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Synthesis, *invitro* and *invivo* anti-hyperglycemic activity of 1,2,4-triazolebenzylidene and 1,3,4-thiadiazole derivatives

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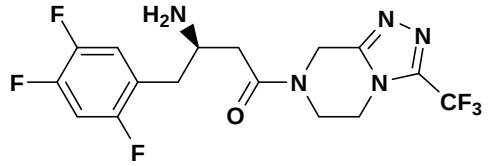
Abstract: New series of 1,2,4-triazole based Schiff base (**5a**-**5i**) and 1,3,4-thiadiazole (**6a**-**6g**) derivatives were synthesized by utilizing 4-amino-5-(4-chloro-2-methylphenyl)-4*H*-1,2,4-triazole-3-thiol (**4**) as active intermediateand evaluated for *invitro* anti-hyperglycemic activity by α-glucosidase enzyme inhibition and *invivo* by streptozotocin (STZ) and nicotinamide induced T2DM rat model. The compounds **5a**, **5c-g** which showed potential DPPH radical scavenging activity with a level of inhibition ranging between 70% and 90% were considered for anti-hyperglycemic activity. The IC_{50} value, for the α-glucosidase inhibition capacity of the compounds **5a** and **5c** was 74.5µg and 113µg respectively. Blood glucose level of test compounds (**5a**, **5c-g**) attenuated the progression of diabetes in a dose dependent manner following 14 days of treatment. The test compounds were given orally at 10mg/kg, 50mg/kg and 100mg/kg body weight of animals. The 14th day data with 10 mg/kg, compounds **5a** and **5c** showed significant decrease in plasma glucose concentration (92mg/dL and 109mg/dL respectively) and for the compounds **5a**, **5c-g** with 100mg/kg, the plasma glucose concentration was 94mg/dL to 111mg/dL.
Key words: anti-hyperglycemic; α-glucosidase; Schiff base; 1,3,4-thiadiazole; 1,2,4-triazolebenzylidene.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a global epidemic. The number of reported cases has doubled over the past 15 years. Recently, dipeptidyl peptidase IV (DPP-4) inhibitors have emerged as a new class of antihyperglycemic agents for the treatment of T2DM and offer several advantages over other existing antidiabetic agents such as lack of body weight gain and decreased incidence of hypoglycemic episodes. Sitagliptin,a trifluoromethylated triazolopiperizine, a selective, potent DPP-4 inhibitor, which recently received

approval for the treatment of type 2 diabetes by the FDA. Blank *et* al. (1972) has reported that certain 4-alkyl-5-aryl-4H-1,2,4-triazole-3-thiols produced hypoglycemia in normal and alloxan-diabetic rats. 1-(mesitylen-2-sulfonyl)-1H-1,2,4-triazole was evaluated for hypoglycemic activity and 1,3,4-thiadiazoles were explored as hypoglycemic agents.Many 1,2,4-triazole and 1,3,4-thiadiazole derivatives have been used as 'privileged' scaffolds to produce active pharmaceutical ingredients. Some of the modern-day drugs like Ribavirin (antiviral agent), Alprazolam (anxiolytic agent), Fluconazole, Itraconazole (antifungal agents) and Rizatriptan (antimigrane agent) are having triazole nucleus in their structure. The ambient nucleophilic centers present in 3-substituted-4-amino-5-mercapto-1,2,4-triazoles render them useful synthons for the synthesis of various N-bridged heterocycles. 1,2,4-triazole, 1,3,4-thiadiazole and thiadiazine are explored to the maximum extent owing to their wide spectrum of pharmacological activities such as antibacterial, anti-fungal[1,2], antitubercular[3], anticancer[4], anticonvulsant[5], anti-inflammatory[6,7], analgesic[8,9], antitumor [10], molluscicidal[7,11], and antiviral[10,12] activities. Among the pharmacological profiles of 1,2,4-triazoles, their antimicrobial, anticonvulsant, and antidepressant properties have been best documented. Recently, some benzoxazoline, trisubstituted triazoles, and 4-benzylidenamino derivatives containing triazole and thiadiazole units have been found to be endowed with excellent free radical scavenging activities[13-15]. Type 2 diabetes mellitus (T2DM) is a global epidemic. The number of reported cases has doubled over the past 15 years[16]. Recently, dipeptidyl peptidase IV (DPP-4) inhibitors have emerged as a new class of antihyperglycemic agents for the treatment of T2DM[17-20] and offer several advantages over other existing antidiabetic agents such as lack of body weight gain and decreased incidence of hypoglycemic episodes. Sitagliptin[21,22], a trifluoromethylated triazolopiperizine, a selective, potent DPP-4 inhibitor, which recently received approval for the treatment of type 2 diabetes by the FDA. Blank *et* al. (1972)[23] has reported that certain 4-alkyl-5-aryl-4H-1,2,4-triazole-3-thiols produced hypoglycemia in normal and alloxan-diabetic rats. 1-(mesitylen-2-sulfonyl)-1H-1,2,4-triazole was evaluated for hypoglycemic activity[24] and 1,3,4-thiadiazoles were explored as hypoglycemic agents[25,26]. Prompted by these observations and in continuation of our research for biologically active heterocyclic compounds[27] it was contemplated to synthesize some N-bridged heterocycles containing 1,2,4-triazole moiety with a view to explore their potency as better chemotherapeutic agents. Herein, we describe the synthesis, free-radical scavenging and *in vitro* and *in vivo* anti-hyperglycemic activity of 1,2,4-triazole benzylidene and 1,3,4-thiadiazole derivatives.



Sitagliptin

2. Materials and Methods

2.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX400 spectrometer operating at 400 MHz, with Me_4Si as internal standard. The chemical shifts are expressed as δ values in parts per million (ppm), and the coupling constants (J) are given in hertz (Hz). Mass spectra were determined by the EPSRC (Engineering and Physical Sciences Research Council) Mass Spectrometry (Swansea, UK). Flash column chromatography was performed with silica gel 60 (Merck), and Thin layer chromatography (TLC) carried out on precoated silica plates (kiesel gel 60 F_{254}). Melting points were determined on an electro thermal instrument and are uncorrected. All reagents involved in the experiments were commercially available and used without further purification. The yields were of purified compounds and were not optimized.

2.1.1. Synthesis of 5-chloro-2-methylphenyl benzoate (1)

To a stirred solution of 2-methyl benzoic acid (73.45 mmol) in dry methanol (100 mL), was added 3-4 drops of con.H_2SO_4. The reaction mixture was refluxed for 8-10 h under nitrogen atmosphere. The reaction was monitored by TLC. After the reaction completion, methanol was distilled off and the residue was dissolved in ethyl acetate (200 mL). The organic layer was washed with 10% sodium bicarbonate solution (50 mL) and water (50 mL) followed by saturated sodium chloride solution. The organic layer was treated with anhydrous

sodium sulfate, filtered and concentrated under vacuum to afford title compound. 5-chloro-2-methylphenyl benzoate, % yield: 76.2, MF: $C_9H_9ClO_2$,MW: 183.67.

