, Ar-H), 7.30 (d, 1H, Ar-H, *J* = 8.00 Hz), 7.20 (d, 2H, ArH; *J* = 8.00 Hz), 7.10 (d, 2H, ArH; *J* = 7.60 Hz), 4.57 (s, 2H, NCH_2N), 4.04 (s, 2H, CH_2Ph), 3.80 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), 3.56 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 2.58 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 1.12 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.45

(Triazole C_5), 150.16 (N=CH), 145.02 (Triazole C_3), [150.82, 139.62, 135.14, 134.84, 133.54, 129.47 (2C), 128.13 (2C), 124.28, 120.73, 111.96] (ar-C), [135.84, 132.53, 129.04 (2C), 128.47 (2C)] (Ar-C linked C-3), 66.03 (CH_2OCH_2 + NCH_2N), 63.98 ($O\underline{C}H_2CH_3$), 49.98 (CH_2NCH_2), 30.59 (CH_2Ph), 20.56 ($PhCH_3$), 14.03 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 118.08 (40), 129.08 (100), 132.06 (85), 159.07 (18), 592.12 (M+1, 18).

2-Ethoxy-4-{[1-(morpholine-4-yl-methyl)-3-(*p*-methoxybenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (5f) White solid; yield: 73 %; m.p. 143-145 °C. IR (cm^{-1}): 1699 (C=O), 1595, 1577 (C=N), 1364 and 1177 (SO_2), 813 (1,4-disubstituted benzenoid ring), 752 and 689 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.61 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.68-7.65 (m, 2H, Ar-H), 7.42-7.39 (m, 2H, Ar-H), 7.30 (d, 1H, Ar-H, *J* = 8.00 Hz), 7.23 (d, 2H, ArH; *J* = 8.80 Hz), 6.86 (d, 2H, ArH; *J* = 8.80 Hz), 4.57 (s, 2H, NCH_2N), 4.02 (s, 2H, CH_2Ph), 3.82 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), 3.71 (s, 3H, OCH_3), 3.56 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 2.58 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 1.12 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.50 (Triazole C_5), 150.16 (N=CH), 144.87 (Triazole C_3), [150.83, 139.62, 135.14, 134.84, 133.54, 129.47 (2C), 128.13 (2C), 124.30, 120.67, 112.08] (ar-C), [158.11, 129.66 (2C), 127.38, 113.92 (2C)] (Ar-C linked C-3), 66.03 (CH_2OCH_2 + NCH_2N), 64.00 ($O\underline{C}H_2CH_3$), 55.02 (OCH_3), 49.98 (CH_2NCH_2), 30.12 (CH_2Ph), 14.03 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 118.09 (44), 129.10 (100), 132.07 (60), 159.06 (22), 173.08 (12), 608.12 (M+1, 12).

2-Ethoxy-4-{[1-(morpholine-4-yl-methyl)-3-(*p*-chlorobenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (5g) White solid; yield: 81 %; m.p. 145-147 °C. IR (cm^{-1}): 1700 (C=O), 1612, 1576 (C=N), 1359 and 1196 (SO_2), 818 (1,4-disubstituted benzenoid ring), 753 and 692 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.62 (s, 1H, N=CH), 7.85-7.83 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.40-7.29 (m, 7H, Ar-H), 4.57 (s, 2H, NCH_2N), 4.11 (s, 2H, CH_2Ph), 3.79 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), 3.56 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 2.58 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 1.12 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.64 (Triazole C_5), 150.15 (N=CH), 144.53 (Triazole C_3), [150.83, 139.65, 135.12, 134.84, 133.47, 129.47 (2C), 128.13 (2C), 124.29, 120.72, 112.02] (ar-C), [134.66, 131.45, 130.52 (2C), 128.42 (2C)] (Ar-C linked C-3), 66.10 (CH_2OCH_2), 66.03 (NCH_2N), 63.99 ($O\underline{C}H_2CH_3$), 49.96 (CH_2NCH_2), 30.12 (CH_2Ph), 14.03 ($OCH_2\underline{C}H_3$);); MS (70 eV): *m/z* (%)132.07 (100), 159.06 (28), 173.07 (36), 612.10 (M, 12); Anal. Calcd. for $C_{29}H_{30}N_5O_6SCl$: C, 56.91; H, 4.94; N, 11.44. Found: C, 56.29; H, 4.96; N. 11.06.

