Abstract

Of

The Thesis Entitled

“Design, Synthesis and Biological Evaluation of Novel GPR 119 Agonist for the Treatment of Type-2 Diabetes Mellitus”

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CHAPTER 1: Introduction

1.1 Diabetes Mellitus & Type 2 Diabetes Mellitus

Diabetes is one of the major causes of death worldwide in this century after heart disease and cancer.[1] According to the International Diabetes Federation (IDF) data, 463 million individuals were suffering from diabetes in the world in 2019, which is expected to significantly increase to 700 million by 2045.[2] If untreated, diabetes also leads to several other complications, such as cardiovascular disease, retinopathy, nephropathy, neuropathy, dental diseases and kidney related diseases.[3-10]

Type 2 diabetes mellitus (T2DM) is a complex chronic disease characterized by metabolic disorder and hyperglycaemia due to insulin resistance, hepatic glucose overproduction and/or insufficient insulin secretion. In addition to genetic predisposition, obesity is an added risk factor associated with growing incidence of type 2 diabetes mellitus and many patients with T2DM are obese. These patients are often accompanied by increased cardiovascular risk factors. Innovative new therapies that could improve glucose metabolism and reduce body weight and excess food intake will provide benefits to such patients. Although there are a number of therapeutic options such as sulfonylureas, metformin, glitazones and glinides, they are unable to get satisfactory glycaemic control without adverse side effects. Thus, there is an urgent need of novel therapeutic approaches for the treatment of T2DM by a good glycaemic control without side effects.[11-12]

1.2 Current Therapies for Type 2 Diabetes:

Type 2 diabetes is characterized by insulin resistance coupled with an inadequate compensatory insulin secretory response. Ultimately, there is a loss of pancreatic islet β-cells and a need for insulin replacement.

As on today, several therapies are available for the treatment of T2DM which are elaborated in **Table 1**.

Table 1: Available therapies for the treatment of T2DM.

Drugs	Mechanism Of Action	Side Effects
Metformin, PPAR-γ Agonist	Insulin sensitizer	GI distress, weight gain, liver toxicity
Sulfonylureas, Meglitinide analogues	Insulin secretion (Glucose-independent)	Hypoglycaemia, weight gain, short acting
GLP-1 Mimetics, DPP-IV Inhibitors, **GPR-119 Agonists**	Insulin secretion (Glucose-dependent)	Injection delivery (BYETTA ™), GI distress, Pancreatitis.

Insulin	Insulin Replacement	weight gain, Hypoglycaemia, Inj.delivery

Among these, the G protein-coupled receptor 119 (GPR119) has received considerable attention from the pharmaceutical industry in recent years. GPR119 may present an attractive drug target for treating T2DM, and its agonists may therefore represent potential new insulin secret agogues free of the risk of causing hypoglycaemia. GPR119 has been described as a class A (rhodopsin-type) orphan GPCR without close primary sequence relative in the human genome.[13] The activation of GPR119 increases the intracellular accumulation of cAMP, leading to enhanced glucose-dependent insulin secretion from pancreatic β-cells and increased release of the gut peptides GLP-1 (glucagonlike peptide 1), GIP (glucose-dependent insulin tropic peptide) and PYY (polypeptide YY).[14] Preclinical and clinical studies with GPR119 agonists in type 2 diabetes support that GPR119 agonists have been proposed as a novel therapeutic strategy for diabetes. These investigations indicate that an orally available potent and selective, synthetic GPR119 agonists can lower blood glucose without hypoglycaemia, can slow diabetes progression and can reduce food intake as well as body weight.

1.3 History of GPR-119 agonist:

1.3.1 Characteristics of GPR119

After the discovery of GPR119 in 1999 using data afforded by the Human Genome Project, it was subsequently described in the peer-reviewed literature as a Class A receptor with no close relatives. Independently, this receptor has been studied and described in the literature under various synonyms, including SNORF25, RUP3, GPCR2, 19AJ, OSGPR116, MGC119957, HGPCR2 and glucose-dependent insulinotropic receptor (GDIR). This potentially confusing nomenclature has now been largely rationalized in favor of the designation “GPR119”. The human receptor is encoded by a single exon with introns located on the short arm of Xchromosome (Xp26.1) (**Figure 1**). GPR119 homologs have been identified in several vertebrate species, including the rat, mice, hamster, chimpanzee, rhesus monkey, cattle and dog. Fredriksson et al. (2003) reported the rat isoform of GPR119 (accession number AY288429) as being 133 amino acids longer than the mouse and human receptors (468 vs. 335 amino acids). In contrast, Bonini et al. (accession number AR240217)

and Ohishi et al. gave identical sequences for the rat receptor, which are 335 amino acids in length and have 96% amino-acid identity with mouse GPR119.[14-23]

1.3.2 GPR119 Receptor Expression

Using methods to detect receptor GPR119 mRNA, it has been proposed that, in human tissues, the pancreas and foetal liver have been consistently identified as major sites of GPR119 mRNA expression, with high expression also being noted in the gastrointestinal tract in several studies, while, in rodents, mRNA was detected in most of the tissues examined, with the pancreas and gastrointestinal tract, in particular the colon and small intestine, again appearing as major sites of expression. GPR119 expression has also been described in certain regions of the rat brain.

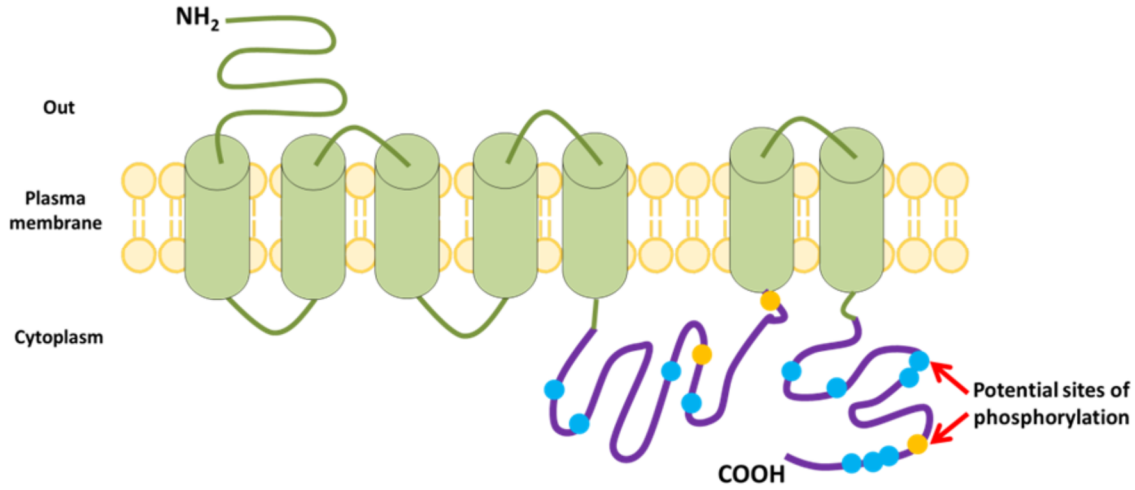


Figure 1. Schematic summary of Human GPR119 membrane topology model.

