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Synthesis ,characterization, biological evaluation of substituted 1,2,3-traizole derivatives as a potential antidiabetic, antibacterial, antioxidant agents and molecular docking studies.

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Abstract
*In the present study, a series of 1,2,3-triazole were synthesized by employing the regioselective copper(I)-catalyzed azide-alkyne via 1,3-dipolar cycloaddition reaction. The synthesized moieties were screened for α-amylase and glucose uptake assay. The moieties displayed moderate to good inhibitory activity in the range of IC_{50} value of 0.290-2.879 µM and 0.288-1.528 µM against α-amylase and glucose uptake assay respectively. Out of all the synthesized compounds, compound **6a** with IC_{50} value of 0.290µM and 0.288µM with methoxy substitution at aryl ring were found to be most active. Further, the moieties were screened for in vitro antibacterial activity. Among the synthesized compounds, compounds **6b**,**6c**,**7b** and **7c** with -Cl and -Br substitution on aryl ring were found to be most active against E.coli and S. aureus bacterial strains. The synthesized moieties also showed moderate DPPH radical scavenging ability when compared to standard ascorbic acid. Furthermore, the docking studies were employed to determine the binding confirmation of the most active compounds.*

Key words: *Antibacterial, antidiabetic, antioxidant, click-chemistry, molecular docking, 1,2,3-triazole.*

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I. Introduction

One of the most common chronic diseases is diabetes mellites(DM) , that is typified by hyperglycemia, which is brought via insufficient insulin secretion or resistance to insulin action, or both. Chronic hyperglycemia raises the risk of cardiovascular diseases [1] and is associated with microvascular issues that affect the kidneys ,nerves and eyes. Owing to the increasing prevalence of DM ,numerous approaches have been developed to handle and regulate the illness and its causes . However ,a typical method to treat or reduce these metabolic disorders [2] is the inhibition of carbolytic enzymes like α-amylase and α-glucosidase. α-Amylase is a key enzyme of hydrolase family bearing calcium atom as a metal co-factor[3] , it hydrolyzes starch to produce glucose and maltose [4] and it is widely distributed across the animals, plants, fungi and bacteria. The human body requires the enzymes α-amylase and α-glucosidase to catalyze the hydrolytic action of starch intake[5]. Therefore, the development of significant α-amylase inhibitors can be implemented as chemotherapeutic agents and it may be a more effective strategy for DM treatment[6] .

Moreover ,there is a close relationship between hyperglycemia and infections[7]. While hyperglycemia in mouse model inferable leukocytes have been explained , particularly infected by *S. aureus*[8]. Moreover, 30% DM patients have high risk for bacterial infections, most often wound infection(WI) and urinary tract infections(UTI) and pneumonia are common compared to non-diabetic. However, the DM patients have cumulative lifetime risk, mainly foot ulcer , this can get infected easily, which leads to gangrene[9]. Moreover ,hyperglycemia initiates auto-oxidation of glucose to form free radicals, these free radicals beyond the scavenging ability of endogenous antioxidants defenses, which leads to macro and micro vascular dysfunction[10]. While, hyperglycemia induced oxidative stress has also been related with increased endothelial cell apoptosis[11]. For instance, most of the hospital acquired, and community acquired infections are caused by Gram-positive pathogen *S. aureus* and Gram-negative pathogen like *E.coli* [12,13]. The world's scientific and public health communities are gravely concerned about the startling rates of new and emerging microbial threats as well as increasing

antimicrobial resistance to current antibiotics[14]. The necessity for developing novel classes of antimicrobial resistance towards present antibiotics. The new agents with distinct chemical structures and modes of action and broad range of medications with less resistance has been necessary at present with their safety and bioavailability[15].

The discovery and development of novel, safe pharmaceutical is the main goal of current research[16]. Nitrogen heterocycles make up a substantial portion of most pharmacological scaffolds. The concept of molecular hybridization[17] approach has been explored in medicinal chemistry, applying this method two or more biologically active compounds can be joined together to form molecular hybrids. Compared with their parent pharmacophoric units , these hybrid molecules are expected to have a novel mechanism of action and high potency. In this scenario , 1,2,3-triazole plays a significant role in medicinal chemistry by their broad spectrum of biological activities such as anti-HIV[18,19], antiviral[20,21], antiallergenic[22], antifungal[23–25], anticonvulsant[26,27], antidiabetic[28], antibacterial[29,30], antioxidant[31], anti-tubercular[32,33] and anti-inflammatory agents[34] and some of the 1,2,3-triazole molecules are listed in **Fig. 1.** 1,2,3-Triazole have high stability in basic, acidic, oxidative, and reductive conditions[35,36]. Moreover, their favorable property is binding with biological targets. Therefore, adding the triazole motif in the synthesized organic compounds is a promising idea. In this context ,we have prepared various 1,2,3- triazole compounds containing ether, ester, and acid groups in order to investigate the effect of these groups on biological activity.

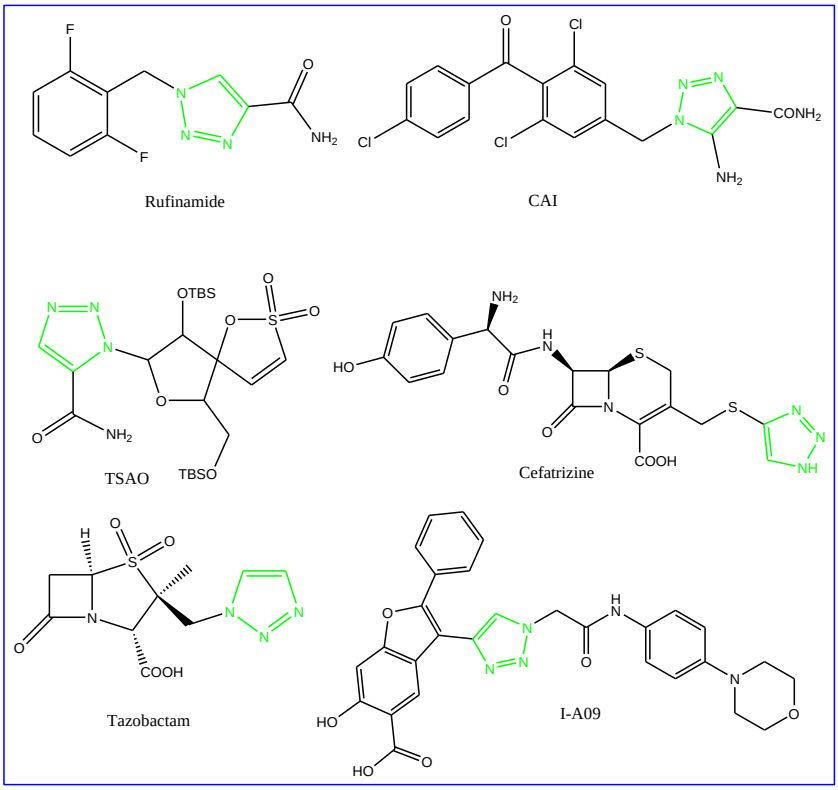


Fig. 1. Triazole validated products on the market.

II. Materials and Methods

The melting points were measured using the open capillary method and are uncorrected. IR spectra were obtained using a Nicolet IN-10 FT-IR spectrophotometer using KBr disc method. The ^{1}H and ^{13}C NMR spectra were captured with a Jeol 400 and 100 MHz spectrometer with solvents DMSO-d_6. Mass spectra were collected using LC-MS/ESI-MS-Waters ACQUITY TQD. CHN elemental analysis of all the products were recorded by LECO TRUSPEC CHN analyser. Unless otherwise noted, all the reagents were of analytical quality and were used without further purification.

Synthesis

Synthesis of substituted(prop-2-ynyloxy)benzene 3(a-e)

To a solution of substituted phenols **1(a-e)**(1.2mmol) in dry acetone, anhydrous potassium carbonate and 3-bromoprop-1-yne**(2)** (1mmol) were added. The resultant mixture was heated under stirring at 50°C for 16 h, the completion of the reaction was monitored by TLC. After cooling the solvent was removed under reduced

pressure. The residue obtained was diluted with water and extracted using $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and was concentrated to get the desired compound **3(a-e).**

Synthesis of methyl 2-(azidomethyl)-3-nitrobenzoate (5)

Compound **(4)** (1mmol) was taken in 15mL of acetone in 50mL round-bottom flask. To this, sodium azide (1.2mmol) in 3 mL of water was added drop wise with stirring, which was continued for 10 h. Atter completion, the reaction mixture was then poured into ice-cold water. Separated solid was filtered and recrystallized using ethanol to get the desired product**(5).**

Synthesis of methyl 2-((4-((substituted)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoate 6(a-e)

The mixture of $CuSO_4.5H_2O$ (0.15 mmol) and sodium ascorbate (0.3 mmol) in water (10 mL) was added to the stirred solution of substituted(prop-2-ynyloxy)benzene **3(a-j)** (1 mmol) in THF (10 mL), then the reaction mixture was kept for stirring at room temperature for about half an hour (till the mixture became lemon-yellow colour), preceded by addition of methyl 2-(azidomethyl)-3-nitrobenzoate **(5**)(1 mmol) which was then refluxed on water bath until the completion of the reaction as monitored by TLC (5:5 ethylacetate: hexane)). The reaction mixture was poured over crushed ice, and the precipitated solid was filtered off, washed with water, and dried to get the desired 1,4 - substituted triazoles **6(a-e)** in pure form.

Methyl2-((4-((4-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoate(6a)

Light yellow solid; Yield: 96%; MP:144-146°C ; FT-IR(cm^{-1}): 1719(C=O stretching, ester) ; MS (EI) *m/z*: Calcd. for $C_{19}H_{18}N_4O_6$ – 398.37, Found- 399.19 [M+1],400.19[M+2]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.25 (d, J = 8.6 Hz, 1H,), 8.19 (d, J = 7.9 Hz, 1H,), 8.12 (s, 1H,), 7.83 (t, J = 8.3 Hz, 1H), 6.94 (d, J = 9.2 Hz, 2H), 6.85 (d, J = 9.2 Hz, 2H), 5.97 (s, 2H), 5.02 (s, 2H), 3.83 (s, 3H), 3.69 (s, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 166.4, 154.0, 152.5, 151.6, 143.0, 135.1, 134.1, 131.2, 128.7, 127.8, 125.4, 116.1, 115.1, 61.9, 55.8, 53.6, 46.2; Anal. Calcd. for $C_{19}H_{18}N_4O_6$: C, 57.28; H, 4.55; N,14.06; Found: C, 57.11; H, 4.49; N,14.00.

