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A-Glucosidase inhibitory potential and hemolytic evaluation of newly synthesized 3, 4, 5-trisubstituted-1, 2, 4-triazole derivatives

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Abstract: A series of 1, 2, 4-triazole derivatives bearing piperidine moiety has been introduced as new anti-diabetic drug candidates with least cytotoxicity. *p*-Chlorophenylsulfonyl chloride (1) and ethyl nipecotate (2) were the starting reagents that resulted into corresponding 3,4,5-trisubstituted-1,2,4-triazole (6) through a series of steps. A series of electrophiles, 9a-e, were synthesized by reacting 4-bromobutyryl chloride (7) with differently substituted aromatic amines (8a-e) under basic aqueous medium. Target derivatives, 10a-e, were synthesized by the reaction of compound 6 with *N*-aryl-4-bromobutanamides (9a-e) in an aprotic solvent. Structures of all the derivatives were verified by spectroscopic analysis using IR, ^{1}H-NMR, ^{13}C-NMR and EIMS. Most of the derivatives revealed moderate to good α-glucosidase inhibitory activity with reference to acarbose. The moderate hemolytic potential demonstrated least toxicity.

Keywords: 1,2,4-Triazoles, anti α-glucosidase, hemolytic activity, sulfonamides.

INTRODUCTION

1,2,4-triazole and its derivatives has grasped considerable attention of medicinal chemists due to their antibiotic (Collin *et al.,* 2003) and antifungal (Papakonstantinou-Garoufalias *et al.,* 2002) potential. 1,2,4-Triazole derivatives were also found in antagonist receptor ligands (Neumann-Schultz *et al.,* 2003; Chen *et al.,*2001; Wadsworth *et al.,* 1992; Jenkins *et al.,* 1992). These are also known to work as mimics (Wadsworth *et al.,* 1992; Jenkins *et al.,* 1992; Burrell *et al.,* 1994; Tully *et al.,* 1991) or isosteres (Thompson *et al.,* 1994; Boyd *et al.,* 1994) of amide bond and enhances the bioavailability of bioactive potent moiety. A variety of methodology have been reported for the synthesis of substituted 1,2,4-triazoles (Duncia *et al.,* 1998; Conde *et al.,* 1974; Buzykin *et al.,*1993; Paulvannan *et al.,* 2000). Furthermore, 1,2,4-triazoles have been reported for a variety of therapeutic activities like hypoglycemic, anti-inflammatory, antidepressant, antimicrobial and anticonvulsant activity (Papakonstantinou-Garoufalias *et al.,* 2002). These also play a key role as corrosion inhibiting agent for metals due to their chelating effect (Neumann-Schultz *et al.,* 2003; Chen *et al.,* 2001).

The newly recognized diseases have focused the need of hour to introduce novel potent molecules to combat these disorders or malfunctions. Triazoles fused with other rings, revealed better pharmacokinetic properties (Wadsworth *et al.,* 1992). 1,2,4-Triazoles act as building block for various synthetic intermediates, synthesis of dyes, agricultural chemicals, photostabilizers, photographic elements etc (Buzykin *et al.,*1993; Paulvannan *et al.,* 2000). Present research work describes the multistep synthesis of *N*-aryl-3,4,5-trisubstituted-1,2,4-triazol-3-ylthio butanamide (10a-e) to inaugurate new anti-diabetic drug candidates. All the synthesized derivatives were evaluated for cytotoxicity through hemolytic activity analysis.

MATERIALS AND METHODS

All Merck and Sigma Aldrich chemical reagents and solvents essential for the designed protocol were purchased from local suppliers. Melting points of all derivatives were recorded by Gallenkamp M.P. apparatus. Completion of reaction and purity of all synthesized molecules were assured by TLC. EtOAc and *n*-Hexane (30:70) ratio was used as mobile phase to develop TLC. On Jasco FTIR spectrometer, all derivatives were subjected to record IR spectra. IR spectra were recorded by KBr pellet method. Bruker spectrometers (600 and 150 MHz) were used to record ^{1}H-NMR and ^{13}C-NMR respectively using deuterated solvent and tetramethylsilane as reference. On JMS-HX 110 spectrometer, EIMS spectra of synthesized derivatives were recorded.

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Synthesis of Ethyl 1-[(4-chlorophenyl) sulfonyl] piperidin-3-carboxylate (3)

Ethyl 1-[(4-chlorophenyl) sulfonyl] piperidin-3-carboxylate (3) was synthesized by stirring ethyl piperidin-3-carboxylate (2; 100 mmol) with 4-chlorobenzenesulfonyl chloride (1; 100 mmol) in a basic media with pH 10-11 for 3-4 hours. By performing TLC, reaction progress was monitored. The product formed was collected by solvent extraction.

Synthesis of 1-[(4-chlorophenyl) sulfonyl] piperidin-3-carbohydrazide (4)

1-[(4-chlorophenyl) sulfonyl] piperidin-3-carbohydrazide (4) was prepared on refluxing ethyl 1-[(4-chlorophenyl) sulfonyl] piperidin-3-carboxylate (3; 80 mmol) with hydrated hydrazine (80 mmol) in methanol (40 mL) as solvent for 2-3 hours. TLC assured the reaction completion. Finally excess methanol was distilled off and cold distilled water was used to quench the precipitates. The precipitates were filtered, washed and dried.

Synthesis of 2-({1-[(4-chlorophenyl) sulfonyl]-3-piperidinyl} carbonyl)-N-phenyl-1-hydrazinecarbothioamide (5)

Compound 4 (60 mmol) and phenyl isothiocyanate (60 mmol) were dissolved in methanol (100 mL) and refluxed for 3-4 hours. Reaction coordinates and purity of compound was confirmed by TLC. On completion of reaction, product was precipitated out by addition of distilled water. Precipitates were filtered, washed and dried.