Clusters of serine (S) and threonine (T) residues in the third intracellular loop and the C-terminus domain could represent potential sites of phosphorylation. *In situ* reveals that pancreatic β cells are the main site of GPR119 expression in pancreatic islets. High expression levels in pancreatic β cell lines NIT-1, MIN6 and RIN5 supports this observation. Consistent with its expression in gut tissues, GPR119 mRNA was strongly expressed in several rodent GLP-1 secreting L-cell lines-including STC-1, FRIC, Hnci-h716 and GLUTag line. GPR119 mRNA has also been found in glucose dependent insulin tropic peptide (GIP)-producing murine intestinal K cells.[24-28]

1.3.3. Mechanism of GPR-119 Agonist

High-level expression of GPR119 in transfected HEK293 cells led to an increase in intracellular cAMP levels via activation of adenylate cyclase, indicating that this receptor couples efficiently to Gαs. In support of these data, potential endogenous

ligands and synthetic small molecule agonists of GPR119 have been shown to increase cAMP levels (**Figure 2**).[16-19]

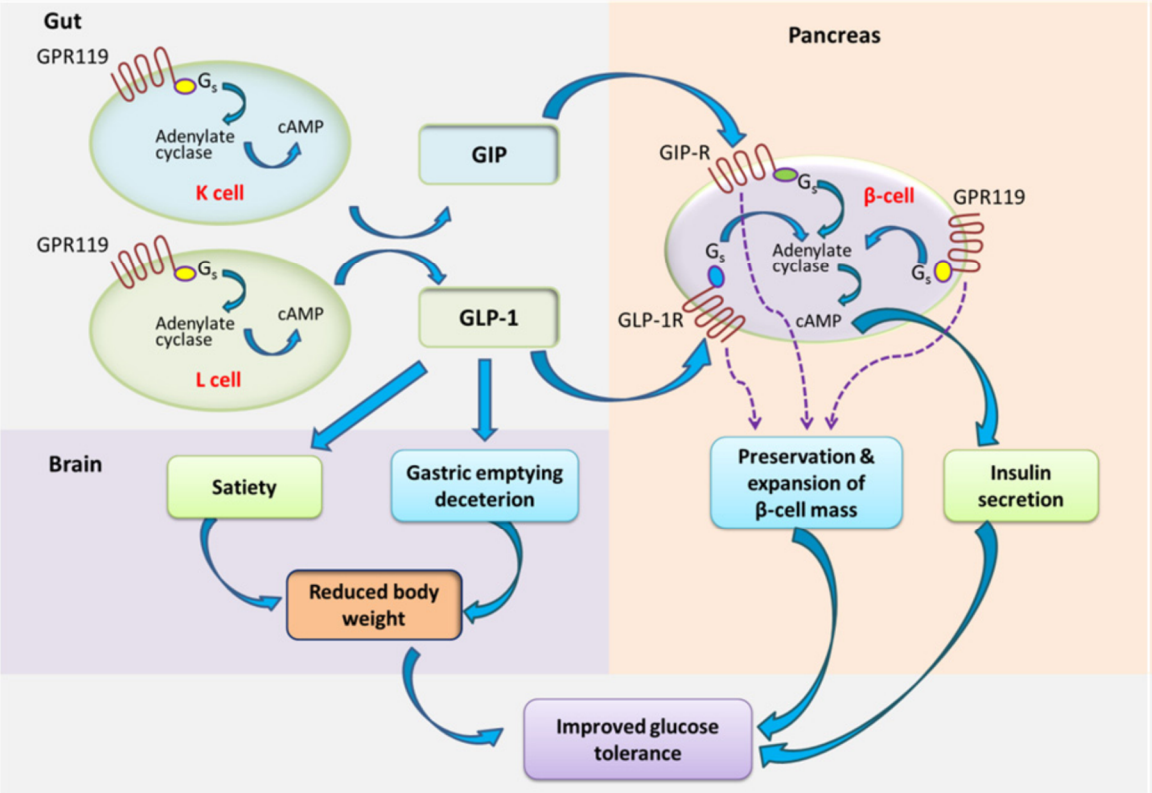


Figure 2. Schematic diagram illustrating the possible actions of GPR119 agonists.[28]

GPR119 is expressed on certain enteroendocrine cells (L and K cells) in the small intestine and by β-cells within the islets of Langerhans of the pancreas. In all three cell types, ligation of GPR119 by an agonist leads to the activation of adenylate cyclase and a rise in cAMP. This triggers the release of glucagon-like peptide 1 (GLP-1), and glucose-dependent isulinotropic peptide (GIP) or insulin from L, K and β-cells, respectively. Additionally, GLP-1 and GIP can both interact with their cognate receptors on the β-cell to elicit insulin secretion. Thus, GPR119 agonists lead to a rise in insulin release by both direct mechanisms. Since GLP-1 (and probably GIP) also promotes β-cell viability, it is possible that orally acting GPR119 agonists may influence both the secretory activity and the viability of β-cells, leading to improved glucose homeostasis in patients with T2DM.

1.3.4 Natural Ligands and synthetic ligands of GPR-119 Agonist:

Several phospholipids and lipid amides such as lysophosphatidylcholine (LPC), oleoylethanolamide (OEA) and oleoyldopamine (OLDA) have been identified as endogenous ligands for GPR119 agonists (**Figure 3**).

