Supplementary Material for

Design and synthesis of novel pyranone-based insulin sensitizers exhibiting *in vivo* hepatoprotective activity

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General experimental

Commercial reagents were used without purification. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz, with DMSO-d_6 as the solvent. Chemical shifts are reported in parts per million (δ-value) from Me_4Si (δ = 0 ppm for ^{1}H) as an internal standard or based on the middle peak of the solvent (DMSO-d_6, 2.5 ppm for ^{1}H and 39.5 ppm for ^{13}C NMR). Signal patterns are indicated as s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constants (*J* values) are given in Hertz (Hz). Infrared (IR) spectra were recorded on a Perkin-Elmer AX-1 spectrophotometer in KBr disc and are reported in wave number (cm^{-1}). For mass spectra analysis, an ESI-MS spectrometer was used.

Experimental procedures and characterization data

General procedure for the synthesis of 6-aryl-4-[(2-hydroxyethyl)-methylamino]-2-oxo-2*H*-pyran-3-carbonitrile (3a-h). A mixture of 6-aryl-3-cyano-4-methylsulfanyl-2*H*-pyran-2-ones (**1**, 1 mmol) and *N*-methylethanolamine (**2**, 1.2 mmol) was refluxed in methanol for 1-4 h. After completion, the reaction was cooled to room temperature and left over night. The crystalline solid was filtered off and washed with methanol. Crude product was purified by silica gel column chromatography using chloroform as eluent.

4-((2-Hydroxyethyl)(methyl)amino)-2-oxo-6-phenyl-2*H*-pyran-3-carbonitrile (3a). The compound was prepared from of 6-phenyl-3-cyano-4-methylsulfanyl-2*H*-pyran-2-ones (**1a**, mmol) and *N*-methylethanolamine (**2a**, 1.2 mmol) was refluxed in methanol for 1-4 h. After completion, the reaction was cooled to room temperature and left over night. The creamy white crystalline solid was filtered off and washed with methanol. Crude product was purified by silica gel column chromatography using chloroform as eluent furnishing **3a** as a Creamy white solid, yield 90%; mp 159-161 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 3021 (OH), 2211 (CN), 1711 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.45 (3H, s), 3.66-3.72 (2H, m), 3.83-3.87 (2H, m), 5.03 (1H, t, *J* 5.4, D_2O exchange), 6.97 (1H, s), 7.51-7.57 (3H, m), 7.88-7.95 (2H, m); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 162.0, 159.9, 158.2, 131.6, 130.6, 129.0, 126.1, 118.2, 95.5, 58.8, 55.7, 41.5; MS (ESI) *m/z* 271 ($[M + H]^+$).

6-(4-Chlorophenyl)-4-((2-hydroxyethyl)(methyl)amino)-2-oxo-2*H*-pyran-3-carbonitrile (3b). The compound **3b** was prepared as described for **3a**, as a creamy white solid, yield 86%; mp 182-184

^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 3423 (OH), 2213 (CN), 1686 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.46 (3H, s), 3.66-3.73 (2H, m), 3.83-3.87 (2H, m), 5.02 (1H, *J* 5.4, OH, D_2O exchange), 7.00 (1H, s), 7.61 (2H, d, *J* 8.6), 7.95 (2H, d, *J* 8.6); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 161.5, 160.2, 157.7, 136.5, 129.3, 129.0, 128.1, 117.6, 95.5, 70.3, 56.3, 53.9; MS (ESI) *m/z* 305 ([M + H]$^+$).

6-(4-Bromophenyl)-4-((2-hydroxyethyl)(methyl)amino)-2-oxo-2*H*-pyran-3-carbonitrile (3c). Following the procedure of **3a**, compound **3c** was obtained as a creamy white solid, yield 88%; mp 184-186 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 3478 (OH), 2211 (CN), 1662 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.46 (3H, s), 3.66-3.72 (2H, m), 3.83-3.88 (2H, m), 5.00 (1H, t, *J* 5.4, OH, D_2O exchange), 7.02 (1H, s), 7.75 (2H, d, *J* 8.6), 7.88 (2H, d, *J* 8.6); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 162.2, 161.2, 157.7, 136.8, 129.3, 129.1, 117.9, 96.5, 70.9, 54.1, 29.9; MS (ESI) *m/z* 349 ([M + H]$^+$).

4-((2-Hydroxyethyl)(methyl)amino)-2-oxo-6-*p*-tolyl-2*H*-pyran-3-carbonitrile (3d). Following the procedure of **3a**, compound **3d** was obtained as a creamy white solid, yield 78%; mp 188-190 ^{o}C, ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 3450 (OH), 2196 (CN), 1640 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.38 (3H, s), 3.45 (3H, s), 3.64-3.74 (2H, m), 3.82-3.86 (2H, m), 4.99 (1H, t, *J* 5.4, OH, D_2O exchange), 6.92 (1H, s), 7.35 (2H, d, *J* 8.1), 7.82 (2H, d, *J* 8.2); MS (ESI) *m/z* 285 ([M + H]$^+$).

4-((2-Hydroxyethyl)(methyl)amino)-6-(4-methoxyphenyl)-2-oxo-2*H*-pyran-3-carbonitrile (3e). The compound **3e** was prepared as described for **3a**, was furnishing as a creamy white solid, yield 72%; mp 190-192 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 3360 (OH), 2207 (CN), 1671 (CO) cm^{-1}; ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.45 (3H, s), 3.64-3.73 (2H, m), 3.82-3.86 (5H, m), 4.99 (1H, t, *J* 5.5, OH, D_2O exchange), 6.85 (1H, s), 7.08 (2H, d, *J* 8.8), 7.89 (2H, d, *J* 8.8); MS (ESI) *m/z* 301 ([M + H]$^+$).

4-((2-Hydroxyethyl)(methyl)amino)-6-(naphthalen-1-yl)-2-oxo-2*H*-pyran-3-carbonitrile (3f). According to the procedure described for **3a**, compound **3f** was furnished as a white solid, yield 69%; mp 160-164 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 3383 (OH), 2207 (CN), 1675 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.46 (3H, s), 3.66-3.82 (4H, m), 5.03 (1H, t, *J* 5.4, OH, D_2O exchange), 6.83 (1H, s), 7.58-7.68 (3H, m), 7.75-7.81 (1H, m), 8.02-8.15 (3H, m); MS (ESI) *m/z* 321 ([M + H]$^+$).

