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Novel hybrids of benzothiazole-1,3,4-oxadiazole-4-thiazolidinone: Synthesis, *in silico* ADME study, molecular docking and *in vivo* anti-diabetic assessment

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ABSTRACT

A series of new benzothiazole-1,3,4-oxadiazole-4-thiazolidinone hybrid analogs (**Tz1-Tz28**) were synthesized in search of potential anti-diabetic agents. Molecular docking study was conducted with binding pocket of peroxisome proliferator activated receptor-gamma to elucidate the binding interactions of newly synthesized targets. Seven selected compounds with best docking scores were further screened for *in vivo* anti-hyperglycemic efficacy by oral glucose tolerance test in non-diabetic rats and on streptozotocin induced diabetic rat models. All the tested compounds demonstrated excellent to moderate reduction in blood glucose levels. Three of the compounds (**Tz21, Tz7 and Tz10**) showed excellent anti-diabetic effect by reducing concentration of glucose to 157.15 ± 1.79 mg/dL, 154.39 ± 1.71 mg/dL, 167.36 ± 2.45 mg/dL, respectively better than the standard drug, pioglitazone, 178.32 ± 1.88 mg/dL. Moreover, three derivatives **Tz21, Tz4 and Tz24** with IC50 values of 0.21 ± 0.01 μM, 9.03 ± 0.12 μM and 11.96 ± 0.40 μM respectively also showed better inhibitory activities on alpha-glucosidase even more than the standard acarbose (IC50 = 18.5 ± 0.20 μM), indicating **Tz21** has the highest inhibitory effect among the seven tested derivatives. Prediction of Drug like properties using molinspiration online software suggests that all the synthesized compounds have potential of becoming the orally active molecules. Thus, these novel hybrids could serve as potential candidates to become leads for the development of new drugs eliciting anti-hyperglycemic effect orally.

1. Introduction

Various compounds incorporating heterocyclic and fused heterocyclic ring plays major role in designing and synthesis of anti-diabetic pharmaceuticals. Among them, nitrogen, sulphur and oxygen containing heterocyclic compounds have attracted the interest of medicinal chemists due to their innumerable biological applications. 1,3,4-oxadiazole derivatives among the family of heterocycles showed many promising pharmaceutical applications. Extensive literature report reveals the anticancer [1–3], antioxidant [4], anti-hyperglycaemic [5–7] anti-tubercular [8] properties of 1,3,4-oxadiazole scaffold. Large number of medicinal drugs used clinically contain 1,3,4-oxadiazole moiety as pharmacophore such as raltegravir an antiretroviral drug for HIV infection, antibiotic furamizole, zibotentan, an antineoplastic agent, tiodazosin and nesapidil as antihypertensive drugs [9].

On the other hand, the benzothiazole (a benzo-heterocycle) scaffold represents an important core template for a vast variety of pharmacologically active compounds, having many pharmaceutical activities. The versatile biological functions shown by benzothiazole nucleus include anti-diabetic [10,11], anti-convulsant [12], anticancer [13] and anti-viral properties [14].

Moreover, 4-thiazolidinone, a 4-carbonyl derivative of tetrahydro form of thiazole, has been found as a basic structure in diverse synthetic pharmaceutical drugs [15,16]. The presence of keto group at 4th position and N—C—S linkage was found accountable for the pharmacological activity of thiazolidinones [17]. 4-thiazolidinone derivatives exhibited abundance of applications such as anti-diabetic [18], antimicrobial [19], antiviral [20], anti-tubercular [21] and anticonvulsant [22].

Diabetes or Diabetes Mellitus is a chronic progressive metabolic disorder characterized by increased levels of blood glucose/impaired insulin secretion/liver or peripheral insulin resistance [23,24]. Type-I, Type-II and gestational diabetes are different types of diabetes, among

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which Type-II diabetes mellitus or non-insulin dependent diabetes mellitus is the most prevalent diabetes occurring in 80% of the affected patients worldwide [25]. The World Health Organization reported that the global prevalence of diabetes has been increased from 108 million people in 1980 which increased to 422 million people in 2014 [26]. The medicinal agents used in the therapy of Diabetes Mellitus comprise insulin and oral anti-hyperglycaemic agents (thiazolidinediones, biguanides, sulphonylureas and α-glucosidase inhibitors [27]. Peroxisome proliferator-activated receptors (PPARs), ligand-activated transcription factors, consisting of three distinct subtypes PPAR-α, PPAR-δ and PPAR-γ, are members of nuclear hormone receptor superfamily (NR1C3) [28]. Among these subtypes, Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists have come up with a prominent role for an orally effective anti-hyperglycaemic agent [29]. Thiazolidinediones (TZDs), such as rosiglitazone and pioglitazone, PPAR-γ agonists, are able to improve insulin sensitization and glucose metabolism in the treatment of type II diabetes, but they have also been related with various side effects [30]. The diverse synthetic oral anti-hyperglycemic medicines exist but most of them are linked with miscellaneous side effects and toxicity [31].

Latest trends in pharmaceutical chemistry research indicated the increased popularity of molecular hybridization for medical drug development, which is based on the combination of two or more pharmacophoric moieties of different biologically active substances to make a new potent hybrid molecule with enhanced efficiency and affinity when compared to standard drug [32].

Recently our research batch has reported the hybrid analogue of oxadiazole clubbed benzothiazole mannich bases as anti-diabetic agents [33]. In view of the aforementioned findings and as a continuation of our effort in exploring hybrid analogues, we hereby in the present study report the synthesis, *in vitro* alpha-glucosidase inhibition study [34] and *in vivo* anti-diabetic evaluation of novel series of hybrid compounds incorporating the benzothiazole, oxadiazole and 4-thiazolidinone heterocyclic ring systems in a single molecule, with an aim to obtain new, safer, potent and relatively low-cost anti-hyperglycemic agents. Molecular docking studies were also done to understand the binding interactions of synthesized hybrids to a receptor (PPAR-γ, PDB ID-1FM9). Also, the comparison of stabilizing energy of compounds against alpha-glucosidase (PDB ID-2QMJ) and inhibiton activity was done.

2. Experimental section

2.1. General

All chemicals and solvents utilized were obtained from commercial sources and were used in their original form without further purification. Thin layer chromatography (TLC) was run on pre-coated silica gel G 60 F254 plates (Merck, Germany) using toluene: ethyl acetate (3:1) and *n*-hexane: ethyl acetate (6:4) as solvent systems. The melting points of synthesized compounds were determined by the open capillary method in an electric melting point apparatus of Icon Instruments, India and are uncorrected. All the IR spectra were recorded on a Bruker Optics Spectrophotometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) Nuclear Magnetic Resonance spectra were recorded on a Bruker advanced model DPX-300 spectrometer in $CDCl_3$/DMSO-d_6. Chemical shifts (δ) are expressed in parts per million (ppm) with reference to Tetra methyl silane (TMS) as an internal standard. The mass spectra of synthesized analogs were recorded using ESI technique on LCMS/LCQ (Agilent, Advantage-Max) instrument. Elemental analysis (C, H, and N) were conducted using Flash 2000 organic elemental analyzer was used for and the values were found within ± 0.4% of theoretical values.

2.2. Synthesis

2.2.1. Synthesis of 5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-amine, 3

A solution of benzothiazole-2-carboxyhydrazide (0.01 mol) synthesized according to the previously reported protocol [35] and cyanogen bromide (0.01 mol) in methanol (50 mL) was stirred at 40 °C for 1.5 h. The solution was then refluxed on parallel synthesizer (Carousel Tech, Radleys, Germany) for 5 h at 70 °C. The reaction mixture was then poured into crushed ice and neutralized with sodium bicarbonate solution. The resultant precipitate obtained was filtered, dried and recrystallised from methanol to give compound, **3** [36]. Yield: 70%.

2.2.2. General procedure for synthesis of Schiff bases, Sb(1–28)

Compound **3** (0.02 mol) was dissolved in ethanol. To this solution appropriate aromatic aldehydes (0.02 mol) were added. 2–3 mL of glacial acetic acid was added to the above reaction mixture and the mixture was refluxed for 14 h. After the completion of reaction, the solvent (ethanol) was distilled off and the residue thus obtained were poured into ice and stirred for 20 min. The resulting precipitates were filtered, washed occasionally with ice cold water, dried and recrystallized from alcohol [37]. Yield: 65–68%.

2.2.3. General procedure for synthesis of 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-substituted thiazolidin-4-one, Tz(1–28)

A reaction mixture of appropriate Schiff base (4a–z) (0.1 mol) and mercaptoacetic acid (0.1 mol) in dimethylformamide (25–30 mL), containing a catalytic amount of zinc chloride was refluxed with stirring for 15 h. After the completion of reaction, the mixture was cooled and poured into crushed ice. The resultant product was filtered, washed with an excess of 10% sodium bicarbonate solution followed by several times washing with cold water, dried and recrystallised from ethanol to get the desired product, **Tz1–Tz28** [38].

2.2.3.1. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-hydroxyphenyl)thiazolidin-4-one (Tz1).

Yield: 75%; M.p. 155–156 °C, R_f = 0.71. IR (KBr, cm^{-1}); 3046 (Ar, C—H), 1604 (C=N), 1706 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.01 (2H, s, SCH_2); 4.62 (1H, s, *N*-CH-S); 5.23 (1H, s, OH), 6.62–7.07 (4H, m, Ar-CH of phenyl); 7.42–7.79 (2H, m, Ar-CH); 8.02 (1H, m, Ar-CH); 8.17 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.4, 65.4, 115.7, 121.3, 121.8, 125.2, 126.1, 130.2, 131.8, 135.4, 153.5, 156.0, 156.6, 171.8. ESI MS (*m/z*): 397 (M + H) $^{+}$. Calcd. Anal. for $C_{18}H_{12}N_4O_3S_2$: C, 54.53; H, 3.05; N, 14.13; found C, 54.76; H, 3.33; N, 14.41.

2.2.3.2. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-fluorophenyl)thiazolidin-4-one (Tz2).

Yield: 63%; M.p. 209–210 °C, R_f = 0.63. IR (KBr, cm^{-1}); 3236 (Ar, C—H), 1564 (C=N), 1720 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.75 (2H, s, SCH_2); 4.87 (1H, s, *N*-CH-S); 6.85–7.04 (4H, m, Ar-CH of phenyl); 7.34–7.86 (2H, m, Ar-CH); 8.07 (1H, m, Ar-CH); 8.14 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.1, 65.2, 115.3, 121.6, 121.9, 125.4, 125.6, 130.7, 134.8, 135.7, 153.0, 156.4, 161.4, 171.1. ESI MS (*m/z*): 399 (M + H) $^{+}$. Calcd. Anal. for $C_{18}H_{11}FN_4O_2S_2$: C, 54.26; H, 2.78; N, 14.06; found C, 54.60; H, 2.35; N, 14.30.

2.2.3.3. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-chlorophenyl)thiazolidin-4-one (Tz3).