Synthesis of 5-{1-[(4-chlorophenyl) sulfonyl]-3-piperidinyl}-4-phenyl-4H-1,2,4-triazole-3-thiol (6)

Compound 5 (20 mmol) was refluxed in 10% NaOH solution (20 mL) for 2-3 hours to cyclize intramolecularly into 1, 2, 4-triazole. TLC was performed to assure completion of reaction. Finally reaction contents were cool down to room temperature. To quench the product in good yield, cold distilled water was added along with dil. HCl to bring pH to 4-5. The obtained white precipitates were filtered, washed and dried.

General procedure for synthesis of N-aryl-4-bromobutanamides (9a-e)

N-Aryl-4-bromobutanamides (9a-e) were synthesized by stirring *N*-aryl amines (8a-e; 9.0 mmol) with 4-bromobutyryl chloride (7; 9.0 mmol) in 5% Na_2CO_3 aqueous solution at pH=9-10 for 1 hour. Reaction contents were vigorously shaken till the emergence of precipitates. Product purity was assured by performing TLC using *n*-hexane and EtOAc. The title compounds, 9a-e, were filtered, washed and got dried.

Synthesis of 3,4,5-Trisubstituted-1,2,4-Triazole Derivatives (10a-e)

5-{1-[(4-Chlorophenyl)sulfonyl]-3-piperidinyl}-4-phenyl-4*H*-1,2,4-triazole-3-thiol (6; 0.2 g, 0.46 mmol) was stirred in DMF (15 mL) for 0.5 hour, in the presence of sodium hydride (0.001 mol) as base. The *N*-aryl-4-bromobutanamides (9a-e; 0.46 mmol) were added and further the mixture was stirred for 4-5 hours. TLC was conducted to verify the reaction completion and product purity. Excess cold distilled water was added to precipitate out the products.

Anti α-glucosidase assay

Anti *α*-glucosidase activity was performed by the method reported in literature (Chapdelaine *et al.*, 1978). 0.05 M phosphate buffer (70μL) to control pH at 6.8 was mixed with enzyme (10μL) and an additional 0.5mM test compound (10μL) was added to the contents. The mixture was incubated for 10 min at 37ºC and absorbance was recorded at 400 nm. 10μL of 0.5 mM substrate was added to reaction contents to start the reaction. Acarbose was the positive reference standard. Final contents were incubated for half an hour at 37ºC and by using Synergy HT microplate reader, again absorbance was noted at 400 nm. Experiment was repeated thrice. The percent inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Where: Control = Absorbance in control
Test = Absorbance in test sample
IC_{50} values of compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Hemolytic activity

All the target derivatives, 10a-e, were analyzed for hemolytic activity by the method (Sharma *et al.*, 2001, Powell *et al.*, 2000) reported in literature. Heparinized bovine blood (3mL) was collected. Blood was centrifuged for 5 min at 1000 x *g*. Plasma was surplus. Sterile and chilled PBS (5mL, pH 7.4) was used to wash the blood cells thrice. 10^8 cells per mL of erythrocytes were maintained for each assay. 10^8 cells/mL of human erythrocyte was mixed with each test compound (100μL) separately. Samples were incubated at 37^{o}C for 35 min and agitated after 10 min. The samples were placed on ice immediately after incubation for 5 min and centrifuged at 1000 x *g* for 5 min. Supernatant 100μL were taken from each tube and diluted 10 time with chilled (4^{o}C) PBS. Triton X-100 (0.1% v/v) was taken as positive control and phosphate buffer saline (PBS) was taken as negative control and pass through the same process. The absorbance was noted at 576 nm using μQuant (BioTek, USA). The % RBCs lysis for each sample was calculated.

STATISTICAL ANALYSIS

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± SEM.

Molecular docking

The inhibitory potential of all derivatives, 10a-e, were analyzed bio-informatically by molecular docking of these derivatives for α-Glucosidase enzyme by using MOE-Dock program. The Chem Draw ultra 12.0 software was used to draw the chemical structures of these ligands. MOE software 2009-2010 was used to open these structures. MMFF94x force field was used to minimize energy up to 0.05 gradients by default parameter of MOE energy minimization algorithm. The MDB file format was used to save these compounds in a separate database. Protein Data Bank was used to download Protein molecule of α-Glucosidase (PDB Code; 3NO4). Water molecules were removed from receptor protein. 3D protonation was brought about by using Protonate 3D Option. Energy of protein molecule was also minimized by the default parameters of MOE 2009-10 energy minimization algorithms (gradient: 0.05, Force Field: MMFF94X). Finally these derivatives were subjected to molecular docking into the binding pocket of enzyme. Re-docking procedure was implemented to validate the results. After 30 conformations of docking analysis for each compound, the best 2D images were saved, indicating their specific interactions. 3D images were drawn along with their bond lengths (Allouche, 2011; Stewart, 2007; Trott and Olson, 2010).

RESULTS

Presented research work explains the synthesis of a series of *N*-aryl-3,4,5-trisubstituted-*4H*-1,2,4-triazol-3-ylsulfanylbutanamide (10a-e) as depicted in scheme-1 and scheme-2. The structures of all derivatives were corroborated by spectral techniques like IR, ^{1}H-NMR, ^{13}C-NMR and EIMS data.