4-((2-Hydroxyethyl)(methyl)amino)-6-(naphthalen-2-yl)-2-oxo-2*H*-pyran-3-carbonitrile (3g). According to the procedure described for **3a**, compound **3g** was furnished as a white solid, yield 65%; mp 160-162 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 3387 (OH), 2204 (CN), 1655 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.49 (3H, s), 3.68-3.77 (2H, m), 3.87-3.91 (2H, m), 5.05 (1H, t, *J* 5.4, OH D_2O exchange), 7.11 (1H, s), 7.57-7.68 (2H, m), 7.96-8.14 (4H, m), 8.52 (1H, s); ^{13}C NMR δ_C (75 MHz, DMSO-d_6) 162.5, 160.4, 158.5, 134.5, 132.8, 129.5, 129.1, 128.5, 128.3, 128.1, 127.6, 126.9, 123.1, 118.7, 96.3, 69.9, 59.4, 56.3, 31.1; MS (ESI) *m/z* 321 ([M + H]$^+$).

6-(Furan-2-yl)-4-((2-hydroxyethyl)(methyl)amino)-2-oxo-2*H*-pyran-3-carbonitrile (3h). According to the procedure described for **3a**, compound **3h** was furnished as a pale yellow solid, yield 60%; mp 154-156 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 3447 (OH), 2210 (CN), 1697 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.43 (3H, s), 3.65-3.80 (4H, m), 5.02 (1H, t, *J* 5.4, OH, D_2O exchange), 6.70 (1H, s), 6.72-6.78 (1H, m), 7.19-7.24 (1H, m), 8.00 (1H, s); MS (ESI) *m/z* 261 ([M + H]$^{+}$).

General procedure for the synthesis of 6-aryl-4-[(2-bromoethyl)-methyl-amino]-2-oxo-2*H*-pyran-3-carbonitrile (4a-h): A mixture of 6-aryl-4-[(2-hydroxy-ethyl)-methyl-amino]-2-oxo-2*H*-pyran-3-carbonitrile (**3a-h**, 1 mmol) and $POBr_3$ (1.2 mmol) was refluxed in presence of K_2CO_3 in dry toluene for 1-3 h. After completion, the excess toluene was removed under vacuum. Crude product was purified by silica gel column chromatography using chloroform as eluent.

4-((2-Bromoethyl)(methyl)amino)-2-oxo-6-phenyl-2*H*-pyran-3-carbonitrile (4a). A mixture of 6-aryl-4-[(2-hydroxy-ethyl)-methyl-amino]-2-oxo-2*H*-pyran-3-carbonitrile (**3a**, 1 mmol) and $POBr_3$ (1.2 mmol) was refluxed in presence of K_2CO_3 in dry toluene for 1-3 h. After completion, the excess toluene was removed under vacuum. the residue obtained was treated with ice and the crude product was purified by silica gel column chromatography using chloroform as eluent affording **4a** as a white solid, yield 78%; mp 164-168 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2208 (CN), 1705 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.45 (3H, s), 3.80 (2H, t, *J* 6.6), 4.22 (2H, t, *J* 6.6), 7.00 (1H, s) 7.53-7.61 (3H, m), 7.94-7.99 (2H, m); MS (ESI) *m/z* 333 ([M + H]$^{+}$).

4-((2-Bromoethyl)(methyl)amino)-6-(4-chlorophenyl)-2-oxo-2*H*-pyran-3-carbonitrile (4b). According to the procedure described for **4a**, compound **4b** was furnished as a creamy white solid, yield 82%; mp 196-198 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2203 (CN), 1684 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.45 (3H, s), 3.79 (2H, t, *J* 6.6), 4.22 (2H, t, *J* 6.6), 7.03 (1H, s), 7.62 (2H, d, *J* 8.7), 8.00 (2H, d, *J* 8.7); ^{13}C NMR δ_C (75 MHz; DMSO-d_6): 161.5, 160.2, 157.7, 136.5, 129.3, 129.1 128.1, 117.6, 95.5, 70.3, 53.9, 41.0, 29.8; HRMS (ESI) exact mass calcd. for $C_{15}H_{13}BrClN_2O_2$: 366.9849 ([M + H]$^{+}$), found: 367.0871 ([M + H]$^{+}$).

4-((2-Bromoethyl)(methyl)amino)-6-(4-bromophenyl)-2-oxo-2*H*-pyran-3-carbonitrile (4c). The compound **4c** was prepared as described for **4a**, as a creamy white solid, yield 86%; mp 204-206 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2201 (CN), 1682 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.45 (3H, s), 3.79 (2H, t, *J* 6.6), 4.21 (2H, t, *J* 6.6), 7.04 (1H, s), 7.76 (2H, d, *J* 8.7), 7.92 (2H, d, *J* 8.7); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 161.9, 160.7, 158.3, 132.5, 130.1, 128.8, 125.9, 118.1, 96.0, 70.9, 54.4, 41.5, 30.3; MS (ESI) *m/z* 413 ([M + H]$^{+}$).

4-((2-Bromoethyl)(methyl)amino)-2-oxo-6-*p*-tolyl-2*H*-pyran-3-carbonitrile (4d). According to the procedure described for **4a**, compound **4d** was furnished as a white solid, yield 74%; mp 200-202 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2205 (CN), 1683 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6;

Me_4Si) 2.38 (3H, s), 3.43 (3H, s), 3.78 (2H, t, *J* 6.7), 4.19 (2H, t, *J* 6.7), 6.92 (1H, s), 7.35 (2H, d, *J* 8.0), 7.86 (2H, d, *J* 8.0): MS (ESI) *m/z* 347 ($[M + H]^+$).

4-((2-Bromoethyl)(methyl)amino)-6-(4-methoxyphenyl)-2-oxo-2*H*-pyran-3-carbonitrile (4e). According to the procedure described for **4a**, compound **4e** was obtained as a white solid, yield 71%; mp 208-210 ^oC ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2203(CN), 1680 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.42 (3H, s), 3.77 (2H, t, *J* 6.7), 3.84 (3H, s), 4.18 (2H, t, *J* 6.7), 6.83 (1H, s), 7.07 (2H, d, *J* 8.9), 7.91 (2H, d, *J* 8.9); MS (ESI) *m/z* 363 ($[M + H]^+$).

4-((2-Bromoethyl)(methyl)amino)-6-(naphthalen-1-yl)-2-oxo-2*H*-pyran-3-carbonitrile (4f). The compound **4f** was prepared as described for **4a**, as a Pale yellow solid, yield 70%; mp 188-190 ^oC ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2203 (CN), 1700 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.46 (3H, s), 3.79 (2H, t, *J* 6.6), 4.16 (2H, t, *J* 6.5), 6.85 (1H, s), 7.58-7.68 (3H, m), 7.77-7.83 (1H, m), 8.02-8.18 (3H, m); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 162.1, 160.9, 160.3, 133.2, 131.5, 129.7, 129.5, 128.6, 128.4, 127.5, 126.6, 125.2, 124.7, 117.5, 100.1, 70.3, 54.0, 40.9, 29.8; MS (ESI) *m/z* 383 ($[M + H]^+$).

