

Design, Synthesis, Characterization, *in silico* Studies and Biological Screening of Novel Triazole Coupled Amino Acid Derivatives

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ABSTRACT

Background: Triazoles produces a wide range of activities like anti-diabetic, anthelmintic, antimicrobial, anti-inflammatory and so on. The study aimed to synthesise triazole derivatives and assess their pharmacological potential. **Materials and Methods:** In this work, 4-hydroxy benzaldehyde or 4-hydroxy acetophenone is treated with amino acids such as glycine, phenyl alanine, tryptophan, and tyrosine in the presence of green catalyst using only lemon juice and a water-ethanol mixture as a solvent to create triazole linked amino acid derivatives. Sulfonamide group is further added to enhance the activities of derivatives. **Results:** All the synthesized compounds were characterized and *in silico* studies were performed prior to *in vitro* biological evaluation. Molecular docking performed against 4yvv for screening anti-diabetic potential, and docking scores of the derivatives ranges from -8.0 to -10.6 kcal/mol. The synthesized compounds TZ1-TZ8 were examined for various activities and possess significant *in vitro* anti-diabetic, anti-inflammatory activities along with anthelmintic and antimicrobial activities. **Conclusion:** Derivatives TZ8 shows significant activities which may be due to presence of additional heterocyclic ring in its structure. There is a correlation between *in vitro* and *in silico* anti-diabetic activity.

Keywords: Anthelmintic, Anti-diabetic, Anti-inflammatory, Anti-microbial, Docking, Green catalyst, Triazole.

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INTRODUCTION

In pharmaceutical chemistry and in the process of drug discovery and development, heterocyclic compounds possess a key role particularly, nitrogenous heterocyclic compounds occupy central position with a variety of biological activities.¹⁻³ Triazole is a 5 membered heterocyclic compound with 3 nitrogen atoms as hetero atoms at non-adjacent positions.⁴⁻⁶ They occur in two isomeric forms i.e., 1,2,3-triazoles and 1,2,4-triazoles. Green synthesis is applied to synthesize the derivatives by employing green catalysts like lemon juice which contain citric acid as a catalyst instead of employing volatile chemicals like sulfuric acid, etc.⁷⁻⁹ Water, used in this reaction further act as a green solvent.¹⁰⁻¹² 1,2,4-triazole on conjugating with amino acid and sulfonamides enhances the antimicrobial, anthelmintic, anti-inflammatory

and anti-diabetic activities.¹³ Sulfonyl urea act on β -cells to elicit anti-diabetic activity and act on enzyme called dihydropteroate synthase to produce antimicrobial activity.

Docking is a computation method which enables us to determine how well the derivative is binding with the protein. Molecular docking helps us to study how two or more molecular structures fit together. In simple, Molecular docking is a molecular modelling technique which helps in predicting the binding efficiency of the ligand with regard to protein which means the interaction of protein and ligand to form supramolecular complex that may antagonize or agonistic its biological function. The behaviour of ligand in the binding pocket of target proteins can be described by molecular docking. It gives an idea prior to performing the *in vitro* activities thereby preventing chemical wastage and time.¹⁴⁻¹⁷ UCSF chimera along with AutoDock Vina is used for performing *in silico* studies for the characterized compounds. The synthesized compounds were screened for *in vitro* anti-diabetic activity by inhibition of α -amylase enzyme, anti-inflammatory activity by employing egg albumin denaturation inhibition method, antibacterial activity against *E. coli* using paper diffusion method and *in vivo* anthelmintic activity.



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MATERIALS AND METHODS

There was no purification performed on any of the compounds utilized in this investigation. The synthesized compounds' melting points were determined using the open capillary tube method, and the Gallen Kamp Melting Point instrument was left uncorrected. Thin Layer Chromatography (TLC), employing analytical plates covered with silica gel GF254 [E-Merck] and ethyl acetate/hexane (3:7) as an eluent, was used to verify the purity of all the produced compounds. The UV chamber allowed for the visualization of the spots. Potassium bromide pellets were used to record IR spectra using a BRUKER FTIR-8400 spectrometer. Utilizing a Bruker Avance III Spectrometer, ¹H and ¹³C NMR data were obtained at 25°C. UCSF Chimera and AutoDock Vina were used for docking studies.

Synthesis of triazole-coupled amino acids

Equimolar mixture of 4-hydroxy benzaldehyde (0.01 mol, 1.22 g) or 4-hydroxy acetophenone 1b (0.01 mol, 1.22g), amino acids 0.01 mol (phenyl alanine 2a, glycine 2b, tyrosine 2c and tryptophan 2d) and thiosemicarbazide 3 (0.01 mol, 0.91 g) are refluxed for 2-3 hr at 110°C in the presence of lemon juice and solvent water: ethanol in the ratio of 7:1. Reaction is monitored at regular intervals using TLC. The triazole coupled amino acid derivatives 4a-h (Scheme 1) were filtered and recrystallized with ethanol.³

Synthesis of triazole coupled aminoacids conjugated with sulphonamides

Title compounds were synthesised at this step in which triazole coupled aminoacids 4a-h (0.01 mol) and p-toluene sulfonyl chloride 5 (0.01 mol) was refluxed in the presence of chloroform and pyridine for 6 hr. Reaction was monitored regularly throughout the process using TLC. Final solid product TZ1-TZ8 obtained from reaction mixture upon cooling at room temperature, were filtered and purified with ethanol.⁴