General procedure for the synthesis of 2-ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonates (6) The corresponding compound **3** (5 mmol) was dissolved in 100 mL of ethanol followed by addition of 2,6-dimethylmorpholine (6 mmol) and formaldehyde (37%, 10 mmol). The reaction mixture was refluxed for 3 hours. After standing at RT overnight, the solid was filtered and crystallized from ethanol. The solid was recrystallized from the same

solvent and purified by drying *in vacuo* to obtain pure compounds **6** as colourless crystals (Özdemir et al. 2018).

2-Ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-methyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (6a) White solid; yield: 71 %; m.p. 136-138 °C. IR (cm^{-1}): 1705 (C=O), 1603, 1577 (C=N), 1375 and 1171 (SO_2), 754 and 695 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.66 (s, 1H, N=CH), 7.85-7.83 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.49-7.46 (m, 2H, Ar-H), 7.31 (d, 1H, Ar-H, *J* = 8.40 Hz), 4.54 (s, 2H, NCH_2N), 3.83 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), [3.55-3.50 (m, 2H, 2CH), 2.75 (d, 2H, CH_2, *J* = 10.40 Hz), 2.01 (d, 2H, CH_2, *J* = 11.20 Hz)] (morpholine-H), 2.31 (s, 3H, CH_3), 1.10 (t, 3H, OCH_2CH_3, *J* = 6.40 Hz), 1.10 (d, 6H, $2CH_3$, *J* = 6.40 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.00 (Triazole C_5), 150.15 (N=CH), 143.13 (Triazole C_3), [150.87, 139.58, 135.12, 134.85, 133.59, 129.48 (2C), 128.14 (2C), 124.30, 120.08, 112.91] (ar-C), 71.02 (2CH), 65.60 (NCH_2N), 64.05 ($O\underline{C}H_2CH_3$), 55.57 ($2CH_2$), 18.92 ($2CH_3$), 14.04 ($OCH_2\underline{C}H_3$), 10.94 (CH_3);); MS (70 eV): *m/z* (%) 116.10 (80), 128.09 (10), 146.08 (56), 157.08 (100), 160.08 (42), 187.07 (22), 530.10 (M+1, 72); Anal. Calcd. for $C_{25}H_{31}N_5O_6S$: C, 56.70; H, 5.90; N, 13.22. Found: C, 56.47; H, 5.80; N. 12.68.

2-Ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-ethyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (6b) White solid; yield: 74 %; m.p. 138-140 °C. IR (cm^{-1}): 1693 (C=O), 1603, 1576 (C=N), 1373 and 1159 (SO_2), 749 and 696 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.62 (s, 1H, N=CH), 7.85-7.82 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.49-7.46 (s, 2H, Ar-H), 7.31 (d, 1H, Ar-H, *J* = 8.00 Hz), 4.55 (s, 2H, NCH_2N), 3.83 (q, 2H, OCH_2CH_3, *J* = 7.20 Hz), [3.54-3.51 (m, 2H, 2CH), 2.75 (d, 2H, CH_2, *J* = 12.40 Hz), 2.00 (d, 2H, CH_2, *J* = 10.80 Hz)] (morpholine-H), 2.72 (q, 2H, CH_2CH_3, *J* = 7.60 Hz), 1.21 (t, 3H, CH_2CH_3, *J* = 7.60 Hz), 1.11 (t, 3H, OCH_2CH_3, *J* = 6.40 Hz), 1.03 (d, 6H, $2CH_3$, *J* = 6.40 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.03 (Triazole C_5), 150.29 (N=CH), 146.84 (Triazole C_3), [150.87, 139.58, 135.14, 134.86, 133.62, 129.49 (2C), 128.14 (2C), 124.33, 118.98, 112.95] (ar-C), 71.02 (2CH), 65.50 (NCH_2N), 64.03 ($O\underline{C}H_2CH_3$), 55.60 ($2CH_2$), 18.93 ($2CH_3$), 18.40 ($\underline{C}H_2CH_3$), 14.03 ($OCH_2\underline{C}H_3$), 10.01 ($CH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 116.11 (64), 128.12 (3), 146.10 (11), 157.09 (100), 187.11 (8), 544.16 (M+1, 40); Anal. Calcd. for $C_{26}H_{33}N_5O_6S$: C, 57.44; H, 6.12; N, 12.88. Found: C, 56.93; H, 5.96; N. 12.74.