Spectral characterization of synthesized compounds

4-[(5-{1-[(4-Chlorophenyl)sulfonyl]-3-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(2,3-dimethylphenyl)butanamide (10a)

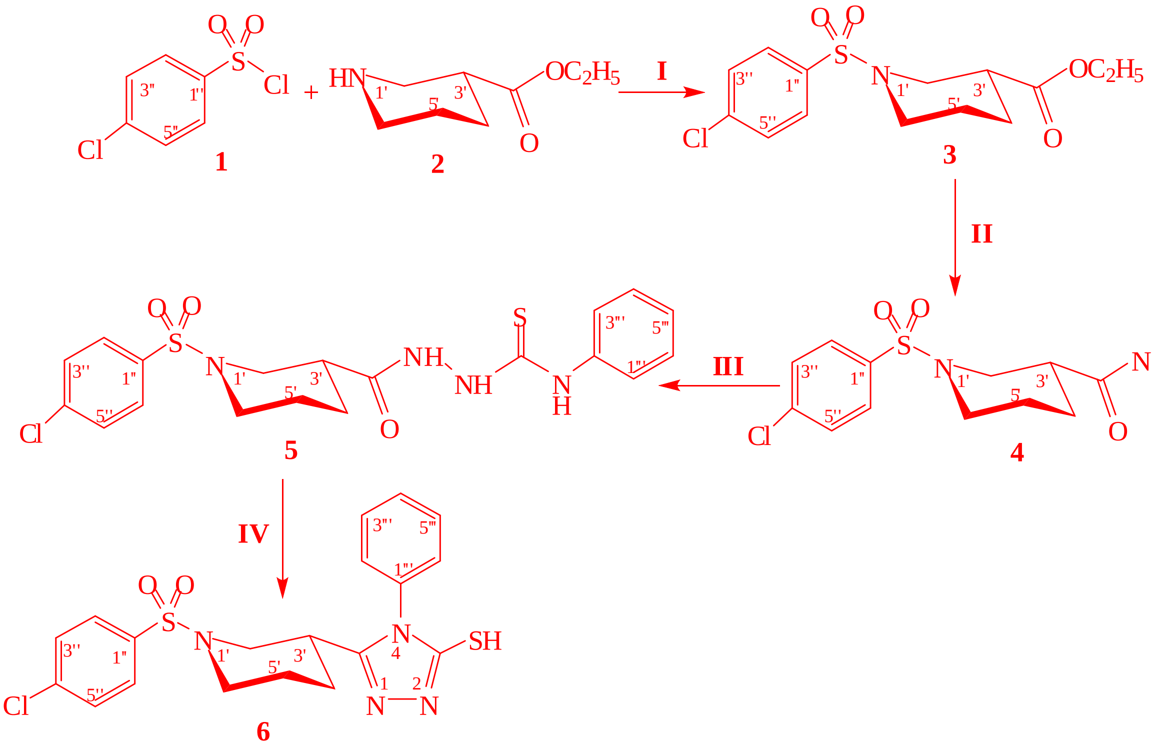
Light brown amorphous solid; Yield: 71%; M.P.: 85-87 °C; Molecular formula: $C_{31}H_{34}ClN_5O_3S_2$; Molecular Mass: 624.0 gmol^{-1}; IR (KBr, cm^{-1}) v_{max}: 3061 (Ar-H), 1573 (C=N), 1558 (Ar C=C), 1373 (-SO$_2$), 1240 (C-N), 828 (C-Cl); ^{1}H-NMR (CDCl$_3$, 600 MHz, δ/ppm): 7.80 (br.s, 1H, -NH), 7.62 (d, *J* = 8.1 Hz, 2H, H-2″ & H-6″), 7.60-7.57 (m, 3H,H-3‴ to H-5‴), 7.47 (d, *J*=8.1 Hz, 2H, H-3″ & H-5″), 7.37 (d, *J* = 8.0 Hz, 1H, H-6⁗′), 7.24-7.23 (m, 2H,H-2‴&H-6‴), 7.07 (t, *J*=7.8 Hz, 1H, H-5⁗′), 7.01 (d, *J*=7.5 Hz, 1H, H-4⁗′), 3.88 (br.d, *J* = 12.0 Hz, 1H, H_e-2′), 3.75 (br.d, *J*=11.7 Hz, 1H, H_a-2′), 3.26 (t, *J* = 6.9 Hz, 2H, H-4⁗), 2.77-2.72 (m, 1H, H-3′), 2.67 (br.t, *J* = 11.2 Hz, 1H, H_e-6′), 2.56 (t, *J* =7.0 Hz, 2H, H-2⁗), 2.28 (s, 3H, CH$_3$-2⁗′), 2.26 (t, *J*=9.3 Hz, 1H, H_a-6′), 2.21 (qui, *J* = 7.0 Hz, 2H, H-3⁗), 2.14 (s, 3H, CH$_3$-3⁗′), 1.84-1.80 (m, 1H, H_e-5′), 1.76-1.73 (m, 1H, H_e-4′), 1.58-1.48 (m, 2H, H_a-4′& H_a-5′); ^{13}C-NMR (CDCl$_3$, 150 MHz, δ/ppm): 170.5 (C-1⁗), 156.1 (C-3), 152.0 (C-5), 139.4 (C-1″), 137.4 (C-4″), 135.3 (C-3⁗′), 134.8 (C-1⁗′), 132.6 (C-1‴), 130.5 (C-2″& C-6″), 130.3 (C-4‴), 129.9 (C-2⁗′), 129.4 (C-3″& C-5″), 128.8 (C-3‴ & C-5‴), 127.4 (C-4⁗′), 127.4 (C-2‴ & C-6‴), 125.7 (C-5⁗′), 122.6 (C-6⁗′), 49.3 (C-2′), 46.0 (C-6′), 35.4 (C-3′), 32.9 (C-2⁗), 31.7 (C-4′), 29.0 (C-4⁗), 26.0 (C-3⁗), 24.3 (C-5′), 20.5 (CH$_3$-3⁗′), 14.0 (CH$_3$-2⁗′); EIMS (*m/z*): 626 $[M+2]^{+}$, 624 $[M]^{+}$, 435 $[C_{19}H_{18}ClN_4O_2S_2]^{\bullet+}$, 375 $[C_{18}H_{18}ClN_3O_2S]^{\bullet+}$, 361 $[C_{18}H_{18}ClN_2O_2S]^{+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^{+}$, 190 $[C_{12}H_{16}NO]^{+}$, 175 $[C_6H_4ClO_2S]^{+}$, 148 $[C_9H_{10}NO]^{+}$, 120 $[C_8H_{10}N]^{+}$, 111 $[C_6H_4Cl]^{+}$.