Methyl2-((4-((4-chlorophenoxy)m-1H-1,2,3-triazol-1-yl)methyl)-3- nitrobenzoate(6b)

Light yellow solid; Yield: 96%; MP: 148-150°C ; FT-IR(cm^{-1}): 1719(C=O stretching, ester) ; MS (EI) *m/z*: Calcd. for $C_{18}H_{15}ClN_4O_5$ – 402.79, Found- 403.14 [M+1],405.14[M+3]; ^{1}H-NMR (400 MHz, DMSO-d_6);) δ8.25 (d, J = 8.6 Hz, 1H), 8.20(d, J = 7.9 Hz, 1H),8.17 (s, 1H), 7.83 (t, J = 7.9 Hz, 1H), 7.33 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 8.6 Hz, 2H), 5.98 (s, 2H), 5.10 (s, 2H), 3.83 (s, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 166.4, 157.4, 151.6, 142.4, 135.1, 134.1, 131.3, 129.8, 128.7, 127.7, 125.6, 125.0, 117.0, 61.7, 53.6, 46.2; Anal. Calcd. for $C_{18}H_{15}ClN_4O_5$: C, 53.67; H, 3.75; N,13.91; Found: C, 53.57; H, 3.55;N,13.71.

Methyl2-((4-((4-bromophenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-nitrobenzoate(6c)

Light yellow solid; Yield: 97%; MP:150-152°C ; FT-IR(cm^{-1}): 1720(C=O stretching , ester) ; MS (EI) *m/z*: Calcd. for $C_{18}H_{15}BrN_4O_5$–447.24, Found- 447.06 [M],449.06[M+2]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ8.25 (d, J = 7.9 Hz, 1H), 8.19 (d, J = 7.4 Hz, 1H),8.16(s,1H) 7.83 (t, J = 8.3 Hz, 1H), 7.45 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 9.2 Hz, 2H), 5.98 (s, 2H), 5.10 (s, 2H), 3.83 (s, 3H)^{13}C-NMR (101 MHz, DMSO-d_6) δ166.4, 157.8, 151.6, 142.4, 135.1, 134.1, 132.6, 131.3, 128.7, 127.7, 125.6, 117.5, 112.8, 61.6, 53.6, 46.2; Anal. Calcd. for $C_{18}H_{15}BrN_4O_5$: C, 48.34; H, 3.38; N,12.53;Found: C, 48.14; H, 3.28; N,12.43.

Methyl2-((4-((3,5-dimethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)- nitrobenzoate(6d)

Light yellow solid; Yield: 95%; MP:146-148°C ; FT-IR(cm^{-1}): 1724(C=O stretching, ester) ; MS (EI) *m/z*: Calcd. for $C_{20}H_{20}N_4O_5$–396.40, Found- 397.21 [M+1],398.21[M+2]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ8.25 (d, J = 7.9 Hz, 1H), 8.19 (d, J = 7.3 Hz, 1H), 8.13 (s, 1H), 7.82 (t, J = 7.9 Hz, 1H), 6.62-6.57 (m, 3H), 5.98 (s, 2H), 5.04 (s, 2H), 3.83 (s, 3H), 2.22 (s, 6H);^{13}C-NMR (101 MHz,DMSO-d_6) δ 166.4, 158.6, 151.6, 143.0, 139.1, 135.1, 134.1, 131.2, 128.7, 127.8, 125.4, 123.0, 112.9, 61.2, 53.6, 46.2, 21.6; Anal. Calcd. for $C_{20}H_{20}N_4O_5$: C, 60.60; H, 5.09; N,14.13; Found; C, 60.40; H, 5.07; N,14.10.

Methyl2-((4-((p-tolyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoate(6e)

Light yellow solid; Yield: 97%; MP: 140-142°C ; FT-IR(cm^{-1}): 1720(C=O stretching, ester) ; MS (EI) *m/z*: Calcd. for $C_{19}H_{18}N_4O_5$–382.37, Found- 383.19[M+1],384.19[M+2]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 7.9 Hz, 1H), 8.14 (d, J = 7.3 Hz, 1H), 8.10 (s, 1H), 7.78 (t, J = 7.9 Hz, 1H), 7.03 (d, J = 7.9 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 5.94 (s, 2H), 5.00 (s, 2H,), 3.79 (s, 3H), 2.18 (s, 3H);^{13}CNMR (101 MHz, DMSO-d_6) δ 166.4, 156.4, 151.6, 142.9, 135.1, 134.1, 131.2, 130.3, 130.0, 128.7, 127.8, 125.4, 115.0, 61.4, 53.6, 46.2, 20.6; Anal. Calcd. for $C_{19}H_{18}N_4O_5$: C, 59.68; H, 4.74; N,14.65; Found; C, 59.48; H, 4.54; N,14.35.

Synthesis of 2-((4-((substituted)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3- nitrobenzoic acid 7(a-e)

Methyl 2-((4-((substituted)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoate **6(a-e)** was taken in 5 mL of 1,4-dioxane in a 15mL round-bottom flask. To this, sodium hydroxide(10%) was added drop wise with stirring, which was continued for 4 h (reaction was monitored by TLC). The reaction mixture was then poured into ice-cold water. Separated solid was filtered and recrystallized using ethanol to get the desired acid derivatives as product **7(a-e).**

2-((4-((4-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoic acid(7a)

White solid; Yield: 96%; MP:154-156°C ; FT-IR(cm^{-1}): 1707(C=O stretching , acid) ; MS (EI) *m/z*: Calcd. for $C_{17}H_{13}ClN_4O_5$–384.34, Found- 385.18[M+1],386.18[M+2]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ8.10 (d, J = 7.9 Hz, 1H), 8.04 (d, J = 7.3 Hz, 1H), 8.00 (s, 1H), 7.67 (t, J = 7.9 Hz, 1H), 6.93 (d, J = 7.9 Hz, 2H), 6.75 (d, J = 8.6 Hz, 2H), 5.83 (s, 2H), 4.90 (s, 2H), 3.68 (s, 3H);^{13}C-NMR(101 MHz, DMSO-d_6) δ 154.0, 152.5, 151.6, 143.0, 135.1, 134.1, 131.2, 128.7, 127.8, 125.4, 116.1, 115.1, 61.9, 55.8, 46.2;Anal. Calcd. for $C_{17}H_{13}ClN_4O_5$: C, 52.52; H, 3.37; N,14.41; Found; C, 52.42; H, 3.22; N,14.38.

2-((4-((4-chlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoic acid(7b)

White solid; Yield: 96%; MP:158-160°C ; FT-IR(cm^{-1}): 1697(C=O stretching , acid) ; MS (EI) *m/z*: Calcd. for $C_{17}H_{13}ClN_4O_5$–388.76, Found- 389.13[M+1],391.12[M+3]; ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.15-8.01 (m, 3H), 7.78 (s, 1H), 7.28 (d, J = 7.9 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H), 6.00 (s, 2H), 5.05 (s, 2H);^{13}C-NMR (101 MHz, DMSO-d_6) δ157.4, 151.8, 142.4, 135.3, 131.2, 129.8, 128.3, 125.6, 125.0, 117.0, 61.7, 46.2;Anal. Calcd. for $C_{17}H_{13}ClN_4O_5$: C, 52.52; H, 3.37; N,14.41; Found; C, 52.42; H, 3.22; N,14.38.

2-((4-((4-bromophenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoic acid(7c)

White solid; Yield: 96%; MP:160-162°C ; FT-IR(cm^{-1}): 1696(C=O stretching, acid)) ; MS (EI) *m/z*: Calcd. for $C_{17}H_{13}BrN_4O_5$–433.21, Found- 433.04[M],435.04[M+2]; ^{1}H-NMR (400 MHz, DMSO-d_6); δ8.21-8.14 (m, 3H), 7.79 (s, ,1H), 7.44 (d, J = 6.7 Hz, 2H), 7.00 (d, J = 6.7 Hz, 2H), 6.05 (s, 2H), 5.08 (s, 2H);^{13}C-NMR (101 MHz, DMSO-d_6) δ167.8, 157.6, 151.6, 142.4, 135.2, 132.5, 130.9, 128.1, 127.4, 125.6, 117.5, 112.8, 61.5, 46.1; Anal. Calcd. for $C_{17}H_{13}BrN_4O_5$: C, 47.13; H, 3.02; N,12.93; Found; C, 47.10; H, 3.00; N,12.73.

2-((4-((3,5-dimethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoic acid(7d)

White solid; Yield: 95%; MP: 154-156°C ; FT-IR(cm^{-1}): 1694(C=O stretching , acid)) ; MS (EI) *m/z*: Calcd. for $C_{19}H_{18}N_4O_5$ –382.37, Found- 383.19[M+1],384.20[M+2]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.15-8.07 (m, 3H), 7.77 (s, 1H), 6.55 (d, J = 20.2 Hz, 4H), 6.00 (s, 2H), 4.99 (s, 2H), 2.17 (s, 6H);^{13}C-NMR (101 MHz, DMSO-d_6) δ 158.9, 151.8, 142.9, 139.4, 135.7, 131.0, 128.2, 125.6, 123.0, 113.0, 61.3, 46.1, 21.6; Anal. Calcd. for $C_{19}H_{18}N_4O_5$: C, 59.68; H, 4.74; N,14.65; Found; C, 59.58; H, 4.54; N,14.55.

2-((4-((p-tolyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-nitrobenzoic acid(7e)

White solid; Yield: 96%; MP:150-152°C ; FT-IR(cm^{-1}): 1703(C=O stretching , acid)) ; MS (EI) *m/z*: Calcd. for $C_{18}H_{16}N_4O_5$ –368.34, Found- 369.17[M+1],370.18[M+2]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ8.19-7.96 (m, 3H), 7.83 (s, 1H), 7.08 (d, J = 6.1 Hz, 2H), 6.91 (d, J = 6.1 Hz, 2H), 6.08 (s, 2H), 5.05 (s, 2H), 2.22 (s, 3H);^{13}C-NMR (101 MHz, DMSO-d_6) δ 156.7, 151.5, 143.7, 135.9, 131.3, 130.3, 130.0, 128.2, 125.5, 115.0, 61.5, 46.1, 20.6; Anal. Calcd. for $C_{18}H_{16}N_4O_5$: C, 58.65; H, 4.38; N,15.21; Found; C, 58.53; H, 4.28; N,15.10.