2.1.2. Synthesis of 5-chloro-2-methylbenzohydrazide (2)

To a stirred solution of 5-chloro-2-methylphenyl benzoate (**1**)(59.93 mmol) in absolute ethanol (90 mL) was added hydrazine hydrate (269.68 mmol). The reaction mixture was refluxed for about 8 h. The reaction was monitored by TLC. After the reaction completion, the solvent was distilled under vacuum, added ice-water (50 mL) and stirred for 15 min. The solid obtained was filtered and dried under vacuum to obtain 5-chloro-2-methylbenzohydrazide (**2**) as off-white solid. % yield: 88; m.p: 163-165°C; MF: $C_8H_9ClN_2O$, MW: 184.62, [m/z]: 184.04.

2.1.3. Synthesis of 2-(5-chloro-2-methylbenzoyl)hydrazinecarbodithiol acid potassium salt (3)

Potassium hydroxide pellets (106.54 mmol) were dissolved in 40 mL absolute ethanol. To this solution, added 5-chloro-2-methylbenzohydrazide (**2**)(53.27 mmol) followed by carbon disulfide (117.19 mmol) and contents were stirred at room temperature for 5 h. The reaction was monitored by TLC. After the reaction completion, added di-ethylether (100 mL) and stirred for 10 min. The solid formed was filtered and dried under vacuum to obtain 2-(5-chloro-2-methylbenzoyl) hydrazinecarbodithiol acid potassium salt (**3**) as off-white solid. % yield: 85; m.p: 169-173°C; MF: $C_9H_8ClKN_2OS_2$; MW: 298.85; [m/z]: 298.2.

2.1.4. Synthesis of 4-amino-5-(5-chloro-2-methyl phenyl)-4H-1,2,4-triazole-3-thiol (4)

Hydrazine hydrate (45.38 mmol) was added to compound **3** (45.38 mmol) and the contents were refluxed for 2 h. The reaction was monitored by TLC. After the reaction completion, the reaction mixture was acidified with con. HCl. The precipitate was filtered and dried under vacuum to obtain compound **4**. White solid, Yield: 87.9%, m.p: 171-174°C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.35 (s, 3H), 5.66 (bs, 2H), 7.13 (t, J = 7.26 Hz, 1H), 7.14 (t, J = 7.26 Hz, 1H), 7.30 (t, J = 7.2 Hz, 1H), 13.05 (s, 1H); MF: $C_9H_9ClN_4S$; MW: 240.7, [m/z]: 240.

2.1.5. General procedure for the synthesis of benzylideneamine derivative (5a-i)

To a stirred solution of compound **4** (4.84 mmol) in ethanol (10 mL), was added benzaldehyde (1 eq.), 2-3 drops of con.H_2SO_4 and the contents were refluxed for 5 h. The reaction was monitored by TLC. After the reaction completion, the solvent was removed under vacuum. To the residue, was added 5 mL of ice-water, stirred for 5 min. and the precipitated solid was filtered and dried under vacuum. The compounds were purified by column chromatography using ethyl acetate and petroleum ether.

4-[(E)-benzylideneamino]-5-(4-chloro-2-methylphenyl)-4H-1,2,4-triazole-3-thiol (5a)

Off-white solid, 70%; m.p 125-129°C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.36 (s, 3H), 7.57 (dd, J_1 = 1.6 Hz, J_2 = 8.2 Hz, 2H), 7.61-7.63 (m, 1H), 7.70 (dd, J_1 = 2 Hz, J_2 = 8.4 Hz, 2H), 7.87 (d, J = 0.8 Hz, 2H), 7.89 (s, 1H), 9.71 (s, 1H), 14.28 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 167.57, 155.45, 149.67, 137.59, 135.48, 133.93, 131.98, 128.40, 129.07, 127.29, 126.39, 119.49, 112.88, 107.54, 102.84, 22.42; IR cm^{-1}, -SH: 2640, -C=N-: 1565, Ar-Me: 2813; Anal. calcd. for $C_{16}H_{13}ClN_4S$: C, 58.23; H, 3.94; N, 6.98%; found: C, 58.25; H, 3.97; N, 6.70%. MW: 328.82, [m/z]$^+$: 329.7.

5-(4-chloro-2-methylphenyl)-4-[(E)-(2,4-difluorobenzylidene)amino]-4H-1,2,4-triazole-3-thiol (5b)

White solid, 76%; m.p 155-158°C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.37 (s, 3H), 7.28 (t, J_1 = 1.6 Hz, 1H), 7.55 (t, J = 1.9 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.70 (dd, J_1 = 1.6 Hz, J_2 = 8.0 Hz, 1H), 7.85 (s, 1H) 8.03 (d, J = 6.8 Hz, 1H), 10.07 (s, 1H), 14.3 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 168.97, 160.45, 151.67, 151.55, 149.44, 137.93, 133.98, 131.42, 129.87, 128.29, 122.39, 120.49, 112.88, 104.54, 103.84, 22.82; IR cm^{-1}, -SH: 2575, -C=N-: 1670, Ar-Me: 2925, C-F: 1200; Anal. calcd. for $C_{16}H_{11}ClF_2N_4S$: C, 52.50; H, 3.0; N, 15.31%; found: C, 52.52; H, 3.03; N, 15.34%. MW: 364.80, [m/z]$^+$: 365.7.

5-(4-chloro-2-methylphenyl)-4-[(E)-(2-fluorobenzylidene)amino]-4H-1,2,4-triazole-3-thiol (5c)

Off-white solid, 81%; m.p 158-164°C;^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.37 (s, 3H), 7.36-7.44 (m, 2H), 7.58 (d, J = 8.4Hz, 1H), 7.67-7.72 (m, 2H), 7.86 (d, J = 1.6 Hz, 1H), 7.95-7.99 (m, 1H), 10.12 (s, 1H), 14.32 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 169.97, 168.45, 161.67, 159.59, 149.48, 137.93, 133.98, 130.40, 129.87, 128.29, 126.39, 120.49, 119.88, 104.54, 103.84, 22.52; IR cm^{-1}, -SH: 2572, -C=N-:

1665, Ar-Me: 2920; C-F: 1250; Anal. calcd. for $C_{16}H_{12}ClFN_4S$: C, 55.18; H, 3.44; N, 16.09%; found: C, 55.21; H, 3.41; N, 16.11%. MW: 346.81, $[m/z]^+$: 347.9.

