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Original article

Novel thiazolidinedione-5-acetic-acid-peptide hybrid derivatives as potent antidiabetic and cardioprotective agents

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ABSTRACT

Thiazolidinediones (TZDs) are one of the important clinically established antidiabetic agents. Amino-acid and peptides have an advantage of better target selectivity and specificity. As hybrids, they also improved absorption and showed better bioavailability, which in turn makes them safer. Hence, here an effort has been made to synthesize hybrids of thiazolidinedione with amino-acids and peptides and evaluate their antidiabetic and cardioprotective effect in streptozotocin-nicotinamide (STZ-NA) induced Type 2 diabetes mellitus (T2DM) rat models.

A series of 14 thiazolidinedione-5-acetic acid hybrids with of different amino-acids and peptide combinations were synthesized, characterized and further screened for antidiabetic and cardioprotective activity.

Among all, six compounds T1 (SSDMA1), T4 (SSDMA4), T5 (SSDMA5), T7 (SDMA13), T9 (SSDMA15) and T13 (SSDMA49) showed better antioxidant activity and comparable % glucose uptake by yeast cells.

Hence, the in vivo antidiabetic screening was done for these six compounds. Among all six T1, T7, T13 showed significant blood glucose level decrease compared to standard pioglitazone HCl. Also T1, T7 and T13 showed better antioxidant activity with lower IC_{50} value than standard ascorbic acid, and hence in vivo cardioprotective studies were done for these. The ECG studies showed that T1 (SSDMA1) and T7 (SSDMA13) were better effective than SDMA49 (T13) in restoring the normal functioning of the heart, thus may help in preventing the development of diabetic cardiomyopathy (DCM) and controlling T2DM.

1. Introduction

Fast growing industrialization and sedentary lifestyles have raised the incidences of Type 2 diabetes mellitus (T2DM), which is engulfing the whole world like an epidemic. T2DM is a chronic metabolic disorder, accompanied with numerous complications which make the management of T2DM a big challenge to the medical field. Prolonged and uncontrolled T2DM accelerates the development of many micro and macrovascular complications which affect major vital organs of the body, the heart being one of them. It causes cellular level changes in the myocardium of the diabetic patients resulting in various structural changes, giving rise to diabetic cardiomyopathy [1]. Thiazolidinediones are the most extensively used oral hypoglycemic agent besides sulfonylureas [2], which control the increasing blood glucose level by binding and activating PPARγ.

Presently the pharmaceutical market is targeted towards amino-acids and peptides as potent therapeutics are being preferred over other drug molecules due to their target specificity, safety profiles, reduced immunogenicity, higher water solubility and improved bioavailability with additional delivery method [3]. Peptides like exenatide (Incretin mimetics), pramlintide (Amylin derivatives) Lixisenatide, Liraglutide have been approved for the treatment of diabetes mellitus [4]. Lixisenatide also showed a cardioprotective effect in rodent models [5]. Kim et al. studied that a hexapeptide (Gly-Ala-Gly-Val-Gly-Tyr) showed improvement in glucose transport and also exerted beneficial lipid metabolic effects [6]. Amino acids like glycine mostly reduce the superoxide anion radical which decreases the protein carbonyl and lipid per-oxidation and protects vascular tissues against oxidative

Abbreviations: BGL, blood glucose level; CPE, chlorophosphate ester; DCM, diabetic cardiomyopathy; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; DNSA, 3,5-dinitro salicylic acid reagent; ECG, electrocardiogram; IR, infra-red; PQRST, wave pattern; STZ, streptozotocin; TLC, thin layer chromatography; TZD, thiazolidinedione.

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stress by restoring the glutathione biosynthesis and which in turn increased the availability of nitric oxide (NO) and provided cardioprotection in sucrose fed rat [7]. Ajjan and Grant concluded that TZDs have a wide range of activity and seemed to be able to alter many CV risk factors by ameliorating insulin resistance and enhancement of hyperglycemia, in addition to its TZDs also provided a positive effect on lipid profile, blood pressure, inflammation and coagulation [8]. Sekhar et al., Bahmani et al., McCarty and DiNicolantonio studied various beneficial effects of glycine [9–11].

The available information on thiazolidinediones, glycine hybrid molecules and their therapeutic capability of treating diabetes mellitus and providing cardioprotection [4,7] made us take this challenge to develop newer hybrids thiazolidinedione-5-acetic acid–amino acid and peptide hybrid methyl esters mostly of glycine, which might be effective as potent antidiabetic with cardioprotection.

2. Materials and method

All the chemicals and solvents used were of Rankem, Merck, and Sigma–Aldrich. Progress in the reactions was monitored by thin-layer chromatography (TLC) on silica gel plates (60 F254) HX617152, Merck, Darmstadt, Germany. Spots were visualized using Ninhydrin solution, ultraviolet light or iodine chamber in different solvent systems. The peptide synthesis was done using Liquid phase method as it is cost effective, more feasible due to use of very common solvents and reagents mentioned in the schemes below and less tedious in laboratory scale to workup. Melting points were determined using automated OPTIMELT apparatus (Stanford Research System, Sunnyvale, CA) and were uncorrected. FTIR spectroscopy was recorded on FT-IR 8400S (SHIMADZU Kosyoto Japan). ^{1}H NMR spectra were determined in DMSO-d6 solution using 400 MHz (Varian). Proton chemical shifts were measured in δ, using tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in ppm. Spin multiplicities are given as singlet (s), doublet (d), triplet (t) and multiplet (m) as well as broad (b), MALDI-TOF was done using UltrafleXtreme, MALDI-TOF/TOF (Bruker, Germany) at CRF, IIT, Kharagpur, India.

Animals were obtained from Central Animal Facility, BIT Mesra. The study was approved by Institutional animal ethical committee, Department of Pharm. Sciences and Technology, Birla Institute of Technology, Mesra (Approval No: BIT/PH/AEC/21/2012).

2.1. Synthesis

The synthesis was performed in three parts:

(a) Synthesis of 2,4-thiazolidinedione-5-acetic acid (I)
(b) Peptide synthesis (II. IIIa–IIIf, IVa–IVf, Va–Vf, VIa–VIf, VIIa–VIIf)
(c) Synthesis of hybrid compounds (VIIIa–VIIIf, IXa–IXb, Xa–Xb).

2.1.1. Synthesis of thiazolidinedione-5-acetic acid (Scheme 1) [12,13]

3.80 g (50 mmol) of thiourea and 4.90 g (50 mmol) of maleic anhydride were reacted in presence of 25 mL of concentrated hydrochloric acid (conc. HCl) refluxed for 5 h, followed by cooling to obtain white crystals of 2-(2,4-dioxothiazolidin-5-yl)acetic acid **(I)**. White crystals, 69.95%, mp: 168 °C, R_f: 0.86 ($CHCl_3$:CH_3OH::4:9); IR (KBr) cm^{-1}: 3047–3117 (ν_{O-H} stretching), 1700 ($\nu_{C=O}$ stretching), 1406 (ν_{H-C-S} stretching), 1047 (ν_{C-N} stretching). ESI-MS (m/z): 174.6 $[M-H]^+$; ^{1}H NMR (400 MHz, δ, ppm. D_2O) 3.09 (d, $J = 5.6$ Hz, 2H), 4.632 (t, $J = 6$ Hz, 1H) **A1**.

2.1.2. Peptide synthesis (Scheme 2) [14,15]

Step 1: Amino protection using phthalic anhydride as carried out by following the reported method of Tella et al. and Zav'yalov et al. to obtain white needle-shaped crystals of phthaloyl glycine [14,15].