Pharmacological screening

α- Amylase inhibitory activity

The α- amylase inhibitory activity was determined according to the method[37] with slight modification. In an Eppendorf tube 0.5mL of phosphate buffer saline (PBS) solution of pH 6.9 was mixed with 0.5mL of different concentrations of samples (10,20,40,80 and 160 µg/mL) with standard solution and 50µL of 0.5mg/mL α- amylase followed by 150 µL of 4µg/mL starch solution and incubated for 10mins at room temperature starch was taken as control. The reaction was stopped by adding 250 µL of dinitro salicylic acid (DNS) solution. The mixture were then incubated on a water bath for 5 mins and cooled to room temperature. The absorbance was measured at 540nm(Labman UV visible spectrophotometer). The percentage(%) of enzyme inhibition was calculated using the following formula.

$$\% \text{ α- amylase Inhibition} = \frac{Ab_C - Ab_S}{Ab_C} \times 100$$

Where Ab_c denotes the absorbance of control and Ab_s denotes the absorption of test compounds at 540nm. (All the experiments were carried out in triplicates)

Glucose uptake activity

The glucose uptake activity was determined according to the method[38] with slight modification. Repeated centrifugation (3000Xg,5min) of the yeast saccharomyces cerevisiae, in distilled water was performed until clear supernatant fluid was prepared in distilled water. Different concentrations of compounds (10,20,40,80 and 160 µg/mL) were placed in test tubes. 1mL of glucose solution (5mM) was added to this and the mixture was incubated at 37°C for 120 mins. The tubes were centrifugated (2500Xg,5min) and glucose content in the supernatant was calculated . The absorbance was measured at 540nm(Labman UV visible spectrophotometer). The standard drug used was Pioglitazone and the percentage(%) increase in glucose uptake by yeast cell was calculated using the following formula.

$$\% \text{ increase in glucose uptake} = \frac{Ab_C - Ab_S}{Ab_C} \times 100$$

Where Ab_c denotes the absorbance of control and Ab_s denotes the absorption of test compounds at 540nm. (All the experiments were carried out in triplicates)

***In vitro* antibacterial activity**

Methods

MIC of the newly synthesized compounds was determined by resazurin based micro-broth dilution method according to the guidelines of CLSI document M07 and M27-A2[39] with slight modifications. 10 µL culture inoculums were added with 180 µL sterile broth in a sterile flat bottom 96 well plate and 10 µL of (20 x concentration) compounds were added to make final concentration from 2-256 µg/mL in a serial dilution manner. Ampicillin (AMP), ciprofloxacin (CIP) was used as standard drug for Gram- positive, Gram- negative bacteria respectively. The plate was incubated for 24 h at 37°C. After incubation period, each of the wells were added with 20 µL of resazurin solution (0.015%) and mixed thoroughly and allowed to stand for half an hour to one hour for colour change.

Microorganisms and media

The synthesized compounds were tested for *in vitro* antibacterial activity against two microbial cultures. Inoculum was prepared by resuspending the pure loop full of exponentially growing culture on LB agar in a 5mL of sterile saline (0.85%). The newly synthesized compounds were vortexed/sonicated in ultrasonic bath till dissolved and the solution become homogenous (final concentration of < 1% DMSO). Using spectrophotometer, the cell density was fixed to 70% transmittance at 530nm (Labman, LMSP UV-1200) which contained 1×10^6 to 5×10^6cells per mL[40]. MIC values for bacteria was determined in Miller Hilton broth as recommended by CLSI[41].

The minimum inhibitory concentration(MIC) and minimum bactericidal concentration (MBC)

The MIC was defined as the lowest concentration of synthesized compounds or standard drug resulting in persistent blue colour. After determining the MIC, an aliquot of 50 µL sample was withdrawn from each blue colour well up to 8 folds of the MIC and spread onto respective agar plates. Inoculated plates were incubated, and MBC was recorded after 36 hrs. The compound is said to be static in action if there's growth at eight folds the MIC whose MBC is not determined (ND). The MBC is defined as the lowest concentration of each compound that resulted in total inhibition or ≥99% inhibition of the growth[42].

***In vitro* antioxidant activity**

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was determined according to the method[43] with slight modification employed to examine the free radical scavenging ability of the synthesized compounds **6(a-e)** and **7(a-e)**. The ability of test samples to donate the hydrogen atoms was governed by the decolorization of methanol solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH). In methanol solution, DPPH produces violet colour and in the presence of antioxidants, it turns to yellow colour. To 0.1 mM methanolic solution of DPPH (300µL), 10 µL of different concentrations of each of the test samples was added and after shaking vigorously the tubes were allowed to stand at room temperature in dark for 30 minutes. Then the changes in absorbance of samples were measured at 517 nm. Using methanol, a control reading was obtained instead of the extract. The radical scavenging ability of the test compounds were compared with the standard drug Ascorbic acid. The percentage of inhibition was measured by the following formula.

$$\% \text{ DPPH radical scavenging activity} = \frac{Ab_C - Ab_S}{Ab_C} \times 100.$$

Where Ab_c denotes the absorbance of control and Ab_s denotes the absorption of test compounds at 517nm. (All the experiments were carried out in triplicates)

Molecular docking

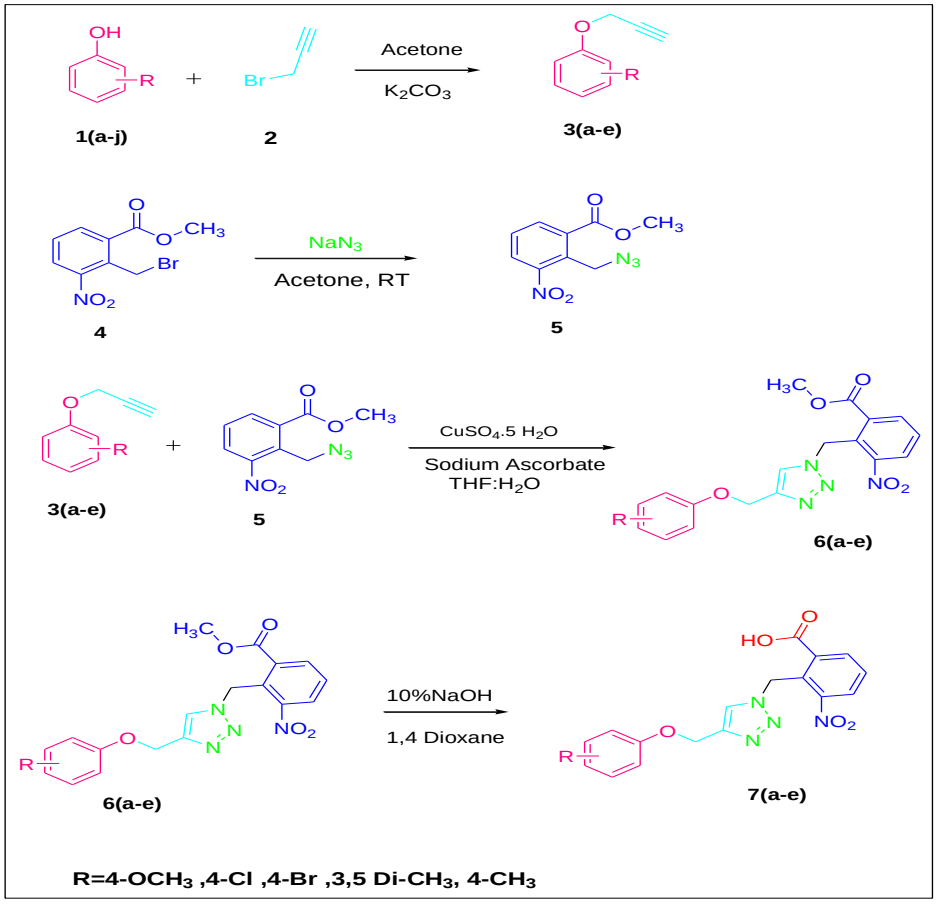
Molecular modeling was carried out using Sybyl-X, version 2.0[44], running on a Intel® Core™ i3-2130 CPU@ 3.40GHz processor using Windows 7 professional workstation. Surflex-Dock algorithm of sybyl-X 2.0 was used to dock designed compounds. The crystral Structure of the Human pancreatic α-amylase in complex with montbretin A was downloaded from the Protein Data Bank (PDB entry code 4W93 (X-Ray Diffraction; 1.35 Å); PDB extracted from the Brookhaven Protein Database http://www.rcsb.org/pdb) and used for initial docking studies. Co-crystalized ligand was removed from the structure, water molecules were removed, essential H atoms were added, and side chains were fixed during protein preparation. The structure was then subjected to an energy refinement procedure. Gasteigere-Huckel charges[45] were calculated for the ligand, while Amber7FF02 were used for the protein. The model was then subjected to energy minimization following the gradient termination of the Powell method for 3000 iterations using Tripos force field with non-bonding cutoff set at 9.0 and the dielectric constant set at 4.0. The binding of the substituted indole derivatives was also estimated using a variety of scoring functions that have been compiled into the single consensus score (C score).

III. Results and Discussion

Chemistry

The desired target structures **6(a-e)** and **7(a-e)** were designed and synthesized stepwise. The required key intermediate compounds **3(a-e)** [46,47]and (**5)**[48] were synthesized as per literature reports and are illustrated in **scheme 1.** Compound **3**(**a-e)** was obtained by the reaction of substituted phenols **1(a-j)**and propargyl bromide**(2)** *via* SN reaction in excellent yield**.** Further, the required one more intermediate structure (**5)** is synthesized by the reaction of methyl 2-(bromomethyl)-3-nitrobenzoate **(4)** with sodium azide in aqueous acetone at room temperature led to compound(**5)**. Finally, the desired 1,4 -substituted 1,2,3-traizole **6(a-e)**was obtained by the reaction of terminal alkynes **3(a-e)** with dipolar azide **(5)** in presence of copper sulphate and sodium ascorbate in THF/H_2O (1:1) under reflux condition, in excellent yield and high purity **scheme 1.** Further, the ester derivatives **6(a-e)** were subject to saponification in presence of sodium hydroxide to obtain respective substituted acid derivatives **7(a-e).**

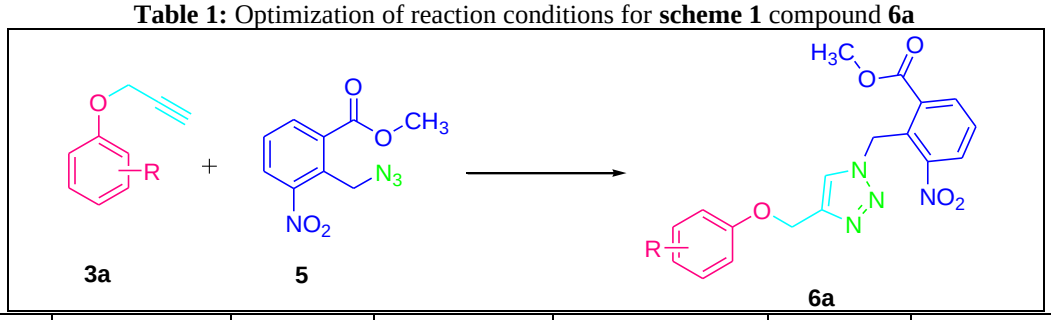
The synthesized molecules were subjected to spectral analysis to validate the formation of the proposed structures. FT-IR spectrum of **6c** showed a band at 1720 cm^{-1} due to the ester carbonyl stretching . The mass of the compound was confirmed by LC-MS analysis where in [M] and [M+2] peak were observed at 447.06 and 449.06 confirming the mass of the compound **6c**. ^{1}H-NMR of **6c** singlet at δ 8.16 ppm indicates triazole ring proton and methylene protons linked to nitrogen and oxygen are resonated as singlet at δ 5.98 and δ 5.10 ppm respectively. Methyl protons of ester are resonated as singlet at δ 3.83 ppm. Signals in the range δ 8.25 to δ 7.00 ppm represents the remaining 7 aromatic protons. In ^{13}C-NMR of compound **6c**, the ester carbonyl carbon resonated at δ 166.4 ppm. Whereas, the carbon attached to nitrogen and oxygen are resonated at δ61.6 and δ53.6 ppm respectively and methyl carbons of ester are resonated at δ46.2ppm . Signals in the range from δ 157.8 to δ112.8 ppm represents the remaining 12 carbons. Further, the saponification of the compound **7a** was confirmed by the decrease in the value of carbonyl stretching frequency in FT-IR spectrum from 1720 to 1696 cm^{-1} and disappearance of singlet at δ 3.83 ppm in ^{1}H-NMR corresponding to methyl protons of ester.