5-(4-chloro-2-methylphenyl)-4-[(E)-(3-fluorobenzylidene)amino]-4H-1,2,4-triazole-3-thiol (5d)

Yellow solid, 75%; m.p 147-152°C;^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.36 (s, 3H), 7.46-7.50 (m, 1H), 7.57-7.64 (m, 2H), 7.68-7.73 (m, 3H), 7.85 (s, 1H), 9.80 (s, 1H), 14.31 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 163.67, 162.45, 160.67, 159.59, 146.48, 137.93, 135.98, 130.40, 129.87, 127.29, 126.39, 120.49, 116.88, 104.54, 103.84, 22.12; IR cm^{-1}, -SH: 2542, -C=N-: 1675, Ar-Me: 2930, C-F: 1300; Anal. calcd. for $C_{16}H_{12}ClFN_4S$: C, 55.18; H, 3.44; N, 16.09%; found: C, 55.20; H, 3.42; N, 16.10%. MW: 346.81, $[m/z]^+$: 347.8.

2-*((E)-{[3-(4-chloro-2-methylphenyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]imino}methyl)benzene-1,4-diol (5e)*

Pale brown solid, 73%; m.p 140-144°C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.36 (s, 3H), 7.19-7.22 (m, 3H), 7.39 (s, 1H), 7.49 (d, J = 2.2 Hz, 1H), 7.62-7.69 (m, 1H), 7.91 (s, 1H), 8.86 (s, 1H), 9.85 (s, 1H), 14.17 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 159.07, 157.45, 155.97, 153.59, 146.48, 142.93, 141.98, 139.40, 136.07, 135.20, 125.37, 119.49, 116.88, 111.49, 107.54, 21.82; IR cm^{-1}, -SH: 2950, -C=N-: 1690, Ar-Me: 2895, Ar-OH: 3365; Anal. calcd. for $C_{16}H_{13}ClN_4O_2S$: C, 53.21; H, 3.60; N, 15.52%; found: C, 53.24; H, 3.63; N, 15.55%. MW: 360.81, $[m/z]^+$: 361.7.

4-[(E)-{[3-(4-chloro-2-methylphenyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]imino}methyl]phenol (5f)

Yellow solid, 81%; m.p 135-139°C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.35 (s, 3H), 6.83-6.93 (m, 3H), 7.54 (d, J = 4.8 Hz, 1H), 7.66-7.75 (m, 3H), 7.86 (s, 1H), 9.38 (s, 1H), 14.19 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 157.07, 155.45, 154.97, 147.59, 146.48, 142.93, 140.98, 139.40, 136.07, 127.20, 125.37, 119.49, 116.88, 111.49, 107.54, 21.72; IR cm^{-1}, -SH: 2940; -C=N-: 1695; Ar-Me: 2890; Ar-OH: 3370; Anal. calcd. for $C_{16}H_{13}ClN_4OS$: C, 55.68; H, 3.77; N, 16.24%; found: C, 55.70; H, 3.80; N, 16.25%. MW: 344.81, $[m/z]^+$: 345.75.

5-(4-chloro-2-methylphenyl)-4-[(E)-(4-methoxybenzylidene)amino]-4H-1,2,4-triazole-3-thiol (5g)

Pale brown solid, 76%; m.p 159-163°C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.37 (s, 3H), 3.81 (s, 3H), 7.03-7.12 (m, 3H), 7.56 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 0.8 Hz, 1H), 7.78-7.86 (m, 2H), 9.5 (s, 1H), 14.22 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 168.57, 165.45, 164.67, 157.59, 156.48, 142.93, 141.98, 137.40, 129.07, 127.29, 125.39, 119.49, 112.88, 110.44, 107.54, 55.84, 22.42; IR cm^{-1}, -SH: 2640, -C=N-:1565, Ar-Me: 2813, Ar-MeO:1150; Anal. calcd. for $C_{17}H_{15}ClN_4OS$: C, 56.72; H, 4.17; N, 15.57%; found: C, 56.75; H, 4.20; N, 15.60%. MW: 358.84, $[m/z]^+$: 359.6.

4-bromo-2-[(E)-{[3-(4-chloro-2-methylphenyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]imino}methyl]phenol (5h)

Pale yellow solid, 80%; m.p 139-143°C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.37 (s, 3H), 6.93 (t, J = 5.2 Hz, 1H), 7.51-7.61 (m, 3H), 7.87-7.90 (m, 2H), 10.05 (s, 1H), 10.90 (s, 1H), 14.22 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 167.07, 165.45, 164.97, 157.59, 156.48, 142.93, 141.98, 139.40, 136.07, 127.29, 125.37, 119.49, 113.88, 111.44, 107.54, 22.42; IR cm^{-1}, -SH: 2740, -C=N-: 1595, Ar-Me: 2893, Ar-OH:3350; C-Br: 580; Anal. calcd. for $C_{16}H_{12}BrClN_4OS$: C, 45.32; H, 2.83; N, 13.21%; found: C, 45.35; H, 2.86; N, 13.25%. MW: 423.71, $[m/z]^+$: 424.65.

5-(4-chloro-2-methylphenyl)-4-{(E)-[2-fluoro-4-(trifluoromethyl)benzylidene]amino}-4H-1,2,4-triazole-3-thiol (5i)

Pale yellow solid, 77%; m.p 120-123°C; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.37 (s, 3H), 7.56-7.60 (m, 1H), 7.70 (dd, J_1 = 1.7 Hz, J_2 = 8.2 Hz, 1H), 7.78 (d, J = 1.6Hz, 1H), 7.83-8.06 (m, 3H), 10.06 (s, 1H), 14.37 (s, 1H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ / ppm): 160.67, 155.45, 149.67, 137.59, 135.48, 132.93, 131.98, 128.40, 128.07, 127.29, 126.39, 120.49, 112.88, 104.54, 103.84, 102.11, 21.42; IR cm^{-1}, -SH: 2540; -C=N-: 1575; Ar- Me: 2830; C-F: 1270; Anal. calcd. for $C_{17}H_{11}ClF_4N_4S$: C, 49.07; H, 2.64; N, 13.47%; found: C, 49.10; H, 2.67; N, 13.50%. MW: 414.80, $[m/z]^+$: 415.7.

2.1.6. General procedure for the synthesis of Thiadiazole derivatives (6a-6g)

To a stirred solution of 4-amino-5-(5-chloro-2-methyl phenyl)-4H-1,2,4-triazole-3-thiol **4** (1 g, 4.84 mmol, 1 eq) in $POCl_3$ (10 mL), was added benzoic acid (1 eq) and the reaction mixture was refluxed about 10 h.