Yield: 65%; M.p. 188–189 °C, R_f = 0.60. IR (KBr, cm^{-1}); 3220 (Ar, C—H), 1540 (C=N), 1667 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.56 (2H, s, SCH_2); 5.21 (1H, s, *N*-CH-S); 6.98–7.28 (4H, m, Ar-CH of phenyl); 7.43–7.60 (2H, m, Ar-CH); 8.03 (1H, m, Ar-CH); 8.15 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.4, 66.0, 121.4, 121.6, 121.7, 125.6, 125.9, 128.8, 130.4, 132.1, 135.0, 137.8, 153.9, 156.0, 171.8. ESI MS (*m/z*): 415 (M + H) $^{+}$. Calcd. Anal. for $C_{18}H_{11}ClN_4O_2S_2$: C, 52.11; H, 2.67; N, 13.50; found C, 52.44; H, 2.45; N, 13.69.

2.2.3.4. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-nitrophenyl)thiazolidin-4-one (Tz4).

Yield: 72%; M.p. 231–232 °C, R_f = 0.58. IR (KBr, cm^{-1}); 3116 (Ar, C—H), 1602 (C=N), 1685 (C=

O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.76 (2H, s, SCH_2); 5.26 (1H, s, *N*-CH-S); 7.32–8.07 (4H, m, Ar-CH of phenyl); 7.34–7.65 (2H, m, Ar-CH); 8.02 (1H, m, Ar-CH); 8.17 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.9, 66.2, 121.4, 121.4, 121.9, 125.4, 125.7, 129.77, 135.2, 145.2, 146.6, 153.0, 156.7, 172.1. ESI MS (*m/z*): 426 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{11}N_5O_4S_2$: C, 50.82; H, 2.61; N, 16.46; found C, 50.47; H, 2.55; N, 16.34.

*2.2.3.5. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-bromophenyl)thiazolidin-4-one (**Tz5**)*. Yield: 68%; M.p. 198–199 °C, R_f = 0.70. IR (KBr, cm^{-1}); 3136 (Ar, C—H), 1610 (C=N), 1735 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.85 (2H, s, SCH_2); 5.02 (1H, s, *N*-CH-S); 6.95–7.14 (4H, m, Ar-CH of phenyl); 7.23–7.68 (2H, m, Ar-CH); 8.09 (1H, m, Ar-CH); 8.12 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.2, 64.9, 121.0, 121.7, 121.9, 124.9, 125.9, 131.2, 131.8, 135.0, 138.2, 153.4, 156.0, 161.4, 171.6. ESI MS (*m/z*): 460 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{11}BrN_4O_2S_2$: C, 47.07; H, 2.41; N, 12.20; found C, 47.30; H, 2.12; N, 12.36.

*2.2.3.6. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(3,4,5-trimethoxyphenyl)thiazolidin-4-one (**Tz6**)*. Yield: 68%; M.p. 168–169 °C, R_f = 0.71. IR (KBr, cm^{-1}); 3145 (Ar, C—H), 1570 (C=N), 1680 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.42 (2H, s, SCH_2); 3.84 (9H, s, 3 × OCH_3); 5.12 (1H, s, *N*-CH-S); 6.08–6.18 (2H, m, Ar-CH of phenyl); 7.34–7.60 (2H, m, Ar-CH); 8.07 (1H, m, Ar-CH); 8.14 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.1, 56.2, 56.5, 65.9, 106.4, 121.4, 120.8, 121.7, 125.0, 125.6, 128.8, 133.7, 135.6, 150.9, 153.7, 156.4, 171.5. ESI MS (*m/z*): 471 (M + H) $^+$. Calcd. Anal. for $C_{21}H_{18}N_4O_5S_2$: C, 53.61; H, 3.86; N, 11.91; found C, 55.36; H, 3.98; N, 11.65.

*2.2.3.7. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(3-hydroxyphenyl)thiazolidin-4-one (**Tz7**)*. Yield: 60%; M.p. 200–201 °C, R_f = 0.69. IR (KBr, cm^{-1}); 3034 (Ar, C—H), 1612 (C=N), 1718 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.80 (2H, s, SCH_2); 5.10 (1H, s, *N*-CH-S); 5.0 (1H, s, OH), 6.53–6.98 (4H, m, Ar-CH of phenyl); 7.52–7.68 (2H, m, Ar-CH); 8.08 (1H, m, Ar-CH); 8.27 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.2, 65.8, 114.4, 114.6, 121.8, 121.9, 125.4, 125.9, 130.8, 135.7, 140.6, 153.9, 155.9, 158.4, 171.1. ESI MS (*m/z*): 397 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{12}N_4O_3S_2$: C, 54.53; H, 3.05; N, 14.13; found C, 54.10; H, 3.28; N, 14.26.

*2.2.3.8. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(3,4-dimethoxyphenyl)thiazolidin-4-one (**Tz8**)*. Yield: 72%; M.p. 175–176 °C, R_f = 0.72. IR (KBr, cm^{-1}); 3152 (Ar, C—H), 1571 (C=N), 1672(C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.78 (2H, s, SCH_2); 3.61 (6H, s, 2 × OCH_3); 5.05 (1H, s, *N*-CH-S); 6.46–6.94 (3H, m, Ar-CH of phenyl); 7.24–7.72 (2H, m, Ar-CH); 8.10 (1H, m, Ar-CH); 8.18 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 32.9, 56.6, 65.8, 113.8, 115.2, 121.7, 121.8, 122.4, 125.7, 125.9, 132.8, 135.6, 148.6, 149.7, 153.0, 156.6, 172.0. ESI MS (*m/z*): 441 (M + H) $^+$. Calcd. Anal. for $C_{19}H_{14}N_4O_3S_2$: C, 54.53; H, 3.66; N, 12.72; found C, 54.35; H, 3.96; N, 12.52.

*2.2.3.9. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-hydroxy-3-methoxyphenyl) thiazolidin-4-one (**Tz9**)*. Yield: 76%; M.p. 212–213 °C, R_f = 0.61. IR (KBr, cm^{-1}); 3267 (Ar, C—H), 1610 (C=N), 1728 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.86 (2H, s, SCH_2); 3.74 (3H, s, OCH_3); 4.96 (1H, s, *N*-CH-S); 6.47–6.84 (3H, m, Ar-CH of phenyl); 7.21–7.45 (2H, m, Ar-CH); 8.09 (1H, m, Ar-CH); 8.12 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.1, 56.3, 66.2, 114.8, 116.3, 121.4, 121.6, 122.4, 125.6, 125.9, 132.7, 135.6, 144.2, 151.3, 153.4, 156.8, 170.8. ESI MS (*m/z*): 427 (M + H) $^+$. Calcd. Anal. for $C_{19}H_{14}N_4O_4S_2$: C, 53.51; H, 3.31; N, 13.14; found C, 53.24; H, 3.27; N, 13.34.

*2.2.3.10. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-[(4-dimethylamino)phenyl] thiazolidin-4-one (**Tz10**)*. Yield: 67%; M.p. 109–110 °C, R_f = 0.68. IR (KBr, cm^{-1}); 3134 (Ar, C—H), 1560 (C=N), 1705 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.40 (2H, s, SCH_2); 6.10 (1H, s, *N*-CH-S); 6.65–6.83 (4H, m, Ar-CH of phenyl); 7.51–7.60 (2H, m, Ar-CH); 8.09 (1H, m, Ar-CH); 8.18 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.8, 40.5, 64.0, 114.4, 121.1, 121.9, 124.9, 126.2, 128.1, 129.4, 135.4, 145.0, 152.9, 156.6, 171.4. ESI MS (*m/z*): 424 (M + H) $^+$. Calcd. Anal. for $C_{20}H_{17}N_5O_2S_2$: C, 56.72; H, 4.05; N, 16.54; found C, 56.94; H, 3.88; N, 16.41.

*2.2.3.11. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(furan-2-yl) thiazolidin-4-one (**Tz11**)*. Yield: 74%; M.p. 165–166 °C, R_f = 0.62. IR (KBr, cm^{-1}); 3010 (Ar, C—H), 1636 (C=N), 1745 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.92 (2H, s, SCH_2); 5.60 (1H, s, *N*-CH-S); 6.26–6.28 (4H, d, 2 × CH_2 of furan), 7.29 (2H, d, CH_2 of furan); 7.54–7.74 (2H, m, Ar-CH); 8.10 (1H, m, Ar-CH); 8.24 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 31.2, 55.6, 57.7, 106.4, 110.6, 116.4, 126.0, 130.2, 135.4, 142.2, 151.8, 163.6, 171.3. ESI MS (*m/z*): 371 (M + H) $^+$. Calcd. Anal. for $C_{16}H_{10}N_4O_3S_2$: C, 51.88; H, 2.72; N, 15.13; found C, 51.40; H, 2.98; N, 15.42.

*2.2.3.12. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(2,4-dimethylphenyl)thiazolidin-4-one (**Tz12**)*. Yield: 73%; M.p. 220–221 °C, R_f = 0.72. IR (KBr, cm^{-1}); 3132 (Ar, C—H), 1570 (C=N), 1695 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.35 (6H, s, 2 × CH3), 3.74 (2H, s, SCH_2); 5.88 (1H, s, *N*-CH-S); 6.74–6.90 (3H, m, Ar-CH of phenyl); 7.32–7.67 (2H, m, Ar-CH); 8.02 (1H, m, Ar-CH); 8.15 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 17.6, 24.6, 33.2, 58.8, 121.8, 121.9, 125.2, 125.7, 126.0, 128.2, 130.7, 135.2, 136.0, 136.4, 136.8, 153.2, 156.5, 171.4. ESI MS (*m/z*): 409 (M + H) $^+$. Calcd. Anal. for $C_{20}H_{16}N_4O_2S_2$: C, 58.80; H, 3.95; N, 13.72; found C, 58.68; H, 3.74; N, 13.89.

*2.2.3.13. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(3-chlorophenyl)thiazolidin-4-one (**Tz13**)*. Yield: 67%; M.p. 132–133 °C, R_f = 0.68. IR (KBr, cm^{-1}); 3225 (Ar, C—H), 1598 (C=N), 1676 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.66 (2H, s, SCH_2); 5.30 (1H, s, *N*-CH-S); 6.94–7.06 (4H, m, Ar-CH of phenyl); 7.43–7.60 (2H, m, Ar-CH); 8.09 (1H, m, Ar-CH); 8.12 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): 34.2, 65.9, 121.8, 122.2, 121.7, 125.1, 126.0, 126.8, 127.4, 128.3, 134.1, 135.8, 140.6, 153.4, 156.6, 171.2. ESI MS (*m/z*): 415 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{11}ClN_4O_2S_2$: C, 52.11; H, 2.67; N, 13.50; found C, 51.84; H, 2.88; N, 13.14.