Scheme 1. Synthesis of 1,4 substituted 1,2,3-triazole derivatives 6(a-e) and acid derivatives 7(a-e).

Furthermore, to synthesis desired series of compounds **6(a-e)** in good yields and purity the optimisation reaction condition has been made for selection of suitable solvent, temperature and amount of copper sulphate, using reactants (**3a)** and (**5) (scheme 1)**. The optimised results obtained are tabulated in **Table 1**. and we noticed that THF-water (**entry-3,4**) found to be excellent. Under optimized reaction conditions a series of compounds were synthesized and are listed in **Fig.2.**

Table 1: Optimization of reaction conditions for **scheme 1** compound **6a**



Entry	Solvent (1:1)	Temperature (^{0}C)	$CuSO_4.5H_2O$ (mM)	Sodium ascorbate(mM)	Time (hours)	Yield (percentage)
1	THF: H_2O	RT	0.10	0.20	10	80
2	THF: H_2O	60	0.10	0.20	7	80
3	**THF: H_2O**	**76**	**0.10**	**0.20**	**4**	**94**
4	**THF: H_2O**	**76**	**0.10**	**0.40**	**4**	**96**
5	THF: H_2O	76	0.10	0.60	4	96
6	DMSO:H_2O	RT	0.10	0.20	10	86
7	DMSO:H_2O	80	0.10	0.40	4	90

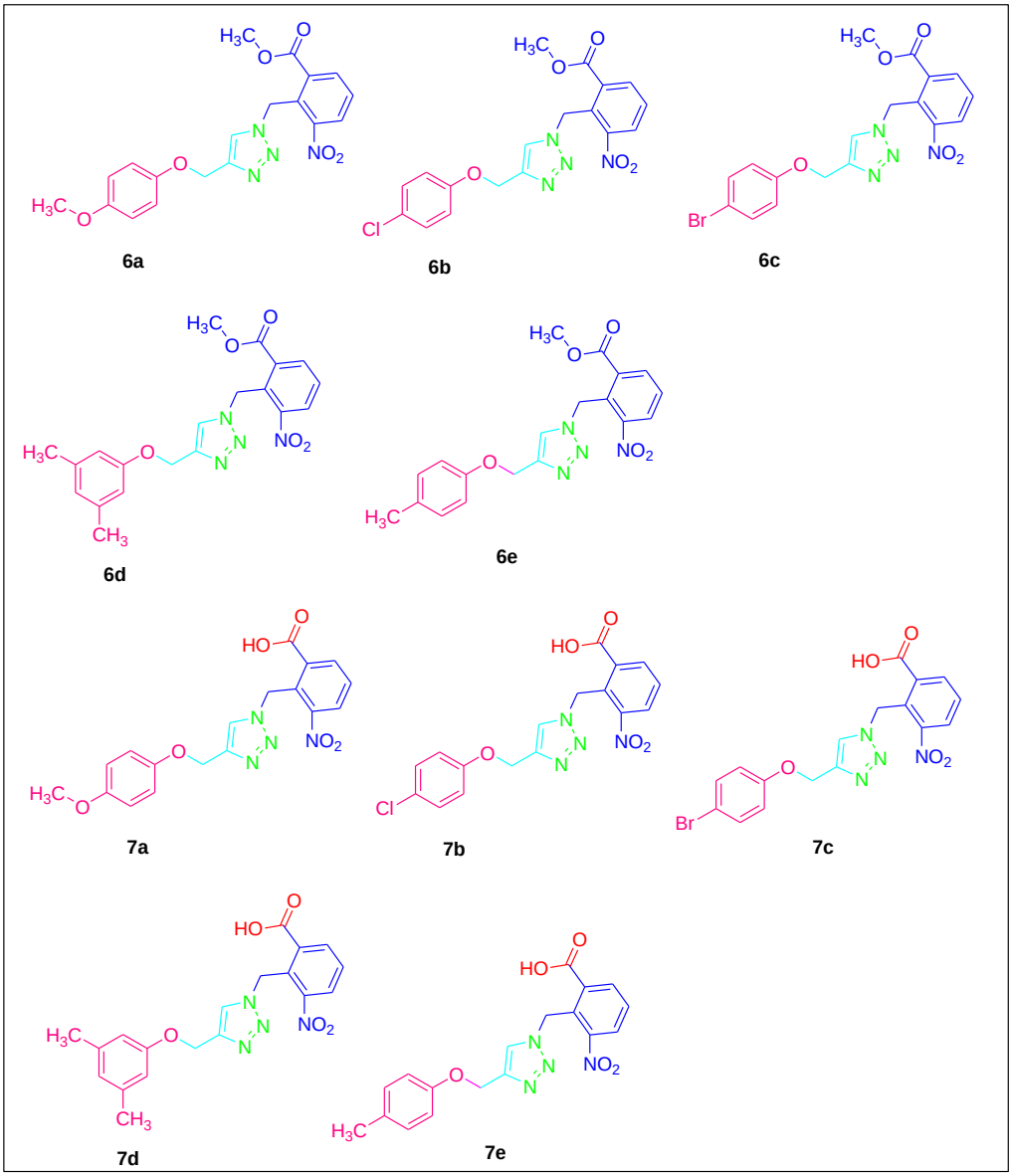


Fig.2. Structures of all the synthesized compounds **6(a-e)** and **7(a-e)**.

Antidiabetic activity

The *in vitro* inhibition of α – amylase and glucose uptake assay were employed for the synthesized compounds **6(a-e)** and **7(a-e)** using standard drug acarbose (ACB) and pioglitazone (PCZ). A drug's half - maximal inhibitory concentration (IC_{50}) was determined, which indicates the amount needed to halve a biological process and also indicates the substance's capacity to prevent a particular biological or biochemical function[49].

***In vitro* α – amylase inhibition activity**

From the **Table 2**, we noticed that the synthesized compounds **6(a-e)** and **7(a-e)** showed varying effects on α – amylase inhibition activity. Among the series **6(a-e)** which having nitro and methyl ester groups are common substituents, compounds **6a** and **6e** having methoxy and methyl groups on aryloxy systems have shown maximum inhibition with IC_{50} values 0.290 µM and 0.363 µM respectively, while standard drug acarbose (ACB) showed 0.144µM. Further the compounds **6d** and **6b** showed moderate inhibition with IC_{50} value 0.393 µM and 0.583µM respectively. Wherein **7(a-e)** series having nitro and carboxy groups are common substituents, compounds **7e** and **7a** having methyl and methoxy groups on aryl systems have shown maximum inhibition with IC_{50} values 0.364 µM and 0.374 µM respectively. Further the compounds **7d** and **7b** showed minimum inhibition with IC_{50} value 0.463 µM and 1.162 µM respectively. The percentage of enzyme inhibition increases with increase in concentration up to 80µg/mL, further increasing the concentration yields the saturated inhibition of enzyme α- amylase and is presented in **Fig.3.**

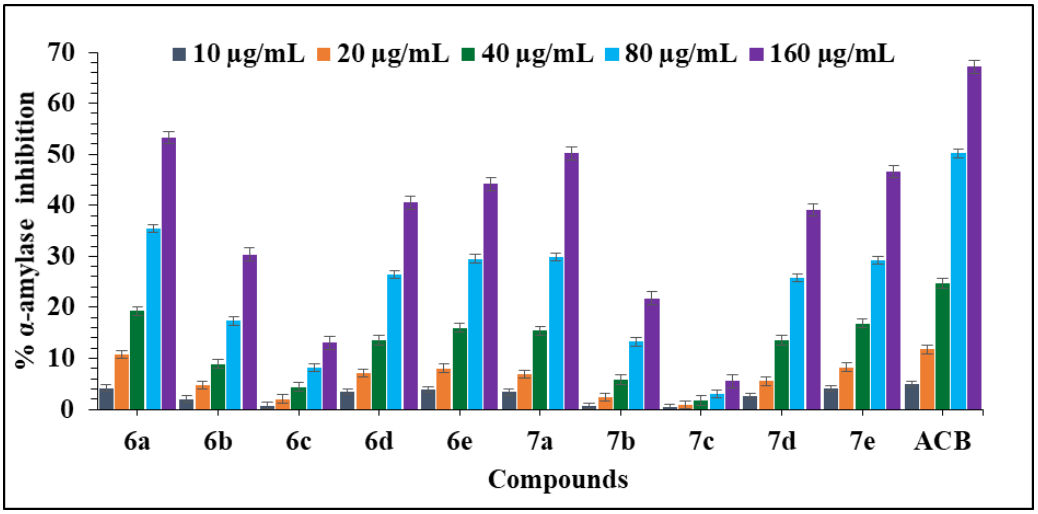


Fig.3. Results of *in vitro* α-amylase inhibitory activity of various concentrations of newly synthesized compounds, and standard drug acarbose (ACB), expressed in % enzyme inhibition. All the experiments were performed in triplicates and mean absorbance was taken for calculating % inhibition. Error bars represent standard error.

***In vitro* glucose uptake activity**

Different concentration of compounds were tested *in vitro* using modified method [38] for glucose absorption ,with yeast acting as a model. The results were compared with standard drug Pioglitazone (PCZ). Among the series, compound **6a** shows maximum glucose absorption with IC_{50} value 0.288µmol/mL which is very close to the standard drug Pioglitazone (IC_{50} = 0.273µM) and the compounds **6e**,**7a**,**7e** and **7d** showed medium glucose absorption with IC_{50} value 0.324,0.327,0.345 and 0.364 µM respectively. Further the compounds **6d**,**6b**,**7b**,**6c** and **7c** showed minimal glucose absorption with IC_{50} value 0.413,0.450,0.614,1.469 and 1.586 µM respectively. The percentage of glucose absorption increases with increase in concentration up to 80µg/mL, further increasing the concentration showed the saturated increase point of glucose absorption and is presented in **Fig.4.**

The results obtained from above two activities, α -amylase inhibition activity and glucose uptake activity provide summary of structure activity relationship (SAR) for this class of compounds. Introduction of electron donating groups (EDG) such as 4-methoxy (**6a**,**7a**), 4-methyl(**6e**,**7e**), 3,5-dimethyl (**6e**,**7e**) into the aryloxy ring results in increased inhibition as well as glucose uptake activity. While the introduction of electronegativity groups such as 4-chloro (**6b** and **7b**) and 4-bromo(**6c** and **7c**) into the aryl ring displayed decreased inhibition as well as glucose uptake activity. In summary, the results suggested that the substitution on the aryl ring has a direct effect on the biological activity of this class of compounds. The present investigation showed that the synthesized compounds **6(a-e)** and **7(a-e)** are effective agents against diabetes.

