CHAPTER NO. 8

Conclusions
8. Conclusions:

The present work was planned as a sequel to earlier studies in our laboratory using thiazolidin-4-ones as antidiabetic and hypolipidemic molecules. The substitutions in the thiazolidine ring were made at C2 and N3. Attached to the latter position was a nicotinamido moiety and the substitution at C2 was either p-methoxyphenyl or 2,5-di-tert-butyl-4-hydroxyphenyl group. The compounds showed significant antidiabetic and hypolipidemic activities\textsuperscript{16,20,31,32}.

In the present study, the substitution at N3 was changed to p-Chloro-phenoxyacetylamino, a group that is similar to clofibrate with a methylene bridge instead of gem-dimethyl substitution. Four compounds out of 21 had a methyl group attached at the C5 of thiazolidine ring, cyclisation being made with thiolactic acid instead of thioglycolic acid. In four other compounds (viz., CLOANI-TGA, CLOBHT-TGA, CLOPFB-TGA, and CLOTRIFLU-TGA), clofibrate was used to make the moiety for substitution at N3. This was done with a view to examining the effect of the gem-dimethyl on the overall activity of the resulting molecule.

While streptozotocin (STZ) induces diabetes in rodents resembling the human Type-1 (Insulin dependent diabetes mellitus or IDDM), the administration of nicotinamide along with STZ affords partial protection of pancreatic β-cells\textsuperscript{52}. The disease condition hence resembles Type-2 (NIDDM). Four thiazolidin-4-ones, viz., ANI-TGA, BHT-TGA, PFB-TGA and TRIFLU-TGA were evaluated for their ability to bring down the elevated blood glucose level in STZ-nicotinamide diabetes. All the four significantly reduced the blood glucose towards normal. Two compounds, BHT-TGA and ANI-TGA also significantly ameliorated the glucose intolerance in OGTT studies performed on these diabetic mice. However, pioglitazone did not reduce the glucose excursions under similar conditions, contrary to expectation.

These early results prompted the evaluation of glucose uptake of all the 21 synthesised thiazolidin-4-ones in an\textit{ in vitro} system using the isolated rat diaphragm. In this experiment, ANI-TGA, CLOANI-TGA, and CLOTRIFLU-TGA significantly raised the amount of glucose uptake by the tissue both in the absence and presence of external insulin. This indicates that these molecules are capable of sensitizing the tissues to insulin. However, TRIFLU-TGA and BHT-TGA failed to enhance the glucose uptake. The lack of activity for these two compounds in this experiment was unexpected as the same molecules showed significant glucose-lowering effect in STZ-nicotinamide diabetes.

From the above experiments it was observed that ANI-TGA has consistent antidiabetic effect. Firstly, it reduced glucose level in diabetic mice. It also reduced the glucose excursions in the OGTT. It enhanced the glucose uptake by isolated rat diaphragm. Hence this compound was chosen along with CLO-ANI-TGA and
ANI-TLA for their effect on a diet-induced model of insulin resistance. All the three compounds have the same p-methoxyphenyl moiety attached to the C2 of the thiazolidine ring. CLO-ANI-TGA has a gem-dimethyl group instead of methylene in the substituent at N3. In ANI-TLA, the thiazolidine ring harbours a methyl group at C5 in place of an H.

Apart from multiple risk factors, diet induced metabolic abnormalities contribute to development of insulin resistance and β-cell failure in Type-2 diabetes. Early detection and treatment with appropriate intervention are considered beneficial for correcting the abnormality. Among the various animal models, induction of diabetes by diet intervention provides more resemblance to human Type-2 diabetes. Chronic intake of diet with high sucrose content has been reported to develop insulin resistance. Similarly in our study, 6 months’ feeding of high-sucrose diet to mice caused metabolic abnormalities like hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia, and hypoadiponectinemia. Occurrence of hyperglycemia and hyperinsulinemia suggests the inability of insulin to sensitize the tissue for glucose uptake, thus diverting glucose toward lipogenesis. This led to hyperlipidemia and, finally to insulin resistance. In addition, adiponectin and leptin, the adipokines secreted from white adipose tissue (WAT), are reported to be involved in the metabolism of glucose and lipid. Presence of hypoadiponectinemia and hypoleptinemia are commonly found to be associated with insulin resistance in Type-2 diabetes. Elevation in plasma leptin level suggests the potential of test drugs in inhibiting the calorie intake as leptin, an endogenous hormone secreted from white adipose tissue, is considered as regulator for food intake by controlling the appetite.

