CHAPTER-II

Antihyperglycemic and Insulin Resistance Reversal Activities of Synthetic Compounds
INTRODUCTION

The knowledge about the etiopathogenesis, treatment and prevention of human diabetes mellitus would not be available today without the support offered by animal experimental diabetes. Diabetes mellitus may be produced experimentally by means of surgery, viral infection, the administration of various hormones and chemical agents and genetic manipulation of selective interbreeding. Both the spontaneous and experimental animal models have been used effectively to study the etiologies, complications, treatments and prevention of diabetes mellitus. There are many advantages to the study of diabetes mellitus in animals. In human populations, for example, genetics are difficult to study and impossible to control. In contrast, rodent colonies allow many generations to be studied accurately in a relatively short period of time. Moreover, researcher has full control on experimental conditions such as nutrition, environment, species and ancestry. The stable incidence of diabetes mellitus in animal colonies affords a powerful tool for the study of preventive therapies for the disease. Diabetes mellitus research in human subjects is impeded by obvious ethical consideration. Provocation of diabetes mellitus is impermissible in man, but constitutes one of the most productive lines of animal study. As a corollary, any new therapeutics either to prevent or cure the disease must first be provided in animals.

Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones there is still a need to look for new drugs as none of the drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications. Current therapies for type 2 diabetes mellitus have inherent problems including non-compliance, ineffectiveness and hypoglycemic episodes with insulin and sulphonylureas. Therefore, here still remains a great need for more effective orally administered antidiabetic agents. Thus, the evaluation of antihyperglycemic and insulin resistance reversal properties in synthetic molecules and search for new antidiabetic molecules have also become one of the major objectives of present day diabetes research. The present study details the antihyperglycaemic activity profile of synthetic compounds and their derivatives belonging to several chemical classes/ series in various animal models for diabetes mellitus and insulin resistance.
**EXPERIMENTAL PROTOCOLS**

**Sucrose Loaded Rat Model**

Male albino rats of Charles Foster/ Wistar strain of average body weight 140 ± 20 g were selected for present study. The blood glucose level of each animal was checked by glucometer using glucostrips (Boehringer Mannheim) after an overnight (16 h) starvation. Animals showing blood glucose level between 3.33 to 4.44 mM (60 to 80 mg/dl) were divided into groups of five to six animals in each. Rats of experimental group(s) were administered suspension of the desired synthetic compound(s) (made in 1.0 % gum acacia) orally at an arbitrary dose of 100 mg/kg-body weight. Animals of control group were given an equal volume of 1.0 % gum acacia. A sucrose load at a dose of 2.5 g/kg was given to each animal orally exactly after 30 min post administration of the test sample/ vehicle. Blood glucose profile of each rat was again determined at 30, 60, 90 and 120 min post administration of sucrose by glucometer using glucostrips (Boehringer Mannheim). Food but not water was withheld from the cages during the course of experimentation. Quantitative glucose tolerance of each animal was calculated by Area Under Curve (AUC) method (Prism Software). Comparing the AUC of experimental and control groups determined the percentage antihyperglycemic activity. Statistical comparison was made by Dunnett's test.

**Sucrose Challenged Streptozotocin-induced Diabetic Rats**

Streptozotocin (STZ) is a broad-spectrum antibiotic, which is produced by *Streptomyces achromogenes* (Rakieten et al., 1963). The chemical structure of STZ comprises a glucose molecule with a highly reactive nitrosourea side chain that is thought to initiate its cytotoxic action. The glucose moiety directs this agent to the pancreatic β-cells where it binds to a membrane receptor to generate structural damage (Johansson and Tjalve, 1978). At the intracellular level, three major phenomena currently held responsible for β-cell deaths are process of methylation, free radical generation and nitric oxide (NO) production. An integrated hypothesis was proposed to explain the mechanism of action of STZ. In this hypothesis, streptozotocin, through production of superoxide, would generate peroxynitrite. The peroxynitrite was dissociate into NO and hydroxyl radicals, thus lading to β-cell DNA damage and apoptosis (Bedoya et al., 1996).
Male Sprague Dawley strain albino rats of average body weight $140 \pm 20$ g and having blood glucose profiles between 3.33-4.44 mM were finally selected for study. Fresh solution of streptozotocin (Sigma, USA) was made in 100 mM citrate buffer (pH 4.5) and calculated amount (60 mg/kg body weight) of freshly prepared solution of streptozotocin was injected intraperitoneally to overnight (16 h) fasted rats. Blood glucose profile was again checked after 48 h by glucometer and animal showing blood glucose profiles between 10.0-15.0 mM were considered suitable for the experiment. These diabetic animals were again divided into groups and their blood glucose profile was again checked on the day of experiment (Day 3). Blood glucose was measured at -20.0 min and 0.0 min (baseline values). Rats showing almost equal or similar blood glucose profiles were divided into groups consisting of five to six animals in each. Rats of experimental group(s) were administered suspension of the desired test sample orally (made in 1 % gum acacia) at an arbitrary dose of 100 mg/kg body weight. Animals of control group received an equal volume of vehicle (1 % gum acacia). A sucrose load at a dose of 2.5 g/kg was given to each animal orally exactly 30 min post administration of the test sample/vehicle. Blood glucose profile of each rat was again determined at 30, 60, 90, 120, 180, 240, 300 min and at 1440 min post administration of sucrose. Food but not water was withheld from the cages during the experimentation. Percent antihyperglycemic activity of the test samples was calculated according to the following formula:

$$\% \text{Antihyperglycemic Activity} = 100 - \left( \frac{\text{Average blood glucose level of test substance treated group at test time}}{\text{Average blood glucose level of control group at test time}} \right) \times 100$$

Statistical analysis was done by Dunnett's test.

**db/db mice Model**

Prior to the development of transgenic and knockout technologies, most rodent studies on the insulin resistance of type 2 diabetes were performed with monogenic models of obesity, especially the ob/ob and db/db mice. Investigations by Friedman and Halaas (1998) uncovered the genetic pathways responsible for obesity in these strains, defect in the adipocytes derived hormone leptin (ob/ob) or its receptor (db/db). Leptin acts primarily in the ventromedial hypothalamus, and lesions in this region of brain are
known to produce hyperinsulinemia and insulin resistance, implicating leptin or other centrally acting peptides in the control of insulin sensitivity.

The db/db mouse is a well-characterised model of type 2 diabetes. The background for the db/db mouse is the C57BL/Ks strain (Coleman, 1978). The major deficiency of the C57BL/KsBom-db mouse (db/db) is lack of a functional leptin receptor. This leads to defective leptin signalling and a complete lack of feedback from leptin. Both hypothalamic NPY content and secretion are consequently elevated, and this result in hyperphagia and decreased energy expenditure, obesity, insulin-resistance, hyperglycemia, hyperinsulinemia and dyslipidemia. The db/db mouse develops NIDDM from around week 10. The disease is stable in mice until week 20 (Coleman, 1978), where destruction of pancreatic β-cells can be recognized clinically as decreasing levels of plasma insulin and very severe hyperglycemia. The male mice are more diabetic, and will normally die earlier than the females. The advantage of using male mice for experimental purposes is that the fluctuations in plasma parameters are less than in the females where the estrogens cycle affects the clinical diabetes. The db/db mouse is suitable for identifying a broad range of compounds that improve different factors of type 2 diabetes and syndrome X.

C57BL/KsBom-db mice bred at Central Drug Research Institute, Lucknow. Optimal age for the experimental study was 12–18 weeks and body weight of each rat was 40-50 g. The male mice are housed in groups of five individuals in a room controlled for temperature (23 ± 2.0°C) and 12/12 hours light/dark cycle (lights on at 6.00 am). Cage size for 5 mice: 425x266x150 mm (LxWxH). All the animals have unrestricted food (normal chow pellets) and water.

To obtain homogenous groups with regards to blood glucose, all animals were marked temporally and their blood glucose was measured by glucometer. The animals were then allocated into groups according to their blood glucose. The mean blood glucose should be the same in all groups after randomization. Grouping to cages must be done at least 3 days before the vehicle training were introduced.

**Feeding Schedule**

All animals had free access to normal chow and fresh water except on the days of the postprandial protocol (day 6) and during the overnight fast before the OGTT
Antihyperglycemic and Insulin Resistance Reversal Activities of Synthetic Compounds

(day 10). Fresh chow was administrated 3 times a week at 9.30 am and the animals always have the free access to fresh water.

Dosing Volume & Vehicle

Drug suspensions/ solutions to a dose volume of 10.0 ml/kg body weight were prepared. The standard vehicles used for synthetic compounds were 0.2 % CMC + 0.4 % Tween 80 + 1.0 % gum acacia in sterile water.

Dosing Schedule

Drug/ test compounds were administered once daily (p.o.) at 9.30 am from day 1 to day 10 and vehicle training from day -3 to day 0.

Blood Glucose

Blood glucose was always measured by glucometer using glucostrips (Boehringer Mannheim).