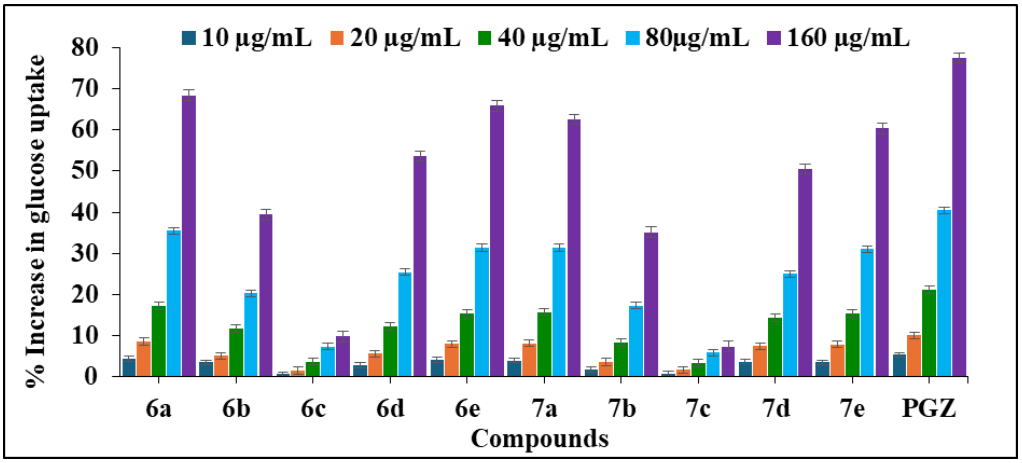


Fig.4.Results of *in vitro* glucose uptake assay of various concentrations of newly synthesized compounds, and standard drug Pioglitazone hydrochloride (PGZ), expressed in % increase in glucose uptake by yeast cells. All the experiments were performed in triplicates and mean absorbance was taken for calculating % increase in glucose uptake. Error bars represent standard error.

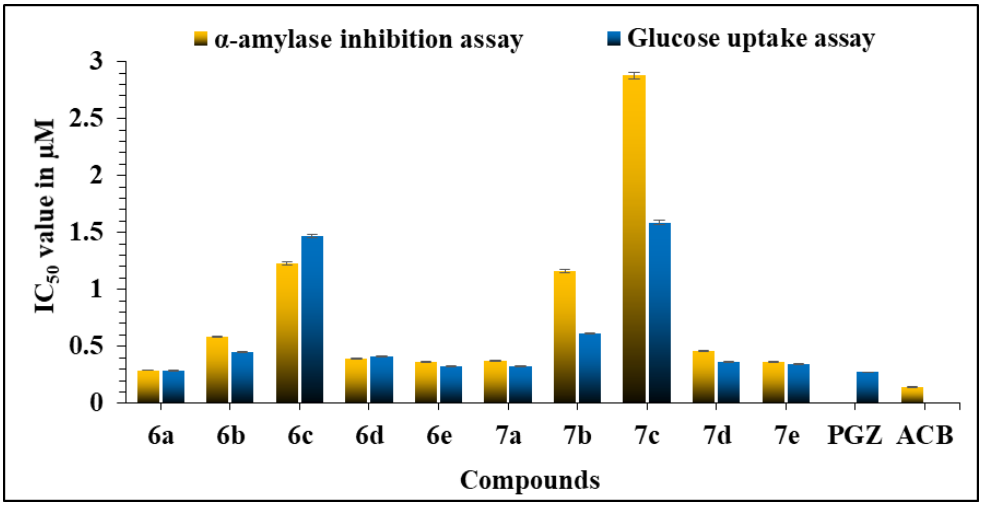


Fig.5. Comparison of mean IC_{50} values (µmol/mL) of α -amylase inhibition and glucose uptake activities of all the synthesized compounds **6(a-e)** and **7(a-e)**. Error bars represent standard error.

Table 2. *In vitro* antidiabetic activities of synthesized compounds.

Compound name	α-amylase inhibition activity	Glucose uptake activity
	IC_{50}(µM)	IC_{50}(µM)
6a	**0.290**	**0.288**
6b	0.583	0.450
6c	1.231	1.469
6d	0.394	0.413
6e	**0.363**	**0.324**
7a	**0.374**	**0.327**
7b	1.162	0.614
7c	2.879	1.586
7d	0.463	**0.364**
7e	**0.364**	**0.345**
Pioglitazone	-	0.273
Acarbose	0.144	-

Data represents in terms of mean of three independent experiments.

Antibacterial activity

All the synthesized compounds **6(a-e)** and **7(a-e)** were analyzed for their *in vitro* antibacterial capacity by resazurin based micro-broth dilution method[39]. The antibacterial assay was studied against Gram-positive bacteria *S. aureus (ATCC 6538),* Gram-negative bacteria *E.coli (ATCC 10799)* .The compounds showed moderate to minimum activity against both bacterial strains. The results are represented in **Table 3.**

From the antibacterial activity results it was revealed that the compound **6b** showed excellent activity against Gram-negative bacteria *E.coli* with MIC value 8µg/mL compared to standard drug ampicillin (AMP). Whereas , compounds **6c** and **7c** showed good activity with MIC value 16µg/mL and compound **7b** showed moderate activity with MIC value 32µg/mL. While , in case of Gram-positive bacteria compound **7b** showed moderate activity with MIC value16µg/mL compared to the standard drug ciprofloxacin (CIP) . Whereas , compounds **6b** and **7c** showed minimum activity with MIC value 32µg/mL compared to the standard drug. Among the ester and acid substituted series , acid substituted series found to be more active than ester substituted series. Antibacterial performed experiments is placed in **Fig.6.** Summarised results from the **Table 3** halogen substituted compounds showed good activity against both bacterial strains compared to the other substitution .

Table 3 . Results of the MIC and MBC of newly synthesized compounds along with standard drug as determined by resazurin based micro-broth dilution method and colony forming units (CFU).

Compound name	Concentration in µg/mL			
	Gram negative *E. coli*		Gram positive *S. aureus*	
	MIC	MBC	MIC	MBC
6a	64	ND	128	ND

6b	**8**	256	**32**	ND
6c	**16**	256	**64**	ND
6d	128	ND	ND	ND
6e	256	ND	256	ND
7a	64	ND	128	ND
7b	**32**	ND	**16**	256
7c	**16**	256	**32**	ND
7d	128	ND	256	ND
7e	256	ND	256	ND
AMP	4	16	ND	ND
CIP	ND	ND	2	8

(ND: Not determined).

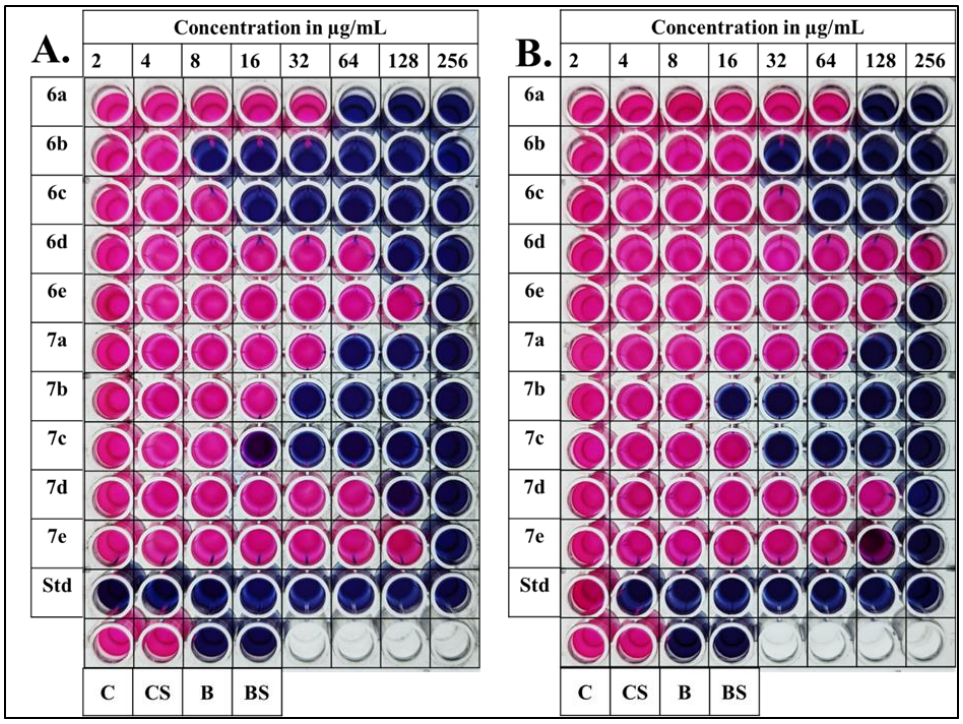


Fig.6. Results of MIC of the newly synthesized compounds along with standard drug as determined by resazurin based micro-broth dilution method against A) *E. coli;* B*) S. aureus*; B: broth; S:solvent; C: culture .

Antioxidant activity

***In vitro* antioxidant activity by DPPH free radical scavenging assay**

In the present work ,the free radical scavenging activity of the synthesized compounds **6(a-e)** and **7(a-e)** was achieved against the stable free radicals of (DPPH) and compared with ascorbic acid(Asco) as a standard antioxidant agent. For more investigations ,the minimum active concentration and IC_{50} (concentration required to scavenge 50% of the radicals) values of the synthesized compounds **6(a-e)** and **7(a-e)** were determined by evaluating their antioxidant activity at five different concentrations (10,20,30,40,50 µg/mL) of each compound. The scavenging activity with respect to different concentrations and IC_{50} values are listed in the **Table 4** and represented in **Fig.7** and **8** respectively. It is evident from the test results that most of the compounds have shown moderate activity with lower IC_{50} values and also noticed that antioxidant capacity increases with increasing the concentration. Compounds **6a** and **7a** with 4-methoxy substitution on the aryloxy ring exhibited good antioxidant activity with IC_{50} values 41.04 and 42.38 µg/mL respectively. Whereas , compounds **7e** and **6e** bearing 4-methyl substitution on aryloxy ring exhibited moderate antioxidant activity with IC_{50} values 51.93 and 53.28 µg/mL respectively. Among the ester and acid substituted series , ester series **6(a-e)** showed good antioxidant activity as that off the acid series **7(a-e)**. Summarised results from the table the electron donating groups bearing compounds showed good antioxidant activities and electronegative groups bearing compounds showed moderate antioxidant activities.

Table 4. Results of DPPH radical scavenging assay of synthesised molecules **6(a-e)** and **7(a-e)**.