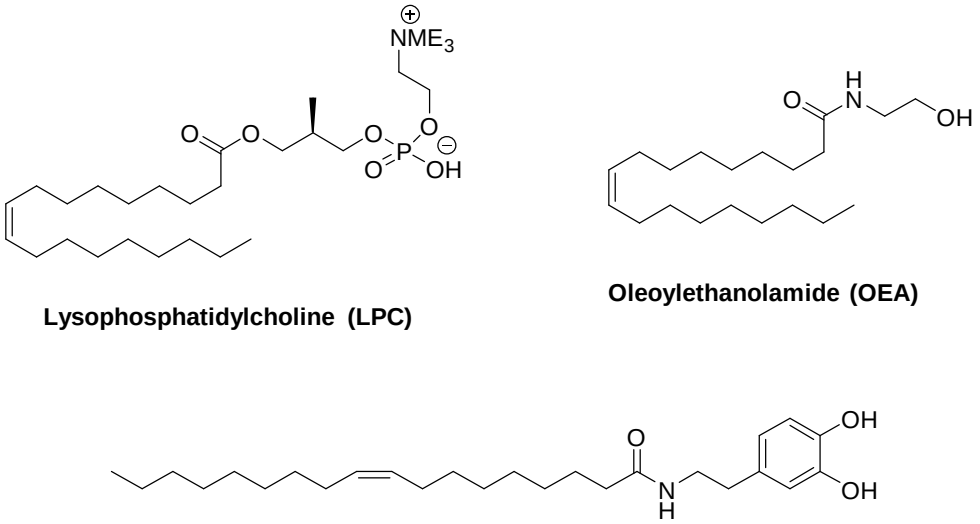


Figure 3: Endogenous ligands of GPR-119

However, due to the low potency and poor selectivity of these endogenous agonists towards GPR119 receptor, many synthetic small molecule GPR119 agonists have been discovered and disclosed by several research groups over the years.[28-31] These intense efforts led to the development of compounds till clinical phase as shown in **Figure 4**.[32-33] However, due to the tachyphylaxis, these compounds failed to show desired efficacy in the clinical trials and none could reach the market.

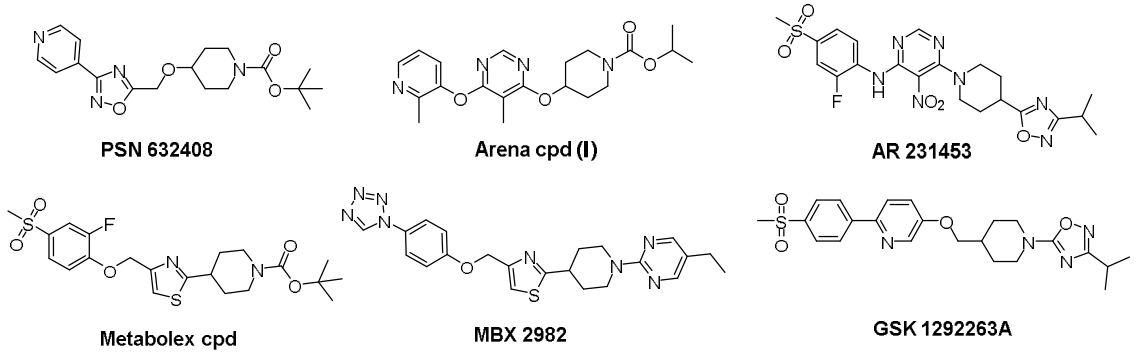


Figure 4: The synthetic ligands of GPR119 Agonists

1.3.5 Current scenario of GPR-119 Agonist [31]

The identification and optimization of GPR-119 agonists has been described in a number of reviews.[31] However, several clinical candidates (**Table 2**) have been discontinued for the reasons not known or undisclosed.

Table 2: Present status of GPR-119 agonists in clinical trials.

Compound	Company	Highest Development Phase (Indication)	Status
PSN821	Astellas (Prosidion)	Phase II (T2DM, obesity)	Discontinued (Nov. 2012)

GSK1292263	GlaxoSmith Kline	Phase II (T2DM, dyslipidemia)	Discontinued
MBX-2982	CymaBay Therapeutics (Metabolex)	Phase II (T2DM)	Search for out licensing
DS-8500a	Daiichi Sankyo	Phase II (T2DM)	Study completed
LEZ763	Novartis	PhaseI/II (T2DM)	Discontinued (Sep. 2014)
APD668	Arena	Phase I (T2DM)	Discontinued (Jan. 2008)
APD597	Arena	Phase I (T2DM)	Discontinued (Aug. 2011)
BMS-903452	Bristol-Myers Squibb	Phase I (T2DM)	Study completed
"NN"	Novartis	Phase I	Discontinued (QTc-prolon-gation)

Hence, there is an unmet need to explore a new molecular targets and strategies. One of the most promising targets among recent for treatment of T2DM is G-Protein Coupled Receptor-119 Agonist (GPR-119 agonist).

1.4 Objective

As the incidence of T2DM is increasing worldwide due to changing lifestyle and prevalence of obesity and associated metabolic syndromes, also available antidiabetic drugs are not adequate for the safe and effective treatment of T2DM. In this project, our aim was to develop, novel class of antidiabetic agents for the prevention and management of T2DM. In this regards, development of new class of potent and selective GPR-119 agonist is found to be very attractive and challenging target. The major advantage of developing GPR-119 agonist could be low risk of hypoglycaemia and no weight gain.

CHAPTER 2: Designing of GPR 119 Agonists

2.1 Novel GPR 119 Agonists

The characteristics of Diabesity (Diabetes and Obesity) and the role of GPR 119 in the pathophysiology of this complex disorder have been described in detail in the previous section. The role of GPR 119 in glucose stimulated insulin secretion (GSIS) from the pancreas and glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) release from the intestine have been scientifically proven. Additionally GPR 119 agonists have been shown to improve insulin resistance associated with obesity. These facts made GPR 119 a promising target for the treatment of T2DM, and since then many synthetic ligands have been discovered and disclosed (see **Figure 4)**. Some of these compounds have been evaluated in clinic for their safety and efficacy in humans. However, none of these candidates entered in to phase III clinical trials.

The challenges in designing GPR119 agonists with acceptable physicochemical properties, the optimization of the pharmacodynamics parameters such as potency at the receptor and extent of activation (maximal intrinsic activity) have proven to be far from trivial. Medicinal chemistry teams have succeeded in designing and synthesizing highly potent GPR119 agonists with activities in the low nanomolar range. However, optimizing maximal receptor activation has been found to be difficult. Also the extent of receptor activation (partial, full or even super agonism) required to achieve an optimal therapeutic effect is still an unresolved question. Poor translation of therapeutic effects from rodents to humans has been a bigger challenge since rodent GPR119 receptor sequences differ from the human receptor as stated earlier. Furthermore, tachyphylaxis, a well-known phenomenon in activation of GPCRs, needs to be considered as a possible limitation for a chronic treatment. These facts made the development of GPR119 agonists a complicated and challenging for the scientific community. Finally, a thorough understanding of the relative contributions of receptor activation at the two major sites of GPR119 expression, i.e. gut and pancreas, to the overall pharmacological effect might help to guide the design of agonists. Taken all together, this leads to the important question whether improved 2nd generation GPR119 agonists will be able to provide diabetic patients with additional benefits, besides glucose lowering, that initially attracted the intense interest.

In an attempt to identify potent GPR119 agonists, designing of new chemical entities with *in-silico* docking studies was initiated.