Postprandial Protocol

Postprandial glucose levels were followed after consecutive dosing of compound on day 6. Food (not water) was removed from the cages just before first blood glucose was measured (around 8:30 am) and given back to the cages after the 6 h blood glucose (around 3 pm). Blood glucose was measured from the tail vein at time -0.30 min and 0.0 min (baseline values) and at 1, 2, 3, 4, 6 and 24 h after dosing of test compound(s)/ standard drug. The compounds were dosed twice daily; they were administered just after the fasting blood glucose at 0 and 4 h. The 24 h blood glucose was taken just before dosing of compound (at 9:00 am) on day 7. The AUC were calculated (Prism Software) and the percent difference between control and test compound treated group of rats were compared with a statistical Dunnett's test.

Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed on day 10 after an overnight (16 h) fasting to rats. Blood glucose was checked from the tail vein at time -30 and 0 min (baseline values) and at 30, 60, and 120 min after an oral glucose load at a dose of 3.0 g/kg body weight. The AUC is calculated and the per cent difference in blood glucose levels between control and test compound/ standard drug treated group of rats was compared with a statistical Dunnett's test.

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Antidiabetic Drugs Used

Glibenclamide
1-[[p-[2-(5-chloro-O-anisamido)ethyl]-phenyl] sulfonyl]-3-cyclohexylurea;
N- [4-1β-(2-methoxy-5-chlorobenzamido) ethyl] benzosulfonfyl]-N1-cyclohexylurea;
N1-[4-[β-(2-methoxy-5-chlorobenzoylamino)-ethyl]benzenesulfonfyl]-N2-cyclohexylurea;
Glibenclamide; glyburide; HB-419; U-26452; Adiab; Azuglucon; Bactiverit; Dia-basan;
Diabeta; Daonil; Duraglucon; Euglucon; Gilemal; Gliben-Puren N; Gli-diabet;
Glimidstda; Glucoremed; Gluco-Tablinen; Glycolande; Lederglib; Libanil; Lisaglucon;
Malix; Maninil; Micronase; Praeciglucon.
C_{23}H_{28}ClIN_{3}O_{5}S  MW 494.01
C 55.92%; H 5.71%; Cl 7.18%; N 8.51%; O16.19%; S 6.49%
Second generation sulfonylurea with hypoglycaemic activity.
Gliclazide
N-[[hexahydrocyclopenta [c] pyrrol-2 (14)-yl amino] carbonyl] -4-
methylbenzenesulfonamide;
1-(hexahydrocyclopenta[c] pyrrol-2 (14)-yl)-3-(p-tolylsulfonyl) urea;
N-(4-methylbenzenesulfonfyl)-N'- (3-azabicyclo [3.3.0]-oct-3-yl)urea
1-(3-azabicyclo[3.3.0] oct-3-yl)-3-(p-tolylsulfonyl)-urea
S-1702; Diamicron; Glimicron; Nordialex;
C_{13}H_{21}N_{3}O_{3}S  MW 323.42
C 55.714%; H 6.55%; N 12.99%; O 14.84%; S 9.91%
Crystal from anhydrous ethanol mp 180-182°C, LD_{50} orally in mice >3 g/kg (Duhault).

Metformin
N,N-Dimethylimidodicarbonimidic diamide
1,1-dimethylbiguanide
N,N- dimethylbiguanide
N', dimethylguanylguanidine (DMGG)
LA-6023
C_{4}H_{11}N_{5}  MW 129.16
C 37.2%; H 8.58%; N 54.22%
Oral hypoglycemic agent

Hydrochloride
Diabetosan; Diabex; Glucophage; Metiguanide;
C_{4}H_{11}N_{5}.HCl  MW 165.63
Prisms from water, mp 232° (Werner, Bell). Crystal from propanol, mp 218-220° (uncover) (Shapiro).
Soluble in water, 95% alcohol. Practically insoluble in ether, chloroform.
LD_{50} in rats (mg/kg): 1000 orally, 300 s.c. (Rx Bulletin).

Rosiglitazone
5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl] methyl]-2,4-thiazolidinedione
5-[[4-[2-[N-methyl-N-(2-pyridinyl) amino] ethoxy] benzyl]-2, 4, - thiazolidinedione
BRL- 49653
C_{18}H_{19}N_{3}O_{3}S  MW 357.43
C 60.49%; H 5.36%; N 11.76%; O 13.43%; S 8.97%
Insulin sensitizer; binds to peroxisome proliferator activated receptor (PPAR-γ).
**Acarbose**

\[ \text{o-4,6-dideoxy-4-[1s-(1\alpha,4\alpha,5\beta,6\alpha)]-4,5,6-tri} \]
\[ \text{hydroxy-3-(hydroxymethyl)-2-cyclop} \]
\[ \text{ropyranosyl-(1-4)-D-glucose;} \]
\[ \text{4",6"-dideoxy-4"-[(1s)-(1,4,6/5)-4,5,6-tri} \]
\[ \text{hydroxy-3-hydroxymethyl-2-cyclohexenylamino]-malt} \]
\[ \text{otrios; Bay g 5421; Glucobay; Prandase; Precose} \]