*2.2.3.14. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(3-methoxyphenyl)thiazolidin-4-one (**Tz14**)*. Yield: 64%; M.p. 187–188 °C, R_f = 0.53. IR (KBr, cm^{-1}); 3165 (Ar, C—H), 1594 (C=N), 1686 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.56 (2H, s, SCH_2); 3.71 (3H, s, OCH_3); 5.16 (1H, s, *N*-CH-S); 6.57–7.06 (4H, m, Ar-CH of phenyl); 7.38–7.65 (2H, m, Ar-CH); 8.07 (1H, m, Ar-CH); 8.13 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.4, 55.9, 65.6, 112.7, 112.8, 121.1, 121.8, 121.9, 125.1, 126.2, 129.8, 135.2, 140.6, 153.6, 156.2, 160.4, 171.4. ESI MS (*m/z*): 411 (M + H) $^+$. Calcd. Anal. for $C_{19}H_{14}N_4O_3S_2$: C, 55.60; H, 3.44; N, 13.65; found C, 55.41; H, 3.16; N, 13.49.

*2.2.3.15. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(3-nitrophenyl)thiazolidin-4-one (**Tz15**)*. Yield: 72%; M.p. 232–233 °C, R_f = 0.74. IR (KBr, cm^{-1}); 3120 (Ar, C—H), 1571 (C=N), 1677 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.36 (2H, s, SCH_2); 5.10 (1H, s, *N*-CH-S); 7.80–7.98 (4H, m, Ar-CH of phenyl); 7.40–7.59 (2H, m, Ar-CH); 8.04 (1H, m, Ar-CH); 8.15 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.2, 65.1, 119.4, 120.3, 121.7, 124.2, 125.1, 126.2, 129.2, 134.2, 135.6, 140.2, 147.8, 153.6, 156.4, 171.4. ESI MS (*m/z*): 411 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{11}N_5O_4S_2$: C, 50.82; H, 2.61; N, 16.46; found C, 50.48; H, 2.75; N, 16.59.

*2.2.3.16. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-p-tolylthiazolidin-4-one (**Tz16**)*. Yield: 69%; M.p. 224–225 °C, R_f = 0.66. IR (KBr, cm^{-1}); 3104 (Ar, C—H), 1606 (C=N), 1705 (C=O). ^{1}H NMR

(DMSO-d_6, δ, ppm): 2.35 (3H, s, CH3), 4.00 (2H, s, SCH_2); 5.02 (1H, s, *N*-CH-S); 6.84–6.92 (4H, m, Ar-CH of phenyl); 7.24–7.60 (2H, m, Ar-CH); 8.08 (1H, m, Ar-CH); 8.12(1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.2, 33.8, 65.3, 121.3, 121.8, 125.7, 125.9, 128.7, 129.1, 135.5, 136.4, 136.7, 153.6, 156.0, 171.1. ESI MS (*m/z*): 395 (M + H) $^+$. Calcd. Anal. for $C_{19}H_{14}N_4O_2S_2$: C, 57.85; H, 3.58; N, 14.20; found C, 57.70; H, 3.82; N, 14.44.

*2.2.3.17. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-methoxyphenyl)thiazolidin-4-one (**Tz17**)*. Yield: 69%; M.p. 208–209 °C, R_f = 0.71. IR (KBr, cm^{-1}); 3260 (Ar, C—H), 1584 (C═N), 1690 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.60 (2H, s, SCH_2); 3.76 (3H, s, OCH_3); 4.81 (1H, s, *N*-CH-S); 6.90–7.18 (4H, m, Ar-CH of phenyl); 7.48–7.59 (2H, m, Ar-CH); 8.06 (1H, m, Ar-CH); 8.18 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.8, 55.1, 64.8, 114.4, 120.8, 120.9, 125.6, 125.9, 128.8, 130.8, 134.7, 151.2, 155.8, 158.6, 170.8. ESI MS (*m/z*): 411 (M + H) $^+$. Calcd. Anal. for $C_{19}H_{14}N_4O_3S_2$: C, 55.60; H, 3.44; N, 13.65; found C, 55.38; H, 3.10; N, 13.51.

*2.2.3.18. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(2,4-dichlorophenyl)thiazolidin-4-one (**Tz18**)*. Yield: 73%; M.p. 198–199 °C, R_f = 0.62. IR (KBr, cm^{-1}); 3230 (Ar, C—H), 1588 (C═N), 1647 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.90 (2H, s, SCH_2); 4.94 (1H, s, *N*-CH-S); 6.93–7.18 (3H, m, Ar-CH of phenyl); 7.34–7.65 (2H, m, Ar-CH); 8.08 (1H, m, Ar-CH); 8.10 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.9, 56.0, 100.6, 121.9, 122.4, 125.1, 125.8, 126.8, 130.8, 131.5, 134.1, 135.4, 135.8, 153.0, 156.6, 171.2. ESI MS (*m/z*): 434 (M + H) $^+$. Calcd. Anal. for $C_{17}H_8Cl_2N_4O_2S_2$: C, 46.91; H, 1.85; N, 12.87; found C, 46.64; H, 1.66; N, 12.96.

*2.2.3.19. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-trifluoromethylphenyl) thiazolidin-4-one (**Tz19**)*. Yield: 76%; M.p. 222–223 °C, R_f = 0.57. IR (KBr, cm^{-1}); 3314 (Ar, C—H), 1610 (C═N), 1703(C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.72 (2H, s, SCH_2); 5.00 (1H, s, *N*-CH-S); 7.06–7.34 (4H, m, Ar-CH of phenyl); 7.20–7.56 (2H, m, Ar-CH); 8.01 (1H, m, Ar-CH); 8.16 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.1, 65.5, 121.6, 121.7, 123.3, 124.6, 125.2, 125.7, 126.4, 129.0, 130.6, 132.5, 135.2, 139.3, 153.5, 156.0, 171.8. ESI MS (*m/z*): 449 (M + H) $^+$. Calcd. Anal. for $C_{19}H_{11}F_3N_4O_2S_2$: C, 50.89; H, 2.47; N, 12.49; found C, 50.37; H, 2.76; N, 12.62.

*2.2.3.20. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(5-chloro-2-hydroxyphenyl) thiazolidin-4-one (**Tz20**)*. Yield: 63%; M.p. 208–210 °C, R_f = 0.58. IR (KBr, cm^{-1}); 3142 (Ar, C—H), 1602 (C═N), 1707 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.90 (2H, s, SCH_2); 5.00 (1H, s, OH); 5.26 (1H, s, *N*-CH-S); 6.54–6.92 (3H, m, Ar-CH of phenyl); 7.32–7.74 (2H, m, Ar-CH); 8.01 (1H, m, Ar-CH); 8.18(1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): 33.8, 54.5, 117.4, 119.5, 121.7, 121.9, 125.8, 125.9, 126.2, 128.6, 130.2, 135.4, 153.7, 154.0, 156.9, 170.8. ESI MS (*m/z*): 431 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{11}ClN_4O_3S_2$: C, 50.17; H, 2.57; N, 13.00; found C, 50.42; H, 2.76; N, 12.84.

*2.2.3.21. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-diethylamino-2-hydroxyphenyl) thiazolidin-4-one (**Tz21**)*. Yield: 69%; M.p. 230–231 °C, R_f = 0.68. IR (KBr, cm^{-1}); 3265 (Ar, C—H), 1640 (C═N), 1721 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.71 (2H, s, SCH_2); 4.98 (1H, s, *N*-CH-S); 5.08 (1H, s, OH); 1.29 (6H, t, 2 × CH_3); 3.18(4H, m, 2 × CH_2); 6.02–6.24 (3H, m, Ar-CH of phenyl); 7.50–7.79 (2H, m, Ar-CH); 8.08 (1H, m, Ar-CH); 8.22 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): 13.7, 33.9, 44.8, 55.2, 99.1, 106.6, 107.4, 121.0, 121.5, 125.1, 125.6, 131.4, 135.7, 149.2, 153.0, 156.0, 156.1, 171.6. ESI MS (*m/z*): 468 (M + H) $^+$. Calcd. Anal. for $C_{22}H_{21}N_5O_3S_2$: C, 56.51; H, 4.53; N, 14.98; found C, 56.78; H, 4.20; N, 14.60.

*2.2.3.22. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-m-tolylthiazolidin-4-one (**Tz22**)*. Yield: 73%; M.p. 205–206 °C, R_f = 0.72. IR (KBr, cm^{-1}); 3120 (Ar, C—H), 1624 (C═N), 1740 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.50 (3H, s, CH_3), 3.98 (2H, s, SCH_2); 5.07 (1H, s, *N*-CH-S); 6.72–7.04 (4H, m, Ar-CH of phenyl); 7.14–7.60 (2H, m, Ar-CH); 8.01 (1H, m, Ar-CH); 8.20 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.6, 33.2, 65.4, 121.6, 121.9, 125.2, 125.6, 125.9, 127.4, 128.6, 130.6, 135.1, 138.4, 139.7, 153.5, 156.7, 171.0. ESI MS (*m/z*): 395 (M + H) $^+$. Calcd. Anal. for $C_{19}H_{14}N_4O_2S_2$: C, 57.85; H, 3.58; N, 14.20; found C, 57.98; H, 3.32; N, 14.38.

*2.2.3.23. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(4-chloro-3-nitrophenyl)thiazolidin-4-one (**Tz23**)*. Yield: 68%; M.p. 215–216 °C, R_f = 0.61. IR (KBr, cm^{-1}); 3134 (Ar, C—H), 1595 (C═N), 1676 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.73 (2H, s, SCH_2); 5.94 (1H, s, *N*-CH-S); 7.39–7.43 (4H, m, Ar-CH of phenyl); 7.24–7.72 (2H, m, Ar-CH); 8.06 (1H, m, Ar-CH); 8.20 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.1, 64.2, 121.8, 121.9, 125.1, 125.7, 125.9, 127.4, 129.4, 135.1, 136.3, 138.3, 148.6, 153.5, 156.2, 171.3. ESI MS (*m/z*): 459 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{10}ClN_5O_4S_2$: C, 47.01; H, 2.19; N, 15.23; found C, 47.34; H, 2.04; N, 15.56.

*2.2.3.24. 4-{3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-4-oxothiazolidin-2-yl} benzaldehyde (**Tz24**)*. Yield: 73%; M.p. 202–203 °C, R_f = 0.56. IR (KBr, cm^{-1}); 3116 (Ar, C—H), 1635 (C═N), 1727 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.71(2H, s, SCH_2); 4.92 (1H, s, *N*-CH-S); 9.50 (1H, s, CHO); 7.10–7.42 (4H, m, Ar-CH of phenyl); 7.55–7.67 (2H, m, Ar-CH); 8.08 (1H, m, Ar-CH); 8.14 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.4, 65.2, 121.4, 121.8, 125.1, 125.7, 129.3, 129.8, 135.2, 135.6, 145.0, 153.0, 156.7, 171.8, 191.2. ESI MS (*m/z*): 409 (M + H) $^+$. Calcd. Anal. for $C_{19}H_{12}N_4O_3S_2$: C, 55.87; H, 2.96; N, 13.72; found C, 55.49; H, 2.67; N, 12.8.