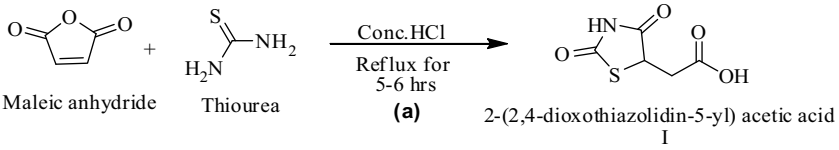
Phthaloyl glycine (II): white fine needle-shaped crystals, 80.5%, mp: 196.6 °C, R_f: 0.85 (BAW::4:1:1), IR (KBr, cm^{-1}) 3564, 1772, 1729, 1715, 1219, 997.

Step 2: Carbonyl protection (IIIa–IIIf) [16].

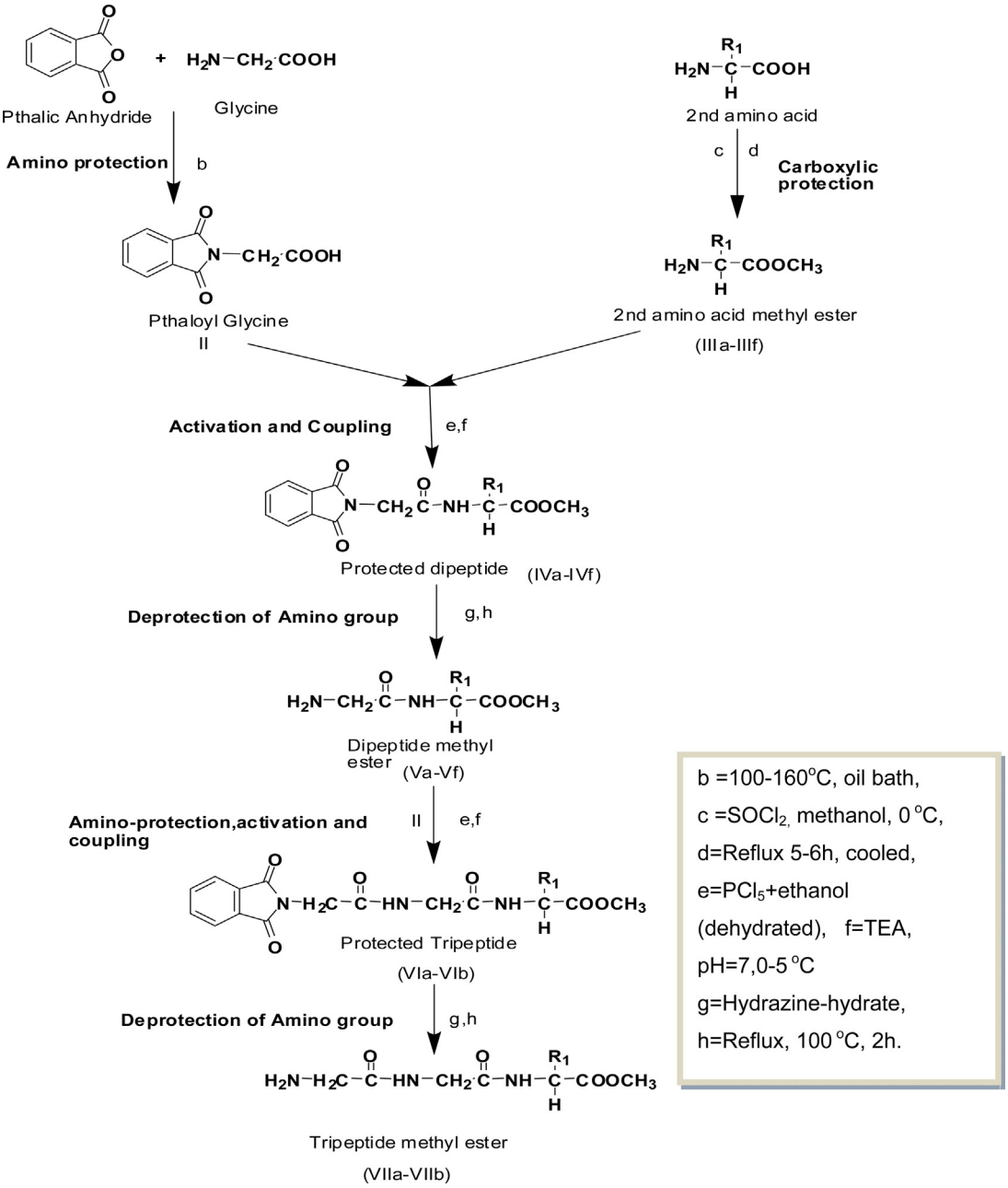
Different amino acids methyl esters were prepared as shown in Fig. 1 and Table 1 following the reported by Li et al. in which amino acids (1 equiv, 13.79 mmol) were dissolved in 30 mL of methanol, followed by addition of $SOCl_2$ at 0 °C with continuous stirring, to form a clear solution, refluxed for 6 h and cooled. The precipitate so obtained was washed with ether:methanol::5:1, and yielded (IIIa–IIIf).

Phe methyl ester HCl (IIIa): white solid, 75%, mp 158–160 °C, $R_f = 0.87$ (50% propanol:water); IR (KBr, cm^{-1}) 3050, 3119, 1752, 1274.
Gly methyl ester HCl (IIIb): white solid, 90%, mp 174–175 °C, $R_f = 0.73$ (50% propanol:water); IR (KBr, cm^{-1}) 3015, 2853, 1744, 1268.
Meth methyl ester HCl (IIIc): yellowish white solid, 65%, mp 148–150 °C, $R_f = 0.83$ (50% propanol:water); IR (KBr, cm^{-1}) 3050, 3119, 1751, 1257, 900.
Leu methyl ester HCl (IIId): white solid, 79%, mp 153–155 °C, $R_f = 0.70$ (50% propanol:water); IR (KBr, cm^{-1}) 3017, 2855, 1742, 1269.
Ser methyl ester HCl (IIIe): white solid, 84%, mp 188–190 °C, $R_f = 0.77$ (50%propanol: water); IR (KBr, cm^{-1}) 3372, 3015, 2852, 1726, 1272.
Tyr methyl ester (IIIf): white solid, 78%, mp 161–163 °C, $R_f = 0.68$ (50% propanol:water); IR (KBr, cm^{-1}) 3216, 3112, 3054–3122, 1735, 1247.

Steps 3–4: Activation of –COOH group, coupling and deprotection of the amino group [17,18] was done using chlorophosphate reagent (CPE) following the method reported by Samanta et al. to obtain dipeptide methyl esters (Va–Vf) and tripeptide methyl ester HCl (VIIa–VIIb). 10 mL of CPE reagent (equimolar quantity of anhydrous ethanol and phosphorous pentachloride 12.50 g, and 0.06 mol were reacted with constant stirring, in anhydrous condition at 0–5 °C) was added to 1.03 g (5 mmol) phthaloyl glycine followed by addition of single amino acid esters (IIIa–IIIf, 5 mmol) to form a clear solution. Triethylamine (TEA) was added to the above mixture (pH 7) and then poured over crushed ice and left for 6 h at 0 °C. to obtained IVa–IVf. To which .0.01 mL of hydrazine hydrate (80%) in 20 mL of ethanol was added and heated for 2 h, cooled, acidified with conc. HCl cooled and left overnight allowing phthaloyl-hydrazide crystals to separate and removed by filtration. Excess solvent was removed by vacuum distillation followed by



Scheme 1. Schematic diagram for the synthesis of thiazolidinedione-5-acetic acid.