C\(_{23}\)H\(_{43}\)NO\(_{18}\)  MW 645.60

C 46.51%; H 6.71%; N 2.17%; O 44.61%

Pseudotetrasaccharide containing an unsaturated cyclitol moiety. An \(\alpha\)-glucosidase inhibitor that reduces sugar absorption in the gastrointestinal tract.

Amorphus powder \([\alpha]_D^{18} +165^\circ \) (c=0.4 in water)
RESULTS AND DISCUSSION

Antihyperglycemic effect of the compounds belonging to nearly ten different series and their derivatives was studied both in sucrose loaded rat model and sucrose challenged streptozotocin-induced diabetic rats. Sucrose loaded rat model was primarily used to identify the lead molecules from various synthetic series having antidiabetic potential. Sucrose challenged streptozotocin-induced diabetic rat model was later used to confirm the antihyperglycemic activity of synthetic compounds in diabetic conditions.

Effect of Synthetic Compounds on Postprandial Hyperglycemia in Normal Rats

Table 7 shows the average antihyperglycemic activity of all synthetic compounds belonging to various chemical classes/series, which were having significant (p<0.05) antihyperglycemic activity in normal rats at fixed single arbitrary dose of 100 mg/kg body weight. A total of 260 synthetic compounds of various chemical class/series and their derivatives were evaluated for their antihyperglycemic property on postprandial hyperglycemia in normal rats; out of which a total of seventy compounds were showing significant (p<0.05) antihyperglycemic activity on postprandial hyperglycemia in sucrose loaded rats. Five known standard drugs (Glibenclamide, Gliclazide, Metformin, Acarbose and Rosiglitazone) were also tested in this animal model. All standard drugs were showing significant (p<0.05) antihyperglycemic activity except roziglitazone, which were having only 11.6 % lowering in their blood glucose levels on postprandial hyperglycemia in normal rats.

Table 7: Synthetic compounds of various class/series and their derivatives showing significant antihyperglycemic activity on postprandial hyperglycemia in normal rats

<table>
<thead>
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<th>Dose (mg/kg)</th>
<th>% Activity</th>
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<td>32.0*</td>
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<tr>
<td>9</td>
<td>S-000-417</td>
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<td>40.8**</td>
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<th>S. No.</th>
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**Chalcone derivatives**

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**Flavone derivatives**

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**Phenylethylurea derivatives**

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**Tetrazole derivative**

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**Flavonoid derivatives**

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**Naphthooxazole derivatives**

