Electronic Supplementary Material (ESI) for MedChemComm.

Construction of phenoxazine ring containing nitro and sulfonic acid group leading to phenoxazine-3-sulfonamide derivatives: Their evaluation as novel and potential insulin secretagogues

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Experimental Section

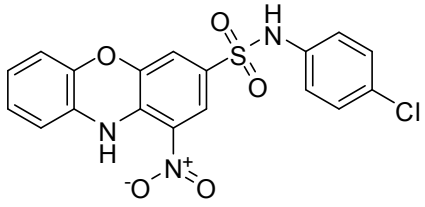
Chemistry

All the reactions were performed under nitrogen atmosphere. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (60-120 mesh) using hexane, ethyl acetate, dichloromethane, and methanol. ^{1}H NMR and ^{13}C NMR spectra were determined in DMSO-d_6 solution by using a 400 MHz spectrometer. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as brs (broad). Coupling constants (*J*) were given in hertz. Infrared spectra were recorded on a FT-IR, Bruker Vertex-70 spectrometer using KBR pellets. Melting points were determined using melting point apparatus and are uncorrected. MS spectra's was recorded on Agilent 6300 Ion Trap LC/MS System.

General procedure for synthesis of *N*-(alkyl/aryl and heteroaryl)-1-nitro-10*H*-phenoxazine-3-sulfonamides (6b-u)

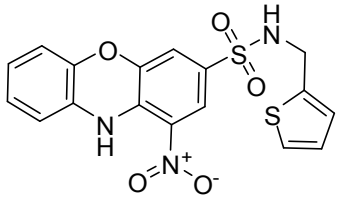
To a solution of alkyl/aryl or heteroaryl amine (1.0 to 1.5 equivalents) and triethylamine (2.0 equivalents) in chloroform (30 mL) was added 1-nitro-10*H*-phenoxzine-3-sulfonylchloride (1.0 equivalent). The mixture was stirred for 30 min under reflux. Completion of the reaction was monitored by TLC (30% chloroform in hexane). The excess of solvent was removed from the reaction mass under reduced pressure and the crude solid was diluted with ethyl acetate (50 mL) and washed with water (100 mL), dilute hydrochloric acid (100 mL), water and saturated sodium chloride solution. Then the organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The compound was purified by column chromatography using 60–120 silica gel and 20 % chloroform and hexane.

N-(4-Chlorophenyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6b)



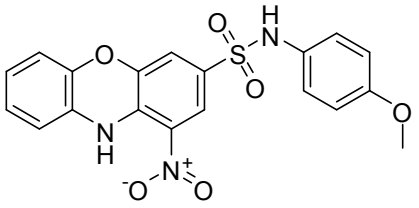
Compound **6b** was synthesized according to above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (2.0 g, 6.1 mmol), 4-chloroaniline (0.93g, 7.3 mmol) and triethyl amine (1.23g, 12.0 mmol) in chloroform (30 mL) to yield 2.0 g (78%), brick red solid, mp 234-237 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 10.46 (br s, 1H), 9.75 (s, 1H), 7.81 (d, 1H, *J*=2.0 Hz), 7.35 (d, 2H, *J*=8.4 Hz), 7.21-7.19 (m, 1H), 7.13 (d, 2H, *J*=8.4 Hz), 6.97 (d, 1H, *J*=2.0 Hz), 6.87- 6.81 (m, 2H), 6.74-6.72 (m, 1H); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 146.0, 142.8, 136.7, 135.4, 130.5, 129.8 (2C), 129.0, 128.7, 127.2, 125.1, 125.0, 122.5 (2C), 120.1, 117.5, 115.5, 115.2; IR (KBr) cm^{-1} v_{max} 3339 (NH), 3238 (NH-SO_2), 3105 (aryl CH), 1639, 1606, 1575, 1532, 1498 (aryl C=C), 1336 (NO_2); MS (ES, -Ve): 417.5 (M-1); Elemental analysis found C, 51.57; H, 2.85; N, 10.23; $C_{18}H_{12}ClN_3O_5S$ requires C, 51.74; H, 2.89; N, 10.06.

***N*-(Thiophen-2-ylmethyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6c)**



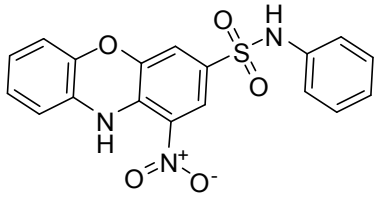
Compound **6c** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (2.0 g, 6.1 mmols), c-thiophene-2yl methylamine (0.62g, 6.1 mmols) and triethyl amine (1.23g, 12.0 mmol) in chloroform (30 mL) to yield 2 g (81%), reddish brown solid, mp 246-248 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.74 (s, 1H), 8.32 (t, 1H, *J*=5.6 Hz), 7.82 (d, 1H, *J*=2.0 Hz), 7.40 (dd, 1H, *J*=1.2, *J*=4.0 Hz), 7.24-7.21 (m, 1H), 7.03 (d, 1H, *J*= 2.0 Hz), 6.94-6.85 (m, 4H), 6.77-6.75 (m, 1H), 4.23 (d, 2H, *J*= 4.0 Hz); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 145.3, 142.5, 140, 130, 127, 126.5, 126.3, 125.7, 124.6, 119.3, 116.9, 115.3, 115.1, 41.2 (CH_2NH); IR (KBr) cm^{-1} v_{max} 3335 (NH), 3292 (NH-SO_2), 3100 (aryl CH), 1574, 1504 (aryl C=C), 1530 & 1338 (NO_2), 1282 & 1154 (SO_2), 1228 (aryl C-O), 870 and 760; MS (ES, -ve): 403 (M-1); Elemental analysis found C, 50.83; H, 3.26; N, 10.23; $C_{17}H_{13}N_3O_5S_2$ requires C, 50.61; H, 3.25; N, 10.42.

***N*-(4-Methoxyphenyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6d)**



Compound **6d** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (2.0 g, 6.1 mmols), *p*-anisidine (1.0g, 7.3 mmols) and triethyl amine (1.23g, 12.0 mmol) in chloroform (30 mL) to yield 2.0 g (77%), reddish brown solid, mp 230-231 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.92 (s, 1H), 9.73 (s, 1H), 7.74 (d, 1H, *J*=2.0 Hz), 7.22-7.20 (m, 1H), 7.1 (d, 2H, *J*=8.8 Hz), 6.94 (d, 1H, *J*=2.0 Hz), 6.87-6.84 (m, 4H), 6.76-6.73 (m, 1H), 3.68 (s, 1H); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 153.5, 145.0, 142.1, 140.0, 134.2, 130.5, 129.2, 127.0, 124.2, 119.3, 117.1, 116.4, 115.4, 115.2, 115.1, 55.9 (OMe); IR (KBr) cm^{-1} ν_{max} 3326 (NH), 3288 (NH-SO_2), 3100 (aryl CH), 1569 & 1500 (aryl C=C), 1521 & 1341 (NO_2), 1281 & 1151 (SO_2), 1228 (aryl C-O), 878, 748; MS (ES, -ve): 413 (M-1); Elemental analysis found C, 55.53; H, 3.65; N, 10.03; $C_{19}H_{15}N_3O_6S$ requires C, 55.20; H, 3.66; N, 10.16.