Compound name	**Scavenging activity (%)**					**IC_{50} µg/mL**
	10 µg/Ml	**20 µg/mL**	**30 µg/mL**	**40 µg/mL**	**50 µg/mL**	
6a	**13.76**	**24.21**	**37.09**	**49.05**	**60.92**	**41.04**

6b	7.17	13.76	23.39	31.00	37.69	66.32
6c	5.65	10.11	15.08	20.76	24.92	100.34
6d	9.40	18.32	27.15	36.58	45.00	55.56
6e	**10.01**	**19.24**	**29.28**	**38.30**	**46.92**	**53.28**
7a	**12.95**	**23.69**	**36.38**	**47.43**	**58.99**	**42.38**
7b	5.85	10.21	16.50	21.47	25.52	97.95
7c	5.24	9.60	14.67	20.05	24.21	103.28
7d	8.18	15.79	24.81	32.32	40.33	61.99
7e	**10.62**	**20.05**	**30.19**	**39.11**	**48.14**	**51.93**
Asco	19.30	38.03	57.00	76.94	94.76	26.38

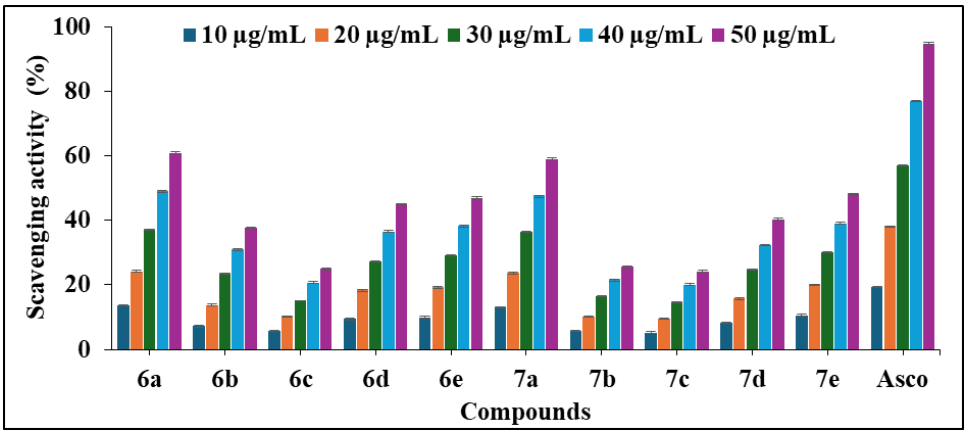


Fig.7. Graphical representation of antioxidant assay of compounds **6(a-e)** and **7(a-e).**

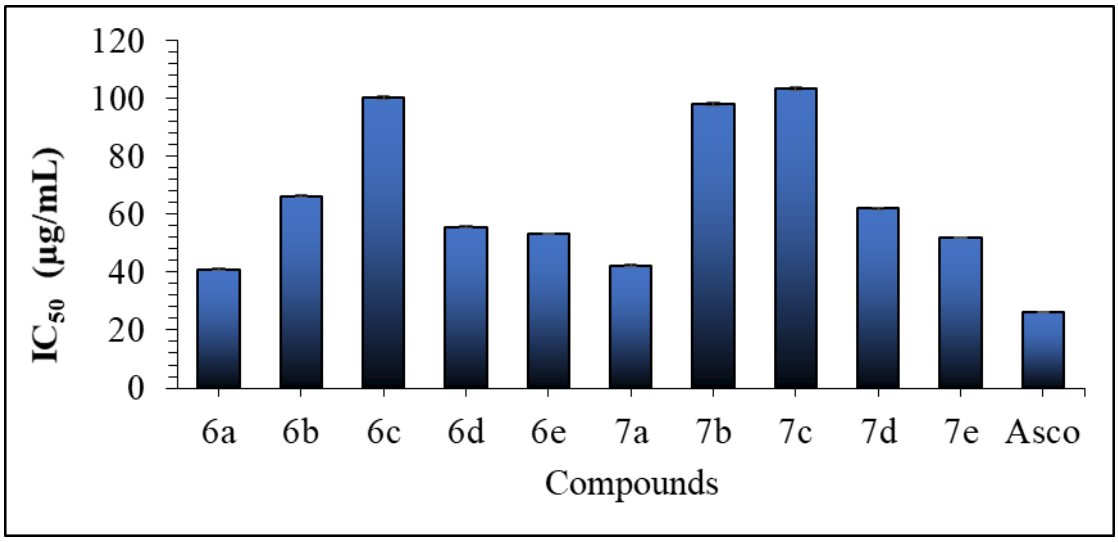


Fig.8. Graphical representation IC_{50} values of compounds6 **(a-e)**and **7(a-e)**.

Molecular docking

To investigate the detailed intermolecular interactions between the ligand and the target protein, a program Surflex-Dock was used. Docking studies give a fair idea related to drug-receptor interactions. Three-dimensional structure information on the target protein was taken from the PDB entry 4W93. Processing of the protein included the deletion of the ligand and the solvent molecules as well as the addition of hydrogen atoms. All the inhibitors were docked into the active site of enzyme as shown in **Fig.9**. The predicted binding energies of the compounds are listed in **Table 5.**

As depicted in the **Fig.10(A-C),** the compound **7b** makes three hydrogen bonding interactions at the active site of the enzyme (PDB ID: 4W93), oxygen atom of C=O of -COOH group of 3-nitro benzoic acid makes a hydrogen bonding interaction with hydrogen atom of LYS200 (C=O----H-LYS200, 2.68 Å), oxygen atom of OH of -COOH group of 3-nitro benzoic acid makes a hydrogen bonding interaction with hydrogen atom of ILE235 (O----H-ILE235, 1.89 Å), and remaining interaction came from the hydrogen atom of OH of -COOH group of 3-nitro benzoic acid with oxygen atom of GLU233 (-OH----O-GLU233, 2.33 Å).

As depicted in the **Fig.11(A-C),** the compound **7c** makes two hydrogen bonding interactions at the active site of the enzyme (PDB ID: 4W93), oxygen atom of C=O of -COOH group of 3-nitro benzoic acid makes a

hydrogen bonding interaction with hydrogen atom of ARG195 (C=O----H-ARG195, 2.15 Å), and remaining interaction came from the hydrogen atom of OH of -COOH group of 3-nitro benzoic acid with nitrogen atom of HIS299 (-OH----N-HIS299, 2.19 Å).

The binding interaction of acarbose with enzyme active sites shows nine bonding interactions and the docked view of the same has been depicted in **Fig.12(A-C)**. As depicted in **Fig.13(A-B)** represents the hydrophobic and hydrophilic amino acids surrounded to the studied compounds **7b & 7c.**

All the compounds showed consensus score in the range 6.32-4.98, indicating the summary of all forces of interaction between ligands and the enzyme and also, we saw that the studied compounds have showed same type of interactions with amino acid residues (LYS200, GLU233 and ARG195) as that of reference drug acarbose. This indicates that molecules preferentially bind to enzyme in comparison to the reference drug acarbose **(Table 5).**

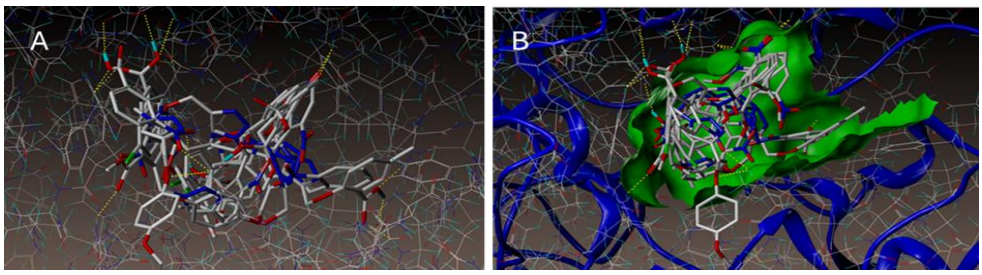


Fig.9. Docked view of all the compounds at the active site of the enzyme PDB ID: 4W93

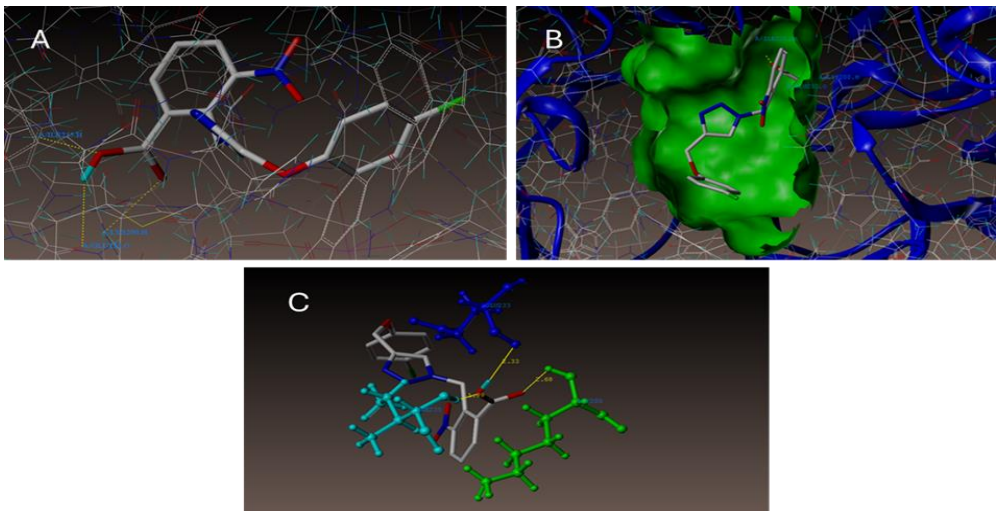


Fig.10. Docked view of compound **7b** at the active site of the enzyme PDB: 4W93

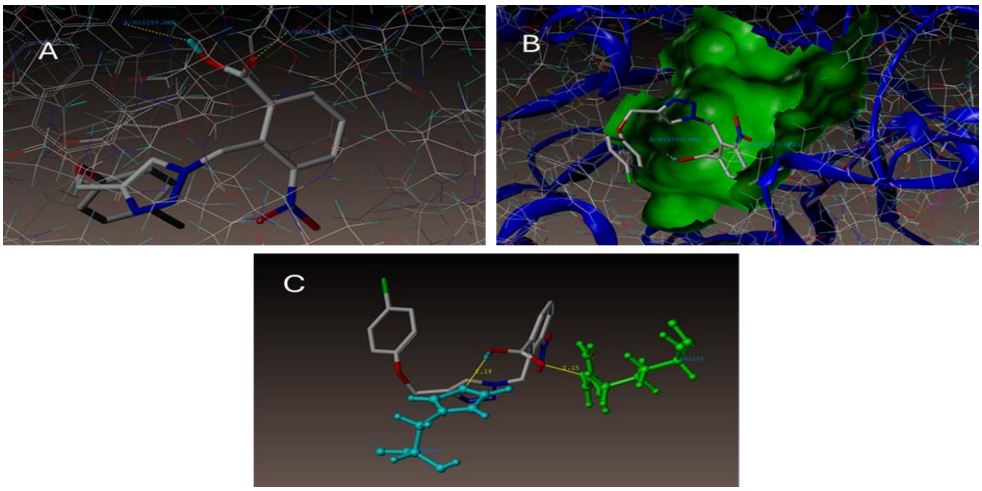


Fig.11. Docked view of compound **7c** at the active site of the enzyme PDB: 4W93