The reaction completion was monitored by TLC. After the reaction completion, the mass was quenched with ice-water, and the product was extracted with ethyl acetate (2× 75 mL). The organic layer was washed 10% sodium bicarbonate solution (50 mL) followed by water and saturated sodium chloride solution. The organic layer was treated with anhydrous sodium sulfate and concentrated under vacuum to afford title compounds. These compounds were purified by column chromatography using ethyl acetate and petroleum ether.

6-(2-bromo 4-fluorophenyl)-3-(4-chloro-2-methylphenyl)[1,2,4]triazole[3,4,b][1,3,4]thiadiazole (6a)

Pale yellow solid, 70%; m.p 136-139°C; H^1 NMR, 400 MHz, DMSO-d_6, δ 2.44 (s, 3H), 7.56 (t, J = 7.7 Hz, 1H), 7.67 (d, J = 8.31 Hz, 1H), 7.96 (dd, J_1 = 2.6 Hz, J_2 = 8.16 Hz, 1H), 8.09 (t, J = 11.2 Hz, 2H), 8.2 (s, 1H); C^{13} NMR, 175.0, 165.1, 148, 138.2, 136.6, 135.4, 134.2, 131.3, 129.3, 128.8, 126.3, 121.8, 121.2, 115.0, 21.2; Anal. calcd. for $C_{16}H_9BrClFN_4S$: C, 45.36; H, 2.14; N, 13.22%; found: C, 45.34; H, 2.14; N, 13.21%. MW: 423.69, [m/z]= 423.94.

6-(2,6-dimethylphenyl)-3-(4-chloro-2-methylphenyl)[1,2,4]triazole[3,4,b][1,3,4]Thiadiazole (6b)

White solid, 75%, m.p 152-154.5°C; H^1 NMR, 400 MHz, DMSO-d_6, δ 2.26 (s, 6H), 2.41 (s, 3H), 7.26 (d, J = 7.6 Hz, 2H), 7.43 (t, J =7.6 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 8.06 (dd, J_1 = 2 Hz, J_2 = 8.2 Hz, 1H), 8.16 (d, J = 1.6 Hz, 1H); C^{13} NMR, 168.0, 148.0, 143.3, 138.7, 138.2, 136.6, 134.2, 132.6, 130.9, 129.3, 126.3, 126.1, 21.8, 21.2; Anal. calcd. for $C_{18}H_{15Cl}N_4S$: C, 60.92; H, 4.26; N, 15.79%; found: C, 60.89; H, 4.26; N, 15.78%. MW: 354.86, [m/z]= 354.07.

6-(3-bromo-5-iodophenyl)-3-(4-chloro-2-methylphenyl)[1,2,4]triazole[3,4,b][1,3,4]thiadiazole (6c)

Yellow solid, 73%; m.p 141-142°C; H^1 NMR, 400 MHz, DMSO-d_6, δ 2.48 (s, 3H), 7.65-7.71 (m, 3H), 8.17 (t, J = 3.6 Hz, 1H), 8.22 (d, J = 4 Hz, 1H), 8.32 (dd, J_1 = 2.1Hz, J_2 = 8.24 Hz, 1H), 8.36 (d, J = 1.9 Hz, 1H); C^{13} NMR, 168.0, 148.0, 143.3, 143.0, 138.2, 136.6, 135.6, 134.5, 134.2, 131.6, 129.3, 128.8, 126.3, 124.8, 96.6, 21.2. Anal. calcd. for $C_{16}H_9BrIClN_4S$: C, 36.15; H, 1.71; N, 10.54%; found: C, 36.14; H, 1.71; N, 10.53%. MW: 531.6, [m/z]= 531.84.

6-(4-fluoro-3-nitrophenyl)-3-(4-chloro-2-methylphenyl)[1,2,4]triazole[3,4,b][1,3,4]thiadiazole (6d)

White solid, 76%, m.p 155-158.5°C; H^1 NMR, 400 MHz, DMSO-d_6, δ 2.41 (s, 3H), 7.67 (d, J = 8.4 Hz, 1H), 7.83-7.87 (m, 2H), 8.04 (s, 1H), 8.20 (d, J = 1.6 Hz, 1H), 8.24 (s, 1H); Anal. calcd. for $C_{16}H_9FClN_2O_5S$: C, 49.30; H, 2.33; N, 17.97%; found: C, 49.28; H, 2.33; N, 17.96%. MW: 389.79, [m/z]= 389.01.

3-(4-chloro-2-methylphenyl)-6-(2,3,4 trifluorophenyl)[1,2,4]triazole[3,4,b][1,3,4]thiadiazole (6e)

Off-white solid, 78%, m.p 139-143°C; H^1 NMR, 400 MHz, DMSO-d_6, δ 2.48 (s, 3H), 7.61-7.68 (m, 2H), 8.08-8.10 (m, 1H), 8.14 (dd, J1 = 2 Hz, J2 = 8.6 Hz, 1H), 8.24 (d, J = 1.6 Hz, 1H); Anal. calcd. for $C_{16}H_8F_3ClN_4S$: C, 50.47; H, 2.12, N, 14.71%; found: C, 50.45; H, 2.12, N, 14.70%. MW: 380.77, [m/z]= 380.01.

6-(4-bromo-3-methyl phenyl)-3-(4-chloro-2-methylphenyl)[1,2,4]triazole[3,4,b][1,3,4]thiadiazole (6f)

Yellow solid, 76%; m.p 134-136°C; H^1 NMR, 400 MHz, DMSO-d_6, δ 2.41 (s, 3H), 2.46 (s, 3H), 7.65 (d, J = 8.34 Hz, 1H), 7.85-7.89 (m, 2H), 8.06 (s, 1H), 8.26 (d, J = 1.75 Hz, 1H), 8.27 (s, 1H); Anal. calcd. for $C_{17}H_{12}BrClN_4S$: C, 48.65; H, 2.88; N, 13.35%; found: C, 48.63; H, 2.88; N, 13.34%. MW: 419.73, [m/z]= 420.

6-(3-bromophenyl)-3-(4-chloro-2-methylphenyl)[1,2,4]triazole[3,4,b][1,3,4]thiadiazole (6g)

White solid, 72%, m.p 149.5-153.5°C; H^1 NMR, 400 MHz, DMSO-d_6, δ 2.45 (s, 3H), 7.61 (t, J = 8.0 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.91 (dd, J_1 = 1.6 Hz, J_2 = 7.8 Hz, 1H), 8.08 (dd, J_1 = 1.6 Hz, J_2 = 8 Hz, 1H), 8.25 (d, J = 1.6 Hz, 2H); Anal. calcd. for $C_{17}H_{10}BrClN_4S$: C, 47.37; H, 2.48; N, 13.81%; found: C, 47.35; H, 2.48; N, 13.80%. MW: 405.7, [m/z]= 406.