4-[(5-{1-[(4-Chlorophenyl)sulfonyl]-3-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(2,4-dimethylphenyl)butanamide (10b)

Lemon yellow amorphous solid; Yield: 67%; M.P.: 93-95°C; Molecular formula: $C_{31}H_{34}ClN_5O_3S_2$; Molecular Mass: 624.0 gmol^{-1}; IR (KBr, cm^{-1}) v_{max}: 3057 (Ar-H), 1570 (C=N), 1555 (Ar C=C), 1373 (-SO$_2$), 1237 (C-N), 825 (C-Cl); ^{1}H-NMR (CDCl$_3$, 600 MHz, δ/ppm): 7.64 (d, *J* = 7.2 Hz, 1H, H-6⁗′), 7.62 (d, *J* = 8.1 Hz, 2H, H-2″ & H-6″), 7.60-7.57 (m, 3H,H-3‴ to H-5‴), 7.52 (d, *J* = 8.2 Hz, 1H, H-5⁗′), 7.48 (d, *J* = 8.5 Hz, 2H, H-3″ & H-5″), 7.23-7.22 (m, 2H,H-2‴&H-6‴), 6.99 (s, 1H, H-3⁗′), 3.88 (br.d, *J* = 11.1 Hz, 1H, H_e-2′), 3.75 (br.d, *J* = 11.6 Hz, 1H, H_a-2′), 3.26 (t, *J* = 6.9 Hz, 2H, H-4⁗), 2.77-2.72 (m, 1H, H-3′), 2.67 (br.t, *J* = 11.3 Hz, 1H, H_e-6′), 2.55 (t, *J* = 6.9 Hz, 2H, H-2⁗), 2.28 (s, 3H, CH$_3$-4⁗′), 2.25 (br.t, *J* = 11.3 Hz, 1H, H_a-6′), 2.21 (s, 3H, CH$_3$-2⁗′), 2.20 (qui, *J* = 7.0 Hz, 2H, H-3⁗), 1.84-1.81 (m, 1H, H_e-5′), 1.76-1.72 (m, 1H, H_e-4′), 1.60-1.47 (m, 2H, H_a-4′& H_a-5′); ^{13}C-NMR (CDCl$_3$, 150 MHz, δ/ppm): 170.4 (C-1⁗), 156.1 (C-3), 151.9 (C-5), 139.4 (C-1″), 135.1 (C-4″), 134.8 (C-1⁗′), 133. (C-2⁗′), 132.6 (C-1‴), 131.1 (C-4⁗′), 130.5 (C-2″& C-6″), 130.3 (C-3″& C-5″), 130.1 (C-3‴ & C-5‴), 129.5 (C-4‴), 129.4 (C-5⁗′), 128.8 (C-2‴ & C-6‴), 127.1 (C-3⁗′), 124.0 (C-6⁗′), 49.3 (C-2′), 46.0 (C-6′), 35.5 (C-3′), 32.9 (C-2⁗), 31.7 (C-4′), 29.0 (C-4⁗), 26.0 (C-3⁗), 24.3 (C-5′), 20.8 (CH$_3$-4⁗′), 17.9 (CH$_3$-2⁗′);EIMS (*m/z*): 626 $[M+2]^{+}$, 624 $[M]^{+}$, 435 $[C_{19}H_{18}ClN_4O_2S_2]^{\bullet+}$, 375 $[C_{18}H_{18}ClN_3O_2S]^{\bullet+}$, 361 $[C_{18}H_{18}ClN_2O_2S]^{+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^{+}$, 190 $[C_{12}H_{16}NO]^{+}$, 175 $[C_6H_4ClO_2S]^{+}$, 148 $[C_9H_{10}NO]^{+}$, 120 $[C_8H_{10}N]^{+}$, 111 $[C_6H_4Cl]^{+}$.

4-[(5-{1-[(4-Chlorophenyl)sulfonyl]-3-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(2,5-dimethylphenyl)butanamide (10c)

Dark brown amorphous solid; Yield: 65%; M.P.: 83-85 °C; Molecular formula: $C_{31}H_{34}ClN_5O_3S_2$; Molecular Mass: 624.0 gmol^{-1}; IR (KBr, cm^{-1}) v_{max}: 3054 (Ar-H), 1567 (C=N), 1552 (Ar C=C), 1370 (-SO$_2$), 1234 (C-N), 822 (C-Cl); ^{1}H-NMR (CDCl$_3$, 600 MHz, δ/ppm): 7.62 (d, *J* = 8.3 Hz, 2H, H-2″ & H-6″), 7.59-7.57 (m, 3H,H-3‴ to H-5‴),7.55 (s, 1H, H-6⁗′),7.48 (d, *J* = 8.1 Hz, 2H, H-3″ & H-5″), 7.23-7.22 (m, 2H,H-2‴&H-6‴), 7.05 (d, *J* = 7.6 Hz,



Scheme 1: Outline for the synthesis of 3,4,5-Trisubstituted-1,2,4-Triazole (6). Reagents & conditions: (I) 5% Na_2CO_3 soln./H_2O/pH=9-10/stirring for 3-4 hours. (II) N_2H_4/MeOH/stirring for 2-3 hours. (III) Phenyl isothiocyanate/ MeOH/refluxing for 3-4 hours. (IV) NaOH/Reflux for 2-3 hours.