The Arena molecule (**AR231453**) was docked into the homology model using the induced fit docking (IFD) protocol[34] where **AR231453** fits snugly to binding site and adopts an extended conformation as shown in **Figure 5**. Further, the fluoro-phenyl ring forms pi-pi interaction with Phe 249 and 1,2,4-oxadiazole ring forms a pi-pi interaction with side chain of Tyr 271. This is also stabilized by cation-pi interaction between nitro group of AR231453 and Phe 168. These three interactions seem to stabilize the molecule in binding site. In addition to these interactions, **AR231453** also forms van der Waals interactions with Phe 182, Trp 246, Val 84, Leu 274 and Gln 66. Supposing that these interactions and similar binding mode might be contributing well to the potent agonistic activity, a general structural architecture which formed the basis for further designing of novel compounds described in following section of the thesis was derived.

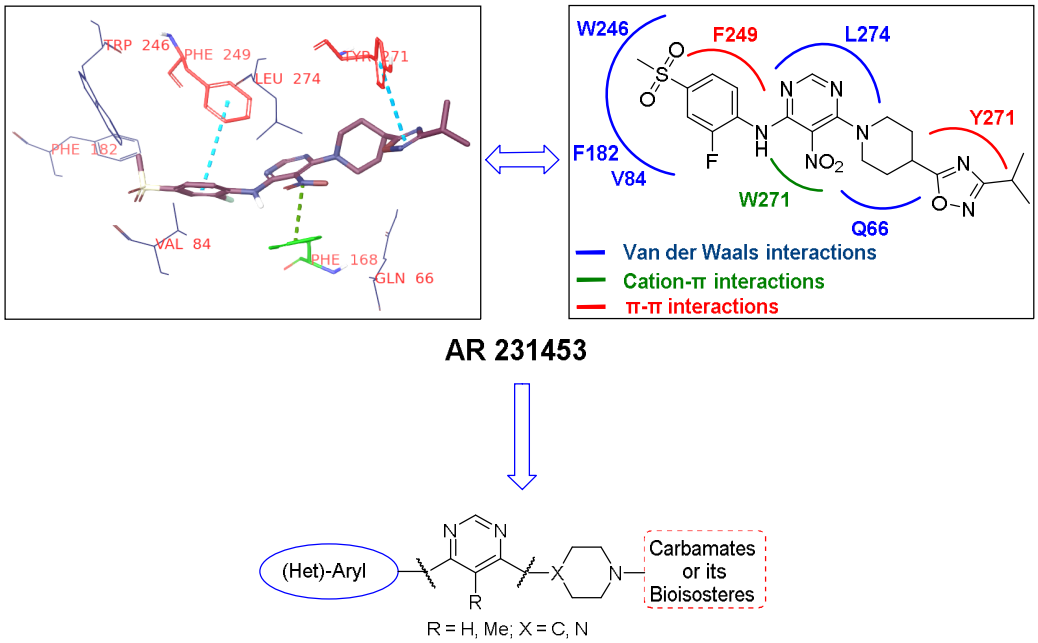


Figure 5: A Typical molecular architecture of synthetic GPR-119 agonists

2.1.1 Rationale for designing *para*-Benzylidene thiazolidinedione's derivatives as GPR 119 agonists

In the foresaid context of high unmet medical needs and the emergence of GPR 119 agonists as a fascinating target for the treatment of diabetes, idea to develop a new class of agonists with distinct biological and safety profile consisting of a novel pharmacophore intrigued us.

A typical structural design of a GPR 119 agonists as shown in **Figure 5** comprises of a (Het-Aryl) as “head group” and “Carbamates or its Bioisosteres” as tail group with a linker (Pyrimidine) in-between. Keeping this scaffold in mind, compound (**II)** was designed (**Figure 6**).

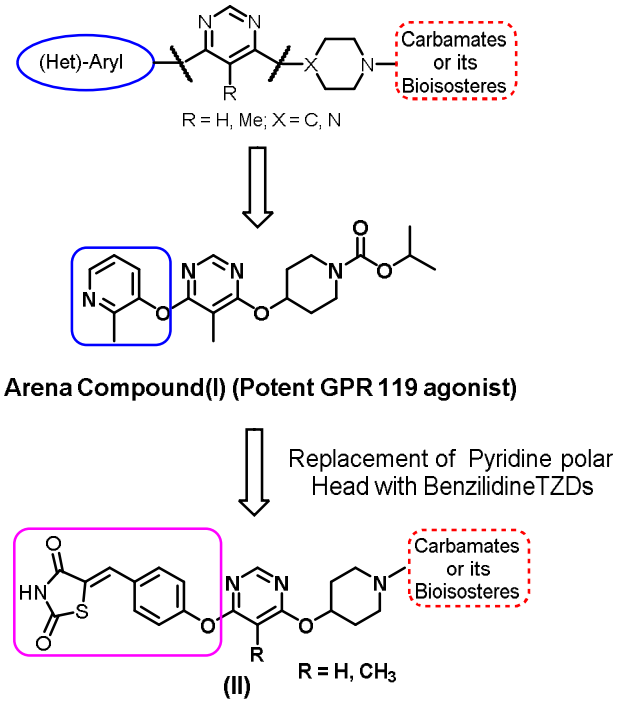


Figure 6: *p*-benzylidene thiazolidinedione containing GPR 119 Agonists.

Arena compound (I) is reported to be a selective and potent agonist of the GPR119 receptor across several species (EC_{50}, melanophore; human: 2 nM; dog: 1 nM; cynomolgus monkey: 35 nM; mouse: 41 nM; rat: 44 nM) and possesses aqueous solubility with no appreciable inhibition of at least five cytochrome P450 enzymes (IC_{50}) (CYP2C9, 15 μM; 1A2 2D6 3A4, > 40 μM; 2C19, 10 μM). **Arena compound (I)** also demonstrated a dose-dependent inhibition of glucose excursion in the oral glucose tolerance test (oGTT) experiment in male SD rats (22%, 0.3 mg/kg p.o.; 24%, 3 mg/kg p.o.; 70%, 30 mg/kg p.o.).[35] However, further development of this compound has been suspended for reasons not disclosed. In the efforts to discover promising and safe GPR119 agonists, 2-methyl pyridine, a polar head of **Arena compound (I)** was replaced with clinically proven pharmacophore benzylidene thiazolidinedione, as a novel heterocyclic head and identified this moiety as a pyridine surrogate (**Figure 6**).[36] Thiazolidinediones (TZDs), also known as glitazones are insulin sensitizers having a pleotropic pharmacology including reduction of insulin resistance, a root cause of diabetes. Importantly these agents also preserve pancreatic *β*-cell function or mass

better than insulin secretagogues such as sulfonylureas. The glitazones (rosiglitazone and pioglitazones) are a new class of anti-diabetic drugs that act by improving sensitivity to insulin and are indicated in the treatment of type 2 diabetes. The glitazones have effects on carbohydrate and lipid metabolism and hold the promise of being able to influence many components of the insulin resistance syndrome seen in type 2 diabetes. It is possible that the glitazones are able to prevent or delay the cardiovascular disease, which accompanies type 2 diabetes. In viewed there the properties of TZDs, benzylidene thiazolidinedione as head group was incorporated to identify novel, potent and safe GPR 119 agonists.