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<td>S-001-8</td>
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<td>40.8**</td>
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<td><strong>Quinazoline derivatives</strong></td>
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<td>1</td>
<td>S-000-448</td>
<td>100</td>
<td>77.0***</td>
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<tr>
<td>2</td>
<td>S-000-449</td>
<td>100</td>
<td>33.4*</td>
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<td>3</td>
<td>S-000-451</td>
<td>100</td>
<td>37.9**</td>
</tr>
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<td>4</td>
<td>S-001-49</td>
<td>100</td>
<td>52.9***</td>
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<td>5</td>
<td>S-001-51</td>
<td>100</td>
<td>57.0***</td>
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<td>6</td>
<td>S-001-220</td>
<td>100</td>
<td>26.3*</td>
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<td></td>
<td><strong>Thiazolidinedione derivatives</strong></td>
<td></td>
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<tr>
<td>1</td>
<td>S-003-309</td>
<td>100</td>
<td>37.4*</td>
</tr>
<tr>
<td>2</td>
<td>S-003-820</td>
<td>100</td>
<td>27.1*</td>
</tr>
<tr>
<td>3</td>
<td>S-003-822</td>
<td>100</td>
<td>23.2*</td>
</tr>
<tr>
<td>4</td>
<td>S-003-823</td>
<td>100</td>
<td>22.2*</td>
</tr>
<tr>
<td>5</td>
<td>S-003-824</td>
<td>100</td>
<td>22.1*</td>
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<td>6</td>
<td>S-003-828</td>
<td>100</td>
<td>26.7*</td>
</tr>
<tr>
<td>7</td>
<td>S-003-1058</td>
<td>100</td>
<td>26.8*</td>
</tr>
<tr>
<td>8</td>
<td>S-003-1062</td>
<td>100</td>
<td>21.6*</td>
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<tr>
<td></td>
<td><strong>Standard drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Gliclazide</td>
<td>100</td>
<td>58.8***</td>
</tr>
<tr>
<td>2</td>
<td>Glibenclamide</td>
<td>100</td>
<td>57.2***</td>
</tr>
</tbody>
</table>
A total of sixty derivatives of carbamate were evaluated for their antihyperglycemic activity in sucrose loaded rat model, out of which only fifteen synthetic compound namely S-000-201, S-000-203, S-000-248, S-000-288, S-000-291, S-000-369, S-000-408, S-000-414, S-000-417, S-000-418, S-000-526, S-000-582, S-000-587, S-001-134 and S-001-406 were showing significant (p<0.05) antihyperglycemic activity on postprandial hyperglycemia in normal rats [Table 7, Fig 7 (a-h) and Fig 8 (a-g)]. A total of 15 chalcone derivatives were tested in sucrose loaded rat model, only eight (99/72, S-001-111, S-001-113, S-001-114, S-001-115, S-001-469, S-001-471 and S-001-472) were found active and showing significant lowering in blood glucose levels on postprandial hyperglycemia in normal rats [Table 7, Fig 9 (a-h)]. Lowering in blood glucose in these cases was evident from 60 min after glucose administration and that continued till 120 min. A total of forty derivatives of flavones based aryloxypropanolamines were evaluated for their antihyperglycemic property in sucrose loaded rats; out of which only twelve compounds (S-000-518, S-002-852, S-002-853, S-002-854, S-002-855, S-002-857, S-003-355, S-003-359, S-003-638, S-003-1126, S-003-1128 and S-003-1130) were showing significant (p<0.05) antihyperglycemic activity in this animal model [Table 7, Fig 10 (a-f) and Fig 11 (a-f)].

Results showing in Table 7, Fig 12 (a-g) having significant antihyperglycemic activity in the synthetic molecules of various series (phenylethylurea, tetrazole, flavonoid, xanthone, naphthooxazole) and their derivatives. A total of twenty-eight derivatives of these classes were evaluated for their antihyperglycemic activity out of which only seven (S-003-20, S-003-318, S-003-353 D2, S-003-354 D1, S-003-834, S-003-1019 and S-003-1020) displayed significant (p<0.05) blood glucose lowering in sucrose loaded rat model.
Fig 7. Antihyperglycemic Effect of Carbamate Derivatives on Postprandial Hyperglycemia in Normal Rats
Fig 8. Antihyperglycemic Effect of Carbamate Derivatives on Postprandial Hyperglycemia in Normal Rats
Fig 9. Antihyperglycemic Effect of Chalcone Derivatives on Postprandial Hyperglycemia in Normal Rats
Fig 10. Antihyperglycemic Effect of Flavone Derivatives on Postprandial Hyperglycemia in Normal Rats

(a) Control
△ S-000-518

(b) Control
△ S-002-852

(c) Control
△ S-002-853

(d) Control
△ S-003-854

(e) Control
△ S-002-855

(f) Control
△ S-002-857
Fig 11. Antihyperglycemic Effect of Flavone Derivatives on Postprandial Hyperglycemia in Normal Rats
Fig 12. Antihyperglycemic Effect of Derivatives of Synthetic Compounds on Postprandial Hyperglycemia in Normal Rats

(a) Phenylethylurea Derivative
(b) Tetrazole Derivative
(c) Flavonoid Derivative
(d) Flavonoid Derivative
(e) Naphthooxazole Derivative
(f) Naphthooxazole Derivative
(g) Xanthone Derivative
A total of twenty synthetic compounds of pregnane and propiophenone derivative (ten from each) were tested for their antidiabetic property in sucrose loaded rats out of which only eight (derivatives of pregnane S-001-284, S-001-285, S-001-286, S-001-289 and derivatives of propiophenone S-002-106, S-002-107, S-002-446, S-002-447) were found significant (p<0.05) active on postprandial hyperglycemia in normal rats [Table 7, Fig 13 (a-d) and Fig 14 (a-d)].

Among ten derivatives of pyrrole evaluated for their antihyperglycemic potential in sucrose loaded rats, six compounds (99/369, 99/370, S-000-166, S-001-6, S-001-7 and S-001-8) were having significant antihyperglycemic activity [Table 7, Fig 15 (a-f)].

A total of thirty quinazoline derivatives were tested for their antihyperglycemic activity on postprandial hyperglycemia in normal rats out of which only six (S-000-448, S-000-449, S-000-451, S-001-49, S-001-51, S-001-220) were showing significant (p<0.05) blood glucose lowering after 60 min of glucose load which were remaining low till 120 min of sucrose administration in normal rats [Table 7, Fig 16 (a-f)].