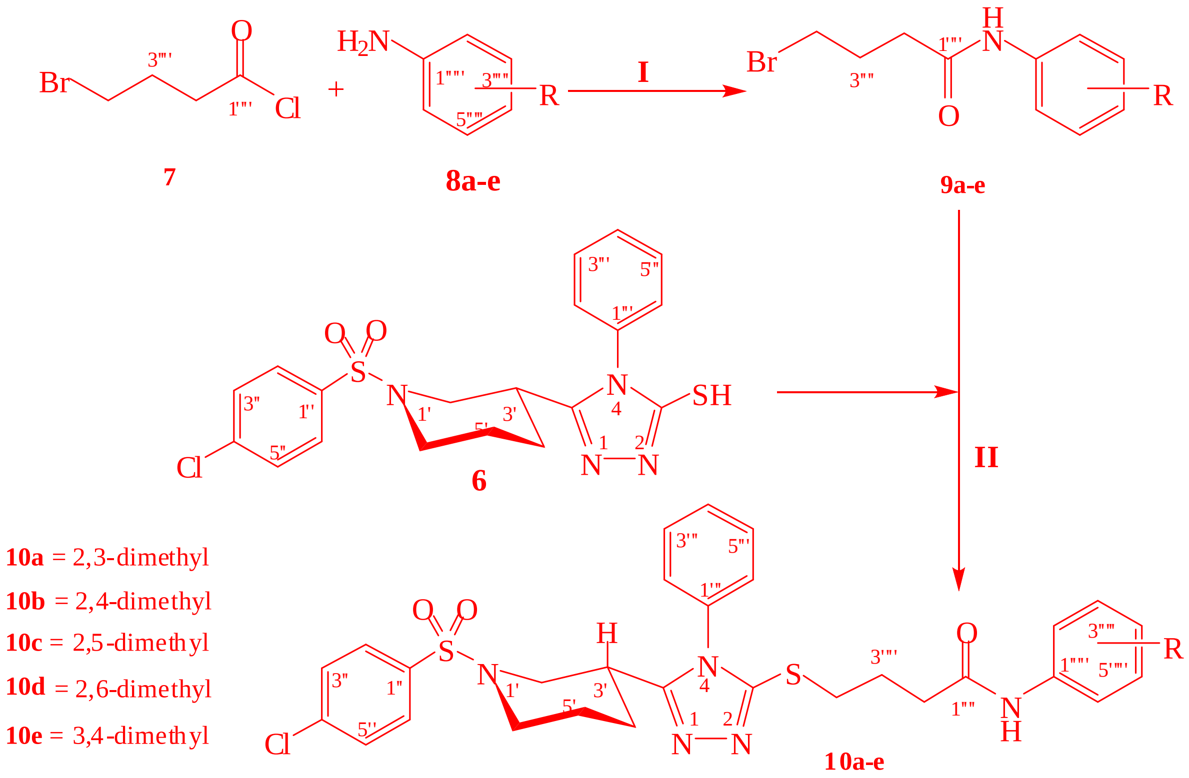
Table 1: Hemolysis, and α-Glucosidase inhibition activities

Compound	% Hemolysis	Anti-α-glucosidase	
		Inhibition (%) at 0.5 mM	IC_{50} (μM)
10a	14.1	85.23±0.16	194.31±0.12
10b	13.5	81.47±0.19	216.71±0.15
10c	14.7	76.32±0.17	239.19±0.11
10d	9.7	85.56±0.15	176.75±0.12
10e	9.5	89.79±0.17	79.42±0.14
Triton-X-100	99.27		
PBS	0.12		
Acarbose		92.23±0.16	37.38±0.12

1H, H-4⁗′), 6.88 (d, *J* = 7.6 Hz, 1H, H-3⁗′), 3.88 (br.d, *J* = 11.4 Hz, 1H, H_e-2′), 3.75 (br.d, *J* = 11.7 Hz, 1H, H_a-2′), 3.26 (t, *J* = 6.9 Hz, 2H, H-4‴′), 2.76-2.72 (m, 1H, H-3′), 2.67 (br.t, *J* = 11.2 Hz, 1H, H_e-6′), 2.55 (t, *J* = 7.0 Hz, 2H, H-2‴′), 2.30 (s, 3H, CH_3-2⁗′), 2.28 (br.t, *J* = 10.6 Hz, 1H, H_a-6′), 2.23-2.18 (m, 5H, CH_3-5⁗′& H-3‴′), 1.84-1.82 (m, 1H, H_e-5′), 1.76-1.73 (m, 1H, H_e-4′), 1.58-1.48 (m, 2H, H_a-4′& H_a-5′); ^{13}C-NMR ($CDCl_3$, 150 MHz, *δ*/ppm): 170.3 (C-1‴′), 156.1 (C-3), 151.9 (C-5), 139.4 (C-1″), 136.3 (C-4″), 135.4 (C-1⁗′), 134.8 (C-1‴), 132.6 (C-5⁗′), 130.5 (C-2⁗′), 130.3 (C-2″& C-6″), 130.2 (C-3″& C-5″), 129.4 (C-3‴ & C-5‴), 128.8 (C-4‴), 127.14 (C-4⁗′), 126.5 (C-2‴ & C-6‴), 126.0 (C-3⁗′), 124.2 (C-6⁗′), 49.3 (C-2′), 46.0 (C-6′), 35.6 (C-3′), 32.9 (C-2‴′), 31.7 (C-4′), 29.0 (C-4‴′), 25.9 (C-3‴′), 24.3 (C-5′), 21.0 (CH_3-5⁗′), 17.5 (CH_3-2⁗′);EIMS (*m/z*): 626 $[M+2]^+$, 624 $[M]^+$, 435 $[C_{19}H_{18}ClN_4O_2S_2]^{\bullet+}$, 375 $[C_{18}H_{18}ClN_3O_2S]^{\bullet+}$, 361 $[C_{18}H_{18}ClN_2O_2S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 190 $[C_{12}H_{16}NO]^+$, 175 $[C_6H_4ClO_2S]^+$, 148 $[C_9H_{10}NO]^+$, 120 $[C_8H_{10}N]^+$, 111 $[C_6H_4Cl]^+$.