2.1.2 Designing of *meta*-Benzylidene thiazolidinedione derivatives as GPR 119 Agonists:

Having studied the SAR of initial compounds, it was decided to optimize the position of thiazolidinedione on phenyl ring. To do so, *meta*-benzylidene thiazolidinedione derivatives (III) were synthesized (**Figure 7**).

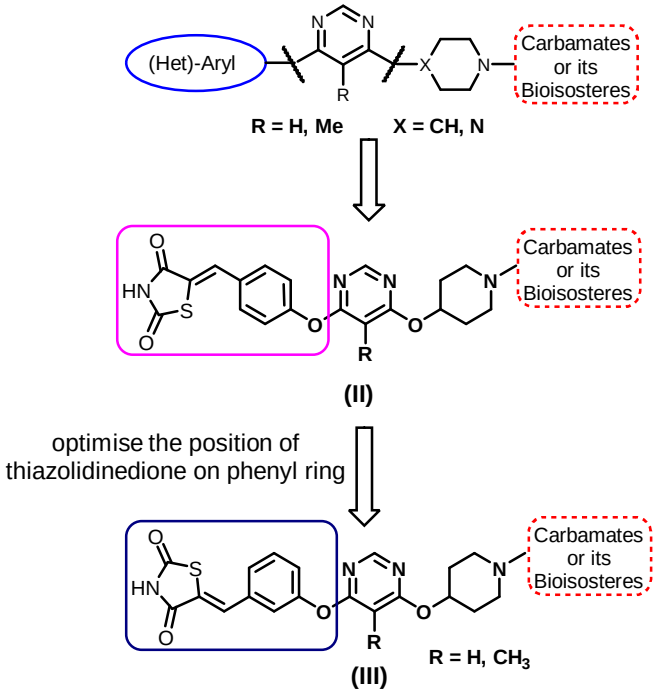


Figure 7: *m*-benzylidene thiazolidinedione containing GPR 119 Agonists

2.1.3 Designing of compounds based on N-substituted *m*-benzylidene thiazolidinedione derivatives as GPR 119 Agonists

The next endeavor in this project was to optimize the lead compound of *meta*-benzylidene thiazolidinedione series. Thus, N-substituted *meta*-benzylidene thiazolidinedione derivatives (IV) were prepared (**Figure 8**).

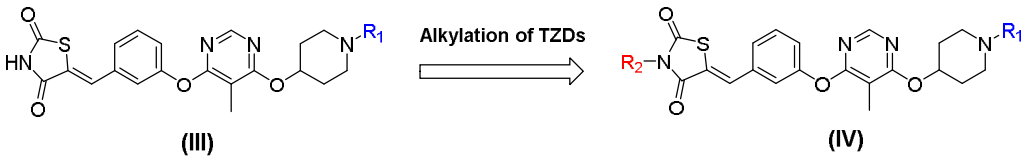


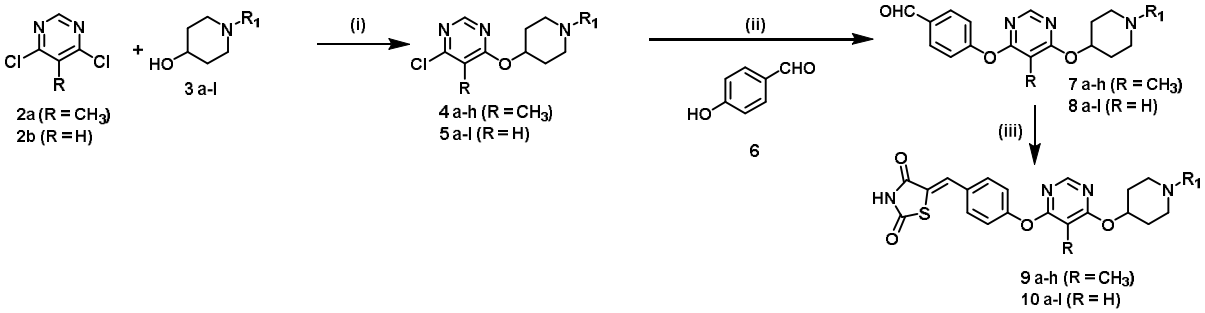
Figure 8: Designing of N-substituted Benzylidene thiazolidinedione as GPR Agonists

CHAPTER 3: Results and Discussion

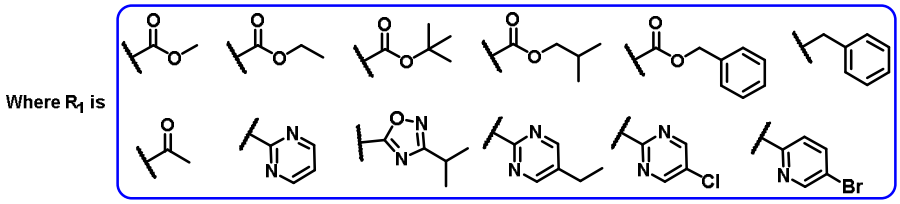
3.1 Synthesis Scheme for preparation of *p*-benzilidine thiazolidine diones derivatives

The general synthetic scheme leading to the compounds of *p*-benzilidine thiazolidine diones derivatives **(9a-h and 10a-l)** is outlined in **Scheme1.**

Scheme 1



Scheme 1. Reagents and Conditions: (i) t-BuOK, THF, 0 ^{o}C, 30 min, (ii) K_2CO_3, DMA, 138 ^{o}C, 3 hrs, (iii) TZD, Piperdine, benzoic acid, Toluene, Reflux, 155 ^{o}C, 3hrs.



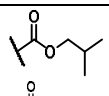
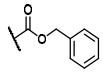
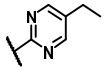
3.1.1 Biological evaluation of p-benzilidine thiazolidine diones derivatives

3.1.1.1 in-vitro evaluation (GPR-119 agonistic activity)

GPR119 agonistic activity of the synthesized compounds was measured using a cAMP assay in the human GPR119 cell line. GPR119 agonist **AR231453** compound was taken as the reference standard and the results are shown in below **Table 3**. cAMP stimulation is measured for all the compounds and the activity is represented as EC_{50} values and % max of stimulation compared to maximal effect at 1µM **AR231453.**

Table 3: GPR-119 agonistic activity of 9a-h and 10a-l.