4-((2-Bromoethyl)(methyl)amino)-6-(naphthalen-2-yl)-2-oxo-2*H*-pyran-3-carbonitrile (4g). The compound **4g** was prepared as described for **4a**, as a pale yellow solid, yield 62%; mp 194-196 ^oC ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2204 (CN), 1684 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.47 (3H, s), 3.82 (2H, t, *J* 6.5), 4.24 (2H, t, *J* 6.6), 7.11 (1H, s), 7.57-7.69 (2H, m), 7.96-8.14 (4H, m), 8.54 (1H, s); MS (ESI) *m/z* 383 ($[M + H]^+$).

4-((2-Bromoethyl)(methyl)amino)-6-(furan-2-yl)-2-oxo-2*H*-pyran-3-carbonitrile (4h). According to the procedure described for **4a**, compound **4h** was furnished as a yellow solid, yield 51%; mp 128-130 ^oC ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{1} 2208 (CN), 1707 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.43 (3H, s), 3.77 (2H, t, *J* 6.6), 4.15 (2H, t, *J* 6.6), 6.69 (1H, s), 6.74-6.80 (1H, m), 7.25-7.30 (1H, m), 8.02 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 160.9, 160.1, 150.7, 147.1, 144.8, 117.6, 114.3, 113.1, 92.7, 69.7, 53.8, 29.6; MS (ESI) *m/z* 323 ($[M + H]^+$).

General procedure for the synthesis of 5-(3/4-hydroxybenzyl)-thiazolidine-2,4-dione (5a/5b). Thiazolidine-2,4-dione (1 mmol) was fused with 3/4-hydroxybenzaldehyde (1 mmol) in presence of sodium acetate (1.2 mmol) at 130-140^oC, which afforded 5-(3/4-hydroxybenzylidene)-thiazolidine-2,4-dione in good yield 88%.

Reduction of 5-(4-hydroxybenzylidene)-thiazolidine-2,4-dione.- 5-(4-Hydroxybenzylidene)-thiazolidine-2,4-dione (0.94 gm, 4.25 mmol) was dissolved in mixture of 3.0 ml of methanol, 4.5 ml of water and 3.39 ml of 1M NaOH solution, resultant solution was stirred for 15 min. Then 0.1 ml of $CoCl_2$-DMG complex solution (42 mg $CoCl_2$. $6H_2O$ and 250 mg of DMG in 5.0 ml of DMF) was added and stirring was continued. After 15 min. $NaBH_4$ (0.2 gm, 5.28 mmol) was added in single portion. The blue-purple solution was stirred for 1h and pH of solution should be maintaining in between 6-7. After completion of the reaction, reaction mixture was acidified with 10% HCl and white

precipitate was formed. White precipitate was filtered and washed with water. Finally crude product was purified by silica gel column chromatography using 1% methanol in chloroform as eluent in 72% yield.

Synthesis of 5-(4-Hydroxybenzyl)-thiazolidine-2,4-dione (5).

White solid; mp 158-168 ^{o}C; IR (KBr): ν_{max}/cm^{-1} 1712, 1742 (CO), 3452 (OH); ^{1}H NMR δ_H (300 MHz, $CDCl_3$) 2.91-3.02 (1H, m), 3.18-3.29 (1H, m), 4.76-4.84 (1H, m), 6.51 (1H, s, OH), 6.67 (2H, d, *J* 8.2), 7.01 (2H, d, *J* 8.2); MS (ESI) *m/z* 224 ([M + H]$^{+}$).

General procedure for the synthesis of 6-aryl-4-({2-[4-(2,4-dioxo-thiazolidin-5-ylmethyl)-phenoxy]-ethyl}-methyl-amino)-2-oxo-2*H*-pyran-3-carbonitrile (6a-h and 7a-h). A mixture of 6-aryl-4-[(2-hydroxy-ethyl)-methyl-amino]-2-oxo-2*H*-pyran-3-carbonitriles (**4a-h**, 1 mmol) and compound **5a/5b** (1.2 mmol) was refluxed in presence of K_2CO_3 in dry acetone for 4-6 h. After completion, the excess amount of acetone was removed under vacuum. Finally the crude product was purified by silica gel column chromatography using 1% methanol in chloroform as eluent.

4-((2-(5-(4-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl) methyl)amino)-2-oxo-6-phenyl-2*H*-pyran-3-carbonitrile (6a). A mixture of 6-phenyl-4-[(2-hydroxy-ethyl)-methyl-amino]-2-oxo-2*H*-pyran-3-carbonitriles (**4a**, 1 mmol) and compound **5a** (1.2 mmol) was refluxed in presence of K_2CO_3 in dry acetone for 4-6 h. After completion, the excess amount of acetone was removed under vacuum. Finally the crude product was purified by silica gel column chromatography using 1% methanol in chloroform as eluent giving **6a** as a white solid, yield 89%; mp 134-136 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2202 (CN), 1670 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.78-2.88 (1H, m), 3.37-3.40 (1H, m), 3.54 (3H, s), 3.85-4.18 (4H, m), 4.87-4.96 (1H, m), 6.76 (2H, d, *J* 8.2), 7.00-7.06 (3H, m) 7.65-7.72 (3H, m), 8.02-8.08 (2H, m), 9.48 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.3, 161.7, 159.9, 158.9, 156.5, 131.9, 130.3, 129.9, 129.1, 126.4, 126.1, 117.6, 115.2, 94.5, 79.1, 70.2, 51.5, 50.6, 41.2, 36.5; HRMS (ESI) exact mass calcd. for $C_{25}H_{22}N_3O_5S$: 476.1280 ([M + H]$^{+}$), found: 476.1260 ([M + H]$^{+}$).

6-(4-Chlorophenyl)-4-((2-(5-(4-hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl) amino)-2-oxo-2*H*-pyran-3-carbonitrile (6b). According to the procedure described for **6a**, compound **6b** was furnished as a white solid, yield 70%; mp 188-192 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2210 (CN), 1690 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.69-2.78 (1H, m), 3.27-3.29 (1H, m), 3.42 (3H, s), 3.78-4.13 (4H, m), 4.76-4.86 (1H, m), 6.65 (2H, d, *J* 8.2), 6.90-6.94 (3H, m), 7.64 (2H, d, *J* 8.6), 7.94 (2H, d, *J* 8.6), 9.34 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 174.3, 171.8, 162.1, 160.3, 158.2, 156.9, 137.2, 130.5, 129.7, 129.6, 128.4, 126.8, 118.1, 115.7, 95.4, 70.8, 51.9, 51.2, 41.7, 36.9, 31.1; HRMS (ESI) exact mass calcd. For $C_{25}H_{21}ClN_3O_5S$: 510.0890 ([M + H]$^{+}$), found: 510.0906 ([M + H]$^{+}$).