2.2. Pharmacology

2.2.1. DPPH free radical and scavenging activity

Free radical-scavenging capacities of test compounds were determined according to the previously reported procedure[28], using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH). This is the most

commonly used method for screening of antioxidant activity of newly synthesized organic compounds. This method is based on the reduction of free radical DPPH by free radical scavengers. The procedure involves the measurement of decrease in absorbance of DPPH at 517 nm, which is proportional to the activity of free radical scavenger added to DPPH[29] reagent solution. A stock solution of test compounds (1 mg/mL) and DPPH (0.004%) was prepared in 95:5 methanol: water. To 3 mL of freshly prepared DPPH solution in a test tube, was added stock solution of test compound (100 µg) and reacted for 15 min and the absorbance was measured at 517 nm using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). BHT was used as a reference standard; Ascorbic acid was used as control sample and 95% methanol served as blank. Free radical inhibition in % (*I* %) was calculated as;

$$I\,\% = (A_{blank} - A_{sample}) / (A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test sample.

2.2.1.1. In vitro α-Glucosidase inhibition assay

The α-glucosidase inhibition activity for the newly synthesized compounds was determined based on the spectrophotometric assay using acarbose as the reference compound[30]. The test compound was dissolved on DMSO to give concentrations from 25, 50, 75 and 100µg/ml. Each well in 96-well plates contained 150µL of 2 mM 4-nitrophenyl α-$_D$-glucopyranoside (PNP-G) in 100 mM potassium phosphate buffer (pH 6.8) and 20µL of the sample in DMSO. The reaction was initiated by the addition of 150µL of the enzyme solution (32mU/mL, from baker's yeast, Sigma Aldrich Chemicals, USA). The plates were incubated at 37°C for 20 min. The absorbance of 4-nitrophenol released from PNP-G at 405 nm was measured spectrophotometrically (iMark Microplate Reader S/N 11638, Bio-Rad Laboratories). The increase in absorbance (ΔB) was compared with that of the control (DMSO) to calculate the inhibition and acarbose was taken as standard reference compound. The inhibitory activity of the test compounds was calculated using the following equation:

$$\text{Inhibition (\%)} = (\Delta B_{control} - \Delta B_{sample}) / \Delta B_{control} \text{ X } 100\%$$

Results were expressed as the mean ± S.E.M. For statistical analysis of the data group, means were compared by one-way analysis of variance (ANOVA). $P < 0.001$ was considered to be statistically significant. The IC_{50} value was calculated by plotting the % inhibition against the concentration and by establishing a logarithmic regression curve.

2.2.1.2. In vivo anti-hyperglycemic activity

Animal housing and maintenance

Adult Wistar rats weighing 200-300 g were used in this study. Animals were taken care as per the Regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The 'Form B' for carrying out animal experimentation was reviewed and approved by the Institutional Animal Ethics Committee (IAEC). Animals were maintained in a controlled environment with 22-25°C temperature, 50 - 60 % humidity, a light/dark cycle of 12 hours each and 15-20 air changes per hour. The animals were fed, *ad libitum*, with certified Irradiated Laboratory Rodent Diet (Nutrilab brand, Tetragon Chemie Pvt. Ltd, Bangalore) except during the fasting & study period.

Hypoglycemic Activity Assay

Test substance and reference item (metformin) suspension was prepared with vehicle containing 0.5% (w/v) of carboxymethyl cellulose and 0.1% Tween 80. Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide in normal saline solution (0.9% NaCl solution). Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of 60 mg/kg of streptozotocin and 120 mg/kg of nicotinamide in Wistar rats (of both sexes)[31]. Hyperglycemia was confirmed by the elevated non fasting glucose levels in blood, determined 48 h after diabetes induction using Glucometer (Accu- check Advantage, Roche diagnostics Mannheim, Germany) and strips. Animals with blood glucose concentrations more than 250 mg/dL were used for the study.Each study group involved 6 diabetic rats. Cage cards indicating the study number, animal number & treatment group details were affixed to the corresponding cages. Standard drug and test compounds were given orally at 10, 50 and 100 mg/kg. The change in the body weight of rats after induction diabetes was noted. The blood sample was collected from caudal vein by the following procedure. The animal tale was cleaned with a cotton swab and the tip was cut using the fine scissor. Tail was gently massaged if required and a drop of

blood was placed in the area specified on the blood glucose measuring strip to record the WBG value. Reduction in blood glucose produced by the compound was recorded on 1st, 2nd, 3rd, 4th, 7th and 14th day. At the end of experiment animals were euthanized by CO_2 asphyxiation and organ weights were recorded.

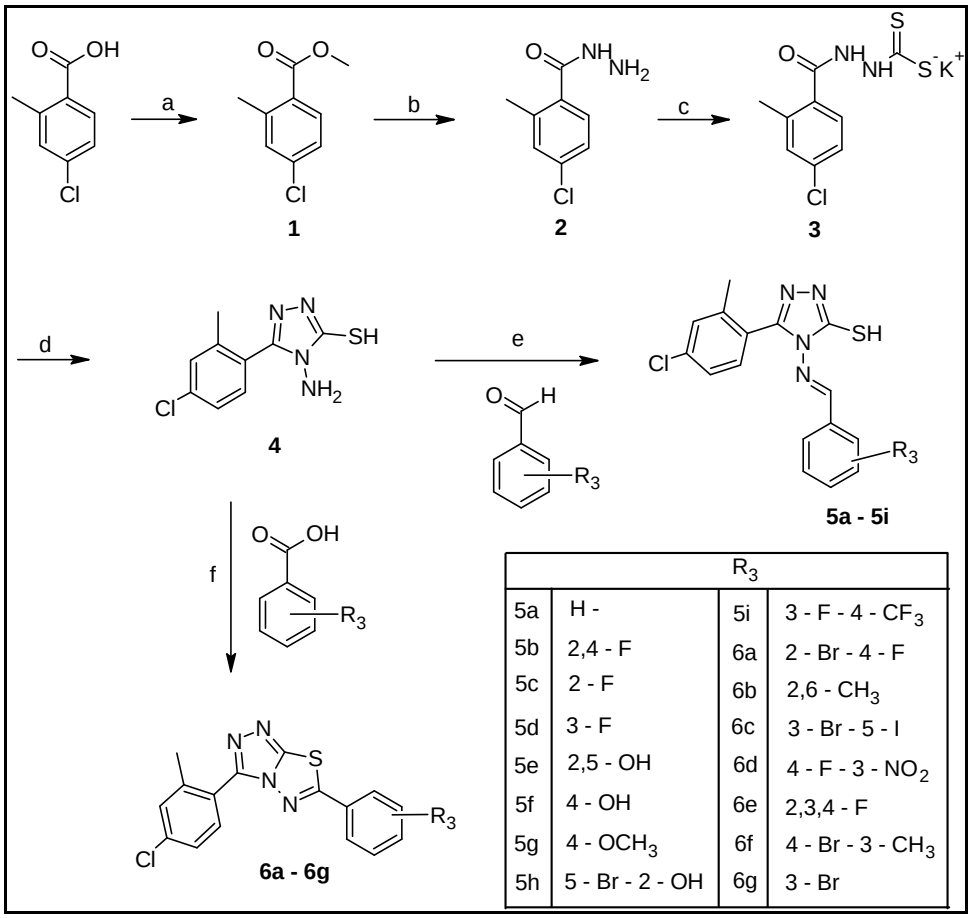
Statistical analysis was performed by One-way ANOVA followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered as a significant change.