2-(5-(4-hydroxyphenyl)-5-methyl-3-((4-methylphenyl) sulfonamido)-1,5-dihydro-4H-1,2,4-triazol-4-yl)-3-phenyl propanoic acid [TZ-1]

Mol. Formula: C₂₅H₂₆N₄O₅S; Mol. Wt.: 494.57 g/mol; % yield: 45.24%; white crystals; M.P: 235-245°C; R_f Value: 0.69; IR (K Br) cm⁻¹: N-S: 818.24, N-N: 1073.58, C-N: 1220.90, Ar C-N: 1302.28, S=O: 1374.86 and 1155.35, Ar C=C: 1499.47, Ar C=N: 1548.03, C=O: 1754.66, Alkyl C-H: 2950.74, Ar C-H: 3032.84, N-H: 3359.60, O-H: 3403.32. ¹H -NMR (DMSO-d₆), δ ppm:- 2.243 (6H, CH₃), 2.365 (2H, CH₂ tolyl), 2.509 (1H, CH), 3.41 (1H, NH), 6.76-7.825 (12H, ArH), 8.18 (2H, Ar-OH), 9.748 (1H, NH thioamide), 10.078 (1H, COOH); ¹³C NMR (DMSO-d₆), δ ppm: 14.29 (1C, methyl), 21.38 (1C, tosyl), 39.32 (CH-COOH), 39.53 (C-N triazole), 39.74 (C=N triazole), 115.47, 118.03,

119.63, 126.06, 126.72, 127.51, 128.68, 129.77, 141.68, 142.34, 148.75 (Ar-C), 40.15 (C-CH₃), 40.36 (C=NH), 40.57 (C=N), 159.17 (C=N), 167.48 (C-S), 178.97 (C=O).

2-(5-(4-hydroxyphenyl)-3-((4-methylphenyl) sulfonamido)-1,5-dihydro-4H-1,2,4-triazol-4-yl)-3-phenyl propanoic acid [TZ-2]

Molecular Formula: C₂₄H₂₄N₄O₅S, Mol. Wt: 480.54 g/mol, % yield: 39.48%, yellow crystals, M.P: 210-215°C, R_f Value: 0.71, IR (K Br) cm⁻¹: Ar C=C: 1506.33, Ar C=N: 1547.57, S=O: 1372.94 and 1154.22, N-S: 816.20, N-N: 1091.28, C=O: 1757.35, N-H: 3356.34, Ar C-H: 3034.18, O-H: 3395.62, C-N: 1230.60, Ar C-N: 1325.40, Alkyl C-H: 2952.48.

2-(5-(4-hydroxyphenyl)-5-methyl-3-((4-methylphenyl) sulfonamido)-1,5-dihydro-4H-1,2,4-triazol-4-yl)acetic acid [TZ-3]

Molecular Formula: C₁₈H₂₀N₄O₅S; Mol. Wt: 404.44 g/mol; % yield: 37.63%; white crystals; M.P: 185-190°C; R_f Value: 0.73; IR (K Br) cm⁻¹: N-S: 815.22, N-N: 1083.58, C-N: 1261.59, S=O: 1150.64, Ar C-N: 1320.82, Ar C=C: 1500.61, Ar C=N: 1596.29, C=O: 1752.28, Alkyl C-H: 2947.43, Ar C-H: 3049.47, N-H: 3320.23, O-H: 3396.14. ¹H -NMR (DMSO-d₆), δ ppm:- 2.24 (6H, CH₃), 2.358 (2H, CH₂), 3.046 (1H, NH), 6.785-7.825 (8H, Ar-H), 8.184 (1H, Ar-OH), 9.76 (1H, NH thioamide), 10.078 (1H, COOH); ¹³C NMR (DMSO-d₆), δ ppm: 21.38 (CH₂), 39.32 (CH-COOH), 39.53 (CH triazole), 39.74 (CH indole), 115.47, 118.03, 119.63, 126.06, 126.72, 127.51, 128.68, 129.77, 141.68, 142.34, 148.75 (m, Ar-C), 40.15 (C-CH₃), 40.36 (C=NH), 40.57 (C=N), 159.17 (C=N), 167.48 (C-S), 178.97 (C=O).

2-(5-(4-hydroxyphenyl)-3-((4-methylphenyl) sulfonamido)-1,5-dihydro-4H-1,2,4-triazol-4-yl)acetic acid [TZ-4]

Molecular Formula: C₁₇H₁₈N₄O₅S, Mol. Wt: 390.41 g/mol, % yield: 37.24%, yellow crystals, M.P: 175-180°C, R_f Value: 0.65, IR (K Br) cm⁻¹: Ar C=C: 1504.83, Ar C=N: 1544.10, S=O: 1321.71 and 1152.40, N-S: 806.23, N-N: 1088.63, C=O: 1746.03, N-H: 3319.50, Ar C-H: 3069.86, O-H: 3355.67, C-N: 1271.62, Ar C-N: 1321.71, Alkyl C-H: 2947.82.