*2.2.3.25. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(3-fluorophenyl)thiazolidin-4-one (**Tz25**)*. Yield: 60%; M.p. 214–215 °C, R_f = 0.66. IR (KBr, cm^{-1}); 3312 (Ar, C—H), 1564 (C═N), 1738 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.90 (2H, s, SCH_2); 5.06 (1H, s, *N*-CH-S); 6.77–7.12 (4H, m, Ar-CH of phenyl); 7.52–7.84 (2H, m, Ar-CH); 8.12 (1H, m, Ar-CH); 8.22 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.9, 65.8, 113.8, 115.4, 121.8, 121.9, 124.4, 125.4, 125.9, 130.6, 135.7, 140.8, 153.5, 156.1, 171.2. ESI MS (*m/z*): 399 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{11}FN_4O_2S_2$: C, 54.26; H, 2.78; N, 14.06; found C, 54.10; H, 2.98; N, 14.40.

*2.2.3.26. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(3-bromophenyl)thiazolidin-4-one (**Tz26**)*. Yield: 69%; M.p. 189–190 °C, Rf = 0.75. IR (KBr, cm-1); 3124 (Ar, C—H), 1608 (C═N), 1702 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.60 (2H, s, SCH_2); 4.86 (1H, s, *N*-CH-S); 7.00–7.24 (4H, m, Ar-CH of phenyl); 7.32–7.56 (2H, m, Ar-CH); 8.07 (1H, m, Ar-CH); 8.10 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.0, 64.6, 121.5, 121.7, 123.0, 125.3, 125.8, 127.7, 130.2, 130.8, 133.8, 135.2, 141.2, 153.8, 156.7, 171.9. ESI MS (*m/z*): 460 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{11}BrN_4O_2S_2$: C, 47.07; H, 2.41; N, 12.20; found C, 47.36; H, 2.76; N, 12.42.

*2.2.3.27. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-phenylthiazolidin-4-one (**Tz27**)*. Yield: 74%; M.p. 198–199 °C, R_f = 0.63. IR (KBr, cm^{-1}); 3065 (Ar, C—H), 1624 (C═N), 1712 (C═O). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.23 (2H, s, SCH_2); 4.98 (1H, s, *N*-CH-S); 6.82–7.14 (4H, m, Ar-CH of phenyl); 7.48–7.78 (2H, m, Ar-CH); 8.00 (1H, m, Ar-CH); 8.22 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.3, 65.6, 121.3, 122.0, 125.9, 127.1, 128.6, 128.5, 135.2, 139.6, 153.6, 156.6, 171.1. ESI MS (*m/z*): 381 (M + H) $^+$. Calcd. Anal. for $C_{18}H_{12}N_4O_2S_2$: C, 56.83; H, 3.18; N, 14.73; found C, 54.72; H, 3.37; N, 14.52.

*2.2.3.28. 3-[5-(benzo[d]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-(2-hydroxyphenyl)thiazolidin-4-one (**Tz28**)*. Yield: 62%; M.p. 188–189 °C, R_f = 0.56. IR (KBr, cm^{-1}); 3126 (Ar, C—H), 1610 (C═N), 1726 (C═O).

^{1}H NMR (DMSO-d_6, δ, ppm): 3.98 (2H, s, SCH_2); 5.00 (1H, s, *N*-CH-S); 5.4 (1H, s, OH), 6.60–6.92 (4H, m, Ar-CH of phenyl); 7.21–7.60 (2H, m, Ar-CH); 8.05 (1H, m, Ar-CH); 8.16 (1H, m, Ar-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.7, 55.4, 115.6, 121.4, 121.6, 121.9, 125.3, 125.8, 128.5, 130.4, 135.6, 153.2, 155.8, 156.2, 171.7. ESI MS (*m/z*): 397 (M +H) $^+$. Calcd. Anal. for $C_{18}H_{12}N_4O_3S_2$: C, 54.53; H, 3.05; N, 14.13; found C, 54.82; H, 3.22; N, 14.32.

2.3. In silico methods

2.3.1. Absorption, distribution, metabolism and excretion (ADME) study

We estimated the pharmacokinetic parameters (ADME) of the novel synthesized compounds for prediction of oral bioavailability using molinspiration online program in compliance with Lipinski's rule of five [39]. According to the rule, for oral absorption of newly designed molecule, it should have a molecular weight < 500, Log P (Octanol-water partition coefficient) < 5, hydrogen bond donors ≤5, hydrogen bond acceptors ≤10, number of rotatable bonds ≤10, the topological polar surface area ≤160 [40]. Drug candidates showing violation of more than one of this rule will lead to difficulty in oral absorption [22]. The percentage of absorption (% Abs) was calculated using topological polar surface area (TPSA) by the formula, % Abs = 109 − [0.345 × TPSA] [41].

2.3.2. Molecular docking study with peroxisome proliferator-activated receptor gamma and alpha glucosidase

Molecular Docking analysis was carried out using X-ray crystal structure of peroxisome proliferator-activated receptor-gamma (PDB code: 1FM9, resolution: 2.1A°) and alpha glucosidase (PDB code: 2QMJ, resolution: 1.9 A°) obtained from Research Collaboratory Structural Bioinformatics protein data bank [42,43]. PPAR gamma receptor is the chief target in the discovery of successful anti-diabetic agents. All the steps of docking i.e. protein selection, protein preparation, grid generation, ligand preparation, docking and docking study analysis were performed by Maestro 9.0 Schrodinger suite [44]. The protein structure was constructed using protein preparation wizard proceeded by optimization and minimization of protein with the 0.3 Å root mean square deviation. Structures of synthesized ligands were drawn and prepared using Lig Prep module. The Optimized Potentials for Liquid Simulations (OPLS) 2005 force field was used for minimization of both the synthesized molecules and protein. The grid box accounting the active site residues was generated. The docking studies of prepared and minimized molecules into the prepared and minimized protein were carried out using glide software with extra precision. The docking score, hydrogen bonding with surrounding amino acids and binding free energy of all the synthesized hits were anticipated to elucidate the binding affinity into the active sites of PPAR gamma.

2.3.3. Prime molecular mechanics-generalized born surface area (MM-GBSA) assessment [45]

The Prime molecular mechanics-generalized born surface area assay, Maestro 9.0, was used to estimate the binding free energy for the set of synthesized small molecules (ligands) to a receptor (PPAR-γ) and alpha-glucosidase enzyme. Protein and ligands were prepared using protein preparation wizard and Lig Prep tool respectively. The results of MM-GBSA may also be obtained by running the MM-GBSA model directly in the document acquired by running the procedure of molecular docking.

2.3.4. Alpha glucosidase assay [46,47]

The inhibitory potential of seven selected analogues was determined by inhibition assay against the standard acarbose [48]. Alpha Glucosidase enzyme was purchased from Sigma-Aldrich which was prepared in potassium phosphate buffer (pH 7.2, 2 mM). The p-nitrophenol-α-D-glucopyranoside substrate (100 µL of pNPG substrate, 2 mM, pH 7.2), various concentrations of synthesized entities and 0.5 IU/mL enzyme were taken in the 96-well plate and incubated at 37° C for 10 min. The reaction was stopped by the addition of Na_2CO_3 solution (50 µL, 2 N). Microplate reader was used to measure the absorbance of the reaction mixture at 405 nm. The percentage of inhibition was calculated according to the formula: % inhibition = (Ac − At)/Ac × 100, where Ac is the absorbance of control and At is the absorbance of the test sample. IC_{50} values were determined using non-linear regression curve.

2.3.5. Enzyme inhibition kinetics study

To determine the mode of inhibition of the synthesized derivatives, kinetics studies were carried out on the highest potent compound **Tz21**. Different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 µM) of most potent compound **Tz21** was added to 20 mL of the enzyme (0.5 IU/mL) and incubated with for 30 °C for 10 min. pNPG at different concentrations (2, 4, and 6 mM) was added as substrate to initiate the reaction. Change in absorbance was noted for 50 min at 405 nm. The type of inhibition is determined by Lineweaver burk plot (1/V versus 1/[*S*]) where V is reaction velocity and [*S*] is substrate concentration was. Ki (EI dissociation constant) and Ki' (ESI dissociation constant) was calculated using the secondary plots of slope versus concentration of inhibitor and secondary plot of intercept versus concentration of inhibitor respectively.

2.4. In vivo biological evaluation

2.4.1. Animals

Albino Wistar rats of either sex weighing 180–240 gm were used for the anti-diabetic study. The animals were acquired from our institute's (Delhi Institute of Pharmaceutical Sciences and Research, New Delhi, India) animal housing facility after the approval by Institutional Animal Ethics Committee (Protocol No. 6, 2016-I). All the animals were housed in polypropylene cages under 12 h light/12 h dark cycle with standard conditions of temperature (24 ± 1 °C) and relative humidity (35–60%). The rats were given standard food and water *ad libitum*.

2.4.2. Experimental procedure of oral glucose tolerance test [49]

On the basis of glide scores obtained from molecular docking study, seven compounds (**Tz4, Tz10, Tz17, Tz19, Tz21, Tz23 and Tz24**) with higher docking scores as compared to the standard drug were selected for *in vivo* hypoglycemic activity by Oral Glucose Tolerance Test in normal rats. Overnight fasted (16 h) normal rats were divided into nine groups of six animals each. Rats of normal control group (Group I) were administered orally with 0.25% (w/v) aqueous carboxymethyl cellulose solution (5 mL/kg). Standard group (Group II) received pioglitazone as a suspension of 0.25% carboxymethyl cellulose at a dose of 36 mg/kg. Groups III-IX was orally fed with selected compounds as 0.25% carboxymethyl cellulose suspension at a dose of 36 mg/kg. Glucose solution (2 g/kg body weight) was given orally to all groups after 30 min of dosing of carboxymethyl cellulose, pioglitazone and tested compounds. Plasma glucose level of each rat in every group was measured at 0 h, 2 h and 6 h using Accu-chek Active TM Test glucometer (Roche Diagnostics, Mannheim, Germany).

2.4.3. Anti-hyperglycemic study on streptozotocin (STZ) induced diabetic rat model [50]

2.4.3.1. Streptozotocin instigation of diabetes. Hyperglycemia was induced in overnight fasted rats by injecting freshly prepared solution of streptozotocin in 0.1 M citrate buffer (pH 4.5) (60 mg/kg bodyweight) intraperitoneally. To overcome the streptozotocin induced hypoglycemic condition the rats were permitted to drink 5% glucose solution whole night and their blood glucose levels were then checked by glucometer. The animals with blood glucose level more than 260 mg/dL after 48 h of STZ injection were considered as diabetic rats and used for the anti-diabetic evaluation.