Compound No	R	R_1	EC_{50} (nM)	% Emax[a]
9a	CH_3		1414	120.4
10a	H		IA	ND
9b	CH_3		157	170.1
10b	H		790	75.0
9c	CH_3		88	68.8
10c	H		130	121.6
9d	CH_3		75	90.2

10d	H		428	63.5
9e	CH_3		805	73.0
10e	H		107	98.1
9f	CH_3		IA	ND
10f	H		IA	ND
9g	CH_3		595	97.6
10g	H		IA	ND
9h	CH_3		124	101.8
10h	H		113.5	92.2
10i	H		916	64.0
10j	H		274	84.6
10k	H		990.6	67.5
10l	H		>1 µM	ND
AR231453			6.0	100

[a] %max: cAMP stimulation % compared to maximal effect at 1µM AR231453.
Where IA is Inactive, ND is not detected

3.1.1.2 Pharmacokinetic Parameters

Based on the above *in-vitro* results, we intended to study the pharmacokinetic parameters of **9c, 10c** and **9h** in *Sprague Dawley* rats at 25 mg/kg/day oral dose and the results of which are expressed in below **Table 4.**

Table 4: Pharmacokinetic parameters of compound **9c**, **10c** and **9h**.

Compound No	C_{max} (ng/ml)	$T_{1/2}$ (hr)	AUC $_{(0-t)}$ (hr.ng/ml)
9c	834	4.37	8108
10c	5961	4.91	34293
9h	1452	6.81	11522

3.1.1.3 in-vivo studies (Glucose lowering activity)

Based on the *in-vitro* results, selected compounds **9c, 10c** and **9h** were subjected for primary *in-vivo* screening for oral glucose tolerance test (oGTT) in C57 BL/N6 mice and db/db mice. For the acute screening assay, we monitored blood glucose for 120 min after oral administration. The vehicle or drug in this study was administered 30 min prior to the challenge and data are presented in below **Table 5.**

Table 5: Glucose excursion of compounds **9c**, **10c** and **9h**.

Compound No	% Improvement in glucose excursion AUC at 50 mg/kg	
	C57 mice	db/db mice
9c	34.6 ± 3.7	56.5 ± 9.2
10c	23.4 ± 5.4	52.4 ± 8.5
9h	29.3 ± 6.1	22.4 ± 7.3

Sitagliptine	39.7±5.0	82±11

3.1.2 Molecular Docking Study

Molecular docking studies were carried out-using Glide, the automated docking program implemented in the Schrodinger package to explain the in-vitro potency activity for GPR 119 receptors of compounds **9c**, **10c** and **9h**. A homology model of GPR119 receptor was constructed based on template protein crystal structure (PDB ID: 2R4R) with sequence identity 26.94 % using the Prime module of Schrodinger. Phenyl ring of Compound **9c** forms π-π interactions with the side chain of PHE 168 and PHE 249 and in addition, thiazolidinedione of compounds **9c** forms H-bonding interactions with the amino acid THR 85. Dashed lines (**Figure 9**) show these interactions. Pyrimidine and phenyl rings of compounds **9h** and **10c** showed π-π interactions with PHE 249 and in addition to these interactions, the compounds **9h** and **10c** possess van der Waals interactions with THR 85 and PHE 168. These interactions and similar binding modes with respect to AR231453 molecule contribute to the potent agonistic activity of the compounds **9c, 9h** and **10c.**

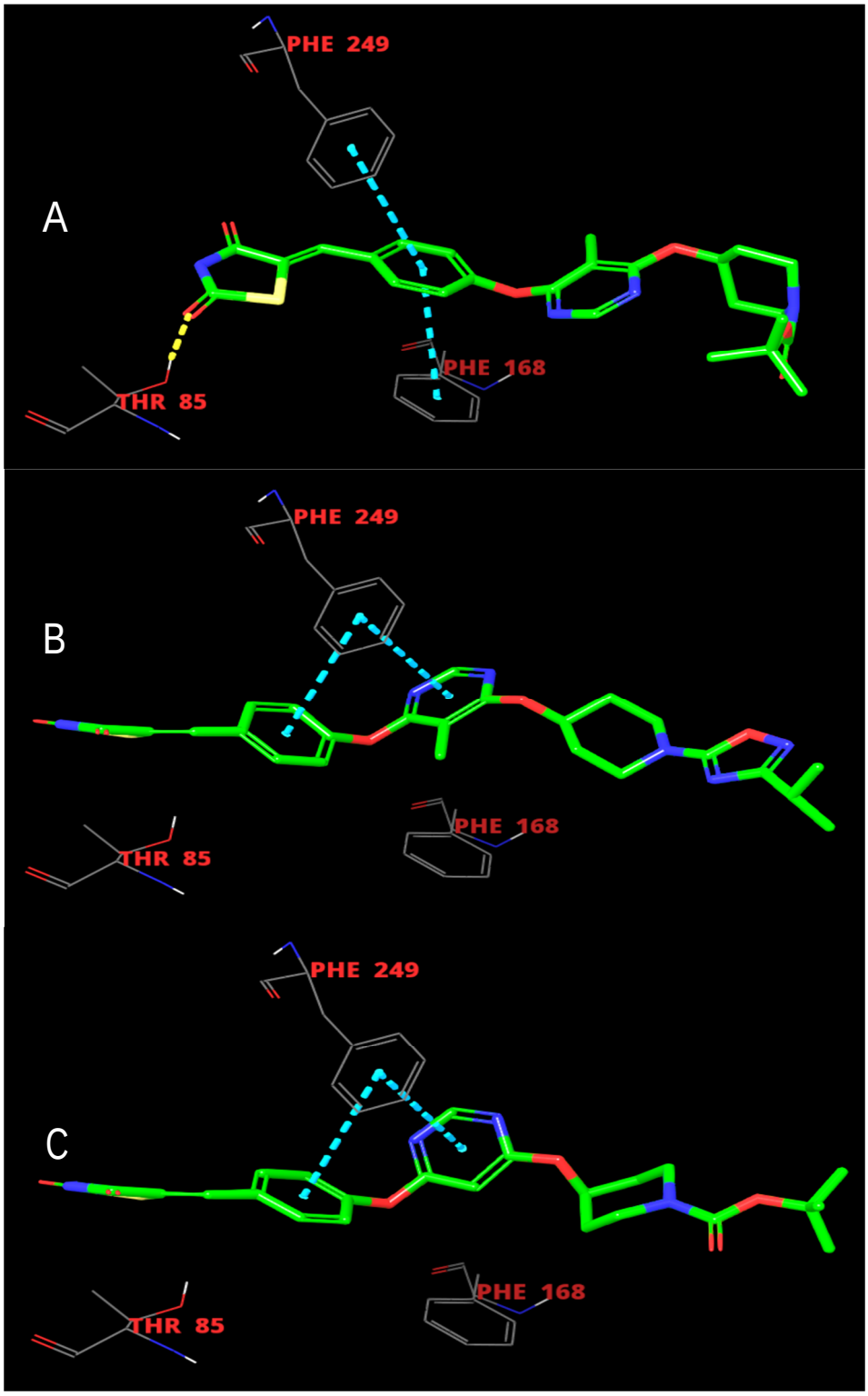
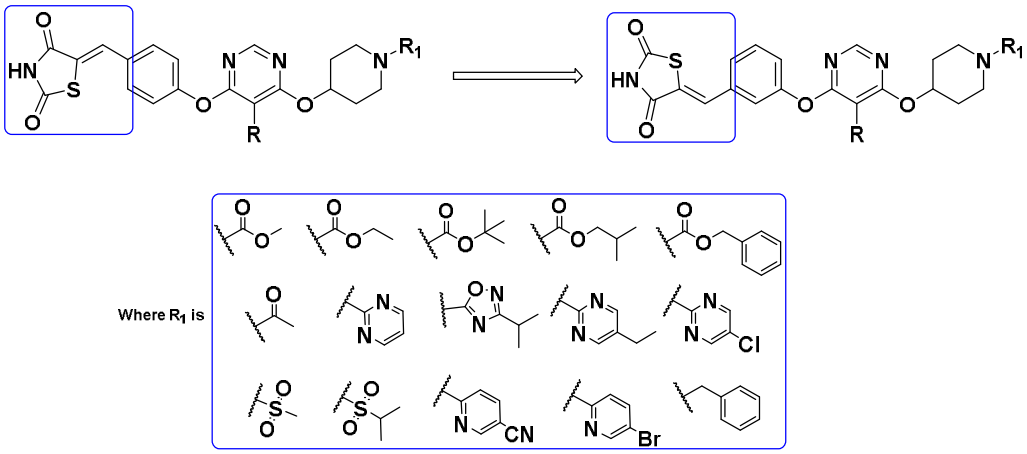