2-Ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-benzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl Furan-2-carboxylate (6d) White solid; yield: 70 %; m.p. 112-114 °C. IR (cm^{-1}): 1708 (C=O), 1587 (C=N), 1391 and 1166 (SO_2), 760 and 698 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.61 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.40-7.37 (m, 2H, Ar-H), 7.32-7.28 (m, 6H, Ar-H), 4.59 (s, 2H, NCH_2N), 4.09 (s, 2H, CH_2Ph), 3.80 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), [3.55-3.51 (m, 2H, 2CH), 2.78 (d, 2H, CH_2, *J* = 10.40 Hz), 2.01 (d, 2H, CH_2, *J* = 11.20 Hz)] (morpholine-H), 1.12 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz), 1.03 (d, 6H, $2CH_3$, *J* = 6.40 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.45 (Triazole C_5), 150.17 (N=CH), 144.86 (Triazole C_3),

[150.81, 139.60, 135.11, 134.86, 133.54, 129.48 (2C), 128.14 (2C), 124.29, 120.68, 112.04] (ar-C), [135.71, 128.60 (2C), 128.47 (2C), 126.78] (Ar-C linked C-3), 71.01 (2CH), 65.60 (NCH_2N), 63.99 ($O\underline{C}H_2CH_3$), 55.64 ($2CH_2$), 30.94 (CH_2Ph), 18.93 ($2CH_3$), 14.03 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 116.09 (64), 146.08 (24, 157.09 (100), 160.06 (15), 187.07 (8), 606.16 (M+1, 18).

2-Ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-(*p*-methylbenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (6e) White solid; yield: 89 %; m.p. 148-150 °C. IR (cm^{-1}): 1709 (C=O), 1589 (C=N), 1349 and 1167 (SO_2), 849 (1,4-disubstituted benzenoid ring), 763 and 697 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.62 (s, 1H, N=CH), 7.85-7.81 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.41-7.38 (m, 2H, Ar-H), 7.30 (d, 1H, Ar-H, *J* = 8.00 Hz), 7.20 (d, 2H, ArH; *J* = 8.00 Hz), 7.10 (d, 2H, ArH; *J* = 8.00 Hz), 4.59 (s, 2H, NCH_2N), 4.03 (s, 2H, CH_2Ph), 3.81 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), [3.55-3.51 (m, 2H, 2CH), 2.78 (d, 2H, CH_2, *J* = 10.40 Hz), 2.01 (d, 2H, CH_2, *J* = 10.80 Hz)] (morpholine-H), 2.25 (s, 3H, $PhCH_3$), 1.12 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz), 1.03 (d, 6H, $2CH_3$, *J* = 6.40 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.38 (Triazole C_5), 150.17 (N=CH), 145.00 (Triazole C_3), [150.81, 139.60, 135.13, 134.84, 133.56, 129.47 (2C), 128.14 (2C), 124.28, 120.68, 112.03] (ar-C), [135.87, 132.57, 129.03 (2C), 128.48 (2C)] (Ar-C linked C-3), 71.00 (2CH), 65.50 (NCH_2N), 63.98 ($O\underline{C}H_2CH_3$), 55.65 ($2CH_2$), 30.55 (CH_2Ph), 20.56 ($PhCH_3$), 18.92 ($2CH_3$), 14.03 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 116.10 (88), 146.08 (20), 157.10 (100), 160.08 (18), 187.09 (8), 620.15 (M+1, 22).