Scheme 2. General scheme for synthesis of peptides.

crystallization from aqueous ethanol to obtain dipeptide methyl esters (Va–Vf) and tripeptide methyl esters (VIIa–VIIb).

Gly-Phe methyl ester HCl (Va): white solid, 73%, mp: 114–116 °C, R_f = 0.58 (50% propanol:water); IR (KBr, cm^{-1}) 3054, 3140, 1615, 1740–1764, 777.

Gly-Gly methyl ester HCl (Vb): White solid, 65%, mp 153–155 °C, R_f = 0.45 (50% propanol:water); IR (KBr, cm^{-1}) 3035–3110, 1590, 1725–1742, 783.

Gly-Meth methyl ester HCl (Vc): white crystals, 60%, mp 142–143 °C, R_f = 0.50 (50% propanol:water); IR (KBr, cm^{-1}) 3058, 3117, 1623, 1735–1740, 861.

Gly-Leu methyl ester HCl (Vd): pale yellowish crystals, 68%, mp 193–195 °C, 0.42 (50% propanol:water); IR (KBr, cm^{-1}) 3150, 1630, 1735, 756.

Gly-Ser methyl ester HCl (Ve): white solid, 54%, mp 164–166 °C, R_f = 0.61 (50% propanol:water); IR (KBr, cm^{-1}) 3078, 1601, 1755, 786.

Gly-Tyr methyl ester HCl (Vf): white solid, 71%, mp 200–202 °C, R_f = 0.46 (50% propanol:water); IR (KBr, cm^{-1}) 3041,31451674,1725, 1762, 864.

Gly-Gly-Phe methyl ester (VIIa): White fluffy crystals, 66%, mp 164–166 °C, R_f = 0.35 (50% propanol:water); IR (KBr, cm^{-1}) 3055, 3210, 1610, 1735, 1747, 798.

Gly-Gly-Gly methyl ester (VIIb): White fluffy crystals, 70%, mp 141–143 °C, R_f = 0.42 (50% propanol:water); IR (KBr, cm^{-1}) 3063, 3195, 1621, 1738, 1754, 801.

2.1.3. Synthesis of Hybrid compounds: (VIIIa–VIIIf), (IXa–IXf) and (Xa–Xb) [14]

1.0 g (1 equiv, 0.005 mmol) of 2,4-thiazolidinedione-5-acetic acid was dissolved in 10 mL of dioxane:water (1:1), 1 mL of 1 N NaOH with stirring for 20 min. Further 0.6 mL (1.6 equiv, 0.008 mmol) of $SOCl_2$ was added at 0–5 °C with continuous stirring for 4 h at room temp till a clear solution was formed. To the

above clear solution single amino acid esters (IIIa–IIIf), dipeptide methyl esters (Va–Vf) and tripeptide methyl esters (VIa–VIb) were added, refluxed for the 1 h excess of solvent was removed to form VIIIa–VIIIf, IXa–IXb and Xa–Xb respectively. The reaction scheme has been outlined in Scheme 3.

SSDMA1 (VIIIa): white crystals, 78%, mp 166–168 °C, R_f = 0.73 ($CHCl_3$:methanol) 4::6, IR (KBr, cm^{-1}) 3054, 3122, 1700, 1735, 1350, 923; ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.02 (s, 2H), 7.2–7.9 (m, 5H), 3.68 (s, 3H), 4.67 (t, 1H), 3.43, 2.99 (d, 2H), 4.08 (t, 1H), 3.04–3.40 (m, 1H), 2.89 (d, 2H).
EI (mass spectroscopy) *m/z*: 337.2 (M+1), 321.1 (M−NH_3), MALDI-TOF 335.5 [M−1], 336.5 [M] and 337.5 [M+1].

SSDMA2 (VIIIb): white solid, 85%, mp 158–160 °C, R_f = 0.74 ($CHCl_3$:methanol) 4::6, IR (KBr, cm^{-1}) 3024, 1699, 1744, 915; ^{1}H NMR (400 MHz, ppm DMSO): δ 12.42 (s,1H), 11.93 (s,1H), 3.61 (s,3H), 4.64 (t,1H), 2.99 (d, 2H); EI (mass spectroscopy) *m/z*: 247.2 (M+1).

SSDMA3 (VIIIc): pale yellowish white solid, 72%, mp 130–131 °C, R_f = 0.68 ($CHCl_3$:methanol) 4::6, IR (KBr, cm^{-1}): 3110, 1746, 1735, 796. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.12 (s,1H), 8.43 (s,1H), 3.85 (s,3H), 4.63 (t,1H), 2.99 (d,2H), 4.25 (t,1H), 2.47 (m,2H), 2.63 (t,2H), 2.01 (s,3H), 3.07 (d,2H); EI (mass-spectroscopy) *m/z*: 321.2 (M+1), 289 (320−OCH_3), 260.1 (289−CO), 185 [260−(CH_2–CH_2–SH–CH)], 156 (185−CO), 128 (158−$NHCH_2$) 164, 116, 102, 74 are due to cleavage of the heterocycle.

SSDMA4 (VIIId): white solid, 65%, mp 143–145 °C, R_f = 0.76 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3391, 1683, 1731, 816; ^{1}H NMR (400 MHz, ppm. DMSO): δ, 12.89 (s,1H), 12.01 (s,1H), 4.65 (t,1H), 4.61 (d,2H), 3.15 (s,3H), 2.42 (t,2H), 1.2 (m,1H), 1.15 (d,3H).; EI (mass spectroscopy) *m/z*: 302 (M), 301.1 (M−1), 271.2 (302−OCH_3), 243 (271−CO), 188 [243−$CH_2CH(CH_3)CH_3$], 145.1 (188−CONH). 174.1, 126.0, 116, 74 are due to cleavage of the heterocycle.

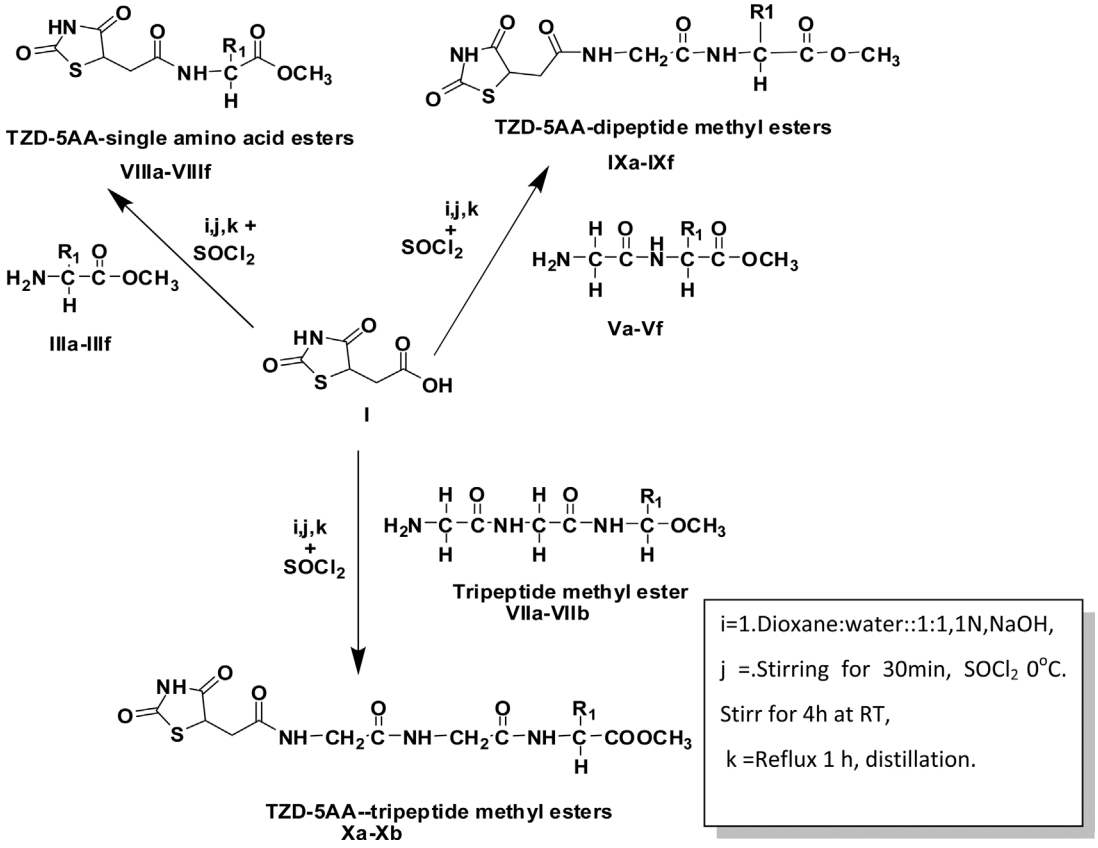
SSDMA5 (VIIIe): white solid, 77%, mp 171–172 °C, R_f = 0.58 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3121, 1620, 1700, 922. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.73 (s,b,1H), 11.95 (s,b, 1H), 7.0–7.9 (m,5H), 4.63 (t,1H), 4.65 (s,1H), 3.512 (s,3H), 3.039 (d,2H), 2.30 (t,1H), 2.95 (d,2H); EI (mass spectroscopy) *m/z*: 275.1 (M−1), 244 (275−OCH_3), 216.4 (244−CO).
MALDI-TOF: 275.4 [M−1] and 276 [M].