2.4.3.2. Experimental design. The diabetic animals were classified into nine groups with six rats in each group. Diabetic control group (Group

I) received 0.25% (w/v) aqueous carboxymethyl cellulose solution (5 mL/kg). Group II diabetic rats received oral dose (36 mg/kg) of standard as 0.25% carboxymethyl cellulose suspension. Groups III-IX was treated with oral dosing (36 mg/kg) of the derivatives (**Tz4, Tz10, Tz17, Tz19, Tz21, Tz23, Tz24**) (as 0.25% suspension), selected on the basis of higher docking scores.

2.4.3.3. Blood glucose level estimation. Blood sample was acquired from the retro orbital plexus of the rat eye utilizing capillary tube. At the 0 h, 2 h, 4 h and 6 h intervals, the blood glucose levels of the rat in every group was monitored by Accu-check Active TM Test meter of Roche Diagnostics.

2.5. Statistical analysis

All the results obtained from *in vivo* study were represented as mean ± SEM. Statistical significance were estimated between the tested derivatives and control by one-way ANOVA followed by Dunnett's multiple comparison test. The values were considered as significant when p is less than 0.01.

3. Results and discussion

3.1. Chemistry

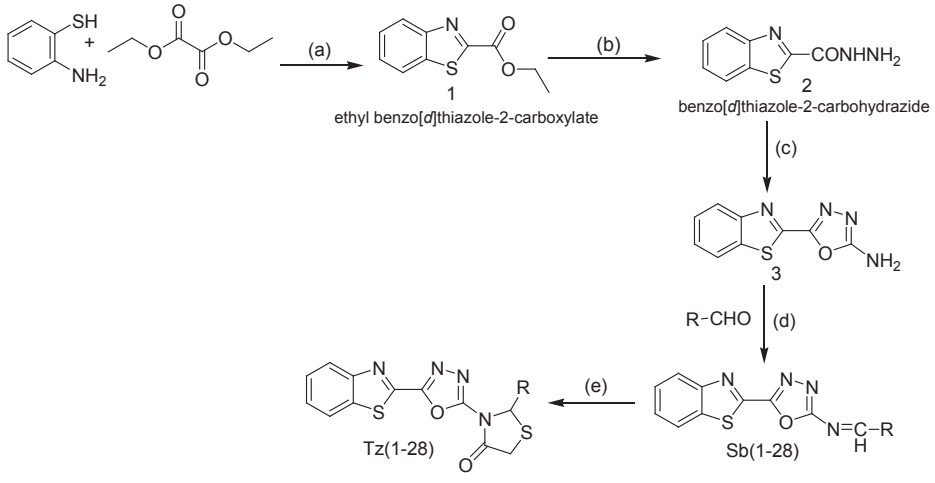
A library of twenty-eight title compounds (**Tz1-Tz28**) was synthesized as depicted in Scheme 1. Refluxing of 2-mercaptoaniline with diethyl oxalate proceeded by pouring the solution into mixture of water, hydrochloric acid and ethanol results in compound 1 which on treatment with hydrazine monohydrate yields intermediate **2**. Compound **3** was prepared by cyclization reaction of 5-(benzo[*d*]thiazol-2-yl)-1,3,4-oxadiazol-2-amine with methanolic cyanogen bromide. Schiff bases (**Sb1-Sb28**) were afforded by reaction of compound **3** with different substituted aromatic aldehydes. Reaction of appropriate Schiff base and mercaptoacetic acid (0.1 mol) in dimethylformamide (25–30 mL), containing a catalytic amount of zinc chloride lead to synthesis of titled derivatives, [5-(benzo[*d*]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-substituted thiazolidin-4-one (**Tz1-Tz28**). The purity of the compounds was checked by thin layer chromatography and all the compounds were obtained in good yield.

The structure of all the synthesized hybrids was confirmed by IR, NMR, Mass and elemental analysis data. The IR spectra displayed bands of aromatic C—H in the region of 3010–3314 cm^{-1}, bands at 1520–1640 cm^{-1} due to C=N group and 1647–1748 cm^{-1} due to carbonyl group of 4-thiazolidinone ring. The mass spectra of the targets exhibited the molecular ion peaks at $[M+1]^+$. The H^1 NMR spectra showed the signal at δ 3.36–4.23 ppm due to $-SCH_2$ and at δ 4.62–6.10 ppm due to *N*-CH-S indicating the generation of 4-thiazolidinone heterocycle. In C^{13} NMR signals appears at appropriate values. C, H and N analysis results were within ± 0.4% for the theoretical values.

3.2. Computer simulation

3.2.1. ADME evaluation

Oral bioavailability is accounted as a crucial characteristic for the discovery of efficacious medicinal drugs. Drug likeliness properties of the prepared benzothiazole-oxadiazole-thiazolidinone hybrids (**Tz1-Tz28**) were calculated by the help of molinspiration online software. The results are presented in Table 1. Molecular weight of all the designed derivatives was found to be less than 500 and thus predicting their easy movement, diffusion and absorption. Molecular aquaphobicity is measured by Log P (Octanol-water partition coefficient) [41]. Log P value of all the synthesized hits were found less than five except **Tz18** (Log P = 5.15), demonstrating good membrane permeability of the compounds. Number of hydrogen bond donors and number of hydrogen bond acceptors in all the hybrid analogues were determined less than 5 and 10 respectively. Topological polar surface area (TPSA) is a very useful parameter for prediction of transport of drug molecule [51] and was observed less than 160 Å i.e. in the range of 72.12–117.95 Å. It can be noticed that all the titled derivatives displayed percentage absorption ranging from 68.31 to 84.12% indicating significant oral bioavailability. All these pharmacokinetic properties, as shown in Table 1, are in accordance with Lipinski's rule of five and thus making all the titled compounds as good orally active antidiabetic agents.



Scheme 1. Synthetic protocol for synthesis of title compounds (**Tz1-Tz28**). Reagent and conditions: (a) 10 h, 130 °C; (b) Hydrazine hydrate, ethanol, 9 h, 80 °C; (c) Cyanogen Bromide, methanol, stirring,1.5 h, 40 °C; reflux, 11 h, 70 °C; (d) Glacial acetic acid, ethanol, 10–12 h, 80 °C; (e) Mercaptoacetic acid, dimethylformamide, 15 h.

R= Tz1 = 4-hydroxyphenyl; **Tz2** = 4-fluorophenyl; **Tz3** = 4-chlorophenyl; **Tz4** = 4-nitrophenyl; **Tz5** = 4-bromo; **Tz6** = 3,4,5-trimethoxyphenyl; **Tz7** = 3-hydroxyphenyl; **Tz8** = 3,4-dimethoxy; **Tz9** = 4-hydroxy-3-methoxyphenyl; **Tz10** = 4-dimethylamine phenyl; **Tz11** = furan-2-yl; **Tz12** = 2,4-dimethylphenyl; **Tz13** = 3-chlorophenyl; **Tz14** = 3-methoxyphenyl; **Tz15** = 3-nitrophenyl; **Tz16** = 4-methylphenyl; **Tz17** = 4-methoxyphenyl; **Tz18** = 2,4-dichlorophenyl; **Tz19** = 3-trifluoromethylphenyl; **Tz20** = 5-chloro-2-hydroxyphenyl; **Tz21** = 4-diethylamine-2-hydroxyphenyl; **Tz22** = 3-methylphenyl; **Tz23** = 4-chloro-3-nitrophenyl; **Tz24** = 4-oxophenyl; **Tz25** = 3-fluorophenyl; **Tz26** = 3-bromophenyl; **Tz27**= phenyl; **Tz28**= 2-hydroxyphenyl

Table 1
ADME study of synthesized compounds.

Compound	%Abs[1]	TPSA[2] (A^0)	n-rotb[3]	MW[4]	MV[5]	miLog Po/w[6]	n-OHNH[7]	n-ON[8]	n violation
Rule				< 500		< 5	< 5	< 10	≤1
Tz1	77.14	92.35	3	396.45	311.36	3.39	1	7	0
Tz2	84.12	72.12	3	398.44	308.28	4.03	0	6	0
Tz3	84.12	72.12	3	414.90	316.88	4.54	0	6	0
Tz4	68.31	117.95	4	425.45	326.68	3.83	0	9	0
Tz5	84.12	72.12	3	459.35	321.23	4.68	0	6	0
Tz6	74.56	99.83	6	470.53	379.98	3.50	0	9	0
Tz7	77.14	92.35	3	396.45	311.36	3.36	1	7	0
Tz8	77.75	90.59	5	440.51	354.44	3.51	0	8	0
Tz9	73.95	101.59	4	426.48	336.91	3.21	1	8	0
Tz10	83.00	75.36	4	423.52	349.25	3.97	0	7	0
Tz11	79.58	85.27	3	370.42	284.91	3.12	0	7	0
Tz12	84.12	72.12	3	408.51	336.47	4.69	0	6	0
Tz13	84.12	72.12	3	414.90	316.88	4.52	0	6	0
Tz14	80.93	81.36	4	410.48	328.89	3.90	0	7	0
Tz15	68.31	117.95	4	425.45	326.68	3.80	0	9	0
Tz16	84.12	72.12	3	394.48	319.91	4.32	0	6	0
Tz17	80.93	81.36	4	410.48	328.89	3.92	0	7	0
Tz18	84.12	72.12	3	449.34	330.42	5.15	0	6	1
Tz19	84.12	72.12	4	448.45	334.64	4.74	0	6	0
Tz20	77.14	92.35	3	430.90	324.90	4.46	1	7	0
Tz21	76.02	95.59	6	467.58	390.87	4.64	1	8	0
Tz22	84.12	72.12	3	394.48	319.91	4.29	0	6	0
Tz23	68.31	117.95	4	459.90	340.22	4.43	0	9	0
Tz24	78.23	89.20	4	408.46	322.33	3.66	0	7	0
Tz25	84.12	72.12	3	398.44	308.28	4.01	0	6	0
Tz26	84.12	72.12	3	459.35	321.23	4.65	0	6	0
Tz27	84.12	72.12	4	448.45	334.64	4.74	0	6	0
Tz28	77.14	92.35	3	396.45	311.36	3.81	1	7	0

[1] Percentage of absorption.
[2] Topological polar surface area.
[3] Number of rotatable bonds.
[4] Molecular weight.
[5] Molecular volume.
[6] Logarithm of octanol/water partition coefficient.
[7] Number of hydrogen bond donors.
[8] Number of hydrogen bond acceptors.

3.2.2. Docking study

The molecular docking study was performed to understand the binding mode of all the synthesized analogs into the active site of PPAR-γ receptor. The docking scores, binding energies and interaction with amino acids of the prepared hits are presented in Table 2. All the compounds in the series were active. Three compounds **Tz21, Tz17 and Tz10** showed the highest docking scores (−9.85, −9.11 and −8.85 respectively) among all the compounds which is higher than pioglitazone (−8.46) in the binding site of the PPAR-γ receptor. Here we discuss the binding mode of three most active compounds (**Tz21, Tz17 and Tz10**). Docking study of compounds **Tz21, Tz17 and Tz10** into the active site of target indicated that several molecular interactions were supposed to be accountable for the remarkable affinity of these compounds.