3. Results and discussion

Chemistry

The key intermediate **4** was synthesized from **1** in three steps. Methyl 4-chloro-2-methylbenzoate **1** was treated with hydrazine hydrate and ethanol to yield 4-chloro-2-methylbenzohydrazide **2.** The condensation of 4-chloro-2-methylbenzohydrazide **2** with carbon disulfide and potassium hydroxide gave potassium dithiocarbazate **3,** which underwent ring closure with an excess of hydrazine to produce aminothiol **4** in good 85% yield. A new series of 4-[(*E*)-substituted-benzylideneamino]-5-(4-chloro-2-methylphenyl)-4*H*-1,2,4-triazole-3-thiol (Schiff base) **5a-5i** were prepared by treating compound **4** with an equimolar amount of the appropriate benzaldehyde derivatives in the presence of catalytic quantity of sulphuric acid and ethanol at reflux condition. Cyclo-condensation of the SH and NH_2 functions of compound **4** with appropriate benzoicacid derivatives in the presence of phosphoryl chloride gave 3-(4-chloro-2-methylphenyl)-6-substituted-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazoles **6a-6g** (**Scheme 1**). The ^{1}H NMR spectra data were consistent with the assigned structures; benzylideneamino CH proton of **5a-5i** was observed at around 10.17 ppm; Triazolethiol SH proton of **5a-5i** was observed at around 14.25 ppm. The ^{13}C NMR spectra data were consistent with the assigned structure; triazole –C=N- carbon of **6a-6g** was observed at around 150.1 ppm; thiadiazole -N=C=N-S carbon was observed at 164.9 ppm.

Scheme 1. Synthesis of 4-[(*E*)-benzylideneamino]-5-(4-chloro-2-methylphenyl)-4*H*-1,2,4-triazole-3-thiol, and 3-(4-chloro-2-methylphenyl)-6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives



R_3			
5a	H -	5i	3 - F - 4 - CF_3
5b	2,4 - F	6a	2 - Br - 4 - F
5c	2 - F	6b	2,6 - CH_3
5d	3 - F	6c	3 - Br - 5 - I
5e	2,5 - OH	6d	4 - F - 3 - NO_2
5f	4 - OH	6e	2,3,4 - F
5g	4 - OCH_3	6f	4 - Br - 3 - CH_3
5h	5 - Br - 2 - OH	6g	3 - Br

Reagents and conditions: (a) MeOH, H_2SO_4, reflux, 8-10 h; (b) hydrazine hydrate, EtOH, reflux, 8 h; (c) CS_2, KOH, EtOH, RT, 5 h; (d) hydrazine hydrate, reflux, 2–6 h; (e) aldehyde, EtOH, H_2SO_4, reflux, 5-6 h; (f) carboxylic acid, $POCl_3$, reflux, 8-10 h.

Pharmacology

DPPH free radical scavenging activity

Newly synthesized compounds were evaluated for their antioxidant activity by DPPH free radical scavenging method. This method was selected to evaluate the antioxidant activity of new compounds because it is one of the most effective methods for evaluating the concentration of radical scavenging materials[32]. The results of free radical scavenging activity of the newly synthesized compounds are given in **Table 1.** Compounds **5e** and **5f** were the strongest free radical scavengers among the entire compounds tested, with the level of inhibition of 89.2%, and 86.8% respectively, followed by **5a** and **5d** with the level of inhibition of 75.8% and 74.3% respectively. The structure activity relationship studies reveal that the antioxidant property of the triazole derivatives is due to the presence of phenethylthio group and 2^{nd} and 5^{th}, or 4^{th} position of benzylideneamino ring (**5e**, **5f**).

Table 1.*Antioxidant activity by DPPH free radical scavenging (assay in %)*

Compounds	Level of inhibition (%)	Compounds	Level of inhibition (%)
5a	75.8	6a	00
5b	60.3	6b	00
5c	72.5	6c	16.1
5d	74.3	6d	22.3
5e	89.2	6e	53.5
5f	86.8	6f	58.7
5g	64.6	6g	43.2
5h	55.4	BHT	90.42
5i	48.7	-	-

In vitro α-Glucosidase inhibition assay

The inhibitory activity of the newly synthesized compounds against α-glucosidase was determined based on the cleavage of α-glucosidic bond in p-nitropheyl-α-D-glucopyranoside[33] releasing p-nitrophenol detected by absorbance at 405 nm. Compounds **5a**, **5c-g** were tested for their inhibitory effect on yeast α-glucosidase; **5a** and **5c** exhibited good inhibition with IC_{50} value of 74.5 µg and 113 µg respectively **(Table 2).** Compound **5d-g** showed lower level of inhibition than **5c**. Compound **5a** with no substitution on the benzylideneamino ring showed activity better than other substituents.

Table 2.*In vitro α-glucosidase inhibition assay*

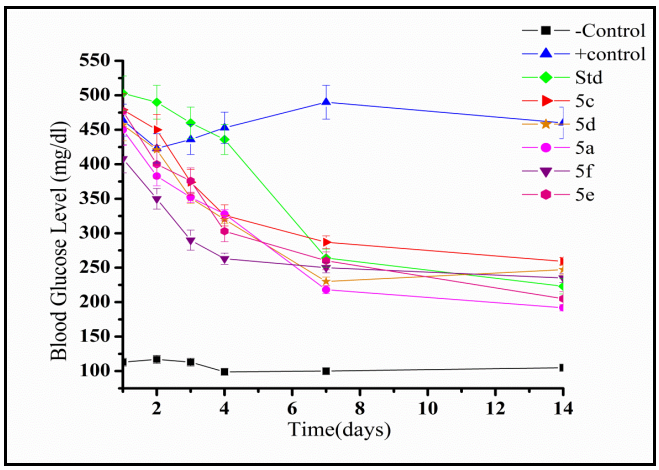
α-glucosidase inhibition assay (%)					
Concentration (µg/ml)	**25µg**	**50 µg**	**75 µg**	**100 µg**	**IC_{50}**
5a	32.5	41.7	50.3	58.7	74.5
5c	19.6	29.1	37.2	45.3	113.0
5d	14.2	19.3	24.7	33.6	168.8
5e	18.4	25.7	30.2	38.6	146.1
5f	2.4	6.4	10.4	18.4	257.7
5g	9.5	12.8	17.4	21.8	271.1
Acarbose	40.3	52.5	68.5	78.3	43.5