A total of thirty thiazolidinedione derivatives were evaluated for their antihyperglycemic activity out of which eight compounds (S-003-309, S-003-820, S-003-822, S-003-823, S-003-824, S-003-828, S-003-1058 and S-003-1062) were showing significant (p<0.05) antihyperglycemic activity on postprandial hyperglycemia in normal rats [Table 7, Fig 17 (a-h)].

Five standard drugs (Glibenclamide, Gliclazide, Metformin, Acarbose and Roziglitazone) known for antihyperglycemic activity were evaluated on postprandial hyperglycemia in normal rats. All were showing significant blood glucose lowering except Roziglitazone in sucrose loaded rats after 60 min of glucose load that remain low till 120 min [Table 7, Fig 18 (a-e)].
Fig 13. Antihyperglycemic Effect of Pregnan Derivatives on Postprandial Hyperglycemia in Normal Rats

Fig. 14: Antihyperglycemic Effect of Propiophenone Derivatives on Postprandial Hyperglycemia in Normal Rats
Fig 15. Antihyperglycemic Effect of Pyrrole Derivatives on Postprandial Hyperglycemia in Normal Rats
Fig 16. Antihyperglycemic Effect of Quinazoline Derivatives on Postprandial Hyperglycemia in Normal Rat
Fig 17. Antihyperglycemic Effect of Quinazoline Derivatives on Postprandial Hyperglycemia in Normal Rat

(a) Control vs S-003-300
(b) Control vs S-003-520
(c) Control vs S-003-622
(d) Control vs S-003-623
(e) Control vs S-003-624
(f) Control vs S-003-828
Fig 18. Antihyperglycemic Effect of Standard Drugs on Postprandial Hyperglycemia in Normal Rats

(a) Control
- Glibenclamide
(b) Control
- Gliclazide
(c) Control
- Metformin
(d) Control
- Acarbose
(e) Control
- Rosiglitazone
Effect of Synthetic Compounds on Sucrose Challenged Streptozotocin-induced Diabetic Rats

The synthetic compounds found active in sucrose loaded rat model were tested in sucrose challenged streptozotocin-induced diabetic rat model for the confirmation of antidiabetic potential of these active molecules in diabetic conditions. Table 8 shows the average antihyperglycemic effect of derivatives of various classes of synthetic compounds having significant antihyperglycemic activity on sucrose challenged streptozotocin-induced diabetic rats.

Table 8: Synthetic compounds of various chemical class / series and their derivatives showing significant antihyperglycemic activity in sucrose challenged streptozotocin-induced diabetic rats

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound Code</th>
<th>Dose (mg/kg)</th>
<th>% Activity</th>
<th>5h</th>
<th>24h</th>
</tr>
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<tbody>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Chalcone derivative</td>
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<td>1</td>
<td>S-001-115</td>
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<td>28.0*</td>
<td>11.5</td>
<td></td>
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<tr>
<td>Flavone derivatives</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S-003-853</td>
<td>100</td>
<td>21.6**</td>
<td>28.7***</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>S-003-857</td>
<td>100</td>
<td>50.4***</td>
<td>48.5***</td>
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</tr>
<tr>
<td>3</td>
<td>S-003-1128</td>
<td>100</td>
<td>16.1</td>
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<tr>
<td>1</td>
<td>S-003-353 D2</td>
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<td>9.87</td>
<td>19.8*</td>
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<td>2</td>
<td>S-003-354 D1</td>
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<td>19.6*</td>
<td>29.9*</td>
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<td>Furanoflavonoid derivative</td>
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<td>S-003-849</td>
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<td>18.0*</td>
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<td>Naphthoazoxole derivative</td>
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<td>S-003-1020</td>
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<td>22.1</td>
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<td>2</td>
<td>S-003-446</td>
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<td>S-001-8</td>
<td>100</td>
<td>24.5</td>
<td>25.1*</td>
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Antihyperglycemic and Insulin Resistance Reversal Activities of Synthetic Compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound Code</th>
<th>Dose (mg/kg)</th>
<th>% Activity</th>
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<td></td>
<td></td>
<td>100</td>
<td>5h</td>
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<tr>
<td>1</td>
<td>S-003-309</td>
<td>100</td>
<td>22.7*</td>
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<tr>
<td>Standard drug</td>
<td>Metformin</td>
<td>100</td>
<td>23.6**</td>
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</table>