6-(4-Bromophenyl)-4-((2-(5-(4-hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl) amino)-2-oxo-2*H*-pyran-3-carbonitrile (6c). According to the procedure described for **6a**, compound

6c was furnished as a white solid, yield 78%; mp 194-196 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2209 (CN), 1680 (CO), 1748 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.67-2.79 (1H, m), 3.25-3.31 (1H, m), 3.41 (3H, s), 3.77-4.07 (4H, m), 4.75-4.84 (1H, m), 6.65 (2H, d, *J* 8.3), 6.90-6.95 (3H, m), 7.77 (2H, d, *J* 8.6), 7.88 (2H, d, *J* 8.6), 9.34 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 174.3, 171.8, 162.1, 160.3, 158.3, 156.9, 132.7, 130.5, 130.1, 128.6, 126.9, 126.2, 118.1, 115.8, 95.6, 70.9, 51.9, 51.2, 41.8, 37.1; MS *m/z* (ESI) 554 ([M + H]$^+$).

4-((2-(5-(4-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl)amino)-2-oxo-6-*p*-tolyl-2*H*-pyran-3-carbonitrile (6d). Following the procedure of **6a**, compound **6d** was synthesized as a white solid, yield 81%; mp 170-172 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2209 (CN), 1680 (CO) 1748 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.38 (3H, s), 2.63-2.75 (1H, m), 3.24-3.30 (1H, m), 3.40 (3H, s), 3.77-4.06 (4H, m), 4.75-4.83 (1H, m), 6.64 (2H, d, *J* 8.5), 6.76 (1H, s), 6.91 (2H, d, *J* 8.4), 7.11 (2H, d, *J* 9.0), 7.90 (2H, d, *J* 9.0), 9.37 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.3, 161.7, 160.0, 159.0, 156.5, 142.2, 130.0, 129.6, 127.5, 126.4, 126.0, 117.8, 115.3, 93.7, 69.9, 51.5, 50.6, 41.1, 36.5, 21.0; MS *m/z* (ESI) 490 ([M + H]$^+$).

4-((2-(5-(4-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl)amino)-6-(4-methoxyphenyl)-2-oxo-2*H*-pyran-3-carbonitrile (6e). Following the procedure of **6a**, compound **6e** was obtained as a white solid, yield 69%; mp 178-180 ^{o}C ($CHCl_3$/MeOH), IR (KBr): ν_{max}/cm^{-1} 2207 (CN), 1671 (CO), 1740 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.64-2.74 (1H, m), 3.25-3.29 (1H, m), 3.41 (3H, s), 3.78-4.05 (4H, m), 3.84 (3H, s), 4.76-4.82 (1H, m), 6.64 (2H, d, *J* 8.4), 6.76 (1H, s), 6.90 (2H, d, *J* 8.4), 7.11 (2H, d, *J* 8.9), 7.90 (2H, d, *J* 8.9), 9.38 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.3, 161.7, 160.0, 159.0, 156.5, 142.2, 130.0, 129.6, 127.5, 126.4, 126.0, 117.8, 115.3, 93.7, 70.0, 51.5, 50.6, 41.1, 36.5, 21.0; HRMS (ESI) exact mass calcd. for $C_{26}H_{24}N_3O_6S$: 506.1386 ([M + H]$^+$), found: 506.3299 ([M + H]$^+$).

4-((2-(5-(4-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)methyl)amino)-6-naphthalen-1-yl)-2-oxo-6-phenyl-2*H*-pyran-3-carbonitrile (6f). Following the procedure of 6a, compound 6f was synthesized as a white solid, yield 68%; mp 166-168 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2210 (CN), 1690 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.70-2.82 (1H, m), 3.25-3.36 (1H, m), 3.39-3.41 (3H, m), 3.78-3.94 (4H, m), 4.77-4.86 (1H, m), 6.62-6.72 (3H, m), 6.93 (2H, d, *J* 8.2), 7.55-7.68 (3H, m), 7.76-7.70 (1H, m), 8.02-8.11 (2H, m), 8.15 (1H, d, *J* 8.2), 9.41 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 174.3, 171.7, 162.5, 161.7, 160.6, 157.0, 133.7, 132.1, 130.5, 130.1, 129.8, 129.1, 128.7, 128.0, 127.1, 126.9, 125.7, 125.1, 118.1, 115.7, 100.1, 70.9, 52.0, 51.2, 41.7, 37.1; HRMS (ESI) exact mass calcd for $C_{29}H_{24}N_3O_5S$: 526.1437 ([M + H]$^+$), found: 526.1377 ([M + H]$^+$).

4-((2-(5-(4-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)methyl)amino)-6-naphthalen-2-yl)-2-oxo-6-phenyl-2*H*-pyran-3-carbonitrile (6g). According to the procedure described for **6a**, compound **6g** was furnished as a white solid, yield 60%; mp 168-170 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2207 (CN), 1670 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.68-2.75 (1H, m), 3.26-3.29 (1H, m), 3.45 (3H, s), 3.82-4.06 (4H, m), 4.76-4.83 (1H, m), 6.59 (2H, d, *J* 8.1), 6.87 (2H, d, *J*

8.2), 7.01 (1H, s), 7.62-7.66 (2H, m), 7.98-8.10 (4H, m), 8.54 (1H, s), 9.33 (1H, s); HRMS (ESI) exact mass calcd. for $C_{29}H_{23}N_3O_5S$: 526.1437 ($[M + H]^+$), found: 526.1367 ($[M + H]^+$).

6-(Furan-2-yl)-4-((2-(5-(4-hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl)amino)-2-oxo-2*H*-pyran-3-carbonitrile (6h). Following the procedure of **6a**, compound **6h** was obtained as a white solid, yield 54 %; mp 182-184 °C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2209 (CN), 1680 (CO), 1748 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.64-2.76 (1H, m), 3.26-3.31 (1H, m), 3.35-3.39 (3H, m), 3.78-3.93 (4H, m), 4.76-4.85 (1H, m), 6.57 (1H, s), 6.66 (2H, d, *J* 8.4), 6.75- 6.80 (1H, m), 6.90-6.97 (2H, m), 7.29 (1H, m) 8.04 (1H, m), 9.384 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 171.2, 161.0, 159.7, 156.4, 150.8, 147.3, 144.7, 130.0, 126.5, 117.6, 115.2, 114.2, 113.1, 92.0, 69.7, 51.5, 50.7, 41.0, 36.5; MS *m/z* (ESI) 466 ($[M + H]^+$).

