5. SUMMARY

The discovery of the crucial role of Peroxisome Proliferator Activated Receptors (PPARs) as regulators of lipid and glucose metabolism has raised interest in the development of synthetic ligands as potential tool for therapeutic intervention in type 2 diabetes mellitus (T2DM) and metabolic syndrome.

An important class of compounds currently under focus in the same category is dual activators of Peroxisome Proliferator Activated Receptors (α,γ). Each of these subtypes appears to be differentiated in a tissue-specific manner and to play a pivotal role in glucose and lipid homeostasis. Special efforts to design multiple activating molecules can be successfully made using computational methods. Knowledge of the 3D structure of all the targeted receptors is of an advantage. PPARγ agonists enhance insulin action and promote glucose utilization in peripheral tissues. PPARα agonists improve insulin sensitivity associated with obesity and mediate their effects on lipid metabolism. Therefore PPARα/γ dual activators provide superior profile toward the control of hyperglycemia and hypertriglyceridemia.

So, we first aimed to work on the hypothesis that PPARα/γ dual agonism must provide additive and positive synergistic pharmacology, and laid down certain objectives to meet and fulfill the issues facing the development of dual activating agonists: a balance between α and γ activity.

The fact that dual PPARα/γ activators though highly potent agonists of PPARα and γ; they lead to various undesirable adverse effects (cardiovascular, hepatic, carcinogenic etc.) came into picture in the due course of time during the research work. Partial agonism simultaneously to both the Receptors may provide solution to the problem of toxic adverse effects of dual activators.

Accordingly, it was planned to synthesize and validate computationally heterocyclyl linked acyclic analogs of isoxazolidinediones \textit{i.e.} 1,3-dicarbonyl compounds in general and β-ketoesters in particular, as was designed on the basis of 3D-QSAR studies, which have almost all the structural features to act as effective insulin sensitizer and are expected to bind and activate PPARα and PPARγ, but will have only partial efficacy at the receptors relative to a full agonist (Partial PPARα and PPARγ agonists) in the management of hyperglycemia and hyperlipidemia.
AIM AND OBJECTIVES

The aim is to work on the hypothesis that partial PPARα/γ dual agonism may provide additive and positive synergistic pharmacology with less or no adverse effects so as to meet and overcome the issues facing with the development of dual acting agonists.

- To build robust and significantly good predictive 3D QSAR (Comparative Molecular Field Analysis- CoMFA) models for prediction of PPAR α, γ activities.
- Design of New Chemical Entities based on the contour analysis resulted from the generated CoMFA models and assisted by CoMSIA studies.
- To predict the activities and binding affinities (Docking studies) of the designed NCEs computationally.
- Final selection of the molecules for synthesis on the basis of prediction of activities and binding affinities obtained computationally with an aim to achieve partial agonism.
- Devising new synthetic strategies, which meet the requirement of “Ideal Synthesis” and also take into consideration the concept of connectivity analysis, thereby, providing new insights towards the skeletal and functionality requirement of these molecules.
- Synthesis and structural characterization of the final selected molecules.

The work compiled in this thesis is presented under the following heads:

1. INTRODUCTION

2. REVIEW OF LITERATURE
   General
   Computational

3. EXPERIMENTAL
   3.1. COMPUTATIONAL
   3.2. SYNTHESIS

4. RESULTS AND DISCUSSION
   4.1. COMPUTATIONAL
   4.2. SYNTHESIS

5. SUMMARY

6. REFERENCES
Peroxisome proliferator-activated receptors (PPAR) have gained importance over the past recent years in the area of type 2 diabetes drug research, due to the discovery of their physiological roles in the regulation of glucose and lipid homeostasis. Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor family. Ligands which bind to PPARα and PPARγ receptors are involved in the pathophysiology of type 2 diabetes mellitus. PPARα/γ dual agonists provide superior therapy through lipid and glucose control simultaneously compared to the individual agonists though the design of such ligands is a challenge. Computational methods like 3D QSAR, can be effectively employed in designing such multiple receptor activating ligands. X-ray crystal structure information of the 3D-geometry of targeted receptors is advantageous for this special effort. Several such ligands (PPARα/γ dual activators) of various chemical classes have been designed and studied extensively by the pharmaceutical companies. Though the effect of the PPARα/γ dual agonists are advantageous over the individual agonists in the therapy of T2DM, the dual agonists give rise to serious and sometimes fatal adverse effects which has substantially reduced the research interests for their development. Partial PPAR agonists have the potential to retain the desired efficacy and beneficial effects of full PPAR agonists while diminishing the unwanted effects. Partial agonism at both PPARα and PPARγ receptors by dual partial activators may provide a solution resulting in the desirable responses and reducing the adverse effects caused by the individual agonists for the treatment of T2DM. Development of such agents is again a challenging endeavor as the molecules should have partial binding affinity simultaneously for all the aimed macromolecular targets with similar binding pockets to obtain the optimum potency that finally may result in desired activity profile and lesser adverse effects as compared to the selective and full dual agonists. Comparative Molecular Field Analysis (CoMFA) along with docking studies enables us to design, computationally validate and finally select the best molecules with expected desired activities. The selected molecules can be taken forward for synthesis and characterization. The present work brings out such an approach. The design of six series of heterocyclyl linked β-ketoester based molecules and; the syntheses and characterization of the selected molecules with expected desired partial PPARα/γ activities are reported.
A set of thirty-four agonists of PPARα as well as PPARγ receptor subtypes including eighteen α-alkoxy propanoic acid and five α-aryloxy propanoic acid derivatives and eleven tyrosine-based molecules with their activities of single enantiomer was selected from the literature by an exhaustive survey as there is limited configuration-based activity data available for PPAR ligands. The structures of the compounds with their corresponding in vitro human PPAR transactivation assay activity in terms of EC$_{50}$ were tabulated. The activity values have been converted into pEC$_{50}$ (-log EC$_{50}$) values in molar terms wherever required and used as the dependent variables in the study. The activities of the compounds at PPARα and PPARγ were used to build the α-model and γ-model respectively. The sum of the pEC$_{50}$ values for PPARα and PPARγ have been taken as dual pEC$_{50}$ value for each compound for developing the dual model. The dataset molecules were sorted into a training set of 26 molecules and a test set of 5 molecules in the process of model refinement and internal prediction of training set and test set for developing all the three models (3 molecules were left out during the process). The training set was so chosen that the molecules cover a wide range of activities and identical set of molecules can be used for building the three significant models.

![Structural alignment of the molecules of the training set](image1)

![Structural alignment of the molecules of the test set](image2)

All the dataset molecules were built onto energy minimised farglitazar (extracted from the Ligand Binding Domain of 1fm9: Crystal structure of PPARγ LBD reported in RCSB PDB) using the ‘Sketch module’ while maintaining the U-shape essential for activity. These were subjected to energy optimisation by Powell method after assigning Gasteiger–Marsili charges. The energy minimised conformers of the training and test
set molecules were aligned and Comparative Molecular Field Analysis (CoMFA) was performed with the aid of the molecular modelling software Sybyl 7.3 (available at in silico Drug Design Lab, Department of Chemistry, Punjabi University, Patiala) to build three 3D QSAR models, namely PPARα, PPARγ and PPAR dual models. The models were well validated by the prediction of the test set and training set.

Crystal structure of PPARγ (1FM9) bound ligand (Farglitazar)- shown in yellow

<table>
<thead>
<tr>
<th>Parameters</th>
<th>α-model</th>
<th>γ-model</th>
<th>d-model</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of molecules in the training set</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>No. of molecules in the test set</td>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$r^2_{cv}$</td>
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<td>No. of components</td>
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<td>$r^2_{ncv}$</td>
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<tr>
<td>SEE</td>
<td>0.316</td>
<td>0.356</td>
<td>0.515</td>
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<tr>
<td>F value</td>
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<td>142.796</td>
<td>98.367</td>
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<tr>
<td>steric field contributions</td>
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<td>0.575</td>
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<tr>
<td>electrostatic field contributions</td>
<td>0.415</td>
<td>0.352</td>
<td>0.425</td>
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</table>

After analysing the resulted steric and electrostatic contour maps; a series of tricyclic heterocycle linked α-alkoxy propanoic acid based molecules and a series of tricyclic
heterocycle linked tyrosine based molecules were designed as potent dual agonists. The activities of both the designed series of molecules were predicted by the developed models and docking studies were carried out using Surflex dock method for these at the crystal structure of active site of PPAR\(\gamma\) (1fm9). The best dual agonists were selected from each series after comparison of the predicted activities and docking scores among the designed molecules and with that of some selected standard molecules. Left hand side fragment as the lipophilic portion and right hand side fragments as the hydrogen bonding part were designed which are to be used for building of the Partial agonists by critical analysis of the structure of standard ligand and the contours resulted from the CoMFA models. The effect of the fragments to impart partial agonism were validated by building molecules by incorporating the designed fragments into standard dual molecules and study of their predicted activities by the developed CoMFA models.

CoMSIA models were also developed using the same dataset molecules, and the hydrogen bond donor and acceptor contours thus obtained helped in the design of the rhs hydrogen bonding fragments for the novel partial agonists.