4-[(5-{1-[(4-Chlorophenyl)sulfonyl]-3-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(2,6-dimethylphenyl)butanamide (10d)

Off white amorphous solid; Yield: 62%; M.P.: 107-109 °C; Molecular formula: $C_{31}H_{34}ClN_5O_3S_2$; Molecular Mass: 624.0 $gmol^{-1}$; IR (KBr, cm^{-1}) ν_{max}: 3051 (Ar-H), 1564 (C=N), 1549 (Ar C=C), 1367 ($-SO_2$), 1231 (C-N), 819 (C-Cl); ^{1}H-NMR ($CDCl_3$, 600 MHz, *δ*/ppm): 7.79 (s, 1H, -NH), 7.63 (d, *J* = 8.1 Hz, 2H, H-2″ & H-6″), 7.61-7.58 (m, 3H,H-3‴ to H-5‴), 7.48 (d, *J* = 8.1 Hz, 2H, H-3″ & H-5″), 7.25-7.24 (m, 2H,H-2‴&H-6‴), 7.11-7.04 (m,



Scheme 2: Outline for synthesis of *S*-substituted derivatives (10a-e) of 6. Reagents & conditions: (I) Aq. Na_2CO_3/stirring for 1 hour. (II) DMF/NaH/stirring for 4-5 hours.

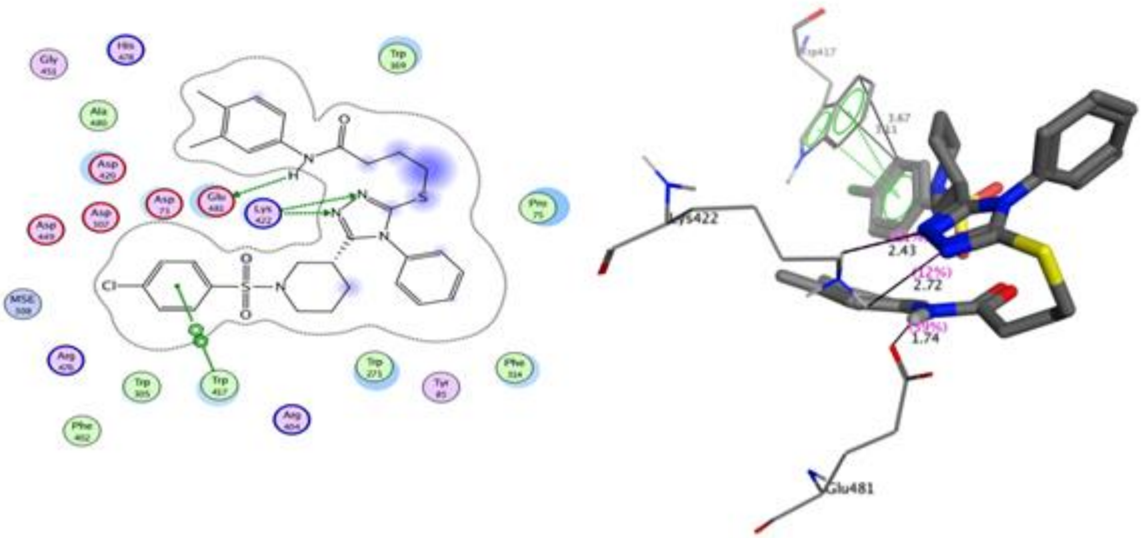


Fig. 1 (2D & 3D): Compound 10e showing three acidic interactions including one of Glu481-amidic proton with bond length of 1.74 Å& two of Lys 422-nitrogens of triazole ring with bond lengths of 2.43 & 2.72Å and arene-arene interactions of Trp417-4-chlorophenyl ringwith bond length of 3.39 Å.

3H, H-3′′′′′ to H-5′′′′′), 3.88 (br.d, *J* = 12.1 Hz, 1H, H_e-2′), 3.74 (br.d, *J* = 11.5 Hz, 1H, H_a-2′), 3.29 (t, *J* = 6.9 Hz, 2H, H-4′′′′), 2.76-2.72 (m, 1H, H-3′), 2.65 (br.t, *J* = 11.2 Hz, 1H, H_e-6′), 2.59 (t, *J* = 7.0 Hz, 2H, H-2′′′′), 2.26 (br.t, *J* = 12.0 Hz, 1H, H_a-6′), 2.24-2.22 (m, 2H, H-3′′′′), 2.21 (s, 6H, CH_3-2′′′′′&CH_3-6′′′′′), 1.85-1.82 (m, 1H, H_e-5′), 1.76-1.72 (m, 1H, H_e-4′), 1.57-1.48 (m, 2H, H_a-4′& H_a-5′); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ/ppm): 170.5 (C-1′′′′), 156.2 (C-3), 152.0 (C-5), 139.4 (C-1″), 135.4 (C-4″), 134.9 (C-1′′′′′), 134.1 (C-1‴), 132.6 (C-2′′′′′& C-6′′′′′), 130.6 (C-2″&