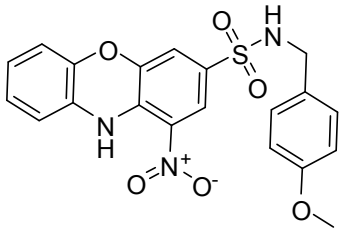
***N*-(Phenyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6e)**



Compound **6e** was synthesized according to the above general procedure by using 1-nitro-10*H*-phenoxazin-3-sulfonyl chloride (1.0 g, 3.0 mmols), aniline (0.34 g, 3.6 mmols) and triethylamine (0.61g, 6.0 mmol) in chloroform (20 mL) to yield 1.0 g (85%), reddish brown solid, mp 233-234 °C; ^{1}H-NMR, 400 MHz, (DMSO-d_6) δ 10.28 (br s, 1H), 9.70 (s, 1H), 7.80 (d, 1H, *J*=2.0 Hz), 7.27 (t, 2H, *J*=8.4 Hz), 7.18-7.16 (m, 1H), 7.11-7.05 (m, 3H), 6.95 (d, 1H, *J*=2.0 Hz), 6.85-6.79 (m, 2H), 6.72-6.70 (m, 1H); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 145.9, 142.8, 137.6, 135.3, 130.5, 129.8 (2C), 129.0,

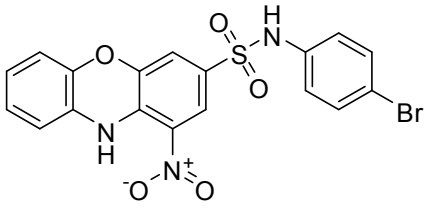
127.2, 125.1, 125.0 (2C), 120.9 (2C), 120.0, 117.5, 115.5, 115.3; IR (KBr) cm^{-1} v_{max} 3304 (NH & NH-SO_2), 1634, 1600, 1592, 1575, 1513, 1500 (aryl C=C), 1524 & 1352 (NO_2), 1277 & 1149 (SO_2), 1232 (aryl C-O); MS (Es, -ve): 382 (M-1); Elemental analysis found C, 56.18; H, 3.45; N, 10.73; $C_{18}H_{13}N_3O_5S$ requires C, 56.39; H, 3.42; N, 10.96.

_N_-(4-Methoxybenzyl)-1-nitro-10_H_-phenoxazin-3-sulfonamide (6f)



Compound **6f** was synthesized according to the general procedure using 1-nitro-10*H*-phenoxazin-3- sulfonylchloride (2.0 g, 6.1 mmol), 4-methoxybenzylamine (0.98g, 7.9 mmol) and triethylamine (1.23g, 12.0 mmol) in chloroform (30 mL) to yield 2.0 g (74%), reddish brown solid, mp 211-213 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.70 (s, 1H), 8.13 (t, 1H, *J*=6.4 Hz), 7.72 (d, 1H, *J*= 2.0 Hz), 7.24-7.22 (m, 1H), 7.12 (d, 2H, *J*=8.8Hz), 6.91-6.74 (m, 6H), 3.97 (d, 2H, *J*=6.4Hz), 3.63(s, 3H); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 161.5, 145.3, 142.4, 135.7, 134.5, 132, 129.9, 129.7, 127, 124.6, 119.9, 116.9, 115.3, 115.1, 114.2, 56.5 (OMe), 49.2 (CH_2); IR (KBr) cm^{-1} v_{max} 3335 (NH), 3292 (NH-SO_2), 3100 (aryl CH), 1574 & 1504 (aryl C=C), 1530 & 1338 (NO_2), 1282 & 1154 (SO_2), 1228 (aryl C-O), 870 and 760; MS (ES, -ve): 427 (M-1); Elemental analysis found C, 56.55; H, 4.00; N, 9.63; $C_{20}H_{17}N_3O_6S$ requires C, 56.20; H, 4.01; N, 9.83.

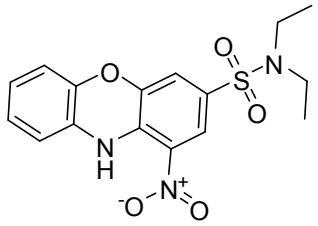
_N_-(4-Bromophenyl)-1-nitro-10_H_-phenoxazin-3-sulfonamide (6g)



Compound **6g** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazine-3-sulfonylchloride (1.8 g, 5.5 mmol), 4-bromoaniline (1.0 g, 6.0 mmol)

and triethyl amine (1.11g, 11.0 mmol) in chloroform (30 mL) to yield 2.0 g (80%), brick red solid, mp 250-252 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 10.46 (br s, 1H), 9.75 (s, 1H), 7.83 (d, 1H, *J*=2.0 Hz), 7.48 (d, 2H, *J*=8.8 Hz), 7.21-7.19 (m, 1H), 7.09-7.05 (d, 2H, *J*=8.8 Hz), 6.99 (d, 1H, *J*=2.0 Hz), 6.87-6.82 (m, 2H), 6.75-6.73 (m, 1H); IR (KBr) cm^{-1} ν_{max} 3340 (NH), 3236 (NH-SO_2), 3105 (aryl CH), 1636, 1606, 1575, 1504 (aryl C=C), 1532 & 1336 (NO_2), 1265 & 1141 (SO_2), 1225 (aryl C-O); MS (ES, -ve): 462 (M-1); Elemental analysis found C, 46.95; H, 2.65; N, 8.83; $C_{18}H_{12}BrN_3O_5S$ requires C, 46.77; H, 2.62; N, 9.09.