3-(4-hydroxyphenyl)-2-(5-(4-hydroxyphenyl)-5-methyl-3-((4-methylphenyl) sulfonamido)-1,5-dihydro-4H-1,2,4-triazol-4-yl)propanoic acid [TZ-5]

Molecular Formula: C₂₅H₂₆N₄O₆S, Mol. Wt: 510.57 g/mol, % yield: 44.56%, white crystals, M.P: 215-225°C, R_f Value: 0.72, IR (K Br) cm⁻¹: Ar C=C: 1500.45, Ar C=N: 1554.26, S=O: 1318.89 and 1148.52, N-S: 815.89, N-N: 1084.35, C=O: 1755.12, N-H: 3323.34, Ar C-H: 3058.47, O-H: 3358.35, C-N: 1262.33, Ar C-N: 1318.89, Alkyl C-H: 2963.54.

3-(4-hydroxyphenyl)-2-(5-(4-hydroxyphenyl)-3-((4-methylphenyl) sulfonamido)-1,5-dihydro-4H-1,2,4-triazol-4-yl) propanoic acid [TZ-6]

Molecular Formula: $C_{24}H_{24}N_4O_6S$, Mol. Wt: 496.14g/mol, % yield: 42.58%, yellow crystals, M.P: 200-210°C, R_f Value: 0.69, IR (K Br) cm^{-1} : Ar C=C:1508.63, Ar C=N: 1580.22, S=O: 1324.44 and 1154.91, N-S: 833.89, N-N: 1100.38, C=O: 1756.56, N-H: 3361.67, Ar C-H: 3056.43, O-H: 3395.28, C-N: 1237.26, Ar C-N: 1324.44, Alkyl C-H: 2936.52.

2-(5-(4-hydroxyphenyl)-5-methyl-3-((4-methylphenyl) sulfonamido)-1,5-dihydro-4H-1,2,4-triazol-4-yl)-3-(1H-indol-3-yl) propanoic acid [TZ-7]

Molecular Formula: $C_{27}H_{27}N_5O_5S$, Mol. Wt: 533.17 g/mol, % yield: 48.75%, white crystals, M.P: 255-265°C, R_f Value: 0.78, IR (K Br) cm^{-1} : Ar C=C:1505.54, Ar C=N: 1556.91, S=O: 1317.66 and 1160.75, N-S: 817.89, N-N: 1084.13, C=O: 1757.35, N-H: 3357.70, Ar C-H: 3172.55, O-H: 3397.65, C-N: 1220.46, Ar C-N: 1317.66, Alkyl C-H: 2958.43.

2-(5-(4-hydroxyphenyl)-3-((4-methylphenyl) sulfonamido)-1,5-dihydro-4H-1,2,4-triazol-4-yl)-3-(1H-indol-3-yl) propanoic acid [TZ-8]

Molecular Formula: $C_{26}H_{25}N_5O_5S$, Mol. Wt: 519.58 g/mol, % yield: 51.10%, yellow crystals, M.P: 220-225°C, R_f Value: 0.82, IR (K Br) cm^{-1} : Ar C=C:1511.60, Ar C=N: 1555.56, S=O: 1347.90 and 1147.87, N-S: 827.56, N-N: 1053.55, C=O: 1755.40, N-H: 3361.46, Ar C-H: 3047.61, O-H: 3399.31, C-N: 1226.37, Ar C-N: 1347.90, Alkyl C-H: 2952.89. ^1H NMR (DMSO- d_6), δ ppm: 2.242 (3H, CH_3 tosyl), 2.369 (2H, CH_2), 2.491 (1H, CH_2 triazole), 2.505 (1H, CH-COOH), 3.046 (1H, NH triazole), 3.452 (1H, NH indole), 6.504 (1H, Ar-CH indole), 6.761 to 7.728 (12H, 3ArH), 7.822 (1H, OH), 9.74 (1H, NH thioamide), 10.177 (1H, COOH). ^{13}C NMR (DMSO- d_6), δ ppm: 14.28 (CH_3 tosyl), 19.03 (CH_2), 21.38 (CH-COOH), 27.42 (CH triazole), 39.75 (CH indole), 109.37, 111.81, 115.48, 115.65, 118.85, 121.47, 124.72, 126.90, 128.67, 128.91, 129.77 (Ar-C), 131.17 (C- CH_3), 136.80 (C-NH), 141.88 (C=N), 142.33 (C=N), 148.09 (C-S), 178.97 (C=O).

In silico Molecular Docking

Preparation of ligand

The designed ligands were drawn in Chem sketch and smiles were generated using open babel 3.1.1. The generated smiles are used to build the structure¹⁸ and minimize the energy with UCSF Chimera 1.16 in PDB format. Then, it was converted into pdbqt using AutoDock 4.0.