C-6″), 130.3 (C-3″& C-5″), 130.2 (C-3‴ & C-5‴), 129.4 (C-4‴), 128.9 (C-3⁗′& C-5⁗′), 128.1 (C-2‴ & C-6‴), 127.1 (C-4⁗′), 49.3 (C-2′), 46.0 (C-6′), 34.8 (C-3′), 32.9 (C-2⁗), 31.8 (C-4′), 29.0 (C-4⁗), 26.2 (C-3⁗), 24.3 (C-5′), 18.5 (CH_3-2⁗′ & CH_3-6⁗′); EIMS (*m/z*): 626 $[M+2]^+$, 624 $[M]^+$, 435 $[C_{19}H_{18}ClN_4O_2S_2]^{\bullet+}$, 375 $[C_{18}H_{18}ClN_3O_2S]^{\bullet+}$, 361 $[C_{18}H_{18}ClN_2O_2S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 190 $[C_{12}H_{16}NO]^+$, 175 $[C_6H_4ClO_2S]^+$, 148 $[C_9H_{10}NO]^+$, 120 $[C_8H_{10}N]^+$, 111 $[C_6H_4Cl]^+$.

4-[(5-{1-[(4-Chlorophenyl)sulfonyl]-3-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(3,4-dimethylphenyl)butanamide (10e)

Light brown amorphous solid; Yield: 60%; M.P.: 116-118 °C; Molecular formula: $C_{31}H_{34}ClN_5O_3S_2$; Molecular Mass: 624.0 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3048 (Ar-H), 1561 (C=N), 1546 (Ar C=C), 1364 (-SO_2), 1228 (C-N), 816 (C-Cl); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ/ppm): 8.65 (s, 1H, -NH), 7.64 (d, *J* = 8.1 Hz, 2H, H-2″ & H-6″), 7.60-7.59 (m, 3H,H-3‴ to H-5‴), 7.49 (d, *J* = 8.1 Hz, 2H, H-3″ & H-5″), 7.47 (s, 1H, H-2⁗′), 7.38 (d, *J* = 8.1 Hz, 1H, H-6⁗′), 7.23-7.22 (m, 2H,H-2‴&H-6‴), 7.05 (d, *J* = 8.1 Hz, 1H, H-5⁗′), 3.91 (br.d, *J*=11.5 Hz, 1H, H_e-2′), 3.76 (br.d, *J* = 11.9 Hz, 1H, H_a-2′), 3.23 (t, *J* = 6.9 Hz, 2H, H-4⁗), 2.79-2.74 (m, 1H, H-3′), 2.70 (br.t, *J*=11.2 Hz, 1H, H_e-6′), 2.53 (t, *J* = 6.7 Hz, 2H, H-2⁗), 2.31 (br.t, *J* = 12.1 Hz, 1H, H_a-6′), 2.23-2.14 (m, 8H, H-3⁗, CH_3-3⁗′&CH_3-4⁗′), 1.86-1.84 (m, 1H, H_e-5′), 1.78-1.76 (m, 1H, H_e-4′), 1.62-1.59 (m, 1H, H_a-5′), 1.55-1.51 (m,1H, H_a-4′); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ/ppm): 170.2 (C-1⁗), 156.2 (C-3), 152.3 (C-5), 139.4 (C-1″), 136.9 (C-3⁗′), 136.0 (C-4″), 134.9 (C-1⁗′), 132.5 (C-1‴), 132.2 (C-2″& C-6″), 130.6 (C-3″& C-5″), 130.3 (C-3‴ & C-5‴), 129.8 (C-4⁗′), 129.5 (C-4‴), 128.8 (C-2‴ & C-6‴), 127.0 (C-5⁗′), 121.2 (C-2⁗′), 117.3 (C-6⁗′), 49.3 (C-2′), 46.0 (C-6′), 35.8 (C-3′), 32.9 (C-2⁗), 31.6 (C-4′), 29.0 (C-4⁗), 26.2 (C-3⁗), 24.3 (C-5′), 19.8 (CH_3-3⁗′), 19.1 (CH_3-4⁗′); EIMS (*m/z*): 626 $[M+2]^+$, 624 $[M]^+$, 435 $[C_{19}H_{18}ClN_4O_2S_2]^{\bullet+}$, 375 $[C_{18}H_{18}ClN_3O_2S]^{\bullet+}$, 361 $[C_{18}H_{18}ClN_2O_2S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 190 $[C_{12}H_{16}NO]^+$, 175 $[C_6H_4ClO_2S]^+$, 148 $[C_9H_{10}NO]^+$, 120 $[C_8H_{10}N]^+$, 111 $[C_6H_4Cl]^+$.

Biological activities

The synthesized compounds were screened for *α*-Glucosidase enzyme inhibition to inaugurate new anti-diabetic agents. Acarbose was used as reference standard. The results are given in table 1. Further more cytotoxicity of these compounds was analyzed through hemolytic activity. Percent hemolysis of each compound was determined and is given in table 1.