***N,N*-Diethyl-1-nitro-10*H*-phenoxazine-3-sulfonamide (6h)**



Compound **6h** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (2.0 g, 6.1 mmol) and diethylamine (1.34 g, 18 mmol) in chloroform (30 mL) to yield 1.8 g (81%), brown solid, mp 237-239 °C; Mass: calculated for $C_{16}H_{17}N_3O_5S$ 363, found 362 (M-H$^+$). ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.76 (s, 1H), 7.81 (d, 1H, *J*=2.0 Hz), 7.24-7.22 (m, 1H), 7.08 (d, 1H, *J*=2.0 Hz), 6.90-6.83 (m, 2H), 6.75 (m, 1H), 3.17 (q, 4H, *J*=7.2 Hz), 1.07 (t, 6H, *J*=7.2 Hz); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 145.6, 142.5, 134.5, 130.3, 129.1, 127.0, 124.6, 119.3, 117, 115.2, 115.0, 41.9 (2C, CH_2), 14.9 (2C, Me); IR (KBr) cm^{-1} ν_{max} 3339 (NH), 3099 (aryl CH), 1575 & 1507 (aryl C=C), 1531 & 1333 (NO_2), 1290 & 1141 (SO_2), 1224 (aryl C-O), 876 and 755; MS (ES, -ve): 363 (M-1); Elemental analysis found C, 52.58; H, 4.73; N, 11.73; $C_{16}H_{17}N_3O_5S$ requires C, 52.88; H, 4.72; N, 11.56.

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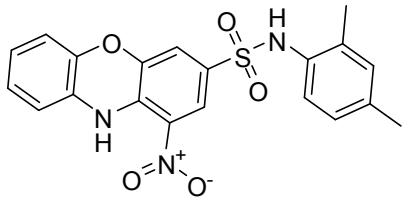
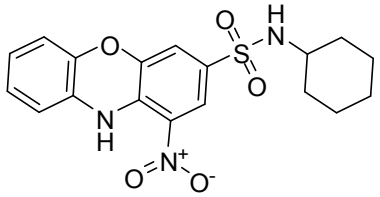
***N*-(2,4-Dimethylphenyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6i)**



Compound **6i** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (2.0 g, 6.1 mmol), 2,4-dimethylaniline (0.89 g, 7.3 mmol) and triethyl amine (1.23g, 12.0 mmol) in chloroform (30mL) to yield 1.7g (67%), reddish color solid, mp 247-249 °C; Mass: calculated for $C_{20}H_{17}N_3O_5S$ 411, found 410(M-H^+). ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.77(s, 1H), 9.52 (s, 1H), 7.69(d, 1H, *J*=2.0Hz), 7.24-7.22 (m, 1H), 7.01 (s, 1H), 6.94-6.84 (m, 5H), 6.76-6.74 (m, 1H), 2.21 (s, 3H), 2.13 (s, 3H); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 146.4, 145.4, 142.5, 134.5, 132.7, 131.9, 130.7, 129.6, 129.4, 127.0, 125.2, 124.6, 119.9, 116.9, 116.8, 115.3, 115.1, 22.3 (Me), 14.2 (Me); IR (KBr) cm^{-1} ν_{max} 3336 (NH), 3293 (NH-SO_2), 3016 (aryl CH), 1574 & 1502 (aryl C=C), 1529 & 1337 (NO_2), 1281 & 1153 (SO_2), 1228 (aryl C-O), 871 and 759; MS (ES, -ve): 411 (M-1); Elemental analysis found C, 58.59; H, 4.15; N, 10.03; $C_{20}H_{17}N_3O_5S$ requires C, 58.38; H, 4.16; N, 10.21.

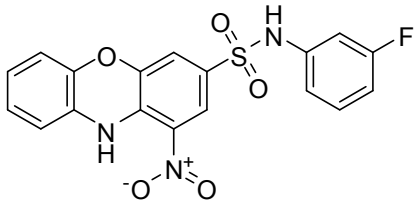
***N*-(Cyclohexyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6j)**



Compound **6j** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazine-3-sulfonylchloride (2.0 g, 6.1 mmol), cylcohexylamine (0.79g, 7.3 mmol) and triethyl amine (1.23g, 12.0 mmol) in chloroform (30 mL) to yield 2.0 g (81%), brick red solid, mp 222-224 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.74 (s, 1H), 7.87 (d, 1H, *J*=2.0), 7.70 (d, 1H, *J*=7.2 Hz), 7.23-7.20 (m, 1H), 7.10 (d, 1H, *J*=2.0Hz), 6.89-6.83 (m, 2H), 6.77-6.75 (m, 1H), 2.94-2.92 (m, 1H), 1.65-1.02 (m, 10H); IR (KBr) cm^{-1} ν_{max} 3331 (NH), 3290 (NH-SO_2), 3102 (aryl CH), 1574, 1500 (aryl C=C), 1528 &

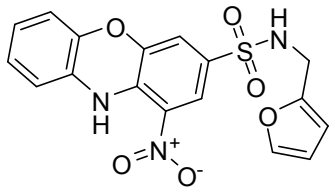
1335 (NO_2), 1279 & 1150 (SO_2), 1225 (aryl C-O), 870 and 760; MS (ES, -ve): 388 (M-1); Elemental analysis found C, 55.79; H, 4.95; N, 10.53; $C_{18}H_{19}N_3O_5S$ requires C, 55.52; H, 4.92; N, 10.79.

***N*-(3-Fluorophenyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6k)**



Compound **6k** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (1.8 g, 5.5 mmol), 3-fluoroaniline (0.73g, 6.6mmol) and triethyl amine (1.11g, 11.0 mmol) in chloroform (30 mL) to yield 1.7 g (77%), brown solid, mp 254-256 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 10.55 (br s, 1H), 9.67 (s,1H), 7.77 (d, 1H, *J*=2.0 Hz), 7.28-7.22 (m, 1H), 7.12-7.09 (m, 1H), 6.92 (d, 1H, *J*=2.0 Hz), 6.89-6.81 (m, 3H), 6.78-6.73 (m, 2H), 6.66-6.63 (m, 1H); IR (KBr) cm^{-1} ν_{max} 3321 (NH & NH-SO_2), 3085 (aryl CH), 1635, 1606, 1591, 1571, 1500 (aryl C=C), 1521 & 1326 (NO_2), 1281 & 1144 (SO_2), 1226 (aryl C-O); MS (ES, -ve): 401 (M-1); Elemental analysis found C, 53.59; H, 3.08; N, 10.63; $C_{18}H_{12}FN_3O_5S$ requires C, 53.86; H, 3.01; N, 10.47.