In our study, we observed that HCD caused reduction in plasma adiponectin level and increase in the size of adipocytes but without any change in plasma leptin levels. Thus, we found a correlation between the adiponectin level and size of adipocytes in HCD model. Further, OGTT results in HCD fed mice correlated well with glucose intolerance with the hyperinsulinemia and hypoadiponectinemia. The authenticity of the model validated by the effect of pioglitazone, which was able to correct the metabolic abnormalities. This thus attenuated impaired glucose tolerance and insulin resistance. None of the tested compounds (ANI-TGA, CLOANI-TGA, and ANI-TLA) attenuated hypoadiponectinemia. However, they corrected the impaired glucose tolerance and insulin resistance in mice. ANI-TGA and CLOANI-TGA by their ability to enhance glucose uptake and to sensitize the tissue for available insulin, attenuated hyperinsulinemia and raised the leptin levels. This would have resulted in better glucose utilization by peripheral tissue. Thus, these compounds reduced the metabolic abnormalities like hyperglycemia, hypertriglyceridemia and hypercholesterolemia. On the other hand, ANI-TLA raised the plasma insulin level without affecting adiponectin and leptin. The enhanced insulin release might have acted to correct metabolic abnormalities.
Histopathological investigation showed no change in liver architecture among the various treatment groups. In HCD control mice, there was an increase in the size adipocytes in white adipose tissue (WAT). The treatments caused a decrease in the size of the adipocytes. However, only pioglitazone-treated animals showed positive correlation between reduced size of the cells and correction of hypoadiponectinemia. ANI-TGA and CLOANI-TGA treatment caused elevation in leptin. This could possibly have contributed towards normalising adipocytes architecture.

The pancreatic islets showed hyperplasia in pioglitazone and other treatment groups. Pioglitazone is a PPAR-gamma agonist activity. This could have been responsible for the proliferation of beta cells of pancreas. The test compounds are thiazolidin-4-ones with some similarity to thiazolidinediones. It is possible that hyperplasia observed in CLOANI-TGA and ANI-TLA might have been due to some agonistic activity on PPAR-γ receptors. This needs to be investigated through relevant assay.

Oxidative stress has been implicated with the occurrence of diabetes and compounds ameliorating the oxidative stress by enhancing endogenous antioxidant status have beneficial role in correcting glucose intolerance and insulin resistance in diabetes\(^{54,61}\). The test compounds and pioglitazone reversed the depletion of endogenous antioxidant enzymes such as such as GSH, CAT, SOD, and GST. Further, they reduced malondialdehyde levels. This suggests the inhibitory effect of these compounds on oxidative stress.

CLOANI-TGA raised the plasma leptin level compared to its structural analogue ANI- TGA suggesting that the presence of gem-dimethyl group instead of methylene could have played a role. But there was no difference between CLOANI-TGA and ANI-TGA in any other parameter measured in the study.

No single mechanism would suffice to explain the beneficial effects of the test compounds. They do not seem to act through insulinotropic activity unlike the sulfonylureas. They have no significant effect on adiponectin levels, ruling out any involvement of this mechanism. The increase in the level of serum leptin might point to the involvement of leptin in the antihyperlipidemic and antidiabetic potentials of these molecules.

In conclusion, thiazolidin-4-one derivatives act through multiple mechanisms to correct the metabolic abnormalities in Type-2 diabetes. In present work, ANI-TGA and CLOANI-TGA were found to be the most effective test compounds to ameliorate insulin resistance and development of Type-2 diabetes. Further detailed work is warranted to unequivocally establish their mechanism(s) of action.