DISCUSSION

The compound 5-{1-[(4-chlorophenyl)sulfonyl]-3-piperidinyl}-4-phenyl-4*H*-1,2,4-triazole-3-thiol (6) was synthesized through a multi step protocol. Primarily sulfonamide linkage was formed by reacting 4-chlorobenzenesulfonyl chloride (1) with ethyl piperidin-3-carboxylate (2) in the presence of sodium carbonate solution as explained in experimental section. Sulfamoyl linkage formed was confirmed from IR data by signal around 1380-1390 cm^{-1}. In 2nd step corresponding carbohydrazide (4) was formed by reaction of hydrated hydrazine with ethyl 1-[(4-chlorophenyl) sulfonyl] piperidine-3-carboxylate (3). Carbohydrazide linkage was confirmed from IR bands appeared around 3140 cm^{-1} for N-H and 1640 cm^{-1} for C=O group.3rd step involved the synthesis of corresponding hydrazine carbothioamide (5) by gearing up corresponding carbohydrazide (4) with phenyl isothiocyanate in methanol as solvent. Compound 5 undergo intramolecular cyclization to result into 3,4,5-trisubstituted-1,2,4-triazole (6) in the presence of 10% NaOH solution and on reflux. Compound 6 was quenched by acidifying reaction mixture by dil. HCl. The stretching bands of IR spectrum around 1560-1570 cm^{-1} for C=N & 1220-1230 cm^{-1} for C-N-C stretching confirmed the presence of 1,2,4-triazole ring presence in all derivatives. A series of electrophiles, *N*-aryl-4-bromobutanamide (9a-e) were synthesized on reaction of aryl amines (8a-e) with 4-bromobutyrylchloride (7) under dynamic pH control. *N*-Aryl-3,4,5-trisubstituted-*4H*-1,2,4-triazol-3-ylsulfanyl-butanamide (10a-e) were synthesized by gearing up compound 6 with the synthesized electrophiles in the presence of catalyst and aprotic solvent. All derivatives produced 60-70% yield in pure form. Structures of all derivatives were assured from spectroscopic techniques like IR, ^{1}H-NMR, ^{13}C-NMR and EIMS. In^{1}H-NMR spectra, *p*-chlorophenylsulfonyl ring revealed characteristic two doublet sat *δ* 7.58-7.70 ppm and 7.45-7.50 ppm each integrated for two protons. Piperidine ring gave *axial* and *equatorial* splitting of protons for different positions in aliphatic region of spectra. Phenyl ring at fourth position of 1,2,4-triazole ring revealed characteristic splitting pattern of monosubstituted phenyl ring, one signal for two ortho protons and a merged signal for remaining three protons around *δ* 7.20-7.34 ppm and *δ* 7.70-7.50 ppm respectively. Different aryl substituents gave their characteristic splitting patterns separately. ^{13}C-NMR data also confirmed the structures of all the derivatives. In ^{13}C-NMR six quaternary carbon signals appeared, most deshielded quaternary signal around *δ* 171.5-169.0 ppm was due to carbonyl carbon of acidamide moiety; other two most deshielded signals at *δ* 155.5-157.0 ppm and *δ* 151.0-152.9 ppm were corresponding to quaternary carbons of triazole ring. Two quaternary carbons of *p*-chlorophenyl ring were appeared around *δ* 139.0-140.0 ppm and *δ* 134.0-139.0 ppm each for two symmetrical carbon atoms. Single quaternary carbon of phenyl ring attached to triazole was appeared around *δ* 132.1-135.0 ppm. Along with these five signals were appeared for tertiary carbon atoms. For *p*-chlorophenyl ring two symmetrical carbons (C-2" & C-

6") revealed relatively deshielded signals around δ 130.0-133.0 ppm due to strong electron withdrawing effect of sulfonyl group and signals for tertiary C-3" & C-5" obtained at δ 130.0-131.0 ppm relatively upfield due to meta position. Phenyl ring attached to 4th position of 1,2,4-triazole ring revealed three signals for tertiary carbon atoms, two signals at δ 129.0-131.0 ppm and δ 126.0-129.0 ppm were allotted to symmetrical C-3''' & C-5''' and C-2''' & C-6''' respectively. Third signal at δ 128.5-129.9 ppmwas corresponding to 4th position of phenyl group. Different electrophilic substituent gave their corresponding signals. EIMS spectra showed the characteristic fragmentation pattern to corroborate the structures of all derivatives.

α-Glucosidase inhibition activity

The compounds 10a-e revealed moderate to good inhibition potential. The four compounds bearing one methyl group at *ortho* position and other at other positions remained least active.

Among these least active compounds, two compounds bearing 2,3-dimethylphenyl and 2,6-dimethylphenyl groups remained relatively more active. Compound 10e revealed strong bonding in the active pocket of enzyme by making four interactions as shown in fig. 1 (2D & 3D). The better activity of this compound might be attributed to the presence of 3,4-dimethylphenyl group. First strongest polar interaction was found between analyl proton & Glu 481of *α*-glucosidase (bond length: 1.74A), two stronger acidic interactions were established among cyclic nitrogen atoms & Lys 422 (bond lengths: 2.43A & 2.72A) and a weak arene-arene interaction was made between 4-chlorophenyl ring and Trp417 (average bond length: 3.39A). These interactions strongly affected the anti-diabetic potential of these drug candidates. These results motivated to synthesize new derivatives that can be more efficient *α*-glucosidase inhibitory agents. The docking results are in concordance with the inhibitory studies Compounds 10d and 10e showed good potency (IC_{50} = 176.75±0.12 and IC_{50} = 79.42±0.14 μM) respectively as compared to Acarbose which is used as a standard IC_{50} =37.38±0.12 μM.

Hemolytic activity

Triton-X-100 was the +ve control while PBS was -ve control. Compound 10c revealed the highest activity having % lysis 14.7 probably due to *N*-substituted 2, 5-dimethylphenyl group. The decreasing order of % lysis for the series is as 10 c> 10a> 10b> 10d> 10e. The lowest % lysis from the series was observed for compounds 10d and 10e as 9.7 and 9.5 respectively attributed to *N*-substituted 2,6-dimethylphenyl and 3,4-dimethylphenyl group, but very much higher than that of -ve control PBS (% lysis = 0.12).

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CONCLUSION

The synthesized 5-{1-[(4-chlorophenyl)sulfonyl]-3-piperidinyl}-4-phenyl-*4H*-1,2,4-triazol-3-thiol (6) was modified structurally by substitution at thiol group and target compounds were synthesized in good yields. After structural elucidation, the synthesized compounds were subjected to *α*-glucosidase enzyme inhibition analysis to inaugurate new drug candidates for type II diabetes. The compound 10e remained better inhibitor of the enzyme among the whole series. The hemolytic activity analysis explained toxicity level of these compounds.

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