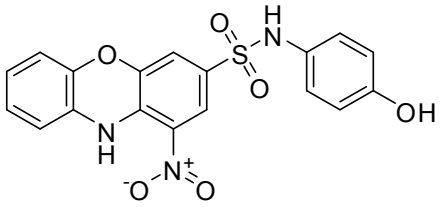
***N*-(Furfuryl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6l)**



Compound **6l** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (2.0 g, 6.1 mmol), furfuraldehyde (0.89g, 9.2mmol) and triethyl amine (1.23g, 12.0 mmol) in chloroform (30 mL) to yield 1.2 g (54%), brown solid, mp 205-207 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.69 (s, 1H), 8.21(t, 1H, *J*=6.0Hz), 7.77 (d, 1H, *J*=2.0 Hz), 7.49 (d, 1H, *J*=1.2 Hz), 7.20-7.18 (m, 1H), 6.99 (d, 1H,

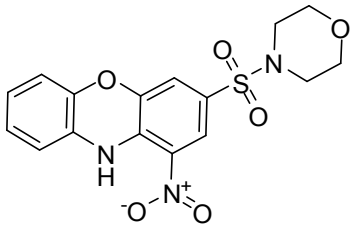
J=2.0 Hz), 6.88-6.81 (m, 2H), 6.75-6.72 (m,1H), 4.03 (d, 2H, *J*=6.0 Hz); IR (KBr) cm^{-1} ν_{max} 3326 (NH), 3289 (NH-SO_2), 3096 (aryl CH), 1635, 1605, 1590, 1570, 1500 (aryl C=C), 1522 & 1332 (NO_2), 1281 & 1154 (SO_2), 1227 (aryl C-O); MS (ES, -ve): 385 (M-1); Elemental analysis found C, 52.56; H, 3.35; N, 10.99; $C_{17}H_{13}N_3O_6S$ requires C, 52.71; H, 3.38; N, 10.85.

_N_-(4-Hydroxyphenyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6m)



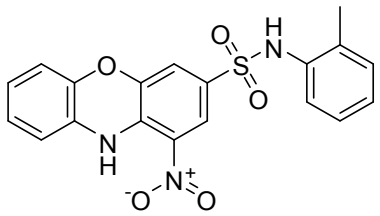
Compound **6m** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (2.0 g, 6.1 mmol), 4-aminophenol (1.0 g, 9.1 mmol) and triethylamine (1.23g, 12.0 mmol) in chloroform (30 mL) to yield 1.5g (61%), brown solid; mp 248-250 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.73 (s, 1H), 9.71 (s, 1H), 9.39 (s, 1H), 7.71 (d, 1H, *J*=2.0 Hz), 7.21-7.19 (m, 1H), 6.91-6.88 (m, 3H), 6.86-6.84 (m, 2H), 6.75 (m, 1H), 6.66 (d, 2H, *J*=8.8Hz); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 155.7, 145.8, 142.9, 135.1, 130.5, 129.3, 128.3, 127.4, 125.2 (3C), 119.9, 117.5, 116.2 (2C), 115.7 (2C); IR (KBr) cm^{-1} ν_{max} 3334 (NH), 3324 (NH-SO_2), 1639, 1609, 1590, 1573, 1510 (aryl C=C), 1533 & 1322 (NO_2), 1279 & 1147 (SO_2), 1227 (aryl C-O); MS (ES, -ve): 398 (M-1); Elemental analysis found C, 54.30; H, 3.25; N, 10.23; $C_{18}H_{13}N_3O_6S$ requires C, 54.13; H, 3.28; N, 10.52.

3-(Morpholinosulfonyl)-1-nitro-10*H*-phenoxazin (6n)



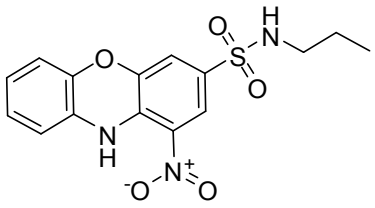
Compound **6n** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (1.5 g, 4.5 mmol) and morpholine (0.6 g,6.8 mmol) in chloroform (25 mL) to yield 1.6g (93%), brick red solid, mp 246-248 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.82 (s, 1H), 7.75 (d, 1H, *J*=2.0 Hz), 7.26-7.24 (m, 1H), 7.01 (d, 1H, *J*=2.0 Hz), 6.90-6.85 (m, 2H), 6.76-6.74 (m, 1H), 3.64 (t, 4H, *J*=4.4 Hz), 2.94 (t, 4H, *J*=4.4 Hz); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 146.2, 143.1, 135.7, 131.0, 127.4, 125.2, 125.1, 124.3, 120.9, 117.6, 116.3, 115.6, 65.7 (2C), 46.3 (2C); IR (KBr) cm^{-1} v_{max} 3337 (NH), 3290 (NH-SO_2), 3101 (aryl CH), 1572, 1502 (aryl C=C), 1530 & 1335 (NO_2), 1281 & 1152 (SO_2), 1225 (aryl C-O), 870 and 760; MS (ES, -ve): 377 (M-1); Elemental analysis found C, 50.59; H, 4.03; N, 11.29; $C_{16}H_{15}N_3O_6S$ requires C, 50.92; H, 4.01; N, 11.13.

***N*-(o-Tolyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6o)**



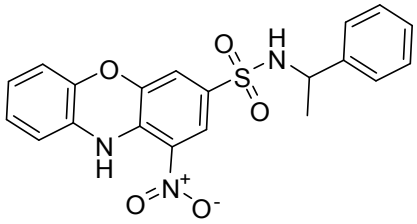
Compound **6o** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (5.0 g, 15 mmol), o-toluedine (2.45 g, 22 mmol) and triethyl amine (3.0g, 30 mmol) in chloroform (60 mL) to yield 4.5 g (74%), brick red solid, mp 224-226 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.78 (s, 1H), 9.65(s, 1H), 7.70 (d, 1H, *J*=2.0 Hz), 7.24-7.19 (m, 3H), 7.15-7.12 (m, 2H), 7.00-6.97 (m, 1H), 6.92 (d, 1H, *J*=2.0 Hz), 6.87-6.83(m, 2H), 6.76-6.74 (m, 1H), 2.17 (s, 3H); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 145.9, 142.9, 135.1, 134.9, 134.8, 131.4, 130.5, 130.3, 127.3, 127.2, 126.9 (2C), 125.1, 125.1, 119.7, 117.5, 115.6, 115.5, 18.3 (Me); IR (KBr) cm^{-1} v_{max} 3322 (NH), 3224 (NH-SO_2), 3105 (aryl CH), 1637, 1606, 1571, 1499 (aryl C=C), 1526 & 1327 (NO_2), 1280 & 1148 (SO_2), 1229 (aryl C-O); MS (ES, -ve): 397 (M-1); Elemental analysis found C, 57.69; H, 3.75; N, 10.33; $C_{19}H_{15}N_3O_5S$ requires C, 57.42; H, 3.80; N, 10.57.