4-((2-(5-(3-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)methyl)amino)-2-oxo-6-phenyl-2*H*-pyran-3-carbonitrile (7a). A mixture of 6-phenyl-4-[(2-hydroxy-ethyl)-methyl-amino]-2-oxo-2*H*-pyran-3-carbonitriles (**4a**, 1 mmol) and compound **5b** (1.2 mmol) was refluxed in presence of K_2CO_3 in dry acetone for 4-6 h. After completion, the excess amount of acetone was removed under vacuum. Finally the crude product was purified by silica gel column chromatography using 1% methanol in chloroform as eluent giving **7a** as a white solid, yield 80%; mp 158-160 °C ($CHCl_3$/MeOH), IR (KBr): ν_{max}/cm^{-1} IR 2202 (CO), 1670 (CN); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.70-2.82 (1H, m), 3.31-3.34 (1H, m), 3.43 (3H, s), 3.73-4.08 (4H, m), 4.81-4.90 (1H, m), 6.50-6.58 (2H, m), 6.59-6.66 (1H, m), 6.89 (1H, s), 7.06 (1H, t, *J* 7.7), 7.50-7.61 (3H, m), 7.90-7.97 (2H, m), 9.41 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.3, 161.8, 160.0, 159.0, 157.3, 137.8, 132.0, 130.3, 129.6, 129.1, 126.1, 119.4, 117.7, 115.7, 114.2, 94.5, 70.2, 50.9, 50.6, 41.2, 37.2; MS *m/z* (ESI) 476 ($[M + H]^+$).

6-(4-Chlorophenyl)-4-((2-(5-(3-hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl)amino)-2-oxo-2*H*-pyran-3-carbonitrile (7b). Following the procedure of **7a**, compound **7b** was furnished as a white solid, yield 72%; mp 194-196 °C ($CHCl_3$/MeOH), IR (KBr): ν_{max}/cm^{-1} 2212 (CN), 1674 (CO), 1746 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.71-2.83 (1H, m), 3.30-3.32 (1H, m), 3.42 (3H, s), 3.79-3.84 (4H, m), 4.81-4.90 (1H, m), 6.51-6.58 (2H, m), 6.63 (1H, d, *J* 8.3), 6.92 (1H, s), 7.07 (1H, t, *J* 7.7), 7.63 (2H, d, *J* 8.7), 7.96 (2H, d, *J* 8.7), 9.39 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6 173.8, 171.3, 161.6, 159.8, 157.7, 157.3, 137.8, 136.7, 129.5, 129.2, 129.1, 128.0, 119.4, 117.6, 115.7, 114.2, 94.9, 79.1, 70.3, 50.9, 50.7, 41.3, 37.2; MS (ESI) *m/z* 510 ($[M + H]^+$).

6-(4-Bromophenyl)-4-((2-(5-(3-hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl)amino)-2-oxo-2*H*-pyran-3-carbonitrile (7c). Following the procedure of **7a**, compound **7c** was obtained as a white solid, yield 74%; mp 200-202 °C ($CHCl_3$/MeOH), IR (KBr): ν_{max}/cm^{-1} 2211 (CN), 1676 (CO), 1746 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.67-2.78 (1H, m), 3.35-3.38 (1H, m), 3.41 (3H, s), 3.74-4.07 (4H, m), 4.80-4.89 (1H, m), 6.49-6.56 (2H, m), 6.63 (1H, d, *J* 8.1), 6.83 (1H, s), 7.06 (1H, t, *J* 7.7), 7.77 (2H, d, *J* 8.1), 7.88 (2H, d, *J* 8.1), 9.44 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.3, 161.6, 159.8, 157.8, 157.3, 137.8, 132.1, 129.5,

128.0, 125.6, 119.4, 117.6, 115.7, 114.2, 94.9, 70.3, 50.9, 50.6, 41.2, 37.2; MS *m/z* (ESI) 554 ([M + H]$^+$).

4-((2-(5-(3-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl)amino)-2-oxo-6-*p*-tolyl-2*H*-pyran-3-carbonitrile (7d) According to the procedure described for **7a**, compound **7d** was furnished as a white solid, yield 68%; mp 192-194 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2209 (CN), 1680 (CO), 1748 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.38 (3H, s), 2.67-2.79 (1H, m), 3.35-3.37 (1H, m), 3.41 (3H, s), 3.74-4.06 (4H, m), 4.80-4.89 (1H, m), 6.49-6.66 (3H, m), 6.84 (1H, s), 7.06 (1H, t, *J* 7.8), 7.37 (2H, d, *J* 8.1), 7.84 (2H, d, *J* 8.1), 9.44 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.3, 161.8, 160.0, 159.1, 157.3, 142.3, 137.8, 129.6, 127.4, 126.0, 119.4, 117.7, 115.7, 114.2, 93.7, 69.9, 50.9, 50.6, 41.1, 37.2, 21.0; MS *m/z* (ESI) 490 ([M + H]$^+$).

4-((2-(5-(3-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl)amino)-6-(4-methoxyphenyl)-2-oxo-2*H*-pyran-3-carbonitrile (7e). According to the procedure described for **7a**, compound **7e** was furnished as a white solid, yield 66%; mp 208-210 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2207 (CN), 1671 (CO), 1740 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.68-2.80 (1H, m), 3.30-3.33 (1H, m), 3.41 (3H, s), 3.84 (3H, s), 3.87-4.05 (4H, m), 4.81-4.88 (1H, m), 6.49-6.57 (2H, m), 6.60-6.66 (1H, m), 6.74 (1H, s), 7.01-7.08 (1H, m), 7.10 (2H, d, 8.9), 7.89 (2H, d, *J* 8.9), 9.37 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 171.4, 162.0, 160.2, 158.8, 156.2, 142.1, 130.2, 129.6, 127.6, 126.2, 123.9, 118.0, 115.0, 94.2, 69.6, 61.6, 52.0, 41.2, 38.1, 21.01; MS *m/z* (ESI) 506 ([M + H]$^+$).

4-((2-(5-(3-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)methyl)amino)-6-(naphthalen-1-yl)-2-oxo-6-phenyl-2*H*-pyran-3-carbonitrile (7f). Following the procedure of **7a**, compound **7f** was synthesized as a white solid, yield 62%; mp 196-198 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2210 (CN), 1690 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.76-2.88 (1H, m), 3.30-3.35 (4H, m), 3.80-3.95 (4H, m), 4.83-4.92 (1H, m), 6.53-6.67 (3H, m), 6.71 (1H, s), 7.07 (1H, t, *J* 7.7), 7.57-7.69 (3H, m), 7.79 (1H, d, *J* 6.9), 8.03-8.10 (1H, m), 8.15 (2H, d, *J* 8.2), 9.42 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.2, 162.1, 161.2, 160.1, 157.4, 137.9, 133.2, 131.7, 129.6, 129.4, 128.6, 128.3, 127.6, 126.7, 125.2, 124.6, 119.4, 117.7, 115.8, 114.2, 99.9, 70.4, 50.9, 50.1, 41.3, 37.3; MS (ESI) *m/z* 526 ([M + H]$^+$).