Preparation of Protein

The protein was optimized using UCSF Chimera 1 after the protein's crystal structure was obtained from the Protein Data

Bank (PDB id: 4yvv). Ultimately, in order to achieve a desirable structural conformation for docking analysis, Gasteiger charges were added using Dock Prep.¹⁹⁻²¹

Docking simulations

AutoDock Vina was employed for the measurement of binding affinity which needs a configure file. It contains information on position of receptor file, ligand file and grid box coordinates X, Y, Z (18.0871, 7.76547, and 7.21775), with the size of 53 × 72 × 78 points. The number of modes and maximum energy difference were set to 9 and 3 kcal/mol respectively. Docking process in Auto Dock Vina has been performed with exhaustiveness 1000 for improving the results accuracy.²²⁻²⁵

Biological screening

In vitro Anti-Diabetic activity by enzyme inhibition assay

To quantify the inhibitory activity of α -amylase, the DNSA method was applied. To make the stock solution at several concentrations (250, 500, and 750 μ g/mL), phosphate buffer was utilized. The derivatives were then made as stock solutions in dimethyl sulfoxide (DMSO). Inoculation took place at 25°C for 15 min after the compounds were combined with 500 μ g/mL α -amylase solutions prepared in 0.02 M sodium phosphate buffer (pH 6.9). In 10 min, 500 μ g/mL of a 1% starch solution in 0.02 M sodium phosphate buffer was added to each tube. The mixture was allowed to sit at 25°C for 10 min. Subsequently, 0.5 mL of DNS reagent- a solution of 12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3,5-dinitrosalicylic acid was added to the reaction mixture to halt it, and the mixture was heated for 5 min. After cooling, the absorbance of the reaction mixture was measured at 540 nm using a UV-visible spectrophotometer (SL-159, Elico, India Ltd.). Gliclazide was used as the standard or positive control.²⁶⁻²⁹ The anti-diabetic effect of the compounds was determined by blocking α -amylase.

The following equation was used to get the inhibition percentage:

$$\% \text{ inhibition} = \frac{AC - AS}{AC} \times 100$$

Where A_c and A_s are the absorbance of the control and sample, respectively.

In vitro Anti-inflammatory activity by protein denaturation assay

Using the inhibition of albumin denaturation technique, the anti-inflammatory activity of the produced compounds is evaluated. The test materials and reference drug were diluted using phosphate buffer (0.2 M, pH 7.4) after being dissolved in a minimum quantity of dimethyl sulfoxide (DMSO). Less than 2.0% of DMSO was the final concentration in each solution. For 15 min, the mixture of 1 mL of a 1% mM egg albumin solution in phosphate buffer and 1 mL of a test solution with varying drug dosages was incubated at 27 $\pm$ 1°C. 10 min were spent keeping

the reaction mixture in a water bath at $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in order to cause denaturation. Using a UV-vis spectrophotometer (SL-159, Elico, India Ltd.), determine the turbidity of the sample at 660 nm after it has cooled. An unmedicated control group was utilized to evaluate the proportion of denaturation inhibition.³⁰⁻³² The average was calculated after each experiment was completed in triplicate. Diclofenac sodium was used as standard drug.

$$\text{Percentage of inhibition (\%)} = [(v_t/v_c) - 1] \times 100 \times 100$$

Where, v_t and v_c are absorbance of test and control.

In vitro Anti-microbial activity by disc diffusion method

Disc diffusion method, also known as the disc plate method, is used to carry out antibacterial activities. By inoculating nutrient broth media with bacteria, a fresh culture of bacteria is generated, which is then cultured for 18 to 24 hr at $37 \pm 20^{\circ}\text{C}$. Using aseptic methods, this culture was combined with nutritional agar medium and then added to petri dishes. Four discs are created using sterile Wattman paper (5 mm diameter) at equal distances following 2 hr of cold incubation, following the media's solidification. The plates were then placed in a refrigerator. Dip these discs into various amounts of liquid. As a control, dimethyl sulfoxide was employed. To ensure that the medications were properly dipped into the media, the plates were refrigerated for 2 hr after the standard pharmaceuticals and synthetic compounds were introduced. The plates were kept at 37°C for 18 to 24 hr after being placed in an incubator after 2 hr. Using a vernier scale, the Petri plates were inspected for the zone of inhibition after the incubation period.³³⁻³⁵

In vitro Anthelmintic activity

Six earthworms of the same type and roughly equal size were inserted in each 9 cm petri dish that held 25 mL of the drug's test solutions, which were generated at doses of 50, 100, and 150 $\mu\text{g/mL}$ in DMSO. The reference standard was albendazole, while the control was DMSO. Before the tests began, all of the standard

and test medication solutions were made from stock. When there was no movement at all, with the exception of the worms being violently shaken, observations were recorded on the amount of time it took for paralysis. The worms' death time was noted once it was determined that they did not move when submerged in warm water (50°C) or when shaken violently.³⁶⁻³⁸

RESULTS

In silico molecular docking

In silico studies shows that compounds TZ2, TZ5 and TZ8 elicits good binding affinity among the characterized compounds with least binding energy of -10.6 kcal/mole followed by TZ6 with -10.4 kcal/mole. The binding energies of all synthesized compounds were less than -8 kcal/mole (Table 1) and graphical representation (Figure 1). The docking interactions of the top 3 ligands displayed along with the gliclazide (Figure 2).

Anti-diabetic activity

Compounds synthesized are screened for anti-diabetic activity using DNSA method at concentrations of 250, 500 and 750 $\mu\text{g/mL}$. Among all TZ8, TZ3 had shown the potent inhibitory activity against α -amylase enzyme. Compounds TZ2, TZ6 had shown moderate inhibitory activity. Compounds TZ4 and TZ7

Table 1: Docking scores of title compounds TZ1-TZ8 against 4YVV.