***N*-(Propyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6p)**



Compound **6p** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchlroride (2.0 g, 6.1 mmol), n-propylamine (0.4 g, 6.7 mmol) and triethyl amine (1.23g, 12.0 mmol) in chloroform (30 mL) to yield 1.5g (71%), reddish brown solid, mp 200-202 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.75 (s, 1H), 7.86 (d, 1H, *J*=2.0 Hz), 7.62 (t, 1H, *J*=6.0 Hz), 7.24-7.22 (m, 1H), 7.08 (d, 1H, *J*=2.0 Hz), 6.90-6.84 (m, 2H), 6.77-6.75 (m, 1H), 2.72 (q, 2H, *J*= 6.8 Hz), 1.41 (sextet, 2H, *J*= 7.2 Hz), 0.82 (t, 3H, *J*= 7.2 Hz); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 145.9, 143.0, 134.9, 130.7, 130.4, 127.5, 125.0 (2C), 119.5, 117.4, 115.7, 115.6, 44.8 (CH_2), 22.8 (CH_2), 11.6 (Me); IR (KBr) cm^{-1} v_{max} 3335 (NH), 3292 (NH-SO_2), 3103 (aryl CH), 1569, 1503 (aryl C=C), 1531 & 1335 (NO_2), 1281 & 1152 (SO_2), 1228 (aryl C-O), 871 and 760; MS (ES, -ve): 349 (M-1); Elemental analysis found C, 51.39; H, 4.35; N, 12.23; $C_{15}H_{15}N_3O_5S$ requires C, 51.57; H, 4.33; N, 12.03.

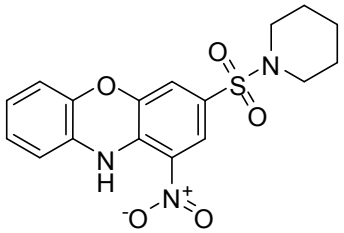
***N*-(1-Phenyl ethyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6q)**



Compound **6q** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (1.0 g, 3.0 mmol), 1-phenylethanamine (0.44 g, 3.6 mmol) and triethyl amine (0.6g, 6.0 mmol) in chloroform (20 mL) to yield 1.0 g (80%), reddish brown solid, mp 236-238 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.63 (br s, 1H), 8.27 (d, 1H, *J*=7.2 Hz), 7.21-7.16 (m, 5H), 7.10-7.04 (m, 1H), 6.88–6.83 (m, 2H), 6.78 (d, 1H, *J*=2.0 Hz), 6.75-6.73 (m, 1H), 4.35(t, 1 H, *J*=6.0 Hz), 1.30 (d, 3H, *J*= 6.8 Hz); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 145.3, 142.9 (2C), 134.4, 131.1, 130.1, 128.4 (2C),

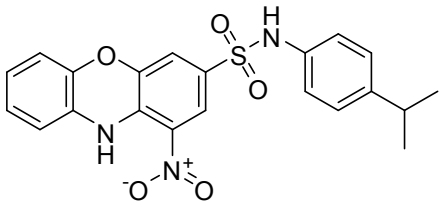
127.4, 127.0, 126.7 (2C), 125.0 (2C), 119.7, 117.4, 115.7, 115.6, 53.8 (CH), 24.1 (CH_3); IR (KBr) cm^{-1} ν_{max} 3330 (NH), 3240 (NH-SO_2), 3107 (aryl CH), 1634, 1605, 1576, 1501 (aryl C=C), 1531 & 1326 (NO_2), 1280 & 1151 (SO_2), 1224 (aryl C-O); MS (ES, -ve): 411 (M-1); Elemental analysis found C, 58.09; H, 4.05; N, 10.43; $C_{20}H_{17}N_3O_5S$ requires C, 58.38; H, 4.16; N, 10.21.

3-(Piperidinosulfonyl)-1-nitro-10*H*-phenoxazine (6r)



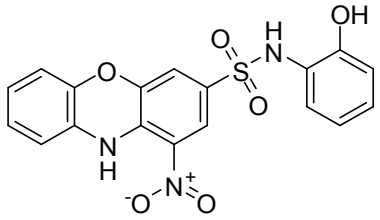
Compound **6r** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (2.0 g, 6.1 mmol) and piperidine (0.77g, 9.1 mmol) in chloroform (30 mL) to yield 1.7 g (73%), reddish brown solid, mp 222-224 °C. ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.78 (s, 1H), 7.74 (s, 1H), 7.24-7.21(m, 1H), 7.00 (s, 1H), 6.89-6.83 (m, 1H), 6.75-6.73 (m, 1H), 2.95-2.91 (m, 4H), 1.55-1.53 (m, 4 H), 1.39-1.37 (m, 2H); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 145.9, 142.9, 134.9, 130.6, 130.4, 127.5, 125.1 (2C), 119.6, 117.4, 115.8, 115.6, 60.2 (2C, CH_2), 45.5 (3C, CH_2); IR (KBr) cm^{-1} ν_{max} 3340 (NH & NH-SO_2), 3105 (aryl CH), 1636, 1607, 1593, 1574, 1502 (aryl C=C), 1530 & 1344 (NO_2), 1284 & 1145 (SO_2), 1227 (aryl C-O); MS (ES, -ve): 375 (M-1); Elemental analysis found C, 54.59; H, 4.45; N, 11.03; $C_{17}H_{17}N_3O_5S$ requires C, 54.39; H, 4.56; N, 11.19.

***N*-(4-Isopropyl phenyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6s)**



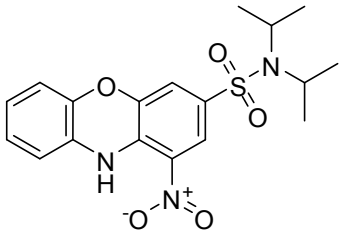
Compound **6t** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonyl chloride (3.0 g, 9.1 mmol), 4-isopropylaniline(1.85 g, 13.7 mmol) and triethyl amine (1.83g, 18.20 mmol) in chloroform (40 mL) to yield 2.8 g (72%), reddish brown solid, mp 255-257 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 10.16 (s, 1H), 9.73 (s, 1H), 7.78 (d, 1H, *J*=2.0 Hz), 7.20-7.18 (m, 1H), 7.15 (d, 2H, *J*= 8.4 Hz), 7.03 (d, 2H, *J*= 8.4 Hz), 6.97 (d, 1H, *J*= 2.0 Hz), 6.86-6.80 (m, 2H), 6.73 -6.71 (m, 1H), 2.80 (septet, 1H, *J*= 6.8 Hz), 1.12 (d, 6H, *J*= 6.8 Hz); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 145.9, 145.3, 142.9, 135.2, 135.1, 130.4, 129.3, 127.6 (2C), 127.2, 125.0 (2C), 121.4 (2C), 119.9, 117.4, 115.5, 115.4, 33.2 (CH), 24.2 (2C, CH_3); IR (KBr) cm^{-1} ν_{max} 3336 (NH), 3240 (NH-SO_2), 3101 (aryl CH), 1636, 1606, 1572, 1505 (aryl C=C), 1528 & 1329 (NO_2), 1280 & 1146 (SO_2), 1228 (aryl C-O); MS (ES, +ve): 426 (M^+); Elemental analysis found C, 59.49; H, 4.35; N, 9.73; $C_{21}H_{19}N_3O_5S$ requires C, 59.28; H, 4.50; N, 9.88.