A total of thirty synthetic compounds belonging to various chemical series were tested in sucrose challenged streptozotocin-induced rat model for diabetes mellitus; out of which fifteen synthetic compounds of various chemical series (S-001-115, S-002-853, S-002-857, S-003-1128, S-003-353 D2, S-003-354 D1, S-003-849, S-003-1020, S-001-284, S-001-285, S-002-106, S-003-446, S-003-447, S-001-8 and S-003-309) and a standard drug metformin were showing significant (p<0.05) antihyperglycemic activity during 1 h to 5 h period [Table 8, Fig 19 (a-h) and Fig 20 (a-h)]. Lowering in blood glucose level in the rats treated with these molecules was evident from 1 h and that continued till 5 h of test compounds/standard drug administration. The peak lowering in most of the cases was obtained at 3 to 5 h post treatment; there was no significant difference in the blood glucose profile of control and synthetic compound treated groups at 24 h of drug administration.

**Effect of Synthetic Compounds on db/db Mice**

Synthetic compounds which were found active in both the above said animal models for diabetes mellitus were also evaluated to check their antidiabetic and insulin resistance reversal activity in a genetic diabetic animal model i.e. db/db mice. Out of twelve evaluated synthetic compounds seven molecules i.e. S-002-853, S-002-857, S-003-1125, S-003-1130, S-001-469, S-001-471 and S-003-447 belonging to three different chemical series were found active in db/db mice and showing significant antihyperglycemic activity on postprandial (Day 6) and oral glucose tolerance after 10 days of feeding [Table 9, Fig 21 (a-d), (a1-d1) and Fig 22 (a-c), (a1-c1)]. Metformin, a known drug available in the market were also tested in this animal model and were found significant active both in postprandial hyperglycemia (day 6) and oral glucose tolerance after 10 days of drug feeding [Table 9, Fig 22 (d), (d1)].
Fig 19. Antihyperglycemic Effect of Derivatives of Synthetic Compounds in Sucrose Challenged Streptozotocin-induced Diabetic Rats

(a) Chalcone Derivative
(b) Flavone Derivative
(c) Flavone Derivative
(d) Flavone Derivative
(e) Flavonoid Derivative
(f) Flavonoid Derivative
(g) Furanoflavonoid Derivative
(h) Naphthooxazole Derivative
Fig 20. Antihyperglycemic Effect of Derivatives of Synthetic Compounds in Sucrose Challenged Streptozotocin-induced Diabetic Rats

(a) Pregnane Derivative

(b) Pregnane Derivative

(c) Propiophenone Derivative

(d) Propiophenone Derivative

(e) Propiophenone Derivative

(f) Pyrrole Derivative

(g) Thiazolidinedione derivative

(h) Metformin
Fig 21. Antihyperglycemic Effect of Flavone Derivatives in db/db Mice

Postprandial blood glucose levels in db/db mice before and up to 24 hours after 6 days of treatment with test compounds

Oral Glucose Tolerance on day 10 after test compounds feeding in db/db mouse
Fig 22. Antihyperglycemic Effect of Derivatives of Synthetic Compounds in db/db Mice

Postprandial blood glucose levels in db/db mice before and up to 24 hours after 6 days of treatment with test compounds/drug

a. Chalcone derivative

b. Chalcone derivative

c. Propiophenone derivative

d. Metformin

Oral Glucose Tolerance on Day 10 of after test compounds/drug feeding in db/db mice

a1. Chalcone derivative

b1. Chalcone derivative

c1. Propiophenone derivative

d1. Metformin
Table 9: Synthetic compounds of various class / series showing significant antidiabetic activity on db/db mice

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound code</th>
<th>Dose (mg/kg)</th>
<th>db/db mouse (% Activity)</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Flavone derivatives</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>S-002-853</td>
<td>10</td>
<td>24.6*</td>
</tr>
<tr>
<td>2</td>
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<td>10</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>S-001-469</td>
<td>10</td>
<td>7.53</td>
</tr>
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<td>2</td>
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<td>10</td>
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<td>Propiophenone derivative</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>S-003-447</td>
<td>10</td>
<td>28.4*</td>
</tr>
<tr>
<td>Standard drug</td>
<td>Metformin</td>
<td>10</td>
<td>32.2**</td>
</tr>
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</table>

The oral route of administration was preferred as it is simple and physiological. In the light of the above reports, an attempt was made to identify the new classes of antihyperglycemic compounds using various animal models for diabetes mellitus like sucrose loaded rats, sucrose challenged streptozotocin-induced diabetic rats and db/db mice. Albino rats have been chosen for experiment because the blood sugar level of rats remains fairly stable during handling, unlike other animals such as rabbits, (Mukherjee and Mukherjee, 1987). Streptozotocin-induced diabetes is reproducible, convenient and can produce diabetes of graded severity suitable for experimental diabetes mellitus (Junod et al., 1969). In experiment with many animal species, streptozotocin produces permanent diabetes with extra pancreatic lesions that mimic the pathological status found in human beings (Arison et al., 1967).