SSDMA6 (VIIIf): white solid, 69%, mp 164–166 °C, R_f = 0.58 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3121, 1620, 1700, 922; ^{1}H NMR (400 MHz, ppm. DMSO): δ 11.9 (s, 1H), 11.5.5 (s, b, 1H), 9.50 (s, b,1H), 7.0–7.9 (m,5H), 4.68 (t, 1H), 2.981 (d,2H), 4.08 (t, 3H), 3.9 (d,2H), 3.432 (s, 3H). EI (mass-spectroscopy) *m/z*: 352.8 (M+1), 322.2 (353−OCH_3), 294.1 (322−CO), 187 (294−$CH_2C_6H_5OH$).

SSDMA13 (IXa): white solid, 71%, mp 153–155 °C, R_f = 0.61 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}) 3046, 1698, 1735, 922. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.01 (s,1H), 10.95 (s,1H), 7.927 (s,1H), 7.855 (m,5H), 3.021 (d,2H), 4.64 (t,1H), 3.671 (s,3H). EI (mass-spectroscopy) *m/z*: 393 (M), 243 [393−(OCH_3,–CO,–$CH_2C_6H_5$)], 213 (243−$NHCH_3$), 186 (213−CO), 113 [186−(–CH_2,–NH_3,–CH_2CO)], 116, 102, are due to cleavage of the heterocycle. MALDI-TOF: 393.7 [M+1].

SSDMA14 (IXb): white solid, 79%, mp 155–157 °C, R_f = 0.58 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3024, 1699, 1744, 915. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.018 (s,1H), 8.319 (s, 1H), 4.63 (t,1H), 3.005 (d,2H), 4.039 (m,1H), 3.63 (s,3H). EI (mass spectroscopy) *m/z*: 303 [M], 226 [303−(OCH_3,CO,H_2O)], 168 [226−($NHCH_2$,CO)], 138 [168−$NHCH_2$], 110 [138−CO], 116, 102, 74 are due to cleavage of the heterocycle.

SSDMA15 (IXc): white solid, 66%, mp 135–137 °C, R_f = 0.70 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3160–3200, 1695,



Scheme 3. General scheme for the synthesis of hybrids [19].

1728, 918. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.015 (s,1H), 10.215 (s,1H), 8.292 (s,1H), 4.64 (m,1H), 3.52 (d,2H), 3.05 (d,2H), 4.59 (t,1H), 2.45 (t,2H), 1.95 (m,2H), 1.99 (s,3H), 3.65 (s,3H). EI (mass spectroscopy) *m/z*: 377 [M], 318 [377−(OCH_3, CO)], 243 [318−$CH_2CH_2SHCH_3$], 226 [243−NH_3], 183 [226−(C_2H_2OH)], 113 [183−(−CONH,−$CHCH_2$)], 116, 102, 74 are due to cleavage of the heterocycle.

SSDMA16 (IXd): white solid, 66%, mp 137–138 °C, R_f = 0.67 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3048, 1696, 1734, 788. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.07 (s,1H), 8.89 (s,b,1H), 8.85 (s, b,1H), 4.68 (t,1H), 4.09 (d,2H), 4.61 (t,1H), 2.48 (d,2H), 4.05 (d,2H), 1.21 (m, 1H), 1.75 (d,3H). EI (mass spectroscopy) *m/z*: 359 [M], 243 [M−(−OCH_3,−CO,−$CH_2CH(CH_3)CH_3$)], 187 [243−(−NHCH,−CO)], 160 [187−(CHN)], 118 [160−(−CH_2CO)], CH_2CO)], 102 [118−(−O)], 104, 74 are due to cleavage of the heterocycle.

SSDMA17 (IXe): white solid, 66%, mp 153–151 °C, R_f = 0.67 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3050–31161, 1699, 1740, 921. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.02 (s, 1H), 8.29 (s, 1H), 7.9 (s, 1H), 4.612 (t, 1H), 3.69 (s, 3H), 3.21 (s, 2H), 3.33 (t, 1H), 4.64 (s, 1H), 3.05 (d, 2H). EI (mass-spectroscopy) *m/z*: 333.1 [M], 274 [M−(−OCH_3,−CO)], 243 [M−(CH_2OH)], 163 [243−($CH_2CONHCH$)], 148 [163−(NH)], 106 [148−(CO,−CH_2)], 116, 104, 102, 74 are due to cleavage of the heterocycle.

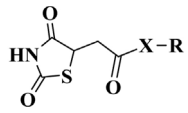
SSDMA18 (IXf): white solid, 66%, mp 166–168 °C, R_f = 0.59 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}) IR (KBr, ν, cm^{-1}): 3050, 1620, 1700, 922. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.01 (s,1H), 8.291 (m,1H), 8.044 (m,1H), 4.64 (t,1H), 3.45 (d,2H), 7.862 (s,1H), 7.63–7.21 (m,5H), 3.05 (s,2H), 3.63 (s,3H), 3.03 (d, 2H,−CH_2 of tyrosine).

EI (mass spectroscopy) *m/z*: 409 [M], 243 [M−(−$COOCH_3$,−$CH_2C_6H_5OH$)], 163 [243−(−NHCH,−C═O,−$NHCH_2$,−C═O)], 115 [163−(C═O)], 102 [115−(−CH)].

SSDMA49 (Xa): white solid, 66%, mp 138–140 °C, R_f = 0.83 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3117, 1599, 1700, 786. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.51 (s,1H), 12.01 (s, 1H), 11.89 (s,1H), 4.627–4.702 (m,1H), 3.01 (d,2H), 4.09 (s,2H), 4.06 (s,2H), 4.52 (t,1H), 3.06 (d,2H), 7.33–7.44-7.19 (m,5H), 3.28 (s,3H).