***N*-(2-Hydroxyphenyl)-1-nitro-10*H*-phenoxazin-3-sulfonamide (6t)**



Compound **6u** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (5.0 g, 15.2 mmol), *o*-aminophenol (2.0 g, 18.3 mmol) and triethyl amine (3.0g, 30.0 mmol) in chloroform (60 mL) to yield 5.0 g (83%), reddish brown solid, mp 261-263 °C. ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.69 (s, 1H), 9.65 (s, 1H), 9.40(s, 1H), 7.74 (d, 1H, *J*= 2.0 Hz), 7.19-7.17 (m, 1H), 7.13-7.11 (m, 2H), 7.01 (d, 1H, *J*=2.0 Hz), 6.99-6.95 (m, 1H), 6.86-6.80 (m, 2H), 6.75-6.71(m, 3H); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 151.5, 145.5, 142.9, 134.9, 130.5, 130.3, 127.5, 127.4, 126.7, 125.1, 125.0, 123.8, 119.8, 119.6, 117.4, 116.2, 116.1, 115.6; IR (KBr) cm^{-1} ν_{max} 3325 (NH), 3259 (NH-SO_2), 3097 (aryl CH), 1634, 1607, 1601, 1591, 1574, 1503 (aryl C=C), 1531 & 1334 (NO_2), 1286 & 1155 (SO_2), 1228 (aryl C-O); MS (ES, -ve): 398 (M-1); Elemental analysis found C, 54.39; H, 3.25; N, 10.63; $C_{18}H_{13}N_3O_6S$ requires C, 54.13; H, 3.28; N, 10.52.

***N,N*-Diisopropyl-1-nitro-10*H*-phenoxazine-3-sulfonamide (6u)**



Compound **6s** was synthesized according to the above general procedure using 1-nitro-10*H*-phenoxazin-3-sulfonylchloride (1.0 g, 3.0 mmol) and *N,N*-diisopropylamine (0.9 g, 9.0 mmol) in chloroform (20 mL) to yield 1.0 g (82%), reddish brown solid, mp 252-254 °C; ^{1}H-NMR, 400 MHz (DMSO-d_6) δ 9.75 (s, 1H), 7.86 (s, 1H), 7.23-7.21 (m, 1H), 7.03 (s, 1H), 6.89-6.83 (m, 2H), 6.77-6.75 (m, 1H), 3.71 (septet, 2H, *J*= 6.8 Hz), 1.19 (d, 12H, *J*= 6.8 Hz); ^{13}C-NMR, 100 MHz (DMSO-d_6) δ 146.0, 143.0, 134.8, 130.6, 127.5, 125.1, 119.9, 117.5, 117.1, 115.7 (3C), 48.7 (2C), 22.0 (4C); IR (KBr) cm^{-1} ν_{max} 3331 (NH & NH-SO_2), 3111 (aryl CH), 1634, 1606, 1591, 1575, 1500 (aryl C=C), 1528 & 1336 (NO_2), 1276 & 1132 (SO_2), 1226 (aryl C-O); MS (ES, +ve): 392 (M^+); Elemental analysis found C, 55.46; H, 5.05; N, 10.53; $C_{18}H_{21}N_3O_5S$ requires C, 55.23; H, 5.41; N, 10.73.

Pharmacology

Drugs and Chemicals

Streptozotocin, Nicotinamide, Glibenclamide were procured from Sigma Aldrich labs, God-pod kits, SGOT, SGPT kits, Total cholesterol and Triglyceride kits were procured from Excel diagnostics Ltd, Hyderabad and all were of analytical grade.

Experimental animals

Wistar albino rats of both sexes weighing 150-200 g were used for study (Mahaveer Enterprises, Hyderabad, Andhra Pradesh, India). All animals were maintained under standard laboratory conditions [temperature (22± 2°c) and humidity 50 ± 15%] with 12 hours day: 12 Hours night cycle. The animals were fed with normal Laboratory diet and

allowed to drink water *ad libitum*. The experimental protocol has been approved by the Institutional animal ethics committee (2012/10/1/5) and by the regulatory body of government of India.

Acute toxicity studies

Acute oral toxicity study was Performed as per organization for economic cooperation and development (OECD) guidelines 423.[1] After the oral administration of test, animals were observed individually at least once during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours and daily Thereafter, for total of 14 days.

Experimental design for hypoglycemic activity

The animals were divided into twenty three groups (n = 6)

Group i: Rats served as normal- control and received 5 % gum acasia

Group ii: Rats served as standard received glibenclamide (10 mg/kg)

Group iii - Group xxiii: Rats were administered (10 mg/kg b.wt) orally with test compounds **6a-u** respectively.

All the animals were fasted for 18 h, before experimentation, but allowed free access to water. For the hypoglycemic activity blood samples were collected for the measurement of blood glucose by puncture of Retro-orbital plexus at 0, 1, 2, 4 and 6 h after feeding the test compounds.

Intraperitoneal glucose tolerance test

The animals were divided into three groups (n = 5)
Group i: Rats served as normal- control and received 5 % gum acasia
Group ii: Rats served as standard received glibenclamide (10 mg/kg)
Group iii - Group x: Rats were administered (10 mg/kg b.wt) orally with [test compounds 1(**6a**), 2(**6b**), 4(**6d**), 7(**6g**), 12(**6l**), 13(**6m**), 15 (**6o**), 19(**6s**)] respectively.

For Intraperitoneal Glucose Tolerance Test[2] over night fasted animals were loaded with glucose (2 g/kg b.wt. i.p), 30 min after the administration of test compounds and the

blood Samples were collected on 0, 30, 60, 90,120 minutes time Interval. The blood glucose levels were determined by using GOD-POD method.[3]

Induction of non-insulin dependent diabetes mellitus (NIDDM)

Non Insulin Dependent Diabetes Mellitus (NIDDM) was induced[4] in overnight fasted adult Wistar strain albino male rats weighing 150–200 g by a single intraperitoneal injection of 60 mg/kg Streptozotocin, 15 min after the i.p. administration of 120 mg/kg of nicotinamide. Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >126 mg/dl.[5] Only rats found with permanent NIDDM were used for the anti-diabetic study.

Experimental design for anti-diabetic activity

The rats were divided into five groups of six (n=5) each randomLy

Group i: Rats served as normal- control and receive5% gum acasia

Group ii: Diabetic rats received 5% gum acasia served as diabetic control

Group iii: Diabetic rats served as standard received glibenclamide (10mg/kg b.wt).

Group iv - Group v: Rats were administered with (10 mg/kg b.wt of test compounds *N*-(4-bromophenyl)-1-nitro-10*H*-phenoxazine-3-sulfonamide (**6g**) and *N*-(4-hydroxyphenyl)-1-nitro-10*H*-phenoxazine-3-sulfonamide (**6m**) orally.