In vivo anti-hyperglycemic activity

The anti-diabetic potential of newly synthesized compounds was evaluated in STZ and nicotinamide induced T2DM in Wistar rats[34].Blood glucose levels of test compounds (**5a**, **5c-g**) attenuated the progression of diabetes in STZ/ nicotinamide model in a dose dependent manner following 14 days of treatment. With the dose of 10 mg/kg, compounds **5a** and **5c** showed significant decrease in plasma glucose concentration (92mg/dL and 109mg/dL respectively) and compounds **5d-f** showed equal or less plasma glucose concentration compared to the standard drug metformin. With the dose of 50 mg/kg, plasma glucose concentration was in the range of 97mg/dL and 144mg/dL against 153mg/dL of standard drug and with 100mg/kg dose, the plasma

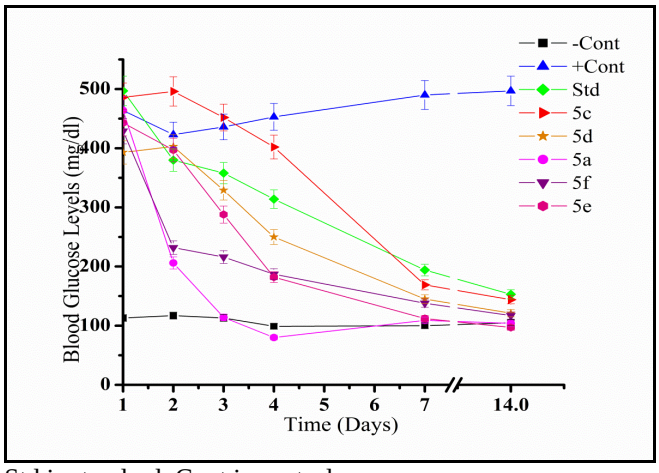
glucose concentration of all the compounds was in normal range (94mg/dL to 111mg/dL for test compounds and 108mg/dL for the standard drug). The lowering plasma glucose in diabetic rats treated with compounds **5a**, **5c-f** at the concentration of 10mg/kg, 50mg/kg and 100mg/kg is presented in **Figures 1**, **2** and **3** respectively. Treatment with test substance for 14 days showed dose dependent increase in body weight compared to diabetic control; no significant increase in liver weight at 100mg/kg; and no change in kidney weight was observed with treatment.

Figure 1: Plasma glucose level in diabetic rats treated with test compounds (10 mg/kg)



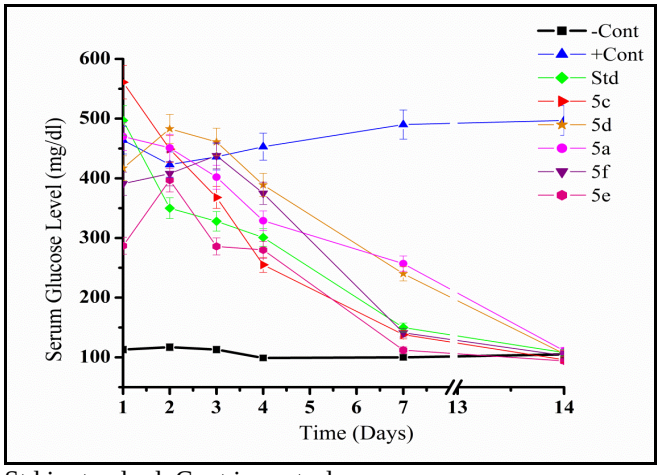
Std is standard, Cont is control

Figure 2: Plasma glucose level in diabetic rats treated with test compounds (50 mg/kg)



Std is standard, Cont is control

Figure 3: Plasma glucose level in diabetic rats treated with test compounds (100 mg/kg)



Std is standard, Cont is control

4. Conclusions

In summary, a series of new 4-[(*E*)-benzylideneamino]-5-(4-chloro-2-methylphenyl)-4*H*-1,2,4-triazole-3-thiol (Schiff base) (**5a-i**) and 3-(4-chloro-2-methylphenyl)-6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**6a-g**) derivatives could be synthesized and evaluated for antimicrobial, free radical scavenging activity by DPPH method and anti-hyperglycemic activity, *invitro* by α-glucosidase enzyme inhibition and *invivo* by STZ/ nicotinamide induced T2DM rat model. 4-[(*E*)-benzylideneamino]-5-(4-chloro-2-methylphenyl)-4*H*-1,2,4-triazole-3-thiol derivatives showed significant decrease in plasma glucose concentration. The study demonstrated that some of the synthesized compounds are potential anti-hyperglycemic agents. Hence, it can be concluded that, this new class of compounds certainly holds a greater promise in discovering a potent anti-hyperglycemic agents.