Sl. No.	Compound Code	Docking score (kcal/mole)
1.	TZ1	-8.2
2.	TZ2	-10.6
3.	TZ3	-10.0
4.	TZ4	-9.7
5.	TZ5	-10.6
6.	TZ6	-10.4
7.	TZ7	-8.1
8.	TZ8	-10.6
9.	Standard	-12.2

Standard- Gliclazide, kcal/mol- kilocalories per mole, 4yv- Aldo keto reductase.

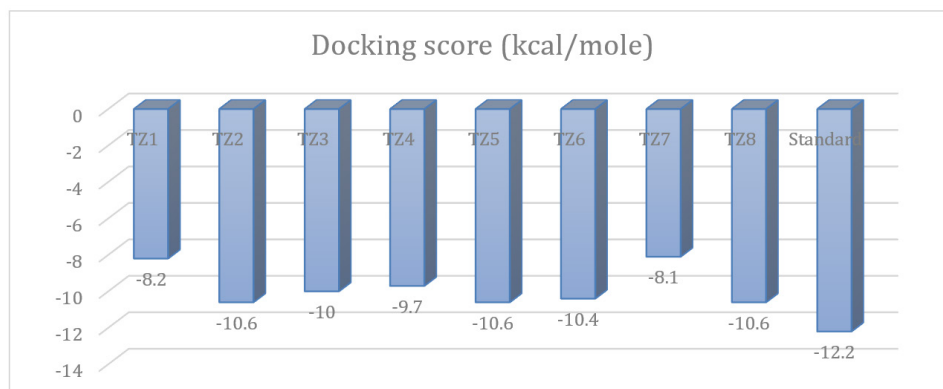


Figure 1: Binding energies of title compounds TZ1-TZ8 and standard drug gliclazide against Aldo-keto reductase.

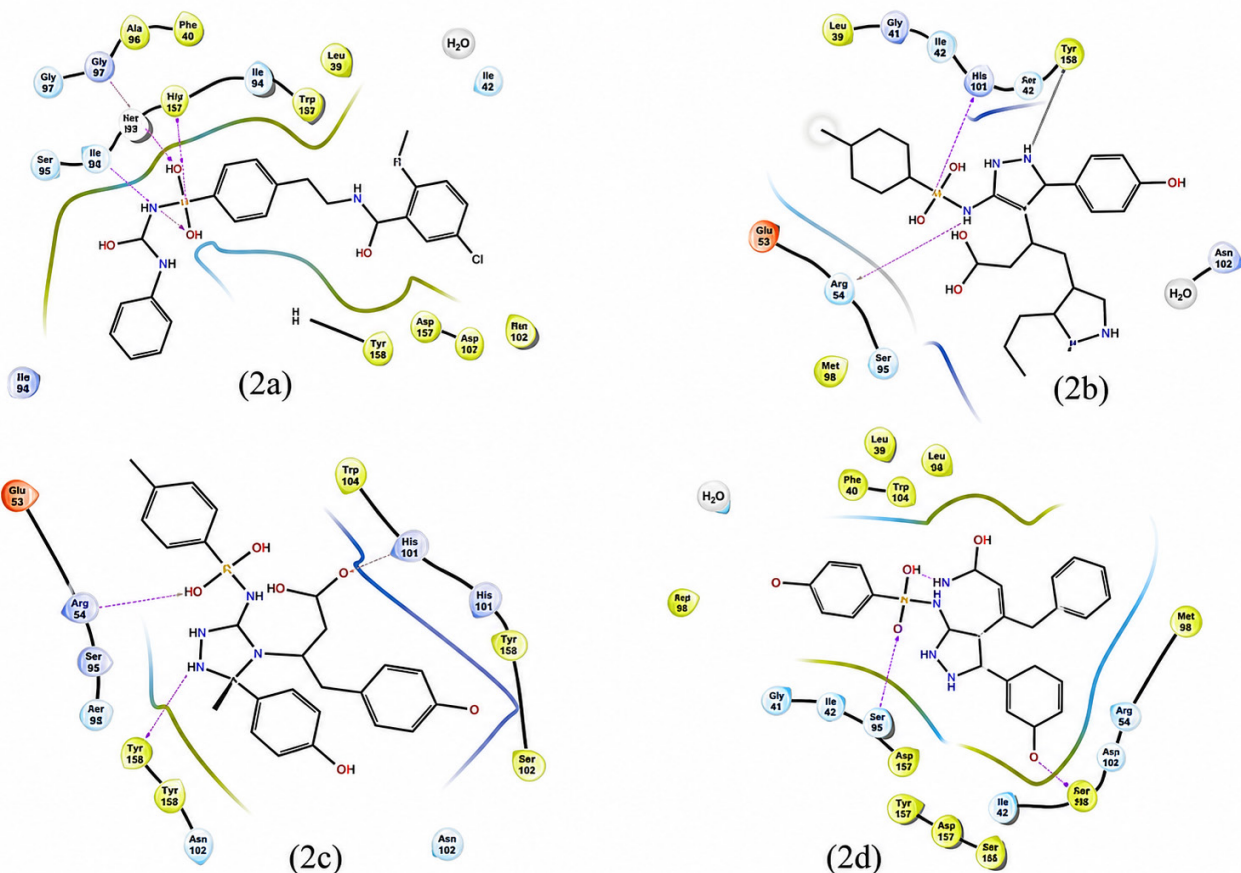


Figure 2: Two-dimensional view of docking interactions against 4yvv. 2a: gliclazide; 2b: TZ8; 2c: TZ5; 2d: TZ2.