Tz21 is the most active compound in the synthesized library. **Tz21** forms four hydrogen bonds with Leu340, Ser342 and Glu343. Oxygen of 4-thiazolidinone ring forms two hydrogen bonds with Glu343 and Ser342. Third hydrogen bond was formed between oxygen of oxadiazole and Ser342 and fourth hydrogen bond was observed between hydrogen of hydroxyl group of benzene and Leu340. In addition, the compound showed hydrophobic interactions with Leu330, Met334, Val339, Met364, Leu356, Ile281, Ala278, Phe260, Phe262, Cys285, Tyr327, Ile326, Leu453, Leu469 and Tyr473. The oxadiazole ring was found to have π-π interaction with Hie449 while the thiazolidinone ring showed polar interaction with Ser289 (Fig. 1.).

In compound **Tz17**, oxygen of thiazolidinone ring showed two hydrogen bonds formation with amino acids Hip323 and Tyr473, in which interaction with Hip323 is similar to the standard drug, pioglitazone. Moreover, third hydrogen bond was observed between nitrogen of oxadiazole ring and Tyr473 amino acid. Additionally it forms hydrophobic contact with Met364, Phe282, Leu356, Phe363, Cys285, Leu330, Leu465, Leu453, and Leu469. π-π stacking appears between the oxadiazole ring and Hie449 and the oxadiazole core also shows polar interaction with Gln286 and Ser289 (Fig. 2).

Compound **Tz10** (third most active compound) shows three hydrogen bond interactions. One hydrogen bond was formed between nitrogen of oxadiazole ring and amino acid Ser342 which is similar to the standard drug, pioglitazone. Second hydrogen bond was observed between nitrogen of oxadiazole ring with amino acid Glu343 and third hydrogen bond was formed between hydrogen of methyl group and Ile326. Futhermore, compound form hydrophobic cloud with Leu255, Ile281, Met348, Met364, Ile341, Leu 340, Leu 340, Leu 333, Val 339, Cys285, Tyr327 (Fig. 3).

3.2.3. MM-GBSA assay

The target protein and ligands were prepared as discussed in experimental section. All the molecules of water were eliminated before operating the Prime MM-GBSA assay, Maestro 9.0. The binding free energy results revealed that all compounds of the series fit well into the PPAR-γ receptor (Table 2). The binding energy of the standard drug, pioglitazone, was found to be −51.58 kcal/mol which is lower than that of most promising synthesized compounds **Tz21, Tz17** and **Tz10** (−57.74, −56.24 and −56.99 kcal/mol respectively). The free binding energies of all other tested ligands lie in the range of −47.80 to −59.80 kcal/mol. Moreover, the binding energy of standard acarbose was found to be −48.59 kcal/mol against alpha-glucosidase which is

Table 2
Glide score, binding free energy and hydrogen bond interactions with amino acids residue of synthesized derivatives and pioglitazone.

Compd.	Glide score	Binding free energy (kcal/mol)	Hydrogen bonds		
			Atom of ligand	Amino acids	Distance (A^0)
Tz1	8.00	−52.20	O	ARG 288	4.727
				SER 342	3.239
			O	SER 342	3.363
				GLU 343	4.453
			O	GLN383	2.091
			H	GLU 259	1.973
Tz2	8.34	−51.98	N	GLU 343	4.574
				SER 342	2.328
			N	SER 342	4.520
Tz3	8.16	−52.50	O	GLU 343	4.203
				SER 342	2.120
			O	SER 342	3.745
Tz4	8.67	−55.20	N	GLU 343	4.667
			N	SER 342	2.337
Tz5	8.02	−55.12	N	GLU 343	4.644
				SER 342	2.367
Tz6	8.37	−55.88	N	GLU 343	4.701
			N	SER 342	2.388
Tz7	8.07	−48.11	N	ARG 288	1.628
			N	ARG 288	4.884
				SER 342	3.219
			O	GLN 383	2.405
			H	GLU 259	1.615
Tz8	8.48	−59.80	O	GLU 343	4.182
				SER 342	2.096
			O	SER 342	3.732
Tz9	8.21	−52.73	O	GLU 343	4.149
				SER 342	2.098
			O	SER 342	3.699
Tz10	8.85	−56.99	O	SER 342	2.096
				GLU 343	4.185
			O	SER 342	3.732
Tz11	8.14	−53.79	N	GLU 343	2.369
				GLY344	4.145
			N	SER 342	2.470
				LEU 288	2.565
Tz12	8.34	−47.80	O	GLU 343	4.120
				SER 342	2.005
			O	SER 342	3.672
Tz13	8.27	−50.94	O	GLU 343	4.196
			O	SER 342	2.108
				SER 342	3.752
Tz14	8.18	−51.22	O	GLU 343	4.196
				SER 342	2.077
			O	SER 342	3.768
Tz15	8.46	−55.37	O	ARG 288	4.241
			O	GLU 343	4.123
				SER 342	2.029
			O	SER 342	3.701
Tz16	8.28	−54.12	N	GLU 343	4.589
				SER 342	2.308
			O	SER 342	3.762
Tz17	9.11	−56.24	S	CYS 285	2.683
			N	TYR 473	4.143
			O	TYR 473	1.975
			O	HIP 323	2.639
Tz18	7.99	−48.71	O	GLU 343	3.726
				SER 342	1.992
			O	SER 342	3.289
Tz19	8.60	−55.11	N	GLU 343	4.657
			N	SER 342	2.364
Tz20	8.05	−53.12	N	ARG 288	4.655
			N	ARG 288	4.913
				SER 342	3.093
Tz21	9.85	−57.74	H	LEU 340	11.89
			O	GLU 343	16.57
				SER 342	14.14
			O	SER 342	13.06
Tz22	8.26	−50.90	O	GLU 343	4.184
				SER 342	2.101
			O	SER 342	3.729
Tz23	8.67	−53.89	O	SER 342	3.713
			O	GLU 343	4.135
			O	SER 342	2.030
			O	ARG 288	4.174
Tz24	8.68	−55.61	N	GLU 343	4.665
			N	SER 342	2.373
Tz25	8.01	−52.17	N	GLU 343	4.652
				SER 342	2.376
Tz26	7.99	−50.74	O	GLU 343	4.181
				SER 342	2.119
			O	SER 342	3.724
Tz27	7.93	−49.99	O	GLU 343	4.176
				SER 342	2.116
			O	SER 342	3.718
Tz28	7.79	−51.23	N	GLU 343	4.276
Pioglitazone	−8.46	−51.58	S	SER 289	2.560
			O	TYR 473	1.961
			O	HIP 323	2.637

lower than the most potent synthetic compound **Tz21** (−49.57 kcal/mol) and the binding energies of all the compounds lie in the range of −35.77 to −49.57 kcal/mol.

3.3. Inhibition study

The *in vitro* inhibition study of the seven selected compounds against α-glucosidase was assessed and compared with standard drug (acarbose). Three derivatives **Tz21** (IC50 = 0.21 ± 0.01 μM), **Tz4** (IC50 = 9.03 ± 0.12 μM), **Tz24** (IC50 = 11.96 ± 0.40 μM) were found to be potent derivatives when compared with standard (IC50 = 18.5 ± 0.20 μM) indicating **Tz21** has the highest inhibitory effect among the seven tested derivatives. **Tz10** (IC50 = 20.36 ± 2.41 μM), **Tz23** (IC50 = 32.6 ± 0.46 μM), **Tz17** (IC50 = 43.63 ± 1.51 μM) and **Tz19** (IC50 = 53.2 ± 0.73 μM) had shown moderate inhibitory activity against α-glucosidase.

On comparing the stabilizing energy obtained through MMGBSA with inhibition analysis it was observed that all the results were compatible being **Tz21** bearing the diethyl amine group at para position and hydroxyl group at ortho position of phenyl ring at second position of 4-thiazolidinone as the most potent compound among the series. Analog **Tz21** has showed the binding energy of −49.57 kcal/mol which is higher than that of standard acarbose (−48.59 kcal/mol) making it more stable towards the alpha glucosidase. In addition, IC50 of **Tz21** (0.21 ± 0.01 μM) is also found to be many folds better than the IC50 of standard (18.5 ± 0.20 μM) which is in relation with the stabilizing energy result obtained from docking study. **Tz4** (second most potent compound, −47.77 kcal/mol) and **Tz24** (third most potent compound, −46.14 kcal/mol) has also shown good correlation between the inhibition activity with IC50 values of 9.03 ± 0.12 μM, 11.96 ± 0.40 μM respectively and binding energy when docked with