EI (mass-spectroscopy) *m/z*: 450 [M], 254 [450−(−OCH_3,−CO,−$CH_2C_6H_5$,−NHCH,−H_2O)], 183 [254−(−$NHCH_2CO$,−CH_2)], 127 [183−(CONH,−CH_2)], 114 [127−(−CH)], 116, 104, 102, 74 are due to cleavage of the heterocycle.

SSDMA50 (Xb): white solid, 66%, mp 141–143 °C, R_f = 0.83 ($CHCl_3$:methanol) 4::6, IR (KBr, ν, cm^{-1}): 3037, 1606, 1696, 903. ^{1}H NMR (400 MHz, ppm. DMSO): δ 12.02 (s,1H), 8.29 (m,1H), 7.45 (s,1H), 7.33 (s,1H), 4.642–4.612 (m,1H), 3.61 (d,2H), 3.911 (s,2H), 3.05 (s,2H), 3.02 (s,2H), 3.6 (s,3H–OCH_3).



X=Single amino acid methyl ester,Dipeptide methyl ester Tripeptide methyl ester

Fig. 1. General structure of hybrids.

EI (mass spectroscopy) *m/z*; 360 [M], 345 [360−(−CH_3)], 183 [360−(−COO,−$NHCH_2CO$,−H_2O,−CONH)], 169 [183−(−CH_2)], 112 [169−(−CONH,−CH_2)], 116, 104, 102, 74 are due to cleavage of the heterocycle.

2.2. Pharmacological activity

Pharmacological activity has been divided into four subparts as follows.

2.2.1. In vitro antioxidant study (DPPH scavenging activity)

The free radical scavenging activity was measured in vitro by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay using different concentrations (10–250 μg/mL) of test drugs (T1–T13) in methanol and 1 mL of the (0.3 mM) DPPH solution. The absorbance was measured at 517 nm following the method described by Blois, and the IC_{50} was reported [20]. All analyses were performed in triplicates.

2.2.2. In vitro antidiabetic activity (glucose uptake by yeast cells)

Yeast cells were prepared according to the method reported by Cirillo in 1963 [21]. Different concentrations of test drug were freshly prepared (10, 20, 40, 80, 100, 200 μL/mL). 3,5-Dinitro salicylic acid (DNSA) reagent was added and absorbance measured at 540 nm, pioglitazone HCl was used as standard drug [22].

2.2.3. Acute toxicity study

The acute toxicity study of the thiazolidinedione-5acetic acid hybrids was performed according to the OECD guidelines in adult female albino mice (Swiss strain) weighing between 25 and 30 g. 6 mice received a single dose of 300 mg/kg body weight (b.w), orally of 3 subseries of synthesized drugs.

2.2.4. In vivo antidiabetic activity

2.2.4.1. Induction of diabetes in Wistar (male) rats. Wistar (male) rats weighing about 160–250 g was used. Animals were maintained at 22 ± 2 °C with 12 h light:12 h dark cycle. The rats were provided commercial standard pelleted diet and tap water ad libitum. Each experimental group consisted of 4 animals each.

Table 1
List of the different amino acids and peptides attached to 2,4-thiazolidinedione-5-acetic acid and their codes as used in the pharmacological activity.

Compound	Codes used in pharmacological activity	Molecular formula	Molecular weight	X	R
SSDMA1 (VIII a)	T1	$C_{15}H_{16}N_2O_5S$	336.36	Phe	−OCH_3
SSDMA2 (VIII b)	T2	$C_8H_{10}N_2O_5S$	246.03	Gly	−OCH_3
SSDMA3 (VIII c)	T3	$C_{11}H_{16}N_2O_5S_2$	320.39	Met	
SSDMA4 (VIII d)	T4	$C_{14}H_{18}N_2O_5S$	302.35	Leu	−OCH_3
SSDMA5 (VIII e)	T5	$C_9H_{12}N_2O_6S$	276.04	Ser	−OCH_3
SSDMA6 (VIII f)	T6	$C_{15}H_{16}N_2O_6S$	352.05	Tyr	−OCH_3
SSDMA13 (IX a)	T7	$C_{17}H_{19}N_3O_6S$	393.41	Gly-Phe	−OCH_3
SSDMA14 (IX b)	T8	$C_{10}H_{13}N_3O_6S$	303.29	Gly-Gly	−OCH_3
SSDMA15 (IX c)	T9	$C_{13}H_{19}N_3O_6S_2$	377.44	Gly-Met	−OCH_3
SSDMA16 (IX d)	T10	$C_{14}H_{21}N_3O_6S$	329.40	Gly-Leu	−OCH_3
SSDMA17 (IX e)	T11	$C_{11}H_{15}N_3O_7S$	333.32	Gly-Ser	−OCH_3
SSDMA18 (IX f)	T12	$C_{17}H_{19}N_3O_7S$	409.41	Gly-Tyr	−OCH_3
SSDMA49 (X a)	T13	$C_{19}H_{22}N_4O_7S$	450.47	Gly-Gly-Phe	−OCH_3
SSDMA50 (X b)	T14	$C_{12}H_{16}N_4O_7S$	360.34	Gly-Gly-Gly	−OCH_3

Selected rats were kept in overnight fasting for 12 h before induction. A single intraperitoneal (i.p) injection of streptozotocin (STZ, 60 mg/kg, body weight (b.w), dissolved in freshly prepared 0.05 M citrate buffer (pH = 4.5)) was given 15 min after i.p administration of nicotinamide (120 mg/kg, b.w, dissolved in normal saline). Blood glucose level was monitored by tail prick method consecutively on day 3 and day 7 using AccuSure Glucometer, Medical Technology Promedt Consulting, Germany. Animals with high blood glucose level between 200 and 350 mg/dL in fasting were considered to have experimental diabetes [23–25].

The animals were divided into five groups ($n = 4$). G-I (Normal Control), G-II (Diabetic Control), G-III (Diabetic+Pioglitazone), G-IV (Diabetic+T1), G-V (Diabetic+T4), G-VI (Diabetic+T5), G-VII (Diabetic+T7), G-VIII (Diabetic+T9) and G-IX (Diabetic+T13). All animals received 30 mg/kg b.w. of the standard and test drugs orally for 14 days and change in the serum blood glucose level was observed on the day 0, day 7, day 14 of treatments.

2.2.5. In vivo cardioprotective activity

Animals from groups I, II, III, IV, VII, and IX were selected for the cardioprotective studies and the ECG was recorded using Biopac Student Lab PRO System. The data and power spectrum analyses were done by AcKnowledge 4 software (Biopac System Incorporation, USA) [26,27].

3. Result and discussion

3.1. Chemistry

As per the SAR of the TZDs, for potent Antidiabetic activity, a molecule should have a polar TZD ring, hydrophobic trunk, 2C atom linker and hydrophobic tail. Here a small library of 14 Thiazolidinedione-5-acetic acids hybrids was synthesized retaining the head and trunk and replacing the hydrophobic tail with highly hydrophobic amino acids (phenylalanine, methionine, and leucine) and less hydrophobic amino-acid (glycine and tyrosine) and also a polar amino acid serine methyl esters.

The synthesis of the hybrids was performed as outlined in Materials and methods following modified Vertesaljai et al. method [19].

The characterization and spectral data of hybrids of peptides are critical and complex. Some have been discussed as under [28–32].