4-((2-(5-(3-Hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)methyl)amino)-6-(naphthalen-2-yl)-2-oxo-6-phenyl-2*H*-pyran-3-carbonitrile (7g). Following the procedure of **7a**, compound **7g** was synthesized as a white solid, yield 56%; mp 198-200 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2207 (CN), 1676 (CO), 1748 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.71-2.83 (1H, m), 3.35-3.37 (1H, m), 3.45 (3H, s), 3.82-4.06 (4H, m), 4.82-4.90 (1H, m), 6.46-6.64 (3H, m), 6.96-7.05 (2H, m), 7.60-7.68 (2H, m), 7.97-8.13 (4H, m), 8.30 (1H, s), 9.40 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 174.4, 171.8, 162.2, 160.4, 159.3, 157.9, 138.7, 134.7, 132.9, 129.9, 129.5, 129.2, 128.7, 128.2, 128.0, 127.7, 127.2, 122.9, 119.8, 118.2, 116.2, 114.7, 95.4, 70.8, 51.4, 51.1, 41.7, 37.7; MS (ESI) *m/z* 526 ([M + H]$^+$).

6-(Furan-2-yl)-4-((2-(5-(3-hydroxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)(methyl)amino-2-oxo-2*H*-pyran-3-carbonitrile (7h). Following the procedure of **7a**, compound **7h** was obtained as a white solid, yield 48%; mp 182-184 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2209 (CN), 1680 (CO), 1748 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.72-2.80 (1H, m), 3.32-3.34 (1H, m), 3.39 (3H, m), 3.80-3.93 (4H, m), 4.80-4.89 (1H, m), 6.52-6.59 (3H, m), 6.60- 6.67 (1H, m), 6.74-6.79 (1H, m), 7.07 (1H, t, *J* 7.9), 7.24-7.28 (1H, m), 8.01-8.03 (1H, m), 9.42 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.2, 161.0, 159.7, 157.4, 150.8, 147.3, 144.8, 137.9, 129.6, 119.4, 117.7, 115.7, 114.3, 114.2, 113.2, 92.1, 69.6, 50.9, 50.7, 41.0, 37.2; MS (ESI) *m/z* 466 ($[M + H]^+$).

2-((3-Cyano-2-oxo-6-phenyl-2*H*-pyran-4-yl)(methyl)amino)ethyl 2-(4-hydroxyphenyl)acetate (9a). A mixture of 4-((2-bromoethyl)(methyl)amino)-2-oxo-6-aryl-2*H*-pyran-3-carbonitrile (**4a,** 1 mmol)**,** p-hydroxy phenyl acetic acid (**8**, 1.2 mmol) and K_2CO_3 stirred in dry DMF for 4-6 h. After completion, the reaction mixture was poured onto crushed ice with vigorous stirring and neutralized with 10% aqueous HCl. The resulting precipitate was filtered, washed with water, dried and purified using silica gel chromatography. Finally the crude product was purified by silica gel column chromatography using 1% methanol in chloroform as eluent giving **9a** as a white solid, yield 82%; mp 238-239 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2202 (CN), 1670 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.37-3.42 (5H, m), 4.03-4.09 (2H, m), 4.28-4.36 (2H, m), 6.61 (2H, d, *J* 8.5), 6.91-6.97 (3H, m), 7.50-7.59 (3H, m), 7.91-7.98 (2H, m), 9.33 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 173.8, 171.3, 162.2, 161.8, 160.1, 159.1, 157.4, 137.8, 129.5, 128.1, 122.5, 119.4, 117.9, 115.7, 114.6, 114.2, 92.7, 69.6, 55.6, 50.9, 50.5, 37.2; MS (ESI) *m/z* 405 ($[M + H]^+$).

2-((6-(4-Chlorophenyl)-3-cyano-2-oxo-2*H*-pyran-4-yl)(methyl)amino)ethyl 2-(4-hydroxyphenyl)acetate (9b). According to the procedure described for **9a**, compound **9b** was furnished as a white solid, yield 79%; mp 182-184 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2202 (CN), 1670 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.39 (3H, s), 3.47 (2H, s), 4.04-4.08 (2H, m), 4.28-4.36 (2H, m), 6.57-6.65 (2H, m), 6.89-6.99 (3H, m), 7.62 (2H, d, *J* 8.7), 7.98 (2H, d, *J* 8.8), 9.29 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 172.3, 162.5, 160.9, 158.3, 157.1, 137.4, 131.0, 130.2, 129.9, 128.9, 127.5, 126.3, 124.8, 118.8, 115.9, 96.4, 62.5, 52.9, 42.2; MS (ESI) *m/z* 439 ($[M + H]^+$).

2-((6-(4-Bromophenyl)-3-cyano-2-oxo-2*H*-pyran-4-yl)(methyl)amino)ethyl 2-(4-hydroxphenyl)acetate (9c). According to the procedure described for **9a**, compound **9c** was furnished as a white solid, yield 84%; mp 180-182 ^{o}C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2208 (CN), 1702 (CO), 1640 (CO); ^{1}H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.38-3.42 (3H, m), 3.47 (2H, s), 4.03-4.08 (2H, m), 4.28-4.36 (2H, m), 6.61 (2H, d, *J* 8.4), 6.90-6.99 (3H, m), 7.74 (2H, d, *J* 8.7), 7.89 (2H, d, *J* 8.7), 9.31 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 171.4, 161.7, 159.9, 157.5, 156.2, 131.9, 130.1, 129.6, 129.0, 128.1, 125.4, 123.9, 117.9, 115.0, 95.4, 70.0, 61.6, 51.9, 41.3; MS (ESI) *m/z* 483 ($[M + H]^+$).

2-((3-Cyano-2-oxo-6-*p*-tolyl-2*H*-pyran-4-yl)-(methyl)amino)ethyl 2-(4-hydroxyphenyl)acetate (9d). The compound was prepared as described for **9a**, compound **9d** was obtained as a white solid, yield 75%; mp 238-239 °C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2202 (CN), 1670 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 2.38 (3H, s), 3.39 (3H, s), 3.47 (2H, s), 4.01-4.08 (2H, m), 4.28-4.36 (2H, m), 6.62 (2H, d, *J* 8.5), 6.90-6.98 (3H, m), 7.36 (2H, d, *J* 8.2), 7.86 (2H, d, *J* 8.2), 9.26 (1H, s); MS (ESI) *m/z* 419 ($[M + H]^+$).

2-((3-Cyano-6-(4-methoxyphenyl)-2-oxo-2*H*-pyran-4-yl)(methyl)amino)ethyl 2-(4-hydroxyphenyl)acetate (9e). According to the procedure described for **9a**, compound **9e** was produced as a white solid, yield 70%; mp 238-239 °C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2202 (CN), 1670 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.35-3.39 (3H, m), 3.46 (2H, s), 3.82 (3H, s), 4.01-4.06 (2H, m), 4.26-4.32 (2H, m), 6.62 (2H, d, *J* 8.2), 6.80 (1H, s), 6.93 (2H, d, *J* 8.2), 7.06 (2H, d, *J* 8.8), 7.89 (2H, d, *J* 8.8), 9.29 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 171.4, 162.1, 160.3, 158.8, 156.2, 130.2, 128.1, 124.0 122.6, 118.2, 115.0, 114.4, 93.2, 69.2, 61.6, 55.5, 51.9, 41.2; HRMS (ESI) exact mass calcd. for $C_{24}H_{23}N_2O_6$ $[M + H]^+$; 435.1556; found: 435.1581 ($[M + H]^+$).