2-Ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-(*p*-methoxybenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (6f) White solid; yield: 90 %; m.p. 104-106 °C. IR (cm^{-1}): 1700 (C=O), 1612, 1576 (C=N), 1365 and 1162 (SO_2), 811 (1,4-disubstituted benzenoid ring), 752 and 688 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.62 (s, 1H, N=CH), 7.85-7.83 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.43-7.40 (m, 2H, Ar-H), 7.31 (d, 1H, Ar-H, *J* = 8.00 Hz), 7.23 (d, 2H, ArH; *J* = 8.80 Hz), 6.86 (d, 2H, ArH; *J* = 8.40 Hz), 4.58 (s, 2H, NCH_2N), 4.02 (s, 2H, CH_2Ph), 3.82 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), 3.71 (s, 3H, OCH_3), [3.55-3.51 (m, 2H, 2CH), 2.77 (d, 2H, CH_2, *J* = 10.00 Hz), 2.00 (d, 2H, CH_2, *J* = 11.20 Hz)] (morpholine-H), 1.12 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz), 1.03 (d, 6H, $2CH_3$, *J* = 6.40 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.47 (Triazole C_5), 150.18 (N=CH), 145.15 (Triazole C_3), [150.82, 139.60, 135.13, 134.84, 133.57, 129.47 (2C), 128.14 (2C), 124.30, 120.60, 112.15] (ar-C), [158.12, 129.68 (2C), 127.43, 113.90 (2C)] (Ar-C linked C-3), 71.01 (2CH), 65.50 (NCH_2N), 63.99 ($O\underline{C}H_2CH_3$), 55.65 ($2CH_2$), 55.02 (OCH_3), 30.10 (CH_2Ph), 18.92 ($2CH_3$), 14.03 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 116.11 (100), 146.07 (40), 157.09 (99), 160.08 (23), 187.10 (12), 636.17 (M+1, 7).

2-Ethoxy-4-{[1-(2,6-dimethylmorpholine-4-yl-methyl)-3-(*p*-chlorobenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethine}-phenyl benzenesulfonate (6g) White solid; yield: 70 %; m.p. 112-114 °C. IR (cm^{-1}): 1705 (C=O), 1577 (C=N), 1373 and 1162 (SO_2), 853 (1,4-disubstituted benzenoid ring), 749 and 694 (monosubstituted benzenoid ring). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.62 (s, 1H, N=CH), 7.85-7.82 (m, 3H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.40-7.34 (m, 6H, Ar-H), 7.29 (d, 1H, Ar-H, *J* = 8.00 Hz), 4.55 (s, 2H, NCH_2N), 4.11 (s, 2H,

CH_2Ph), 3.79 (q, 2H, OCH_2CH_3, *J* = 6.80 Hz), [3.55-3.51 (m, 2H, 2CH), 2.77 (d, 2H, CH_2, *J* = 10.40 Hz), 2.00 (d, 2H, CH_2, *J* = 11.20 Hz)] (morpholine-H), 1.11 (t, 3H, OCH_2CH_3, *J* = 6.80 Hz), 1.03 (d, 6H, $2CH_3$, *J* = 6.40 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.60 (Triazole C_5), 150.17 (N=CH), 144.53 (Triazole C_3), [150.82, 139.63, 135.10, 134.86, 133.49, 129.48 (2C), 128.14 (2C), 124.30, 120.68, 112.10] (ar-C), [134.73, 131.45, 130.53 (2C), 128.41 (2C)] (Ar-C linked C-3), 71.01 (2CH), 65.50 (NCH_2N), 63.99 ($O\underline{C}H_2CH_3$), 55.62 ($2CH_2$), 30.29 (CH_2Ph), 18.92 ($2CH_3$), 14.04 ($OCH_2\underline{C}H_3$); MS (70 eV): *m/z* (%) 116.12 (100), 146.08 (25), 157.09 (99), 160.10 (23), 187.09 (12), 640.13 (M+1, 10).