1. In the case of hybrids, a much stronger band was observed around 3113–3310 cm^{-1} due to –NH stretching of 2,4 thiazolidinedione moiety and –NH stretching of the secondary amides. Strong absorption bands were seen at 1585–1700 cm^{-1} due to –C═O stretch of secondary amide and strong and intense bands at 1720–1770 cm^{-1} were noticed due to the –C═O stretch of esters as well as –C═O stretch of the 2,4-thiazolidinedione-5-acetic acid moiety. Sharp bands at 1565–1475 cm^{-1} were seen distinctly due to –NH deformation of secondary amides. In hybrids containing methionine a weak band was seen at 2482 cm^{-1}.
2. δ values of 11.5–12.5 correspond to the singlet, broad peak of –NH proton of thiazolidinediones. δ 8.00–10.0 correspond to the proton of the amide bonds (–CONH).

   Phenolic protons were observed at δ 9.56 and 11.0 as in SSDMA6 and SSDMA18 respectively. Sharp peaks at δ 4.64 correspond to the proton of –OH in SSDMA5 and SSDMA17 due to the presence of serine combinations.

   Aromatic protons were seen as multiplets around δ 7.1–7.8 in SSDMA1, SSDMA13, SSDMA49, SSDMA6, and SSDMA18. The protons of the methyl ester (–OCH_3) were merged and observed as sharp peaks at δ 3.5 to 3.9.
3. Fragmentation pattern of peptides are unique and follow a specific pattern as under:

   Here the –COOH is protected as methyl ester, hence firstly –OCH_3 (MW = 31) is lost, followed by loss of –CO (MW = 28), if any branching is present, then the loss of the –CH_2R' (R′ = aliphatic chain, aromatic chain,-SH, etc.) occurs. Further there was loss of –NH = CHR″ (R″ = H,-alkyl, etc.), then –CO (MW = 28) and this process get repeated. Sometimes small molecules like: H, H_2O, and NH_3 were also released. Dipeptides and tripeptides followed the above fragmentation pattern as shown in Fig. 2.

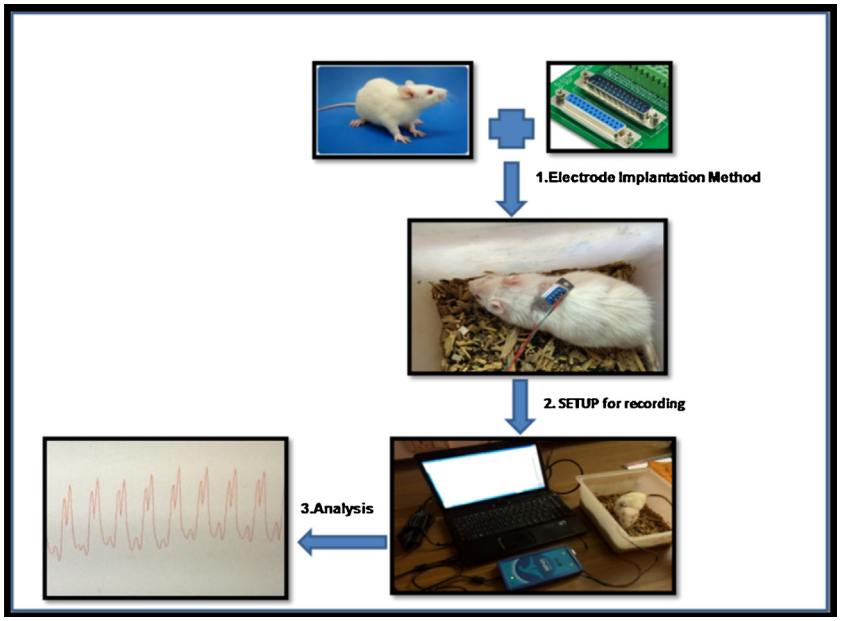


Fig. 2. The ECG recording for normal control, diabetic control, standard and the three test groups (T1, T7, T13) for 1800 s using BIOPAC MP 45 data acquisition unit and associated Laboratory software (BSL PRO), Biopac Systems Incorporation (MS) U.S.A.

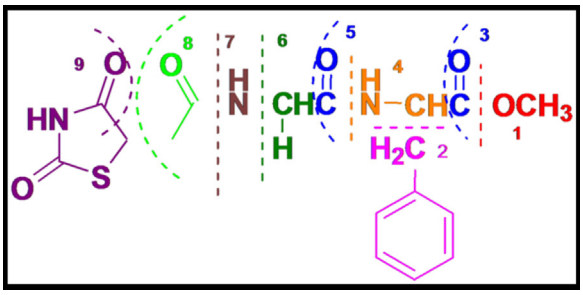


Fig. 3. Fragmentation pattern of SSMA13 (T-Gly-Phe methyl ester).

Fig. 5. Color changes observed during in vitro antidiabetic studies.

3.2. Pharmacological activity

3.2.1. In vitro antioxidant activity

Antioxidants are compounds which prevent the process of oxidation by intervening with the initiation, propagation, and termination of the free radicals (ROS). The increase in the availability of ROS can create oxidative stress which may lead to many degenerative diseases. The harmful effects of these ROS could be balanced by the endogenous as well as exogenous antioxidant compounds. The most common method determination of antioxidant activity is the DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay. It gives a measure of the H-donating capability of newer

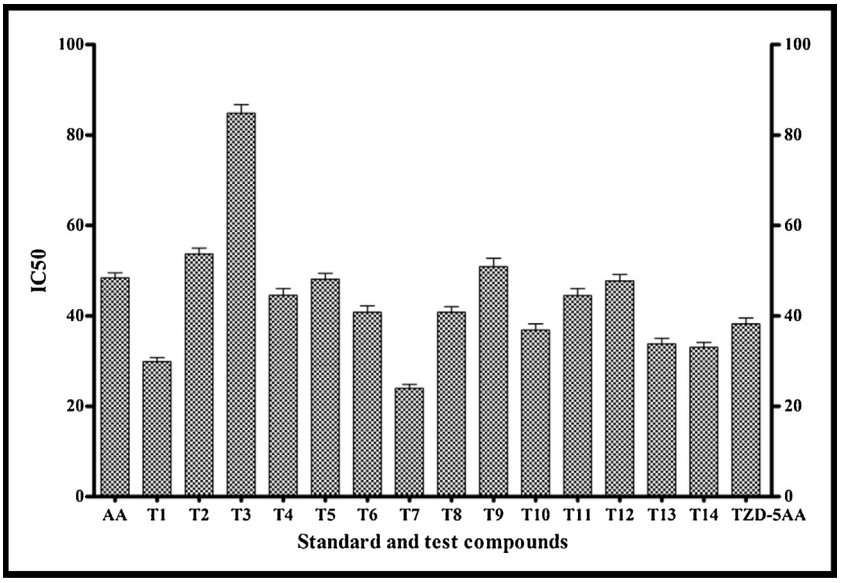


Fig. 4. Bar Graph showing the comparative IC 50 values for Antioxidant Activity, All data are expressed as mean ± SEM. Note: AA = ascorbic acid, T1–T15 = test drugs selected from SSDMA1 to SSDMA50.

Table 2
Effect of treatment of the synthesized drugs on fasting plasma glucose level in T2DSTZ-NA induced T2DM rat models for 14 days at 30 mg/kg body weight.