2-((3-Cyano-6-(naphthalen-1-yl)-2-oxo-2*H*-pyran-4-yl)(methyl)amino)ethyl 2-(4-hydroxyphenyl)acetate (9f). According to the procedure described for **9a**, compound **9f** was produced as a white solid, yield 64%; mp 238-239 °C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2202(CN), 1670(CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.37-3.40 (3H, m), 3.42-3.46 (2H, m), 4.03-4.09 (2H, m), 4.29-4.36 (2H, m), 6.60 (2H, d, *J* 8.4), 6.94 (2H, d, *J* 8.4), 7.05 (1H, s), 7.53-7.67 (2H, m), 7.93-8.09 (5H, m), 9.34 (1H); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 171.4, 160.8, 160.1, 156.2, 133.1, 131.5, 129.7, 129.5, 128.6, 128.4, 127.5, 126.7, 125.2, 124.6, 123.9, 115.1, 100.0, 70.0, 61.7, 52.0, 41.3; MS (ESI) *m/z* 455 ($[M + H]^+$).

2-((3-Cyano-6-(naphthalen-2-yl)-2-oxo-2*H*-pyran-4-yl)(methyl)amino)ethyl 2-(4-hydroxyphenyl)acetate (9g). According to the procedure described for **9a**, compound **9g** was obtained as a white solid, yield 58%; mp 200-202 °C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2202 (CN), 1670 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.40 (3H, s), 3.47 (2H, s), 4.04-4.09 (2H, m), 4.29-4.36 (2H, m), 6.60 (2H, d, *J* 8.4), 6.94 (2H, d, *J* 8.4), 7.05 (1H, s), 7.53-7.67 (2H, m), 7.93-8.09 (4H, m), 8.51 (IH, s), 9.25 (1H); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 171.4, 161.8, 160.0, 158.4, 156.2, 134.1, 132.4, 130.2, 129.0, 128.6, 128.1, 127.6, 127.1, 126.6, 123.8, 122.6, 117.9, 115.0, 95.3, 70.0, 61.6, 52.0, 41.3; MS (ESI) *m/z* 455 ($[M + H]^+$).

2-((3-Cyano-6-(furan-2-yl)-2-oxo-2*H*-pyran-4-yl)-(methyl)amino)ethyl 2-(4-hydroxyphenyl)acetate (9h). According to the procedure described for **9a**, compound **9h** was produced as a white solid, yield 55%; mp 176-178 °C ($CHCl_3$/MeOH); IR (KBr): ν_{max}/cm^{-1} 2212 (CN), 1674 (CO); ^1H NMR δ_H (300 MHz; DMSO-d_6; Me_4Si) 3.37-3.42 (3H, m), 3.48 (2H, s), 3.97-3.99 (2H, m), 4.28-4.35 (2H, m), 6.62 (2H, d, *J* 8.5), 6.67 (1H, s), 6.74-6.80 (1H, m), 6.95 (2H, d, *J* 8.5), 7.23-7.28 (1H, m), 8.00-8.03 (1H, m), 9.30 (1H, s); ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 171.4,

161.2, 159.9, 156.2, 150.5, 147.0, 144.9, 130.1, 123.9, 117.9, 115.0, 114.1, 113.1, 92.6, 69.4, 61.5, 52.0, 41.0; MS (ESI) *m/z* 395 ($[M + H]^+$).

Material and methods

Biological methods: Reagents and chemicals

Silymarin was purchased from Sigma (St. Louis, MO, USA), dissolved in DMSO and administered orally. Thioacetamide was dissolved in water and was administered orally. Rosiglitazone was purchased from promega. DMEM was purchased from Gibco. Dexamethasone, IBMX and Oil-Red-O was purchased from Sigma.

Cell culture and adipogenic differentiation

3T3-L1 mouse embryo fibroblasts cell line was obtained from the American Type Culture Collection. Cells were cultured in a humidified atmosphere at 37ºC and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum and antibiotic penicillin and streptomycin. For adipogenesis induction 50000 cells were seed in 24 multi-well plates. After 2 days of when cells achieved near complete confluence, culture media was replaced with adipogenesis media I (containing Insulin 5 µg/ml, IBMX 0.5 mM and Dexamethasone 250 nm in culture medium). This media was then replaced after 72 hours with adipogenesis media II (Insulin 5 µg/ml in DMEM with 10% FBS). After replacement of this media, cells were then maintained next 2 days in 10% FBS containing DMEM medium. Lipid globules in the adipogenic cells starts forming from day 4^{th} onwards after treatment, and fully developed adipocytes were observed after day 6-8^{th} of adipogenesis treatment. More than 90% cells do have lipid globules at this stage.

Western blotting

Cells were lysed in ice-cold mammalian PE-LB lysis buffer (G-Biosciences cat no-783-180) (containing 0.5M EDTA, protease inhibitor. Protein concentrations were measured by Bicin-choninic acid method (Sigma). Protein lysates were denatured by heating at 65 °C for 10 minutes in Laemmli sample buffer supplemented with 10% ß-mercaptoethanol. Equal quantity of protein were loaded and resolved by 8-12% SDS-PAGE and transferred to Nitrocellulose paper using MiniProtean III Electrophoresis and blotting system (Bio-Rad) overnight at 35 V. The membranes were blocked for 1 hr at room temperature in 5% skimmed milk (Sigma) in Tris buffered saline containing 0.05% Tween-20 (TBS-T). After washing with TBS-T, the membranes were incubated with target protein specific antibodies for 14 hours at 4 °C, followed by incubation with appropriate HRP-conjugated secondary antibodies for 1 hour. The target proteins were detected using Immobiline western Chemiluminescence detector (Millipore) according to the manufacturer's protocol. To validate equal loading in each lane and normalize the blots for protein levels, Actin was used as internal loading control.

Triglyceride assay and Oil Red O staining

To study effect of compound on adipogenic differentiation, cells were differentiated as mentioned in above protocol along-with compound at stated concentrations. Fully differentiated 3T3-L1 (with or without compound) adipocytes were rinsed in phosphate buffered saline (pH 7.4). The adipocytes lipid globules were stained with Oil Red O (0.36% in 60% Isopropanol) for 20 min. Unstained Oil Red O was removed by rinsing wells twice with phosphate buffer saline. After complete removal of PBS, finally, 100% Isopropanol was used to extract the dye from the cells and extracted dye absorbance was measured at 492 nm.