Table 2: *In vitro* anti-diabetic activity by alpha-amylase inhibition assay of TZ1-TZ8.

Sl. No.	Derivatives	% of Inhibition		
		250 µg/mL	500 µg/mL	750 µg/mL
1.	Gliclazide	71%	83%	91%
2.	TZ1	48%	57%	63%
3.	TZ2	57%	61%	79%
4.	TZ3	61%	70%	81%
5.	TZ4	37%	49%	57%
6.	TZ5	40%	53%	61%
7.	TZ6	51%	63%	77%
8.	TZ7	41%	47%	59%
9.	TZ8	64%	79%	85%

had shown lesser anti-diabetic activity. The percentage inhibition was recorded (Table 2) and graphical presentation (Figure 3).

Anti-inflammatory activity

Compounds synthesized are screened for anti-inflammatory activity by using albumin denaturation technique. These compounds were screened for albumin denaturation inhibition activity at 100 µg/mL ranges from 55.68% to 73.86% using diclofenac sodium as standard (90.9%). The turbidity of

compounds was calculated at 660 nm. Among the screened compounds TZ8 showed more activity than the other derivatives. Compounds TZ7, TZ6 shows moderate activity. Compound TZ3 shown the lesser activity over the standard. Values are recorded (Table 3) and graphical representation (Figure 4). The results found that, percentage of inhibition of protein denaturation increases with increase in absorbance values which means the compounds having high activity have high absorbance values and less the protein denaturation nothing but the protein remains in its original state.

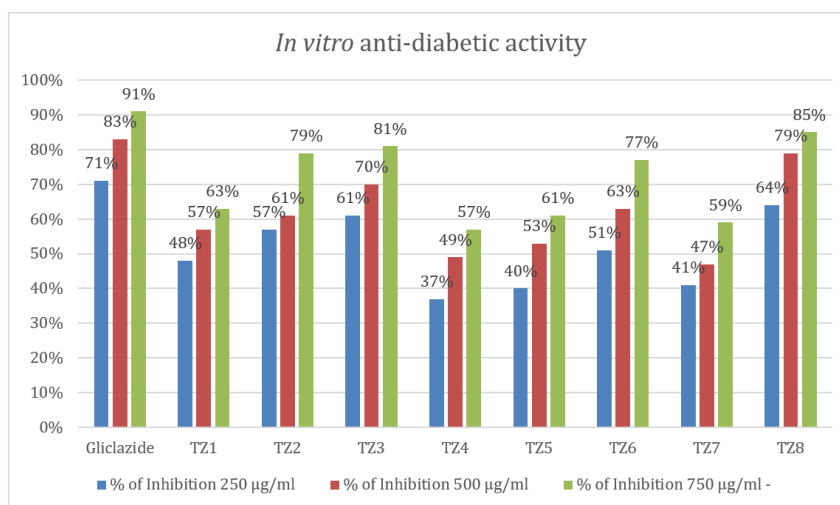


Figure 3: Percentage of inhibition of alpha amylase of title compounds against gliclazide.

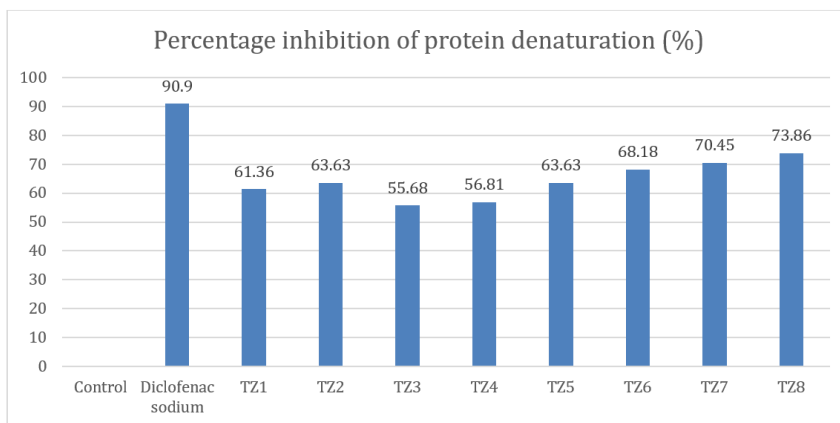


Figure 4: *In vitro* anti-inflammatory activity: egg albumin denaturation inhibition of title compounds and standard drug diclofenac were measured.

Anti-bacterial activity

Compounds synthesized are screened for antibacterial activity using disc plate method at concentrations 20, 40, 60 and 80 µg/mL and the strains used for the screening are *Escherichia coli*. Among the synthesized screened compounds, TZ-5 and TZ-7 had shown the potent activity against the standard. Compounds TZ-1, TZ-6 and TZ-2 had shown moderate activity. Compound TZ-4 had shown lesser activity (Table 4).