Fasting plasma glucose (mg/dl) mean ± SEM				
Group No.	Groups	Day 0	Day 7	Day 14
Group I	Normal Control	101 ± 3.11	102 ± 1.85	98 ± 5.69
Group II	Diabetic Control	309 ± 7.70 a****	336.73 ± 10.59 a****	360 ± 8.66 a****
Group III	Diabetic+Pioglitazone	301 ± 5.19 b^{ns}	179.25 ± 11.78 b****	137.76 ± 4.79 b****
Group IV	Diabetic+T1	284 ± 7.77 b^{ns}	204.75 ± 5.56 b****	142 ± 5.51 b****
Group V	Diabetic+T4	301 ± 8.87 b^{ns}	280 ± 7.63 b**	271 ± 10.18 b****
Group VI	Diabetic+T5	310 ± 9.02 b^{ns}	281 ± 4.31 b**	265 ± 10.21 b****
Group VII	Diabetic+T7	307 ± 9.57 b^{ns}	222.23 ± 2.75 b****	144.47 ± 5.69 b****
GroupVIII	Diabetic+T9	305 ± 9.39 b^{ns}	281 ± 10.88 b**	269.25 ± 10.18 b****
Group IX	Diabetic+T13	298 ± 9.02 b^{ns}	276.37 ± 10.11 b***	156 ± 6.98 b****

All data are expressed as mean ± SEM ($n = 4$). All groups compared with diabetic control.
a-group II-IX, compared with control.
b-group III-IX, compared with group II (diabetic control).
**** $P < 0.0001$.
*** $P < 0.001$.
** $P < 0.01$.
ns $P > 0.05$.

compounds which is accepted by DPPH (purple color) and is reduced to its hydrazine which results in the yellow color (Fig. 3).

T1 (SSDMA1), T7 (SSDMA13) and T13 (SSDMA49) showed lower IC_{50} value of 29.88±, 23.99 ± 0.88 and 33.83 ± 1.21 respectively as compared to 48.41 ± 1.12 of ascorbic acid and 38.21 ± 1.31 of thiazolidinedione-5-acetic acid (TZD-5AA) proving that they have good scavenging ability better than the standard and heterocycle, hence can act as good antioxidants to prevent the oxidation process in cells (Fig. 4).

3.2.2. In vitro antidiabetic activity was carried out using yeast cells and compounds

Endogenously in T2DM condition the cellular uptake of glucose is reduced, which increases the blood glucose level. The in vitro glucose uptake assay by yeast cells (*Saccharomyces cerevisiae*) resembles the in vivo diabetic condition which has been used here to screen the hypoglycemic effect of all synthesized drugs in comparison of standard pioglitazone.HCl. The glucose in the external solution reduces the 3,5-dinitrosalisylic acid to 3-amino,

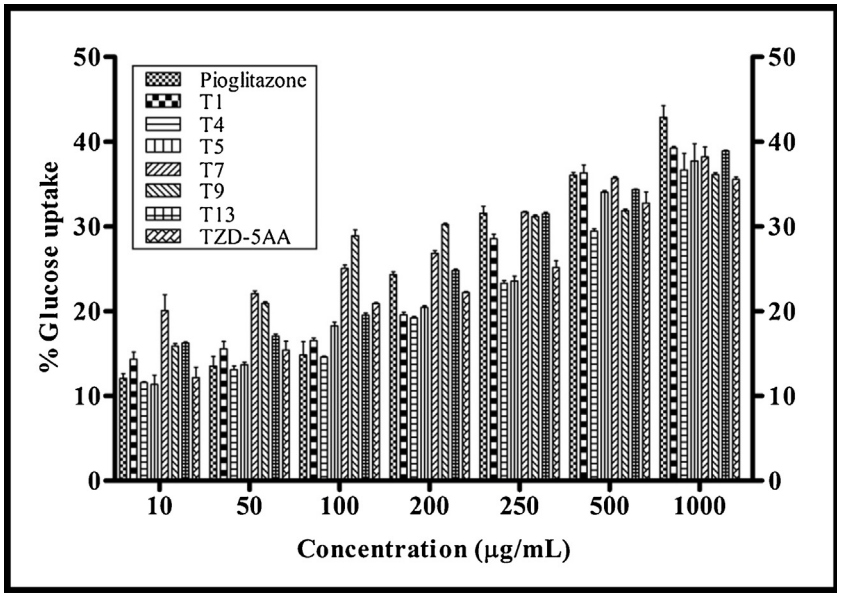


Fig. 6. Bar graph showing the % glucose uptake by yeast cells at different drug concentration by the test drugs T1, T4, T5, T7, T9 and T13 (SSDMA1, SSDMA4, SSDMA5, SSDMA13, SSDMA15 and SSDMA49). All data are expressed as mean ± SEM.

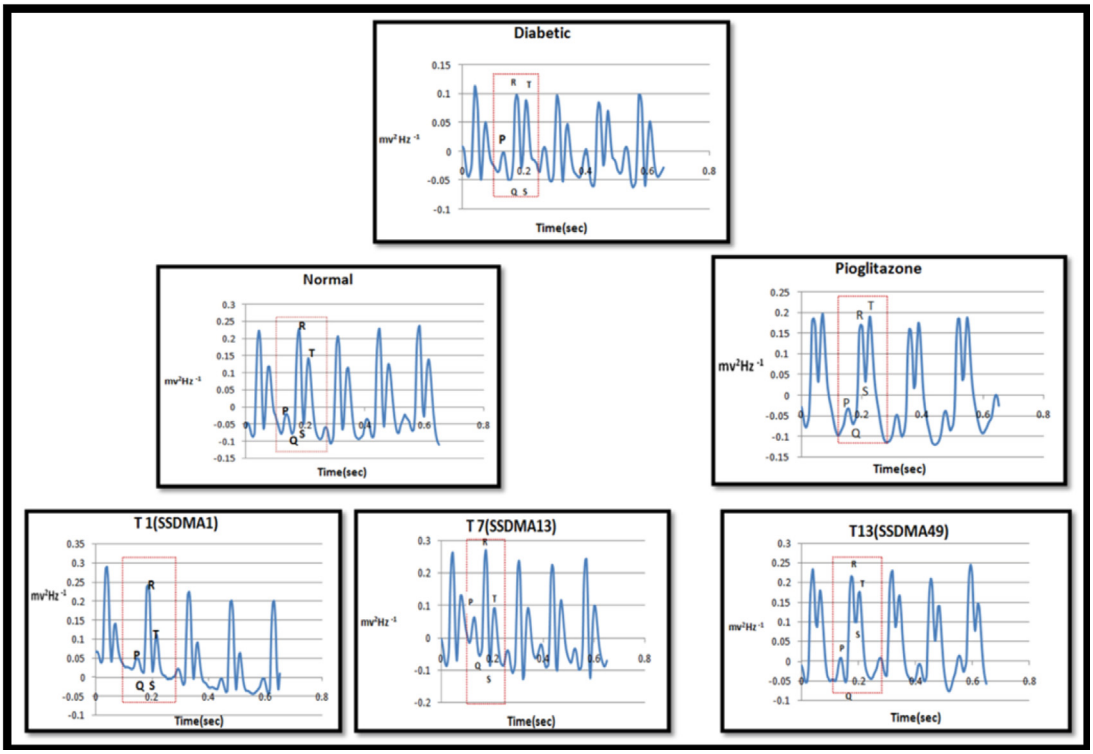


Fig. 7. ECG wave pattern of the treated groups.

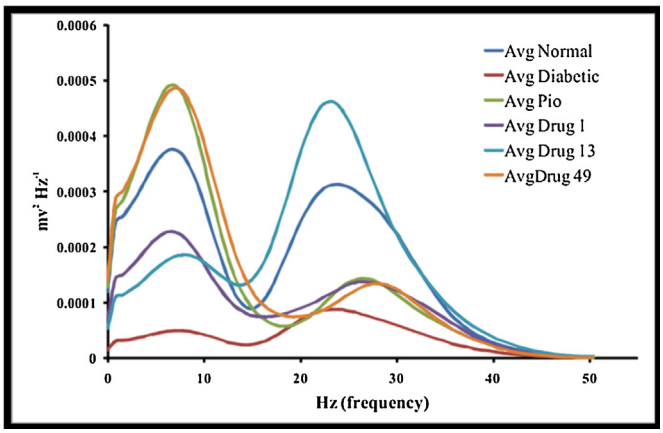


Fig. 8. EGC spectrum analysis of the treated groups.