Radio-labeled glucose uptake assay

After 8 days of induction of differentiation, test compounds were added for an additional 24 hours. The compounds used in the study were solubilized in dimethysulfoxide (DMSO), control cells were treated with matching concentrations of DMSO. Cells were then serum starved for 2 hours in low glucose (5mM) serum free DMEM supplemented with 0.5% fatty acid free BSA with compound. Then the cells were washed twice with freshly prepared Krebs–Ringer–Phosphate buffer. After that cells were treated with KRH buffer alone as control and insulin (100nM)-KRH as treated for 30 min. Cells was washed and pulsed with 100 µM 2-deoxy D-glucose and 1 µCi 2-deoxy-D-[3H]-glucose/ml. Finally cells were washed three time with ice cold PBS to stop the further uptake and lysed using 0.1 N NaOH. 5µl of lysates were taken for protein estimation by BCA (sigma) method with bovine serum albumen (BSA) as standard. Remaining 170µL lysate was added in 3ml cocktail W (SRL) scintillation fluid. After overnight, lysate solublization in fluid reading were taken on scintillation counter (Perkin Elmer).CPM count of radioactivity was normalized with protein content and expressed in pmol/mg.min.

Chronic insulin induced insulin resistance

To validate 3T3-L1 adipocytes as a model for analyzing insulin resistance, as the compounds used in the study were solubilized in Dimethyl sulfoxide (DMSO), control cells were treated with matching concentrations of DMSO with compound. After differentiation of 8 days cells, adipocytes were treated with 10 nM insulin for 24 h along-with compound/DMSO, in a defined time frame [12 PM, 4 PM, 6PM and 8AM] as sufficient to complete the desensitization process. Sample processing was initiated at 12 PM. It was imperative to reestablish basal transport after chronic insulin, extensive washing was performed using Krebs Ringer HEPES (KRH) buffer supplemented with 5mM D-glucose and 0.05% BSA. The cells were washed once with freshly prepared Krebs–Ringer–Phosphate buffer. After the cells were treated with KRH buffer alone as control and insulin (100nM)-KRH as treated for 30 min. Cells were washed and pulsed with 100 µM 2-deoxy D-glucose and 1 µCi 2-deoxy-D-[3H]-glucose/ml. Finally cells were washed three time with ice cold PBS to stop the reaction and lysed using 0.1 N NaOH. 5µl of lysates were taken for protein estimation by BCA (sigma) method with bovine serum albumen (BSA) as standard. Remaining 170µL lysate was added in 3ml cocktail W (SRL) scintillation fluid. After overnight, lysate solublization in fluid reading were taken on scintillation counter (Perkin Elmer).CPM count of radioactivity was normalized with protein content and expressed in pmol/mg.min.

Animals and Drug administration

10-12 week old male Swiss albino mice, weighing about 25-30g were procured from Animal Division of the Institute and randomly allocated to following 6 groups comprising of six animals each. The work with these animals was cleared by institutional ethics committee for animal study, and was conducted in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964. The animals were acclimatized to laboratory conditions of optimal temperature (25±2°C) and light/dark cycle (12 h each) prior to drug administration. Food and water was supplied ad libitum throughout the experiment. All animal procedures were performed in compliance to institutional animal ethics guidelines. Dosage for the study is decided on the basis of dose-deciding pilot study (data not shown) which was carried to mark out the minimum dose of Thioacetamide required to achieve the appreciable change in the serum markers of hepatotoxicity and silymarin to achieve the protection against Thioacetamide treatment.

Group I: Controls, DMSO treated

Group II: 200 mg/kg Thioacetamide (a known hepatotoxicant) treatment on day 1.

Group III: Thioacetamide (200 mg/kg) treatment on the first day and then silymarin (20 mg/kg, a known hepatoprotectant) for the next 7 consecutive days.

Group IV: Thioacetamide (200 mg/kg) treatment on the first day and then with 4b (20 mg/kg) for the next 7 consecutive days.

Group V: Thioacetamide (200 mg/kg) treatment on the first day and then with 7e (20 mg/kg) for the next 7 consecutive days.

Group VI: Thioacetamide (200 mg/kg) treatment on the first day and then with 9e (20 mg/kg) for the next 7 consecutive days.

All animals were sacrificed by cervical dislocation on the 8^{th} day of treatment.

Blood collection and serum biochemistry

At autopsy blood was withdrawn from each animal and allowed to stand undisturbed for 30 min. The serum was separated by centrifugation at 2,000 rpm for 15 min in a minifuge (Sigma). The activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), and Total Bilirubin (T-Bill) in serum were estimated using an automated biochemical analyzer (Beckman, Coulter, California, USA).

Tissue preparation for liver histology

A part of liver tissue was fixed in 10% formal saline for histological investigations. Fixed liver tissue was washed overnight, dehydrated through graded alcohols and embedded in paraffin wax. Serial sections of 4-5µm thickness were stained with hematoxylin and eosin (H&E) for histological examinations.

Table S1. Augmentation of Glucose uptake in presence of Insulin

	Control	Insulin	Insulin + Rosi	Insulin + 4b	Insulin + 7e	Insulin + 9e
Average	7.4722	14.4328	15.9196	17.7696	15.7309	17.4970
SD	0.5298	0.1657	0.0956	0.1422	0.0939	0.4055
P-value			0.0082	0.0021	0.0106	0.0101
			***	***	**	**

Table S2. Insulin resistance prevention

	Control	Insulin	CI-con	CI-Insulin	Insulin - Rosi	Insulin (4b)	Insulin (7e)	Insulin (9e)
Average	4.3902	12.2586	4.8302	10.5345	14.4152	16.5195	10.0979	14.8404
SD	0.0354	0.1649	0.0298	0.6441	0.4711	0.4642	0.0543	0.4601
P-value					0.0205	0.0087	0.4403	0.0165
					**	***	NS	**

Table S3. Hepatoprotective activity profile with percentage protection

Biochemical parameters	ALT (IU L^{-1})	AST (IU L^{-1})	ALP (IU L^{-1})	TBil (IU L^{-1})
Control (Group I)	42.2 ± 2.15	89.67 ± 1.23	354.5 ± 13.99	0.26 ± 0.011
Thioacetamide (Group II)	104.4 ± 11.29	353.1 ± 17.84	595.3 ± 44.59	0.5 ± 0.041
Thioacetamide + Silymarin	46.67 ± 4.86 (92.83)***	102.6 ± 8.07 (95.1)	208.1 ± 12.53 (>100)	0.24 ± 0.024 (>100)***
Thioacetamide+ 4b	27.53 ± 0.59 (>100)***	123.2 ± 10.40 (87.28)	295.1 ± 52.75 (>100)	0.18 ± 0.043 (>100)***
Thioacetamide + 7e	48.73 ± 8.92 (89.51)***	154.1 ± 25.38 (75.55)	237.4 ± 54.79 (>100)	0.25 ± 0.033 (>100)***
Thioacetamide + 9e	46.87 ± 8.96 (92.5)***	194.7 ± 15.98 (60.13)	255.2 ± 21.55 (>100)	0.073 ± 0.013 (>100)***

Values are mean ± SE. % Protection is given in the parenthesis. The percentage of protection of the test compounds is calculated in comparison the positive control.