Figure 9: Compounds 9c (A), 9h (B) and 10c (C) docked into GPR 119 binding site.

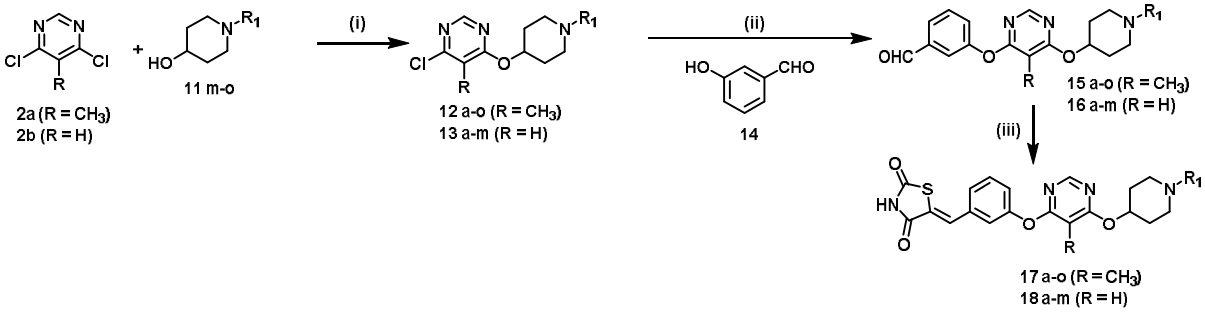
H-bonding and π-π interactions with amino acids are shown as dashed lines.

3.2 Design, synthesis and biological evaluation of compounds based on m-benzilidine thiazolidine dione derivatives.

Our next task in this endeavour was to optimise the position of thiazolidinedione on phenyl ring. To do so we have synthesized *meta*-benzylidene thiazolidinedione derivatives **17a-o** and **18a-m** and the general synthetic procedure leading to the compounds of *m*-benzilidine thiazolidine diones derivatives is outlined in **Scheme 2.**



Scheme 2



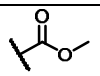
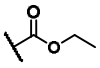
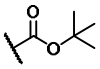
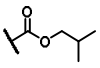
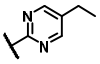
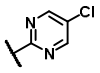
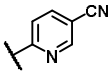
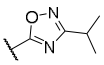
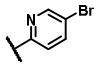
Scheme 2. Reagents and Conditions: (i) t-BuOK, THF, 0 ^{o}C, 30 min, (ii) K_2CO_3, DMA, 138 ^{o}C, 3 hrs, (iii) TZD, Piperdine, benzoic acid, Toluene, Reflux, 155 ^{o}C, 3hrs.

3.2.1 Biological evaluation of 17a-o and 18a-m:

3.2.1.1 in-vitro evaluation (GPR-119 agonistic activity)

GPR119 agonistic activity of the synthesized compounds was measured using a cAMP assay in the human GPR119 cell line. GPR119 agonist **AR231453** compound was taken as the reference standard and the results are shown in below **Table 6**. cAMP stimulation is measured for all the compounds and the activity is represented as EC_{50} values and % max of stimulation compared to maximal effect at 1µM **AR231453.**

Table 6: GPR-119 agonistic activity of 17a-o and 18a-m.

Compound No	R	R_1	EC_{50} (nM)	% Emax[a]
17a	CH_3		IA	ND
18a	H		187	90.7
17b	CH_3		IA	ND
18b	H		819	70
17c	CH_3		43	160
18c	H		125.3	92.5
17d	CH_3		IA	ND
18d	H		720	75
17e	CH_3		105.7	99.2
18e	H		153.4	119.7
17f	CH_3		IA	ND
18f	H		IA	ND
17g	CH_3		IA	ND
18g	H		IA	ND
17h	CH_3		184.4	81.1
18h	H		1102	70.7
17i	CH_3		1005	68.2
18i	H		702	77.6
17j	CH_3		92.2	112
18j	H		104.2	102.8
17k	CH_3		63.4	51
18k	H		343	ND
17l	CH_3		715	72.1
18l	H		977	ND
17m	CH_3		71.09	130
18m	H		134.7	144.6
17n	CH_3		139.6	93.8
17o	CH_3		122.1	101.8
AR231453			6.0	100

[a] %max: cAMP stimulation % compared to maximal effect at 1μM AR231453.
Where IA is Inactive, ND is not detected

3.2.1.2 Pharmacokinetic Parameters

Based on the above *in-vitro* results, we intended to study the pharmacokinetic parameters of **17c, 17j** and **17m** in *Sprague Dawley* rats at 25 mg/kg/day oral dose and the results of which are expressed in below **Table 7.**

Table 7: Pharmacokinetic Parameters of compounds **17c**, **17j** and **17m**.