5-nitro salicylic acid which is observed by the change in color. The unutilized glucose present in the external reacts with DNSA reagent to give brownish orange color, intensity of this color decreases as more glucose is taken up by the drug, thus decreasing the amount of free glucose in the external solution [33].

T1, T4, T5, T7, T9 and T13 (SSDMA1, SSDMA4, SSDMA5, SSDMA13, SSDMA15 and SSDMA49) showed % glucose uptake of 39.23 ± 0.18, 36.683 ± 1.92, 37.731 ± 2.00, 38.19 ± 1.16, 36.11 ± 0.23 and 38.90 ± 0.09 as compared to 42.87 ± 1.36 of standard pioglitazone hydrochloride and 33.33 ± 1.16 of heterocycle TZD-5AA, which was confirmed by the change in the intensity of color as seen in Figs. 5 and 8. As the % glucose uptake by TZD-5AA was less so it was excluded from in vivo studies and, rest T1, T4, T5, T7, T9 and T13 these were included in the in vivo antidiabetic activity.

3.2.3. *In vivo antidiabetic activity*

Study was done in STZ-NA-induced T2DM rat models for 14 days at 30 mg/kg body weight dose of each drug.

From Table 2 it is inferred that all the six compounds were significantly effective in lowering the blood glucose level on the 14th day of the study. T1, T7, and T13 show a consistent and significant decrease in BGL in both 7th day and 14th day of the study (Fig. 6).

3.2.4. *In vivo cardioprotective activity*

3.2.4.1. ECG wave pattern analysis. The ECG wave pattern analysis as depicted in Fig. 7 showed that the diabetic rat when treated with T1 (SSDMA1) and T7 (SSDMA13) showed similar wave pattern as normal control rat. It indicates that the molecule T1 and T7 help the diabetic heart to function almost similarly as the normal heart and thus may have a cardioprotective effect. It is also evident from the PQRST wave that these molecules help to maintain and complete the normal cardiac cycle as the wave patterns of T1 and T7 are similar to wave pattern of a normal rat. T13 shows ECG wave pattern similar to pioglitazone treated group, where the R and T are almost of the same amplitude, which is different from the normal PQRST wave pattern.

3.2.4.2. EGC spectrum analysis. The digitized ECG spectra were analyzed in frequency domain to compare the frequency spectrum of normal, diabetic and different drug treated groups. As the recording setup considered for the evaluation was at the frequency band width of 0.5–35 Hz, so it was not filtered further for the removal of high-frequency noise. However before analyzing the spectrum, the complete set of recorded waveform was passed through 1 Hz high pass filter to remove the motion effects. Five ECG waves were considered for spectral analysis. Ten such spectra were calculated, analyzed and compared.

It was observed from Fig. 8 that almost in all the conditions two peaks are obtained. One at the low-frequency range of about 8 Hz and the other at a higher range of 25 Hz. In diabetic condition the spectrum is very much depressed while the high energy pattern was achieved by all the drugs similar to normal. T1 and T7 are similar to the normal spectra where the high-frequency component is less, while T13 has a spectrum similar to the standard treated.

3.2.4.3. Heart rate variability (HRV) spectrum. Fig. 9 shows the HRV spectrum of a normal rat with one high broad peak towards the low frequency and a small notch towards the high frequency. Almost similar spectral pattern were observed in all the subjects in the experiment. However in T7 and T1 treated groups, the pattern tends towards lowering the energy. T7 (SSDMA13) has a high peak in the low-frequency region with a very small notch in the high-frequency region.

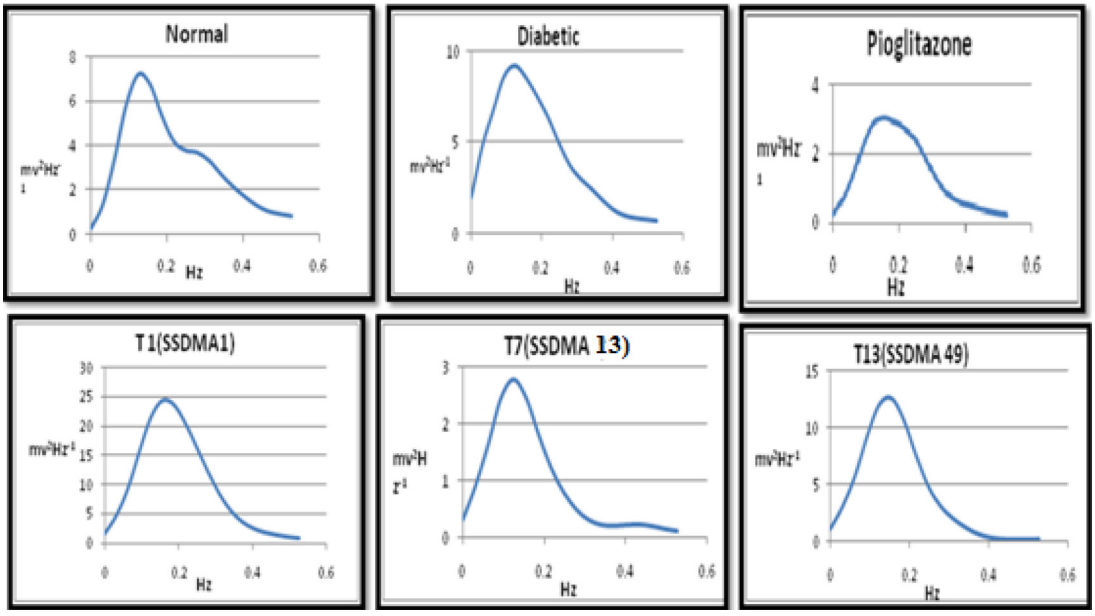


Fig. 9. Heart rate variability of ECG wave pattern of (a) normal rat, (b) diabetic rat, (c) pioglitazone-treated rat, (d) T1 treated rat, (e) T7 treated rat, (f) T13 treated rat.

3.2.4.4. Heart rate analysis. The heart rate analysis was done by manually counting the number of the waveform in 30 s in at different time intervals. Diabetic rat showed a maximum heart rate of 343.19 ± 11.19, while T7 showed a heart rate of 182 ± 5.77 which was similar to normal rat with a heart rate of 175 ± 3.80.T1 and T13 showed heart rate 210.42 ± 7.80 and 210.40 ± 11.15 similar to the standard (pioglitazone) treated with a heart rate of 195 ± 4.54.

4. Conclusion

Among the 14 synthesized hybrids, SSDMA1 (T1, TZD-5AA-Phe methyl ester), SSDMA13 (T7, TZD-5AA-Gly-Phe methyl ester) and SSDMA49 (T13, TZD-5AA-Gly-Gly-Phe methyl ester) showed significant antidiabetic activity while SSDMA1 and SSDMA13 showed both antidiabetic activity and were proposed to show cardioprotective activity. Pioglitazone HCl has been used as the control molecule without the peptide fragment and compared with the hybrids in the biological studies. Thus, it was evident from the study that incorporation of glycine and phenylalanine to the thiazolidinediones moiety retains its antidiabetic property, at the same time restores the ECG towards normal in diabetic rats, hence may be helpful in preventing development of diabetic cardiomyopathy.

Thus, this study motivates us to explore further various amino acid and peptide hybrids which may be effective dually with fewer side effects and toxicity and thus provide robust medicines to the ailing diabetic patients.

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