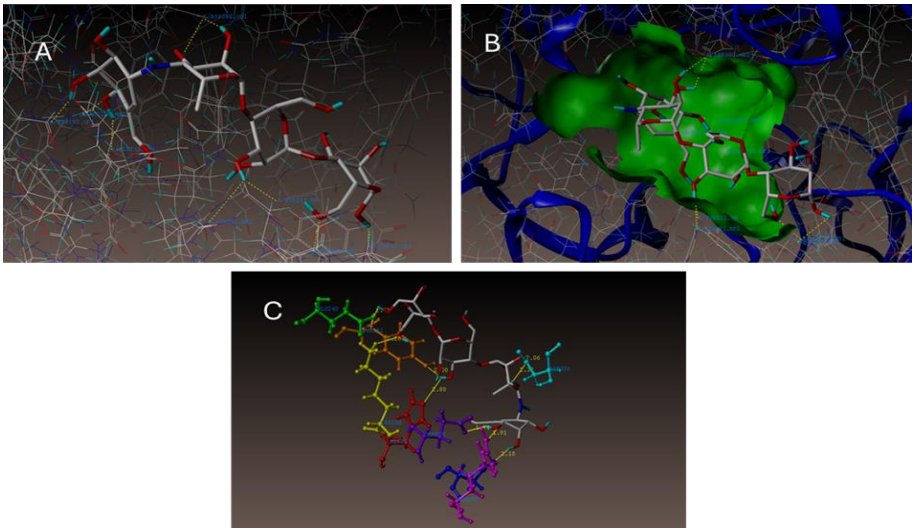


Fig.12. Docked view of compound **acarbose** at the active site of the enzyme PDB: 4W93

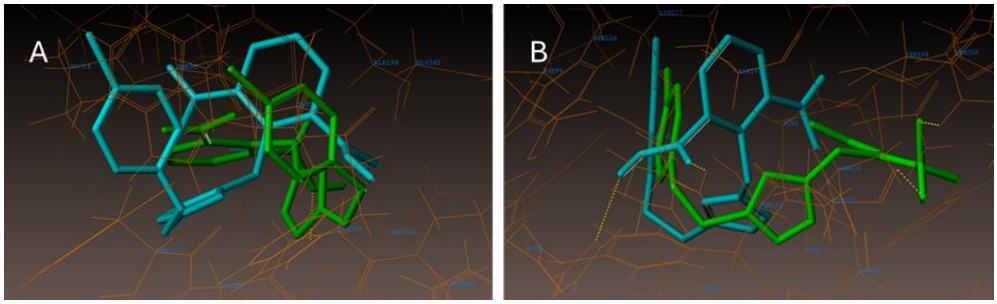


Fig.13. A) Hydrophobic amino acids surrounded to compounds **7b** (green colour) and **7c** (cyan colour). B) Hydrophilic amino acids surrounded to compounds **7b** and **7c.**

Table 5. Surflex Docking score (kcal/mol) of the derivatives on PDB ID: 4W93.

Compounds	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
7b	6.32	-1.34	1.39	-142.425	-74.804	-190.306	-23.157
7c	6.04	-2.13	2.45	-143.329	-86.665	-215.404	-26.202
7d	5.96	-1.08	3.15	-117.309	-90.123	-159.112	-28.109
6c	5.77	-0.91	1.14	-141.730	-89.810	-186.098	-22.849
6d	5.75	-1.63	1.35	-153.996	-125.858	-213.080	-27.795
6b	5.71	-1.05	2.34	-150.933	-109.057	-196.587	-25.588
6a	5.42	-1.40	2.36	-160.552	-97.694	-191.797	-23.232
7a	5.14	-0.83	1.66	-108.670	-38.011	-137.850	-20.754
6e	5.00	-1.27	0.30	-138.885	-81.866	-218.665	-25.124
7e	4.98	-0.54	1.74	-135.710	-118.369	-145.570	-24.654
Acarbose	4.15	-2.72	6.98	-164.918	-159.389	-279.622	-10.393

[a] C Score (Consensus Score) combines a number of widely used scoring methods to rank the affinity of ligands attached to a receptor's active site and provides the total score.

[b] The inadequate penetration into the binding site is revealed by the crash-score. Crash scores of less than 0 are considered favourable. Penetration is indicated by negative values.

[c] The contribution of polar interactions to the total score is indicated by the polar score. The polar score can help to rule out docking outcomes that don't make any hydrogen bonds.

[d] Charge and van der Waals interactions between the protein and the ligand are given as D-score.

[e] The Helmholtz free energy of interactions for protein-ligand atom pairs are indicated by the PMF-score (Potential of Mean Force, PMF).

[f] Hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies are shown in as G-score.

[g] H-bonding, lipophilic contact, and rotational entropy are all given chem-score points, as well as an intercept term.

IV. Conclusion

We have synthesized biologically more potent 1,2,3-triazole moieties in excellent yield with high purity and their antidiabetic activities were evaluated. Compound **6a** exhibited potent antidiabetic activity against α-amylase and glucose uptake assy. Further, the molecules have shown good antibacterial activity and also moderate antioxidant activity in DPPH assay. The molecular docking studies of the molecules at the active site of α-amylase enzyme have shown similar interactions as that of standard acarbose drug.

Acknowledgments

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References

[1] Li Wl, Zheng Hc, Bukuru J, De Kimpe N. Natural Medicines Used In The Traditional Chinese Medical System For Therapy Of Diabetes Mellitus. J Ethnopharmacol 2004;92:1–21. Https://Doi.Org/10.1016/J.Jep.2003.12.031.

[2] Zaharudin N, Staerk D, Dragsted Lo. Inhibition Of A-Glucosidase Activity By Selected Edible Seaweeds And Fucoxanthin. Food Chem 2019;270:481–6. Https://Doi.Org/10.1016/J.Foodchem.2018.07.142.

[3] Sundarram A, Pandurangappa T, Murthy K. Cite This Article: Ajita Sundarram, And Thirupathihalli Pandurangappa Krishna Murthy, "A-Amylase Production And Applications: A Review. J Appl Environ Microbiol 2014;2:166–75. Https://Doi.Org/10.12691/Jaem-2-4-10.

[4] Taha M, Tariq Javid M, Imran S, Selvaraj M, Chigurupati S, Ullah H, Et Al. Synthesis And Study Of The A-Amylase Inhibitory Potential Of Thiadiazole Quinoline Derivatives. Bioorg Chem 2017;74:179–86. Https://Doi.Org/10.1016/J.Bioorg.2017.08.003.

[5] Asgher M, Asad Mj, Rahman Su, Legge Rl. A Thermostable A-Amylase From A Moderately Thermophilic Bacillus Subtilis Strain For Starch Processing. J Food Eng 2007;79:950–5. Https://Doi.Org/10.1016/J.Jfoodeng.2005.12.053.

[6] Gunawan-Puteri Mdpt, Kato E, Kawabata J. A-Amylase Inhibitors From An Indonesian Medicinal Herb, Phyllanthus Urinaria. J Sci Food Agric 2012;92:606–9. Https://Doi.Org/10.1002/Jsfa.4615.

[7] Kawahito S, Kitahata H, Oshita S. Problems Associated With Glucose Toxicity: Role Of Hyperglycemia-Induced Oxidative Stress. World J Gastroenterol 2009;15:4137–42. Https://Doi.Org/10.3748/Wjg.15.4137.

[8] Pettersson Us, Christoffersson G, Massena S, Ahl D, Jansson L, Henriksnäs J, Et Al. Increased Recruitment But Impaired Function Of Leukocytes During Inflammation In Mouse Models Of Type 1 And Type 2 Diabetes. Plos One 2011;6. Https://Doi.Org/10.1371/Journal.Pone.0022480.

[9] Richard J-L. New Insights In Diabetic Foot Infection. World J Diabetes 2011;2:24. Https://Doi.Org/10.4239/Wjd.V2.I2.24.

[10] Mooradian Ad. Antioxidants And Diabetes. Nestle Nutr Workshop Ser Clin Perform Programme 2006;11. Https://Doi.Org/10.4103/2230-8210.104057.

[11] Ovadia H, Haim Y, Nov O, Almog O, Kovsan J, Bashan N, Et Al. Increased Adipocyte S-Nitrosylation Targets Anti-Lipolytic Action Of Insulin: Relevance To Adipose Tissue Dysfunction In Obesity. Journal Of Biological Chemistry 2011;286:30433–43. Https://Doi.Org/10.1074/Jbc.M111.235945.

[12] Xu Z, Zhao Sj, Lv Zs, Gao F, Wang Y, Zhang F, Et Al. Fluoroquinolone-Isatin Hybrids And Their Biological Activities. Eur J Med Chem 2019;162:396–406. Https://Doi.Org/10.1016/J.Ejmech.2018.11.032.

[13] Dhanda G, Sarkar P, Samaddar S, Haldar J. Battle Against Vancomycin-Resistant Bacteria: Recent Developments In Chemical Strategies. J Med Chem 2019;62:3184–205. Https://Doi.Org/10.1021/Acs.Jmedchem.8b01093.

[14] Butler Ms, Cooper Ma. Antibiotics In The Clinical Pipeline In 2011. Journal Of Antibiotics 2011;64:413–25. Https://Doi.Org/10.1038/Ja.2011.44.

[15] Ziemska J, Rajnisz A, Solecka J. New Perspectives On Antibacterial Drug Research. Cent Eur J Biol 2013;8:943–57. Https://Doi.Org/10.2478/S11535-013-0209-6.

[16] Küçükgüzel G, Çikla-Süzgün P. Recent Advances Bioactive 1,2,4-Triazole-3-Thiones. Eur J Med Chem 2015;97:830–70. Https://Doi.Org/10.1016/J.Ejmech.2014.11.033.

[17] Yu B, Qi Pp, Shi Xj, Huang R, Guo H, Zheng Yc, Et Al. Efficient Synthesis Of New Antiproliferative Steroidal Hybrids Using The Molecular Hybridization Approach. Eur J Med Chem 2016;117:241–55. Https://Doi.Org/10.1016/J.Ejmech.2016.04.024.

[18] Giffin Mj, Heaslet H, Brik A, Lin Yc, Cauvi G, Wong Ch, Et Al. A Copper(I)-Catalyzed 1,2,3-Triazole Azide-Alkyne Click Compound Is A Potent Inhibitor Of A Multidrug-Resistant Hiv-1 Protease Variant. J Med Chem 2008;51:6263–70. Https://Doi.Org/10.1021/Jm800149m.

[19] Whiting M, Tripp Jc, Lin Y-C, Lindstrom W, Olson Aj, Elder Jh, Et Al. Rapid Discovery And Structure-Activity Profiling Of Novel Inhibitors Of Human Immunodeficiency Virus Type 1 Protease Enabled By The Copper(I)-Catalyzed Synthesis Of 1,2,3-Triazoles And Their Further Functionalization 2006. Https://Doi.Org/10.1021/Jm060754.

[20] Pålhagen S, Canger R, Henriksen O, Van Parys Ja, Rivière M-E, Karolchyk Ma. Rufinamide: A Double-Blind, Placebo-Controlled Proof Of Principle Trial In Patients With Epilepsy. Epilepsy Res 2001;43:115–24. Https://Doi.Org/10.1016/S0920-1211(00)00185-6.