Antimicrobial Activity

All bacterial and yeast strains were obtained 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 (Perez et al. 1990; Ahmad et al. 1998). All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare extract stock solution of 1 mg/ml.

Each microorganism was suspended in Mueller-Hinton Broth and diluted to 106 colony forming unit (cfu) per ml. They were "flood-inoculated" onto the surface of Mueller Hinton Agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 250–5000 μg/50 μl of the chemical substances were delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin, neomycin and streptomycin were standard antibacterial and antifungal agents, DMSO was used as solved control.

Aldose Reductase Activity Assay and Inhibition Studies

In vitro activity analysis of aldose reductase enzyme inhibition was performed according to the method described by Cerelli (Cerelli et al. 1986). The assay mixture containing 1 M sodium phosphate buffer (pH 5.5), 0.11 mM NADPH, enzyme solution, and distilled water was prepared. The reaction was started by adding 4.7 mM DL-Glyceraldehyde to the reaction medium, and the change in absorbance at 340 nm due to NADPH oxidation was measured with the aid of a UV-Visible Spectrophotometer. In order to determine the inhibition effects of the synthesized triazole derivatives, inhibitors were added to the reaction medium at different concentrations and activity measurements were performed. Control activity without inhibitor was considered as 100% and inhibitor concentrations that halve enzyme activity were determined as IC_{50}. IC_{50} values were calculated from the equation of the graph obtained by calculating the activity% of the enzyme measured in different inhibitor concentrations. In order to calculate K_i values of triazole derivatives showing the inhibitory effect on aldose reductase enzyme, activity values were determined with 3 different inhibitor concentrations

against 5 different substrate concentrations. Lineweaver-Burk plots of each compound were drawn from the activity values obtained and K_i values and inhibition types were determined from graphics equations (Alim et al. 2017; Taslimi et al. 2018).

Molecular Docking Studies

Molecular docking method was used to determine ligand-receptor interactions. For this purpose, high resolution X-ray crystal structure of aldose reductase enzyme was retrieved from RCSB Protein Data Bank (PDB code: 2FZD, Resolution: 1.08 Å). The obtained structure from PDB was pre-processed using Protein Preparation Wizard (Sastry et al. 2013) in Schrödinger Maestro software (Maestro Version 12.4.072, Release 2020-2). Protein Preparation Wizard includes the proper assignment of bond orders and ionization states, simplifying multimeric structures, adding and optimizing hydrogen bonds, converting selenomethionines into methionines, locating and removing unnecessary water molecules, the assignment of partial charges, forming disulfide bonds, aligning and closing terminal amides. In addition, the addition of missing atoms and side-chain residues accomplished with the Prime module. Amino acids were ionized at physiological pH using Propka module. Water molecules at a distance of 3 Å from the receptor were removed. Energy minimization was done using OPLS-2005 force field. 3D structures of the ligands were obtained with the help of LigPrep program. Epik module and OPLS-2005 force field were used to obtain correct molecular geometries and protonation states of the ligands at pH 7.0 ± 2.0. Using the Receptor Grid Generation platform, the possible binding site of the ligands to be docked was determined. Then, using Glide, the prepared ligands were docked to the target enzyme. Docking calculations were performed by keeping the ligand flexible using the Glide XP (extra precision) algorithm. In order to verify the docking procedure, docking validation was also performed (Yuriev et al. 2011). For this purpose, the ligand bound to the receptor was extracted and re-docked to the protein. After docking, the best pose of ligand was aligned with the co-crystallized ligand and the RMSD (Root Mean Square Deviation) value was calculated. RMSD value less than 2 Å proves the accuracy of the docking protocol (Bal et al. 2020).

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