Among the two hundred sixty synthetic compounds screened only seventy demonstrated antihyperglycemic activity in sucrose loaded rat model at 100 mg/kg dose level. The derivatives of carbamate and chalcone series were found very effective in lowering blood glucose level as evidenced from the fact that it inhibits the rise in
postprandial hyperglycemia in sucrose loaded rats; these also prevent the rise in blood glucose level even in streptozotocin-induced diabetic rats post sucrose challenge. Some of the derivatives of other chemical classes/series (derivatives of phenylethylurea, tetrazole, flavonoid, naphthooxazole, xanthone, pregnne and propiophenone) were also showing significant (p<0.05) blood glucose lowering in sucrose loaded rat model.

Flavones are among the most ubiquitous group of polyphenolic compounds in foods of plant origin. Recently flavones and their derivatives have also been reported for antidiabetic (Rong Min et al., 2002) and antihyperlipidemic (Jeong et al., 2003) activities. The antioxidant property of flavones attracted us to explore hybrid structures as hypoglycemic and hypolipidemic agents, as oxidative stress also play an important role in diabetic patients leading to vascular complications (Faure et al., 1997). Here we report the antihyperglycemic activity of flavone-based aryloxypropanolamines. Our results correlate with the earlier findings.

Pyrrole being an integral part of many natural products displays wide spectrum pharmacological properties such as hepatoprotective (Chin et al., 2003), antifugal (El-Gaby et al., 2002), antioxidant (Asad, et al., 2001) and antibacterial (Periers et al., 2000). Recently, 5-octadecylpyrrole-2-carboxaldehyde (I) isolated from a marine organism (Mycale mytilorum) has been reported for antidiabetic activity (Reddy and Dhananjaya, 2000). The therapeutic importance and fascinating chemistry of pyrroles prompted us to design and synthesize variety of molecules to explore their antidiabetic activity. It is evident from the results that derivatives of flavones having significant lowering at a dose of 100 mg/kg body weight on postprandial hyperglycemia in normal rats and also helps in the decline of blood glucose level on sucrose challenged streptozotocin-induced diabetic rats. Our results support the previous findings in this direction.

Interestingly, quinazolines have also been reported for antihyperglycemic activity (Liverton, et al., 1998). Quinazoline binds non-covalently with receptor sites and helps in the glucose absorption into the cells. Several new quinazoline derivatives have been evaluated for their antihyperglycemic activity. It is evident from the results that derivatives of quinazoline significantly reduce the blood glucose in both the above said animal models of diabetes mellitus. Our findings confirm the antihyperglycemic activity in the derivatives of quinazoline. These agents may be useful for the treatment of
diabetes mellitus as these molecules are having significant blood sugar lowering in sucrose loaded rats and sucrose challenge streptozotocin-induced diabetic rats.

Thiazolidinedione are new class of antidiabetic agent to be identified as nuclear peroxisome proliferator activated receptor-γ (PPAR-γ) ligand. They exert their antidiabetic effects by binding to PPAR-γ, which in turn, activates insulin sensitive genes that regulate carbohydrate and lipid metabolism (Willson et al., 2000). Glitazone type therapeutic agents are in the market but some of them have been reported to have hepatotoxicity (Saleh et al., 1999). The present study clearly indicates that these newly synthesized derivatives of thiazolidinediones are the potent blood glucose lowering agents on postprandial hyperglycemia in normal rats. It is therefore, these molecules may be added unequivocally to the growing list of hypoglycemic and antihyperglycemic agents. However, controlled clinical trials will be required to confirm their antihyperglycemic action and general safety.

Plasma insulin levels were always found higher in db/db mice but blood glucose remained high, suggesting that these animals are resistant towards insulin. Antidiabetic agent S-002-853, S-002-857, S-003-1125, S-003-1130 (all are flavone derivatives), S-001-469, S-001-471 (chalcone derivatives) and S-002-853 (propiophenone derivative) prevented and attenuated the increase in both blood glucose and plasma insulin suggest that these molecules promote insulin sensitivity. Therefore, it seems that these molecules exert insulin resistance reversal activity by improving insulin sensitivity as observed with certain antidiabetic agents like metformin (Verma et al., 1994), thiazolidinedione (Suzuki et al., 1997; Verma et al., 1998) and angiotensin converting enzyme inhibitors (Limura et al., 1995).