[21] Jordão Ak, Ferreira Vf, Souza Tml, De Souza Faria Gg, Machado V, Abrantes Jl, Et Al. Synthesis And Anti-Hsv-1 Activity Of New 1,2,3-Triazole Derivatives. Bioorg Med Chem 2011;19:1860–5. Https://Doi.Org/10.1016/J.Bmc.2011.02.007.

[22] Buckle Dr, Rockell Cjm, Smith H, Spicer Ba. Studies On 1,2,3-Triazoles. 13. (Piperazinylalkoxy)-[1]Benzopyrano[2,3-D]-1,2,3-Triazol-9(1h)-Ones With Combined H1-Antihistamine And Mast Cell Stabilizing Properties. J Med Chem 1986;29:2262–7. Https://Doi.Org/10.1021/Jm00161a022.

[23] Sindhu J, Singh H, Khurana Jm, Bhardwaj Jk, Saraf P, Sharma C. Synthesis And Biological Evaluation Of Some Functionalized 1h-1,2,3-Triazole Tethered Pyrazolo[3,4-B]Pyridin-6(7h)-Ones As Antimicrobial And Apoptosis Inducing Agents. Medicinal Chemistry Research 2016;25:1813–30. Https://Doi.Org/10.1007/S00044-016-1604-0.

[24] Govindaiah S, Sreenivasa S, Ramakrishna Ra, Rao Tmc, Nagabhushana H. Regioselective Synthesis, Antibacterial, Molecular Docking And Fingerprint Applications Of 1-Benzhydrylpiperazine Derivatized 1,4-Disubstituted 1,2,3-Triazoles. Chemistryselect 2018;3:8111–7. Https://Doi.Org/10.1002/Slct.201801364.

[25] Mazzotta S, Cebrero-Cangueiro T, Frattaruolo L, Vega-Holm M, Carretero-Ledesma M, Sánchez-Céspedes J, Et Al. Exploration Of Piperazine-Derived Thioureas As Antibacterial And Anti-Inflammatory Agents. In Vitro Evaluation Against Clinical Isolates Of Colistin-Resistant Acinetobacter Baumannii. Bioorg Med Chem Lett 2020;30. Https://Doi.Org/10.1016/J.Bmcl.2020.127411.

[26] Dehestani L, Ahangar N, Hashemi Sm, Irannejad H, Honarchian Masihi P, Shakiba A, Et Al. Design, Synthesis, In Vivo And In Silico Evaluation Of Phenacyl Triazole Hydrazones As New Anticonvulsant Agents. Bioorg Chem 2018;78:119–29. Https://Doi.Org/10.1016/J.Bioorg.2018.03.001.

[27] Dawood Km, Abdel-Gawad H, Rageb Ea, Ellithey M, Mohamed Ha. Synthesis, Anticonvulsant, And Anti-Inflammatory Evaluation Of Some New Benzotriazole And Benzofuran-Based Heterocycles. Bioorg Med Chem 2006;14:3672–80. Https://Doi.Org/10.1016/J.Bmc.2006.01.033.

[28] Deswal L, Verma V, Kumar D, Kaushik Cp, Kumar A, Deswal Y, Et Al. Synthesis And Antidiabetic Evaluation Of Benzimidazole-Tethered 1,2,3-Triazoles. Arch Pharm (Weinheim) 2020;353. Https://Doi.Org/10.1002/Ardp.202000090.

[29] Demaray Ja, Thuener Je, Dawson Mn, Sucheck Sj. Synthesis Of Triazole-Oxazolidinones Via A One-Pot Reaction And Evaluation Of Their Antimicrobial Activity. Bioorg Med Chem Lett 2008;18:4868–71. Https://Doi.Org/10.1016/J.Bmcl.2008.07.087.

[30] Xu Z. 1,2,3-Triazole-Containing Hybrids With Potential Antibacterial Activity Against Methicillin-Resistant Staphylococcus Aureus (Mrsa). Eur J Med Chem 2020;206. Https://Doi.Org/10.1016/J.Ejmech.2020.112686.

[31] Tan W, Li Q, Li W, Dong F, Guo Z. Synthesis And Antioxidant Property Of Novel 1,2,3-Triazole-Linked Starch Derivatives Via "Click Chemistry." Int J Biol Macromol 2016;82:404–10. Https://Doi.Org/10.1016/J.Ijbiomac.2015.10.007.

[32] Costa Ms, Boechat N, Rangel Éa, Da Silva F De C, De Souza Amt, Rodrigues Cr, Et Al. Synthesis, Tuberculosis Inhibitory Activity, And Sar Study Of N-Substituted-Phenyl-1,2,3-Triazole Derivatives. Bioorg Med Chem 2006;14:8644–53. Https://Doi.Org/10.1016/J.Bmc.2006.08.019.

[33] Patpi Sr, Pulipati L, Yogeeswari P, Sriram D, Jain N, Sridhar B, Et Al. Design, Synthesis, And Structure-Activity Correlations Of Novel Dibenzo[B,D Furan, Dibenzo[B,D]Thiophene, And N-Methylcarbazole Clubbed 1,2,3-Triazoles As Potent Inhibitors Of Mycobacterium Tuberculosis. J Med Chem 2012;55:3911–22. Https://Doi.Org/10.1021/Jm300125e.

[34] De Simone R, Chini Mg, Bruno I, Riccio R, Mueller D, Werz O, Et Al. Structure-Based Discovery Of Inhibitors Of Microsomal Prostaglandin E 2 Synthase-1, 5-Lipoxygenase And 5-Lipoxygenase-Activating Protein: Promising Hits For The Development Of New Anti-Inflammatory Agents. J Med Chem 2011;54:1565–75. Https://Doi.Org/10.1021/Jm101238d.

[35] Whiting M, Muldoon J, Lin Yc, Silverman Sm, Lindstrom W, Olson Aj, Et Al. Inhibitors Of Hiv-1 Protease By Using In Situ Click Chemistry. Angewandte Chemie - International Edition 2006;45:1435–9. Https://Doi.Org/10.1002/Anie.200502161.

[36] Vatmurge Ns, Hazra Bg, Pore Vs, Shirazi F, Chavan Ps, Deshpande M V. Synthesis And Antimicrobial Activity Of B-Lactam-Bile Acid Conjugates Linked Via Triazole. Bioorg Med Chem Lett 2008;18:2043–7. Https://Doi.Org/10.1016/J.Bmcl.2008.01.102.

[37] Ranilla Lg, Kwon Yi, Apostolidis E, Shetty K. Phenolic Compounds, Antioxidant Activity And In Vitro Inhibitory Potential Against Key Enzymes Relevant For Hyperglycemia And Hypertension Of Commonly Used Medicinal Plants, Herbs And Spices In Latin America. Bioresour Technol 2010;101:4676–89. Https://Doi.Org/10.1016/J.Biortech.2010.01.093.

[38] Harish M, Ahmed F, Urooj A. In Vitro Hypoglycemic Effects Of Butea Monosperma Lam. Leaves And Bark. J Food Sci Technol 2014;51:308–14. Https://Doi.Org/10.1007/S13197-011-0496-8.

[39] Elshikh M, Ahmed S, Funston S, Dunlop P, Mcgaw M, Marchant R, Et Al. Resazurin-Based 96-Well Plate Microdilution Method For The Determination Of Minimum Inhibitory Concentration Of Biosurfactants. Biotechnol Lett 2016;38:1015–9. Https://Doi.Org/10.1007/S10529-016-2079-2.

[40] Cruz Rc, Werneck Smc, Oliveira Cs, Santos Pc, Soares Bm, Santos Da, Et Al. Influence Of Different Media, Incubation Times, And Temperatures For Determining The Mics Of Seven Antifungal Agents Against Paracoccidioides Brasiliensis By Microdilution. J Clin Microbiol 2013;51:436–43. Https://Doi.Org/10.1128/Jcm.02231-12.

[41] Santos Da, Hamdan Js. Evaluation Of Broth Microdilution Antifungal Susceptibility Testing Conditions For Trichophyton Rubrum. J Clin Microbiol 2005;43:1917–20. Https://Doi.Org/10.1128/Jcm.43.4.1917-1920.2005.

[42] Sortino M, Delgado P, Juárez S, Quiroga J, Abonía R, Insuasty B, Et Al. Synthesis And Antifungal Activity Of (Z)-5-Arylidenerhodanines. Bioorg Med Chem 2007;15:484–94. Https://Doi.Org/10.1016/J.Bmc.2006.09.038.

[43] Wong Cc, Li H Bin, Cheng Kw, Chen F. A Systematic Survey Of Antioxidant Activity Of 30 Chinese Medicinal Plants Using The Ferric Reducing Antioxidant Power Assay. Food Chem 2006;97:705–11. Https://Doi.Org/10.1016/J.Foodchem.2005.05.049.

[44] Joshi Sd, Dixit Sr, More Ua, Raju Kvsn, Narayan R, Aminabhavi Tm, Et Al. 3d-Qsar And Molecular Docking Studies Of 1,3,4-Oxadiazoles Containing Substituted Phenoxy Fragment As Inhibitors Of Enoyl-Acyl Carrier Protein Reductase From Escherichia Coli. Medicinal Chemistry Research 2014;23:4542–58. Https://Doi.Org/10.1007/S00044-014-1013-1.

[45] Gasteiger J, Marsili M. Iterative Partial Equalization Of Orbital Electronegativity—A Rapid Access To Atomic Charges. Tetrahedron 1980;36:3219–28. Https://Doi.Org/10.1016/0040-4020(80)80168-2.

[46] Batool T, Rasool N, Gull Y, Noreen M, Nasim Fuh, Yaqoob A, Et Al. A Convenient Method For The Synthesis Of (Prop-2-Ynyloxy)Benzene Derivatives Via Reaction With Propargyl Bromide, Their Optimization, Scope And Biological Evaluation. Plos One 2014;9. Https://Doi.Org/10.1371/Journal.Pone.0115457.

[47] Anand A, Naik Rj, Revankar Hm, Kulkarni M V., Dixit Sr, Joshi Sd. A Click Chemistry Approach For The Synthesis Of Mono And Bis Aryloxy Linked Coumarinyl Triazoles As Anti-Tubercular Agents. Eur J Med Chem 2015;105:194–207. Https://Doi.Org/10.1016/J.Ejmech.2015.10.019.

[48] Hebbar NU, Patil AR, Gudimani P, Shastri SL, Shastri LA, Joshi SD, Et Al. Click Approach For Synthesis Of 3,4-Dihydro-2(1h) Quinolinone, Coumarin Moored 1,2,3-Triazoles As Inhibitor Of Mycobacteria Tuberculosis H37rv, Their Antioxidant, Cytotoxicity And In-Silico Studies. J Mol Struct 2022;1269. Https://Doi.Org/10.1016/J.Molstruc.2022.133795.

[49] Doddagaddavalli MA, Kalalbandi VKA, Seetharamappa J. Synthesis, Characterization, Crystallographic, Binding, In Silico And Antidiabetic Studies Of Novel 2,4-Thiazolidinedione-Phenothiazine Molecular Hybrids. J Mol Struct 2023;1276. Https://Doi.Org/10.1016/J.Molstruc.2022.134625