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References

1. HacerB, AhmetD, SengulAK, NeslihanD:Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities.*Eur J Med Chem*2009, 44:1057-1066.
2. NurayUG, OmerK:Synthesis and antimicrobial activity evaluation of new 1,2,4-triazoles and 1,3,4-thiadiazoles bearing imidazo[2,1-*b*]thiazole moiety.*Eur J Med Chem* 2010, 45:63-68.
3. ForoumadiA, KianiZ, SoltaniF:Antituberculosis agents VII: Synthesis and in *vitro* antimycobacterial activity of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetates.*IL Farmaco* 2003, 8:1073-1076.
4. HollaBS, VeerendraB, ShivanandaMK, PoojaryB: Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles.*Eur J Med Chem* 2003, 38:759-767.
5. MohammadM, TahminehA, VahidS, MaryamA, LoghmanF, SayyedAT et al.:Synthesis of two novel 3-amino-5-[4-chloro-2-phenoxyphenyl]-4*H*-1,2,4-triazoles with anticonvulsant activity.*Iran J Pharm Res* 2010, 9:265-269.
6. SchenoneS, BrulloC, BrunoO, BondavalliF, RaniseA, FilippelliW et al.:New 1,3,4-thiadiazole derivatives endowed with analgesic and anti-inflammatory activities.*Bioorg Med Chem* 2006, 14:1698-1705.
7. El ShehryMF, Abu-HashemAA, El-TelbaniEM:Synthesis of 3-((2,4-dichlorophenoxy)methyl)-1,2,4-triazolo(thiadiazoles and thiadiazines) as anti-inflammatory and molluscicidal agents.*Eur J Med Chem* 2010, 45:1906-1911.
8. HosamAS, NermenAO, AhmedHM:Synthesis and Analgesic Activity of Some New Pyrazoles and Triazoles Bearing a 6,8-Dibromo-2-methylquinazoline Moiety.*Molecules* 2011, 16:10187-10201.
9. AneesAS, RavineshM, RajivK, MohdR, SomilaK:Synthesis, spectral characterization, and pharma cological screening of some 4-[{1-(aryl)methylidene}-amino]-3-(4-pyridyl)-5-mercapto-4*H*-1,2,4-triazole derivatives. *J Pharm Bioallied Sci* 2010, 2:109-112.
10. Al-SoudYA, Al-DweriMN, Al-MasoudiNA:Synthesis, antitumor and antiviral properties of some 1,2,4-triazole derivatives.*IL Farmaco* 2004, 59:775-783.
11. RadwanMA, El-ZemitySR:Synthesis and molluscicidal structure-activity relationships of some novel 1,2,4-triazole *N*-methyl carbamates.*Pest Manag Sci* 2001, 57:707-712.
12. Abdel-RahmanF, ErikDC, El-KashefH:Synthesis and antiviral activity of novel [1,2,4]triazole[3,4-*b*][1,3,4]thiadiazoles, [1,2,4]triazole[3,4-*b*][1,3,4]thiadiazines and [1,2,4]triazolo[3,4-*b*][1,3,4]thiadia zepines. *Arkivoc* 2006, x:137-151.
13. KemalS, YaseminU, DilekU, EsraD, GulcanK, FatihC, EmrahB:Synthesis, characterization, and antioxidant activities of new trisubstituted triazoles.*Turk J Chem* 2012, 36:457-466.
14. SamiyeYC, NesrinGK, UmutSG, GulberkU:Free-Radical Scavenging Activities of 2-Benzoxazolinone Derivatives Containing Thiosemicarbazide, Triazole, Thiadiazole and Hydrazone Units.*J Facul Pharm* 2011, 31:27-50.

15. HaydarY, SevgiK, MuratK, OzgunYM, UmmuhanO, EsraS, EfecanS, MiracO:Synthesis and antioxidant activities of some 4-benzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives.*Indian J Chem* 2006, 45B:715-718.
16. International Diabetes Federation (IDF):*Diabetes Atlas*, 3rd Edition, 2006. Available from:http:// www.iotf.org/diabetes.asp.
17. Ahren B, Landin-Olsson M, Jansson PA, Svenson M, Holmes D, Schweizer A:Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes.*J Clin Endocrinol Metab* 2004, 89:2078-2084.
18. Gwaltney SL II, Stafford JA:Inhibitors of dipeptidyl peptidase 4.*Annu Rep Med Chem* 2005, 40:149-165.
19. Weber AE:Dipeptidyl peptidase IV inhibitors for the treatment of diabetes.*J Med Chem* 2004, 48:4135-4141.
20. Thornberry NA, Weber AE:Discovery of JANUVIA (Sitagliptin), a selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes.*Curr Topics Med Chem* 2007, 7:557-568.
21. Kim D, Wang L, Beconi M, Eiermann GJ, Fisher MH, He H et al.:(2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes.*J Med Chem* 2005, 48:141-151.
22. Herman GA, Stein PP, Thornberry NA, Wagner JA:Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes: focus on sitagliptin.*Clin Pharmacol Therap* 2007, 81:761-767.
23. Blank B, Nichols DM, Vaidya PD:Synthesis of 1,2,4-triazoles as potential hypoglycemic agents.*J Med Chem* 1972, 15:694-696.
24. Anton-Fos GM, Garcia-Domenech R, Perez-Gimenez F, Peris-Ribera JE, Garcia-March FJ, Salabert-Salvador MT:Pharmacological studies of the two new hypoglycaemic compounds 4-(3-methyl-5-oxo-2-pyrazolin-1-yl)benzoic acid and 1-(mesitylen-2-sulfonyl)-1H-1,2,4-triazole.*Arzneimittelforschung* 1994, 44:821-826.
25. Ameya AC, Nandini RP:Sulfonylureas as Dual Acting Agents_Synthesis and Biological Activity. *J Chin Chem Soc* 2007, 54:771-777.
26. Deliwala CV, Mhasalkar MY, Shah MH, Pilankar PD, Nikam ST, Anantanarayan KG:Synthesis and hypoglycaemic activity of 3-aryl(or pyridyl)-5-alkyl amino-1,3,4, Thiadiazole and some sulfonyl ureas derivatives of 4*H*-1,2,4 triazoles.*J Med Chem* 1971, 14:1000-1003.
27. Chandrashekar A, Eswarappa B, Bodke YD, Venkatesh KB, Raghu N, Shivakumar MC et al.:5-phenyl-1-benzofuran-2-yl derivatives: synthesis, antimicrobial, and antioxidant activity.*Med Chem Res* 2012, 19:617-716.
28. Bari LD, Braca A, Morelli I, Pizza C, Politi M, Tommasi ND:Antioxidant principles from Bauhinia tarapotensis.*J Nat Prod* 2001, 64:892-895.
29. Brand-WilliamsW, CuvelierME, BersetC:Use of free radical method to evaluate antioxidant activity. LWT - Food Science and Technology. 1995, 28:25-30.
30. Matsui T, Yoshimoto C, Osajima K, Oki T, Osajima Y:*In vitro* survey of α-glucosidase inhibitory food components.*Biosci Biotech Biochem* 1996, 60:2019-2022.
31. Shirwaikar A, Rajendran K, Barik R:Effect of aqueous bark extract of *Garuga pinnata Roxb.* in streptozotocin-nicotinamide induced type-II diabetes mellitus.*J Ethnopharmacol* 2006, 107:285-290.
32. Hajji M, Masmoudi O, Souissi N, Triki Y, Kammoun S, Nasri M:Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxidant and antimicrobial activities of essential oil from *Periploca laevigata* root barks.*Food Chem* 2010, 121:724-731.
33. Oluseyi AA, Elizabeth AB, Sarafadeen OK, Olakunle SM:Partial purification and some properties of α–glucosidase from Trichoderma longibrachiatum.*Biokemistri* 2012, 24:31-37.
34. Watcho P, Achountsa JHG, Mbiakop CU, Wankeu-Nya M, Nguelefack TB, Kamanyi A:Hypoglycemic and hypolipidemic effects of Bersama engleriana leaves in nicotinamide/streptozotocin-induced type 2 diabetic rats.*BMC Complementary and Alternative Medicine* 2012, 12:264-269.

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