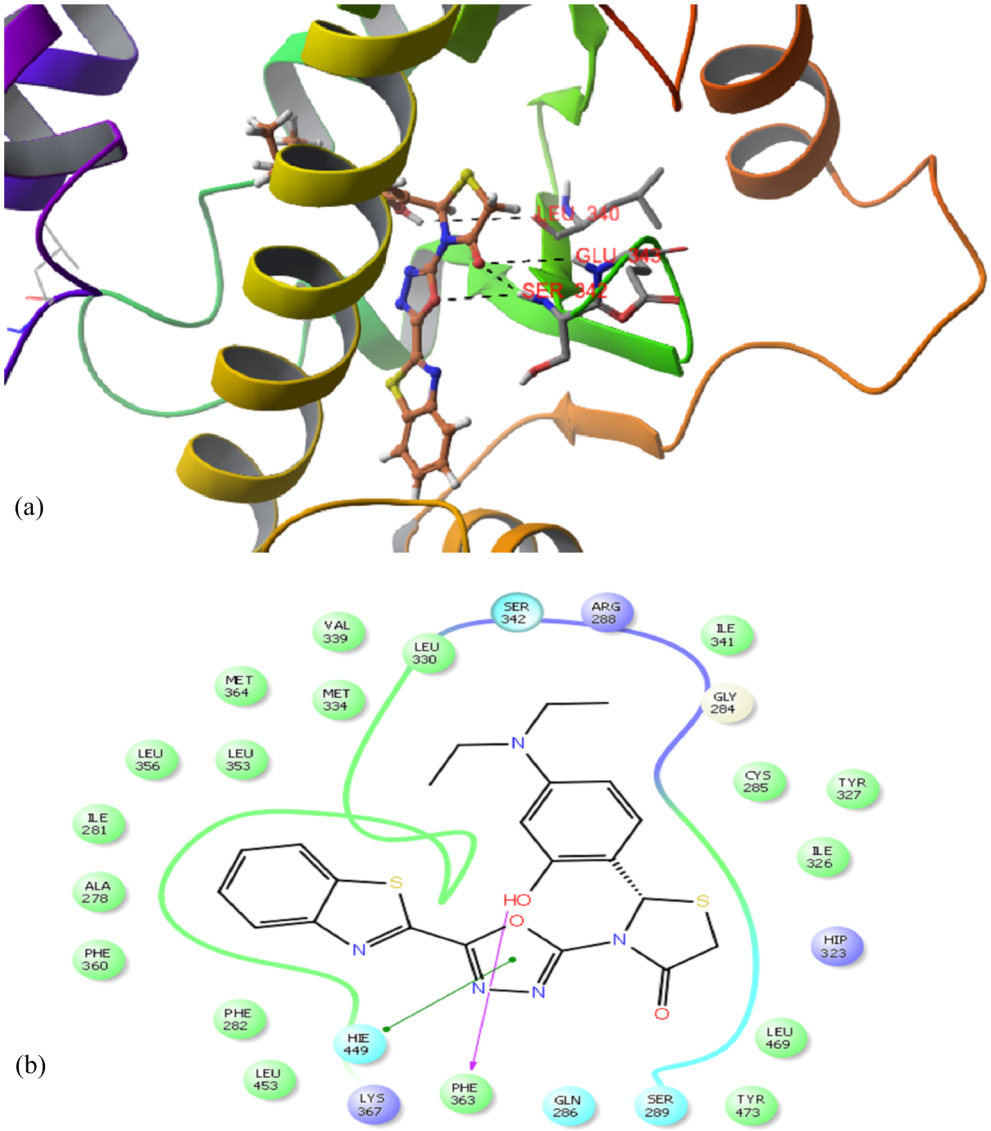


Fig. 1. (a) 3D docked pose of compound **Tz21** showing hydrogen bonds in black dotted line with Leu340, Ser342 and Glu343. Coloured by atom: Carbons: brown; Nitrogen: blue; Oxygen: red; Hydrogen: white. (b) 2D docked pose of compound **Tz21** denoting hydrophobic interactions by green amino acid residues and pie-pie interactions by green solid line.

alpha-glucosidase target.

3.3.1. Kinetics study

Inhibitory kinetic studies were performed on the most potent derivative **Tz21**. The study of lineweaver-burk plot (LB) in the presence of different concentrations of compound indicate that all the straight lines obtained intersect in second quadrant specifying mixed type of inhibition (Fig. 4.). The value of dissociation constants (Ki and Ki′) were obtained from the slope and Y-intercept from LB-plot against the different concentrations of inhibitor was 0.07 μM and 0.12 μM respectively. Thus compound **Tz21** with Ki and Ki′ values of 0.07 μM and 0.12 μM respectively showed a mixed mode of inhibition with unchanged Km, while there is decrease in Vmax. A lower value of Ki than Ki′ suggests stronger affinity of compound towards enzyme which is in relation with the stabilizing energy (−49.57 kcal/mol).

3.4. Oral glucose tolerance test

The results of oral glucose tolerance test (OGTT) of selected compounds, normal control and pioglitazone in normal rats are presented in Table 3 and Fig. 5. An increase in blood glucose level was observed in normal control group, pioglitazone and selected synthesized compounds after 2 h of oral administration of glucose (2 g/kg body weight). At 4 h, all the compounds had shown significant reduction in blood glucose level at a single dose of 36 mg/kg. Out of the seven tested derivatives, three synthesized hits i.e. **Tz10** (101.05 ± 0.70 mg/dL), **Tz17** (95.43 ± 1.33 mg/dL) and **Tz21** (95.35 ± 1.38 mg/dL) were found to cause more lowering in blood glucose level than the pioglitazone (100.53 ± 1.35 mg/dL). Other compounds (**Tz4, Tz19, Tz23 and Tz24**) have reduced the blood glucose level comparable to the standard drug, pioglitazone.

3.5. Anti-diabetic study on streptozotocin (STZ) induced diabetic rat model

Streptozotocin leads to hyperglycemic impact by destruction of pancreatic beta cells, resulting in decrease in insulin exudation and increase in blood glucose levels. Seven analogs were selected on the basis of their molecular docking scores and were investigated for their anti-diabetic activity in streptozotocin induced diabetic wistar rats. Results indicating effect on blood glucose concentration levels are

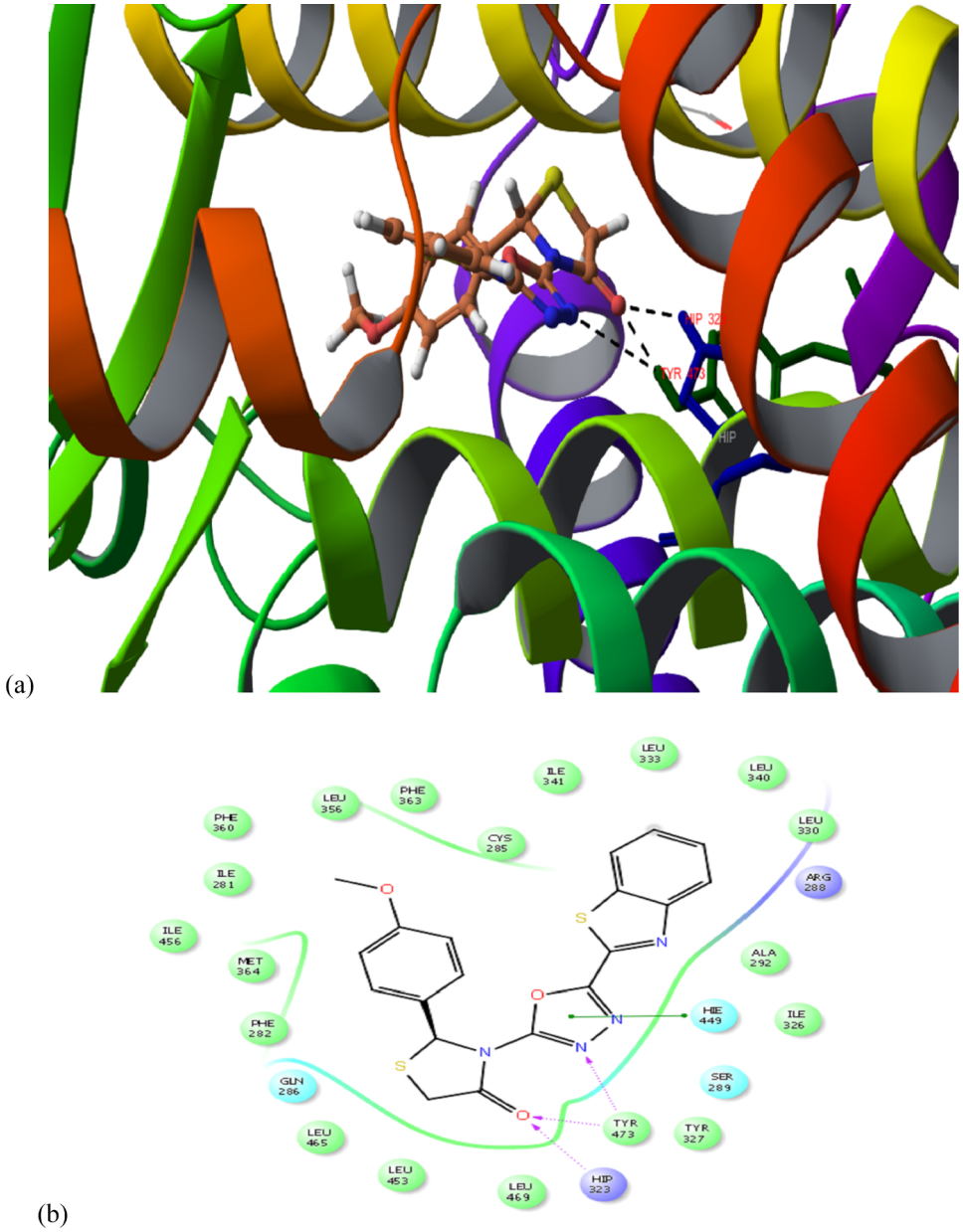


Fig. 2. (a) 3D docked pose of compound **Tz17** showing hydrogen bonds in black dotted line with Hip323 and Tyr473. Coloured by atom: Carbons: brown; Nitrogen: blue; Oxygen: red; Hydrogen: white. (b) 2D docked pose of compound **Tz17** denoting hydrophobic interactions by green amino acid residues and pie-pie interactions by green solid line.

summarized in Table 4 and Fig. 6. It was revealed that all the compounds exhibited excellent to moderate anti-hyperglycemic activity after 6 h when compared to standard drug at a single oral dose of 36 mg/kg. Three synthesized analogs **Tz21, Tz17 and Tz10** were found to be potent anti-diabetic agents then pioglitazone. Compounds **Tz21** (with diethyl amine group at para position and hydroxyl group at ortho position of phenyl ring at 2nd position of 4-thiazolidinone), **Tz17** (having methoxy substituent at para position of phenyl ring at 2nd position of 4-thiazolidinone), **Tz10** (with dimethyl amine group at para position of phenyl ring at 2nd position of 4-thiazolidinone) caused significant lowering in blood glucose level i.e. 157.15 ± 1.79 mg/dL, 154.39 ± 1.71 mg/dL, 167.36 ± 2.45 mg/dL more than the standard, 178.32 ± 1.88 mg/dL. The derivative **Tz24** showed good anti-hyperglycemic activity (134.32 ± 2.67 mg/dL) comparable to the standard drug. Three of the tested compounds, **Tz4** (160.45 ± 2.05), **Tz19** (175.50 ± 2.16 mg/dL) and **Tz23** (151.07 ± 3.92 mg/dL) exhibited moderate blood glucose level reduction.

The pharmacological results indicated that all the synthesized hybrid compounds exhibited excellent to moderate anti-diabetic effect, thus are capable of acting as a lead molecule for further development of oral anti-diabetic medicaments. Compounds **Tz21, Tz17 and Tz10** emerge as the most potent derivatives than standard and furthermore consideration of these compounds may lead to generation of more promising safer anti-hyperglycemic drugs.