Anthelmintic activity

Compounds synthesized are screened for Anthelmintic activity by using Earth worms. These compounds are screened for the activity at 50 and 100 µg/mL using albendazole as standard. Among the screened compounds, TZ8 had shown potent activity against the standard. TZ10, TZ7 and TZ6 Compounds had shown moderate activity. TZ3 and TZ4 compounds had shown the lesser activity over the standard (Table 5) and graphical representation of data (Figure 5).

DISCUSSION

The binding affinity of title compounds at the active pocket of 4yvv ranges from -8.1 to -10.6 kcal/mol and for Gliclazide -12.2 kcal/mol. There are no common interactions between title compounds and standard drug but TZ8, TZ7, TZ1 having hydrogen bonding in common with amino acid ARG 199. Along with these TZ1, TZ7, TZ8 showed hydrogen bonding in common with amino acid ASN 302. The docking interactions were presented (Figure 2). The results of *in vitro* anti-diabetic activity revealed that all the 8 derivatives TZ1-TZ8 showed same pattern of enzyme inhibition i.e. the enzyme inhibition increases with increase in concentration. But standard drug Gliclazide showed more percentage of inhibition than the title compounds. Among all the 8 derivatives TZ5 exhibited significant anti-bacterial potential against gram negative microbe *E. coli*. The anthelmintic activity results indicated that as the time of paralysis and death of parasite decreases with increase in concentration from 50 to 100 µg/mL which means that the anesthetic effect increases with increase in concentration.

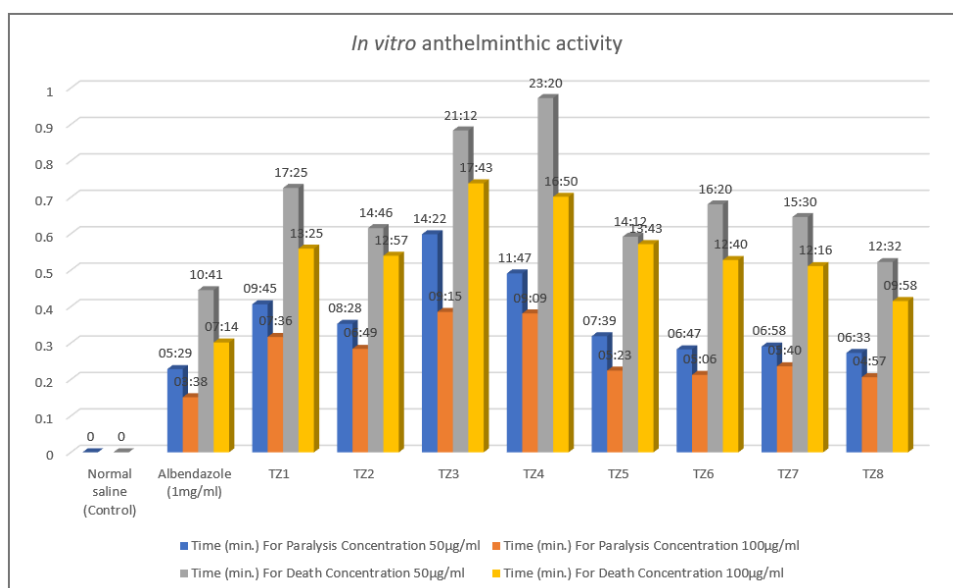
Table 3: Anti-inflammatory activity by egg albumin denaturation assay of TZ1-TZ8.

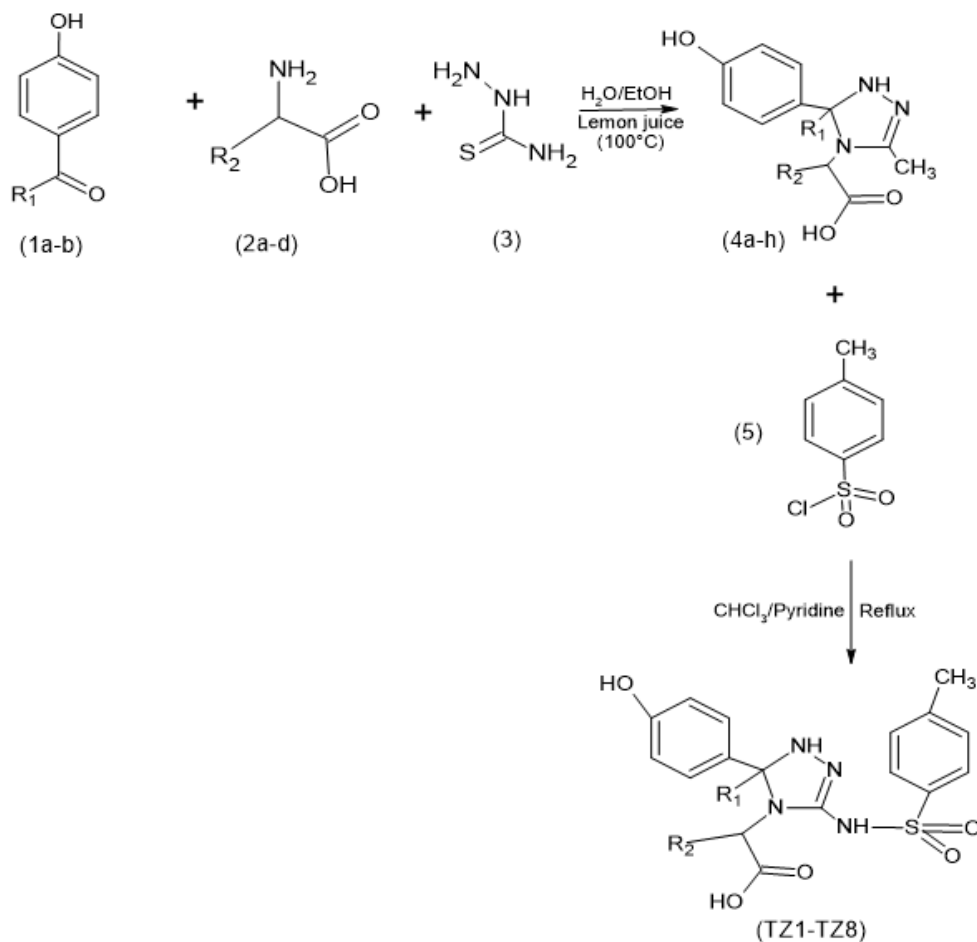
Sl. No.	Name of the Compound	Absorbance Value	Percentage inhibition of protein denaturation (%)
1.	Control	0.088	-
2.	Diclofenac sodium	0.168	90.90
3.	TZ1	0.142	61.36
4.	TZ2	0.144	63.63
5.	TZ3	0.137	55.68
6.	TZ4	0.138	56.81
7.	TZ5	0.144	63.63
8.	TZ6	0.148	68.18
9.	TZ7	0.150	70.45
10.	TZ8	0.153	73.86