After an overnight fast, the test compounds suspended in 5% gum acasia was fed by gastric intubations with the syringe. Blood samples were collected by puncture at retro-orbital plexus on at 0, 1, 2, 4, 6 and 8 h and the blood glucose levels were determined by using GOD-POD method. More active compound is taken for the sub acute study.

Sub acute study

The test, standard compounds are administered for 14 days. Blood samples were collected by Retro-orbital puncture at 1, 7, 14 day at 0, 1, 2, 4, 6 and 8 h and the glucose levels were estimated by GOD-POD kit. On 14th day, plasma Lipid profiles and Liver

enzyme levels were estimated by the method of Reitman and Frankel (1957) using biochemical kits and histopathological studies of pancreas is performed[6] and plasma insulin levels were estimated.

Statistical analysis

The results are expressed as mean±sd. Comparison between the groups was made analysis of variance (ANOVA), followed by dunnet's test as per suitability.

Histology of pancreas

Histological examinations of pancreas of diabetic and *N*-(4-hydroxyphenyl)-1-nitro-10*H*-phenoxazine-3-sulfonamide (**6m**) treated rats are shown in **Fig 13**. In diabetic pancreas, the islet cells were dilated and degranulated. These changes were restored by the treatment with test compound and glibenclamide.

PPAR Transactivation Assay

The reporter construct used for luciferase assay included (UASGAL4 x 5) response element upstream of pFR-Luc reporter under the Simian virus 40 early promoter. GAL4 fusions were made by fusing human PPAR*y* ligand binding domain (amino acids: 174-475) to the C-terminal end of yeast GAL4 DNA binding domain (amino acids: 1-147) of pM1 vector. pAdVantage vector was used to enhance luciferase expression.

HEK 293T cells were transfected with relevant plasmids by Superfect as per the instruction manual.[7] Forty-two hours after transfection, cells were treated for 18 h with test compounds. DMSO (0.1%) was used as blank. Luciferase activity was determined as "fold activation" relative to untreated cells using the Luclite kit (Packard Instrument Co, Meriden, CT) in a Packard Top Count (Packard Instrument Co.)

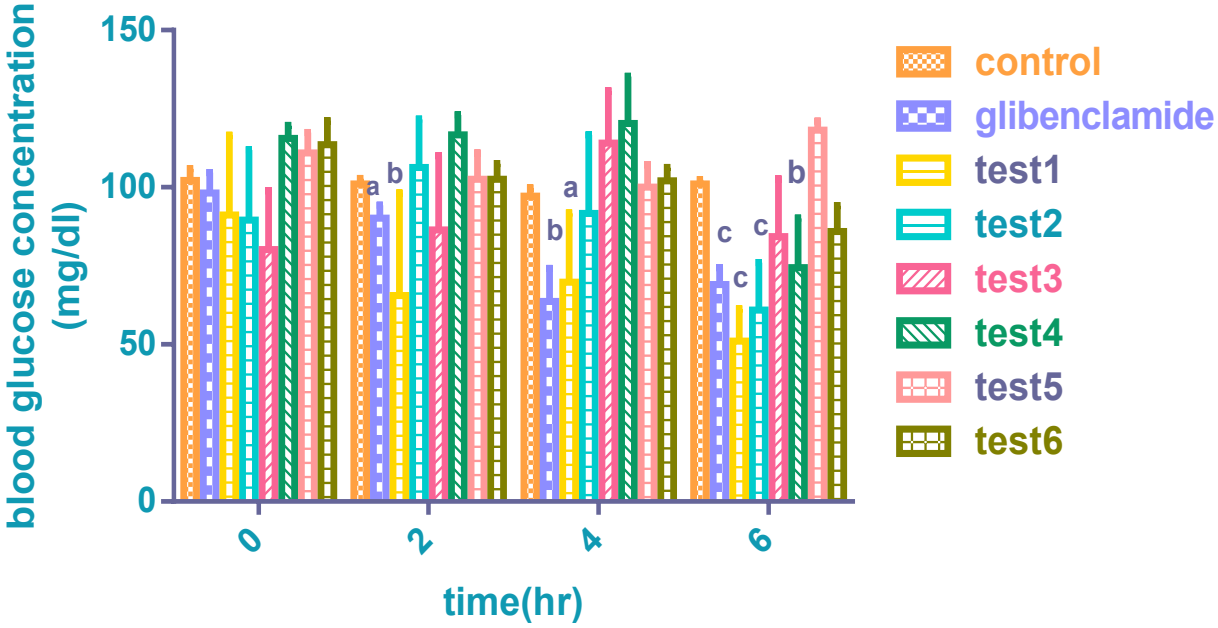


Fig S-1. Hypoglycemic effect of test compounds (**6a-f**, test 1-6 respectively)

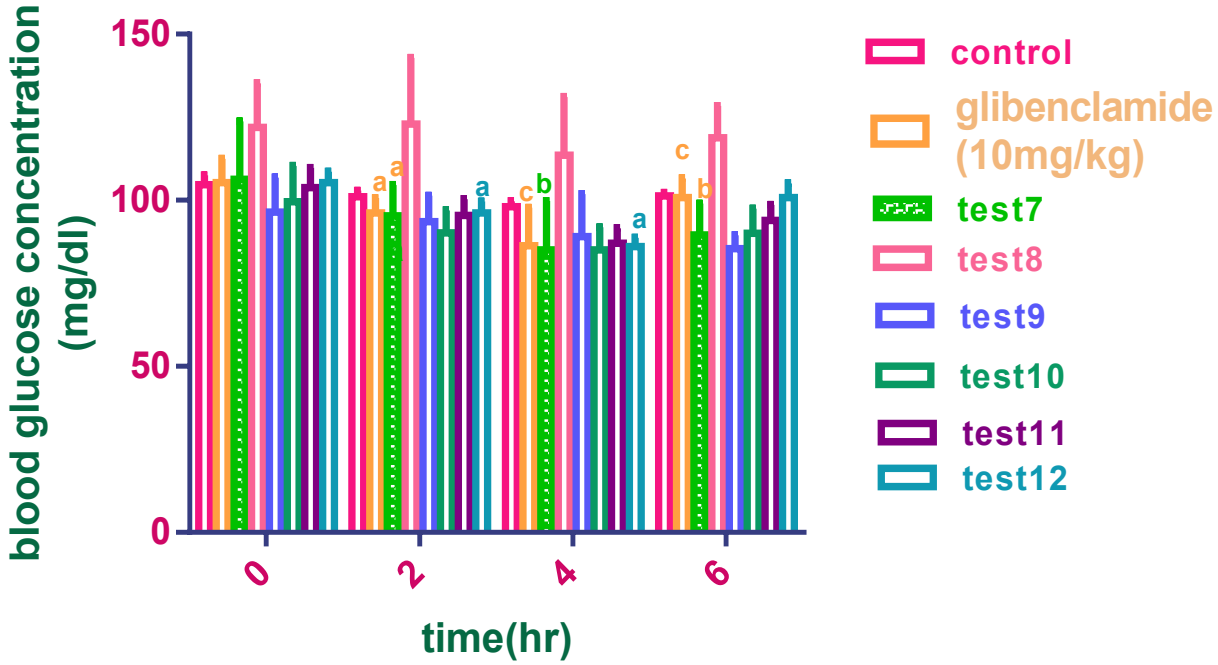


Fig S-2. Hypoglycemic effect of test compounds (**6g-l**, test 7-12 respectively)