3.6. Structure-activity relationship (SAR)

SAR study was done on the basis of *in vivo* anti-hyperglycemic results of the newly developed series. SAR was explored depending on the nature of substituent of phenyl ring attached to the 4-thiazolidinone scaffold. Compound **Tz21** containing electron donating groups i.e. diethyl amino at para position and hydroxyl group at ortho position showed most promising lowering in blood glucose level even more than the standard. Presence of electron donating groups i.e. methoxy and

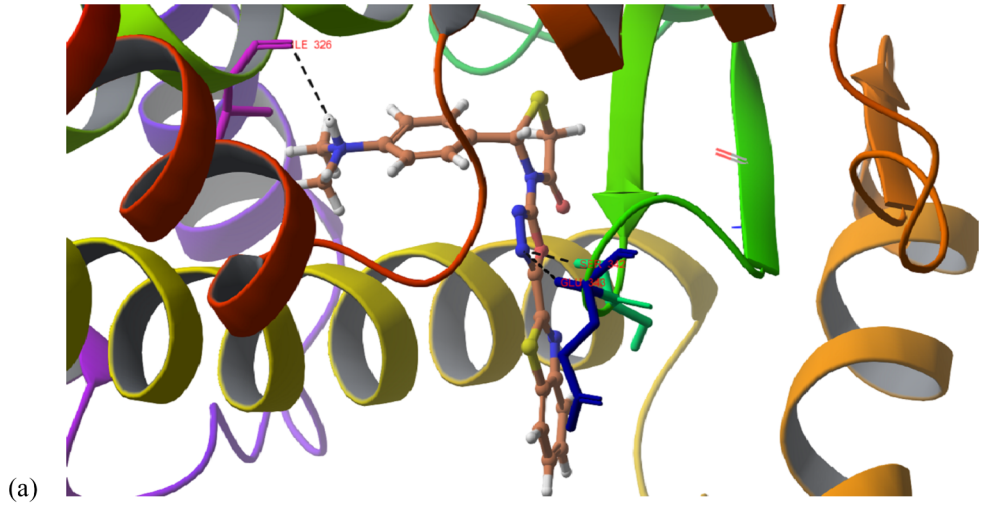


Fig. 3. (a) 3D docked pose of compound **Tz10** showing hydrogen bonds in black dotted line with Ser342, Glu343 and Ile326. Coloured by atom: Carbons: brown; Nitrogen: blue; Oxygen: red; Hydrogen: white. (b) 2D docked pose of compound **Tz10** denoting hydrophobic interactions by green amino acid residues.

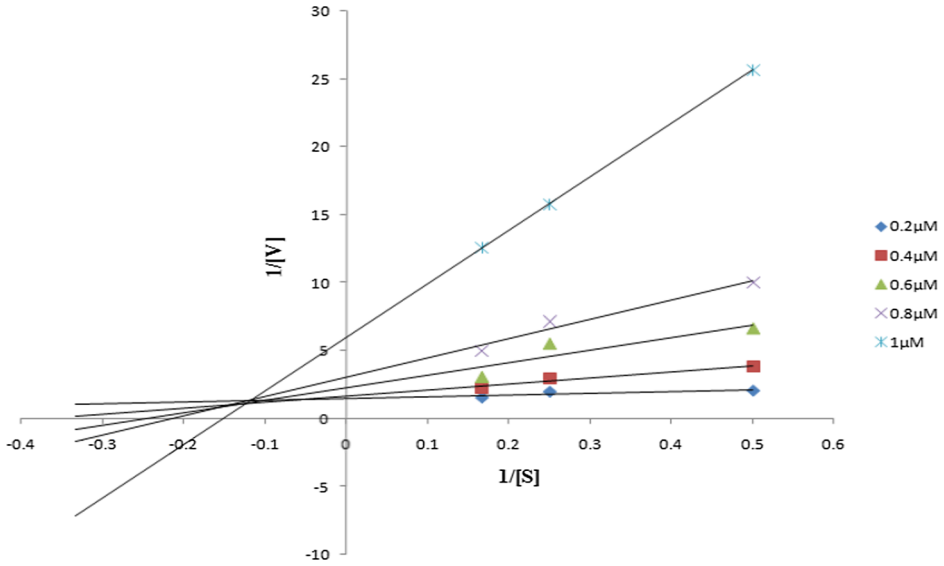


Fig. 4. Lineweaver Burk Plot of most potent compound **Tz21** in different concentrations against alpha glucosidase.

Table 3
Hypoglycemic effect of synthesized compounds and standard drug on Oral glucose Tolerance Test.

Group	Dose	0 h	2 h	4 h
Normal control	0.25%CMC	84.68 ± 1.22	182.35 ± 3.78	128.49 ± 3.57
Pioglitazone	36 mg/kg	78.41 ± 1.76	165.85 ± 3.28	100.53 ± 1.35**
Tz4	36 mg/kg	78.93 ± 0.62	164.29 ± 4.16	103.14 ± 1.27**
Tz10	36 mg/kg	77.20 ± 0.96	167.16 ± 3.05	101.05 ± 0.70**
Tz17	36 mg/kg	80.01 ± 0.86	161.75 ± 2.85	95.43 ± 1.33**
Tz19	36 mg/kg	77.66 ± 1.32	165.72 ± 2.44	106.76 ± 2.69**
Tz21	36 mg/kg	79.41 ± 1.18	163.35 ± 4.32	95.35 ± 1.38**
Tz23	36 mg/kg	80.23 ± 0.83	169.05 ± 3.16	105.21 ± 2.29**
Tz24	36 mg/kg	80.45 ± 0.22	162.67 ± 2.91	99.36 ± 0.90**

All data values are expressed as mean ± SEM from six observations (n = 6); analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, ** $p < 0.01$ versus normal control is considered as significant.

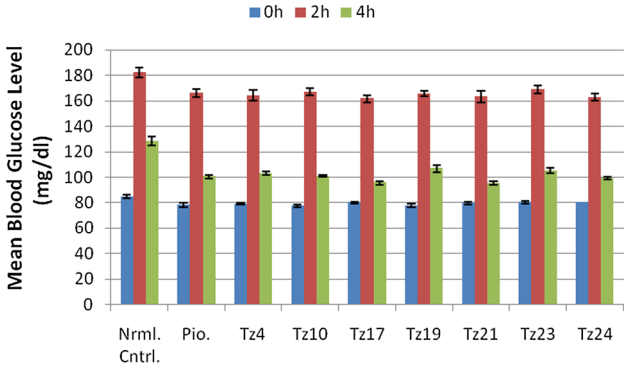


Fig. 5. Effect of the selected seven compounds and pioglitazone on OGTT in normal rats. Data are analyzed by one way ANOVA followed by Dunnett's multiple comparison test and expressed as mean ± SEM from six observations; ** indicates $p < 0.01$vs normal control, Nrml. Cntrl. Normal control, Pio. Pioglitazone.

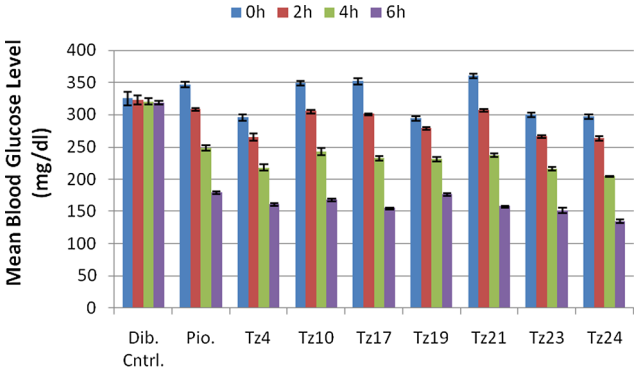


Fig. 6. Effect of the selected seven compounds and pioglitazone on streptozotocin induced diabetic rats. Data are analyzed by one way ANOVA followed by Dunnett's multiple comparison test and expressed as mean ± SEM from six observations; ** indicates $p < 0.01$vs diabetic control, Dib. Cntrl. Diabetic control, Pio. Pioglitazone.

dimethylamino at para position of phenyl ring respectively results in excellent anti-diabetic activity as shown by the prepared derivatives **Tz17** (second most potent compound) and **Tz10** (third most potent compound). Compound **Tz24** containing an oxo group as a substituent which is an electron withdrawing group also results in diminishing the blood glucose level and decrease is found to be comparable with pioglitazone. Derivative **Tz23** possessing a halogen group and an electron withdrawing group on phenyl ring showed moderate anti-diabetic effect but its effect is less when compared with standard. Moreover, presence of electron withdrawing groups in analogues **Tz4** and **Tz27** demonstrated well anti-diabetic effect but less when compared with standard and most potent compound **Tz21** of the synthesized series. Thus we can say that compounds containing electron withdrawing groups such as 4-chloro, 4-nitro and 4-trifloromethyl also displayed good anti-diabetic effect but decrease in blood glucose level was less as compared to compounds having substitution of electron donating groups on phenyl ring of 4-thiazolidinone.

4. Conclusion

A new series of twenty-eight, 3-[5-(benzo[*d*]thiazol-2-yl)-1,3,4-oxadiazol-2-yl]-2-substituted thiazolidin-4-one derivatives was synthesized and evaluated for their PPAR-gamma agonist action and inhibition activity against α-glucosidase. Molecular docking study was also performed on PPAR-gamma to understand the ligand-binding interactions of synthesized analogs. In-vivo anti-hyperglycemic activity was determined of the seven selected compounds on the basis of higher docking scores. It is noteworthy that all the compounds displayed moderate to excellent anti-diabetic effect. Three of the compounds **Tz21, Tz7 and Tz10** showed most potent activity better than of

Table 4
Antidiabetic activity of synthesized analogs and standard drug in streptozotocin induced rat model.

Group	Dose	0 h	2 h	4 h	6 h
Diabetic control	0.25%CMC	325.01 ± 10.86	323.09 ± 6.83	320.40 ± 4.92	318.73 ± 2.47
Pioglitazone	36 mg/kg	346.63 ± 4.08	308.29 ± 2.32	248.32 ± 4.53	178.32 ± 1.88**
Tz4	36 mg/kg	295.77 ± 4.82	265.19 ± 5.09	217.52 ± 5.02	160.45 ± 2.05**
Tz10	36 mg/kg	349.00 ± 4.07	305.03 ± 2.66	242.24 ± 5.73	167.36 ± 2.45**
Tz17	36 mg/kg	351.99 ± 5.45	300.75 ± 1.63	232.33 ± 3.48	154.39 ± 1.71**
Tz19	36 mg/kg	294.68 ± 3.50	278.83 ± 2.46	231.21 ± 3.64	175.50 ± 2.16**
Tz21	36 mg/kg	360.18 ± 3.90	307.19 ± 2.20	237.10 ± 3.25	157.15 ± 1.79**
Tz23	36 mg/kg	299.54 ± 3.29	266.05 ± 2.11	216.00 ± 2.20	151.07 ± 3.92**
Tz24	36 mg/kg	297.37 ± 3.66	262.84 ± 3.86	203.63 ± 0.93	134.32 ± 2.67**

All data values are expressed as mean ± SEM from six observations (n = 6); analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. ** $p < 0.01$ versus diabetic control is considered as significant.

standard drug. ADME screening based on Rule of five demonstrated that all the synthesized hybrid analogs possessed drug-like properties to become biologically active molecules. SAR developed indicated that compounds with electron donating groups as substitution on phenyl ring of 4-thiazolidinone display pronounced anti-diabetic effect as compared to compounds with electron withdrawing group. The seven compounds selected (based on the basis of higher docking scores against 2QMJ (alpha-glucosidase) were also studied for their inhibition activity. Results of assay demonstrated analogue **Tz21** to be the most promising compound. Enzyme kinetics study of most potent compound i.e. **Tz21** revealed its mode of inhibition as mixed type. Also, on comparison of stabilizing energy with inhibition affinity compound **TZ21** was found to be more stable than standard. The benzothiazole-oxadiazole-4-thiazolidinone hybrids discovered in this study seem to have the potential to become as a valuable lead molecule in designing new compounds with potential anti-diabetic activity.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.025.

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