Table 4: Anti-bacterial activity by the cup plate method of TZ1-TZ8.

Compound Code	Mean zone of inhibition (mm)			
	<i>E. coli</i>			
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL
TZ1	4	6	7	11
TZ2	5	7	8	10
TZ3	4	5	7	9
TZ4	4	6	8	7
TZ5	7	9	9	13
TZ6	4	5	8	10
TZ7	6	8	10	12
TZ8	4	5	6	9
Standard	23			
Control	10			

Standard drug: Streptomycin, control: DMSO.

**Figure 5:** Anthelmintic activity: paralysis time and time taken for death of the parasite by title compounds TZ1-TZ8.



Scheme 1: R₁-methyl; 1b: R₂-hydrogen; 2a: R₂-benzyl; 2b: R₂-hydrogen; 2c: R₃- 4-hydroxy benzyl; 2d: R₄- 3-methyl indole; TZ1: R₁-methyl, R₂-benzyl; TZ2: R₁-hydrogen, R₂-benzyl; TZ3: R₁-methyl, R₂-hydrogen; TZ4: R₁-hydrogen, R₂-hydrogen; TZ5: R₁-methyl, R₂-4-hydroxy benzyl; TZ6: R₁-hydrogen, R₂-4-hydroxy benzyl; TZ7: R₁-methyl, R₂- 3-methyl indole; TZ8: R₁-hydrogen, R₂- 3-methyl indole.

Table 5: Anthelmintic activity of the title compounds TZ1-TZ8.

Sl. No.	Name	Time (min.)			
		For Paralysis		For Death	
		Concentration		Concentration	
		50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
1.	Normal saline (Control)	-		-	
2.	Albendazole (1 mg/mL)	5:29	3:38	10:41	7:14
3.	TZ1	9:45	7:36	17:25	13:25
4.	TZ2	8:28	6:49	14:46	12:57
5.	TZ3	14:22	9:15	21:12	17:43
6.	TZ4	11:47	9:09	23:20	16:50
7.	TZ5	7:39	5:23	14:12	13:43
8.	TZ6	6:47	5:06	16:20	12:40
9.	TZ7	6:58	5:40	15:30	12:16
10.	TZ8	6:33	4:57	12:32	9:58

min.- minutes.

CONCLUSION

Recently, there has been a considerable interest in the development of triazole derivatives eliciting anti-diabetic activities. The search for compounds with greater efficiency and lesser toxicities continues in Medicinal Chemistry. In our work, we have synthesized novel derivatives of triazoles coupled with amino acids, which shows a significant anti-diabetic, anti-inflammatory, anthelmintic as well as antibacterial activity. Among the synthesized compounds, TZ8 showed significant biological activities against different standard drugs which might be due to presence of additional heterocyclic ring (indole) in its structure. The *in silico* and *in vitro* results of anti-diabetic activity were correlated. This study needs further investigations by other *in vitro*, *in vivo* and toxicity studies to emphasise the TZ8 potency.

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ABBREVIATIONS

TLC: Thin Layer Chromatography; **DNSA:** 3,5-dinitrosalicylic acid; **DMSO:** Dimethyl sulfoxide; **UV:** Ultraviolet; **FTIR:** Fourier-Transform Infrared Spectroscopy; **PDB:** Protein Data Bank; **NMR:** Nuclear Magnetic Resonance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The summary of the current study is that all the 8 synthesised novel derivatives of triazole coupled amino acids showed good anti-diabetic, anti-inflammatory, anthelmintic and anti-bacterial activity. Out of which, TZ8 exhibited significant biological activities compared to different standard drugs due to presence of indole ring in its structure and TZ4 had showed least biological activity.

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