![Steric and electrostatic solid contours (CoMFA) of the PPAR\(\alpha\) model (Farglitazar shown as the standard ligand, special features taken into account for the design of the NCEs as partial agonists have been indicated)](image-url)
Steric and electrostatic solid contours (CoMFA) of the PPARγ model (Farglitazar shown as the standard ligand, special features taken into account for the design of the NCEs as partial agonists have been indicated)

CoMFA steric and electrostatic contours (transparent) of the PPAR dual model (contributions from each of PPAR α and γ models are indicated)
Hydrogen bond donor and acceptor solid contours (CoMSIA) of the PPAR dual model (Farglitazar shown as the standard ligand, special features taken into account for the design of the NCEs as partial agonists have been indicated)

Benzimidazolyl, indolyl and acridonyl linked benzyl and benzylidene β-ketoester based molecules were designed as novel PPARα/γ partial agonists. The PPAR activities of the designed NCEs were predicted by the developed models and then they were subjected to docking studies at the crystal structure of PPARα (PDB code 1k7l) and PPARγ (PDB code 1fm9) to predict the binding affinities and interactions of the ligands at the active sites of the receptors.

After the activity prediction and docking studies of the designed NCEs and the selected standard molecules; the results obtained were analyzed exhaustively by comparison of the predicted PPARα, PPARγ, PPAR dual activities and, the total scores, Gold score energies for docking studies at the respective active sites of PPARα (1k7l) and PPARγ (1fm9) also taking into account the crash values (this value expresses the housability of the ligand within the cavity at the receptor site) among the NCEs in each series, with the standard ligands and among the series of NCEs with the three different heterocycles. The six series of designed NCEs were assigned ranks (in each series) for each kind of predicted activities and; the total score and the Gold score energies (express the binding affinity of the ligand at the receptor site) for docking at the two crystal structures (1k7l and 1fm9) in each series separately. The highest activity was assigned rank one and then in a
descending order for decreasing order of activities. The total scores and G-scores for docking at each receptor for each series of designed NCEs were also arranged in a similar fashion. The higher sum of the three predicted activities and the total scores for docking at the two receptors was set as the criterion for the first filter to screen out four best designed NCEs from each series. The higher sum of the three predicted activities and the Gold scores for docking at the two receptors was set as the criterion for the second filter to screen out four best designed NCEs from each series. The molecules showing crash values lesser than -5.00 were ignored (crash values near to zero indicates good housability of the ligand within the cavity at the active site of the receptor) to be taken into account for final selection for synthesis even though fulfilling higher sum criterion and the molecule showing next higher sum was given preference over the former for final selection for synthesis. After comparing the activities, docking scores and crash values for the screened molecules by both the filters four NCEs as best partial agonists from each series were selected for synthesis. The hydrogen bonding interactions of the selected molecules at the active sites of the two receptors (PPARα and γ) were also determined and studied comparatively with that of the selected standard molecules.

Targeted Benzimidazolyl and Indolyl linked benzylidene containing β-ketoester based NCEs Selected for synthesis
Targeted Benzimidazolyl and Indolyl linked benzyl containing β-ketoester based NCEs
Selected for synthesis

Synthesis of targeted ketoester based NCEs were achieved by synthesising the left hand side (lhs) heterocyclyl linked alcohol units and the right hand side (rhs) phenolic units followed by Mitsunobu coupling of both the units, a convergent strategy. The β-ketoesters as the starting material for the synthesis of the rhs units were prepapred according to a common synthetic route.

N-methyl-1, 2-phenylene diamine dihydrochloride was cyclised with glycolic acid to furnish the corresponding hydroxy methyl compound (L1) in excellent yield. The indolyl linked methanol (L2) was prepared by N-methylation and subsequent reduction of the commercially available starting material S2. The β-keto ethyl esters were prepared by condensation of the corresponding acid chloride with monoethyl malonate in the presence of a strong base at very low temperature.

The phenolic β-keto ester based rhs units (benzylidenes: R2a-d and benzyls: R4a-d) were synthesised as shown in scheme 4. Starting with the condensation of p-anisaldehyde and the prepared β-keto esters followed by demethylation of the methoxy benzylidenes (R1a-d) thus obtained gave the hydroxy benzylidene based β-keto ester moieties (R2a-d). Reduction of the methoxy benzylidenes followed by subsequent demethylation of the
methoxy benzyis (R3a-d) thus obtained gave the hydroxy benzyl based β-keto ester moieties (R4a-d).

General Scheme for the Synthesis of Designed and Selected NCEs

The hydroxy benzyl and benzylidene based β-keto ester rhs units were coupled with the lhs units L1 and L2 via Mitsunobu reaction to furnish the heterocyclyl linked benzylidene and benzyl β-ketoester based NCEs (F1-F16). The confirmation of the structures of the molecules was authenticated by spectral (IR, PMR and MS) characterization.