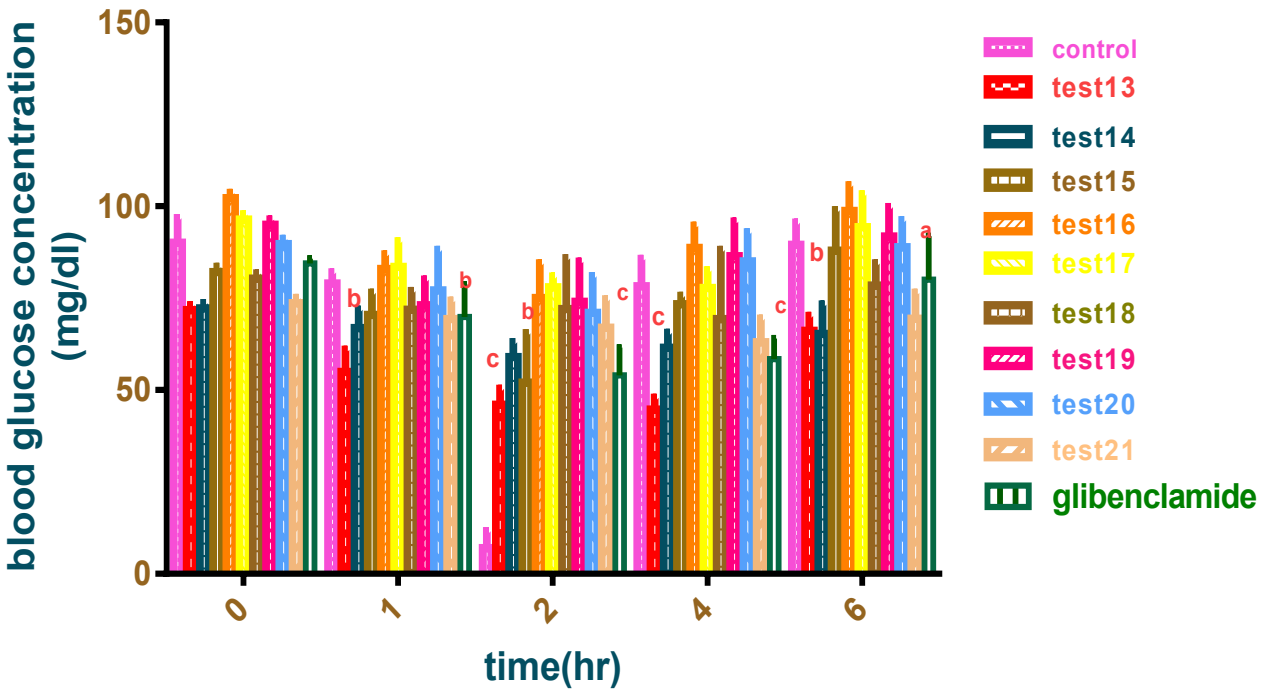


Fig S-3: Hypoglycemic effect of test compounds (**6m-u**, test 13-21 respectively)

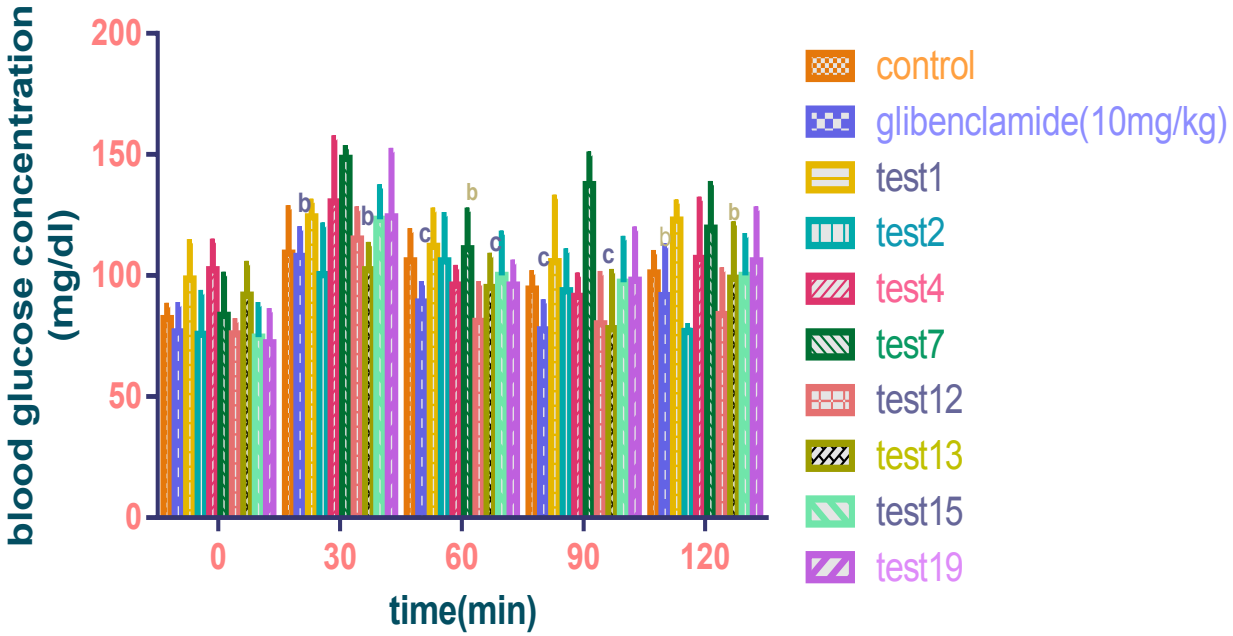


Fig S-4. Intraperitonial glucose tolerance test of test compounds **6a** (test 1), **6b** (test 2), **6d** (test 4), **6g** (test 7), **6l** (test 12), **6m** (test 13), **6o** (test 15) and **6s** (test 19).

References

1. OECD, guideline for testing of chemicals 423, Acute oral toxicity (acute toxic class method) December 2001.
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