Compound No	C_{max} (ng/ml)	$T_{1/2}$ (hr)	AUC $_{(0-t)}$ (hr.ng/ml)
17c	4955	3.09	19055
17j	2379	5.27	22852
17m	875	3.27	3909

3.2.1.3 in-vivo studies (Glucose lowering activity)

Based on the pharmacokinetic parameters, selected compound **17c** was subjected to primary *in-vivo* screening for oral glucose tolerance test (oGTT) in C57 BL/N6 mice

and db/db mice. For the acute screening assay, we monitored blood glucose for 120 min after oral administration. The vehicle or drug in this study was administered 30 min prior to the challenge and data are presented in below **Table 8.**

Table 8: Glucose excursion of compound **17c.**

Compound No	% Improvement in glucose excursion AUC at 50 mg/kg	
	C57 mice	db/db mice
17c	32.4 ± 5.9	69 ± 10.3
Sitagliptine	39.7±5.0	82±11

3.2.2 Docking study of compound 17c

To explain the GPR119 agonist activity of compound **17c**, docking studies have been carried out using Glide, the automated docking program implemented in the Schrodinger package. A homology model of GPR119 receptor was constructed based on template protein crystal structure (PDB ID: 2R4R) with sequence identity 26.94 % using Prime module of Schrodinger 5-methyl pyrimidine of Compound **17c** forms a pi-pi interaction with the side chain of Phe 168. As shown in **figure 10**. In addition to this interaction, compound **17c** forms Van der Waals interactions with Phe 172, Phe 249, Arg 270, Ty2 271 and Gln 66. These interactions and similar binding mode with respect to AR231453 molecule might be contributing to the potent agonistic activity of **17c** molecule. The glide score and binding energies of 17c and Arena compound as mentioned below **Table 9**.

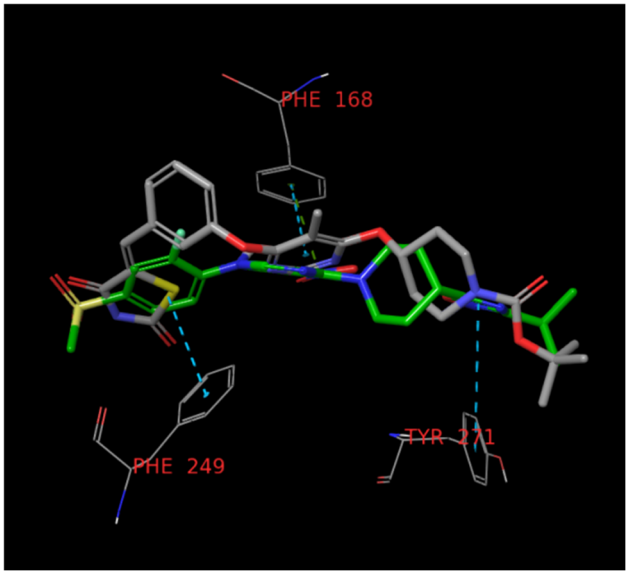


Figure 10: AR 231453 (green) superposed with 17c (grey) in the GPR119 binding site.

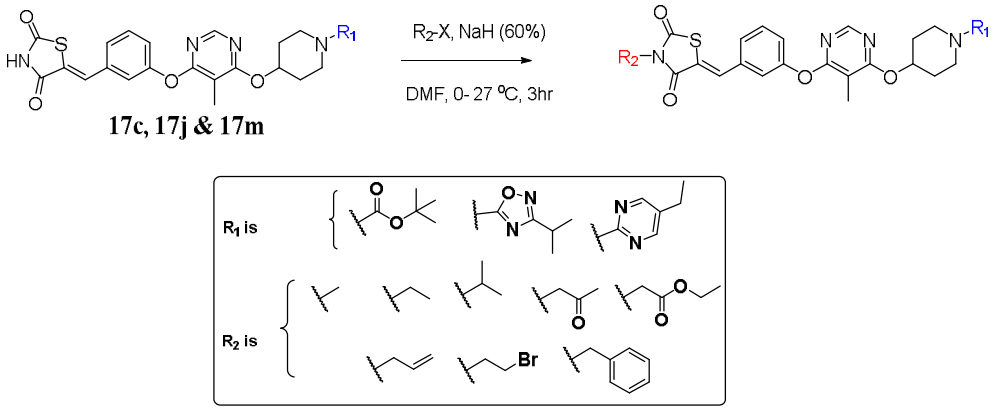
Table 9: Glide score and binding energies of compound **17c**.

Compound No	Glide Score	MMGBSA dG Bind (Energy in kcal/mol)
AR231453	-7.72	-113.3
17c	-6.65	-105.30

3.3 Design, synthesis and biological evaluation of compounds based on N-substituted *m*-benzilidine thiazolidine dione derivatives.

Our next task in this programme was to optimise the lead compound **17c** of *m*-benzilidine thiazolidine dione series. To do so we have synthesized N-substituted *meta*-benzylidene thiazolidinedione derivatives **19 - 29** and the synthesis scheme is outlined in **Scheme 3.**

Scheme 3

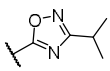
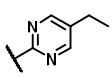


3.3.1 Biological evaluation of Compounds 19-29:

3.3.1.1 in-vitro evaluation (gpr-119 agonistic activity)

GPR119 agonistic activity of the synthesized compounds was measured using a cAMP assay in the human GPR119 cell line. GPR119 agonist AR231453 compound was taken as the reference standard and the results are shown in below **Table 10**. cAMP stimulation is measured for all the compounds and the activity is represented as EC_{50} values and % max of stimulation compared to maximal effect at 1μM AR231453.

Table 10: GPR-119 agonistic activity of **19-29**.

Compound	R_1	R_2	EC_{50} (nM)
19			100
20			94
21			107.4
22			1025
23			79
24			586
25		CH_3	120.6
26			821
27			160.9
28			102.5
29			240.3

Compounds **19**, **20, 21, 23** and **28** were exhibited potent agonistic activity in *in-vitro* study. However, compound **17c** has more potent agonistic activity than above compounds. Based on the overall profile we then decided to select compound **17c** for further detailed biological studies.

3.4 Developmental studies of lead compound 17c

In-vivo efficacy:

Encouraged with the positive results from in-vitro and primary in-vivo studies, we then conducted an oGTT experiment to study time and dose dependent effects of compound **17c** on glucose excursion in *db/db* mice. Compound **17c** in doses ranging from 1 to 50 mg/kg were administrated orally for 14 days and blood glucose levels were measured for 0-120 min after 30 min of the compound administration and AUC was calculated for Day14. Compound **17c** showed significant and dose dependent reduction of glucose excursion at all the tested doses as shown in **Figure 11.**

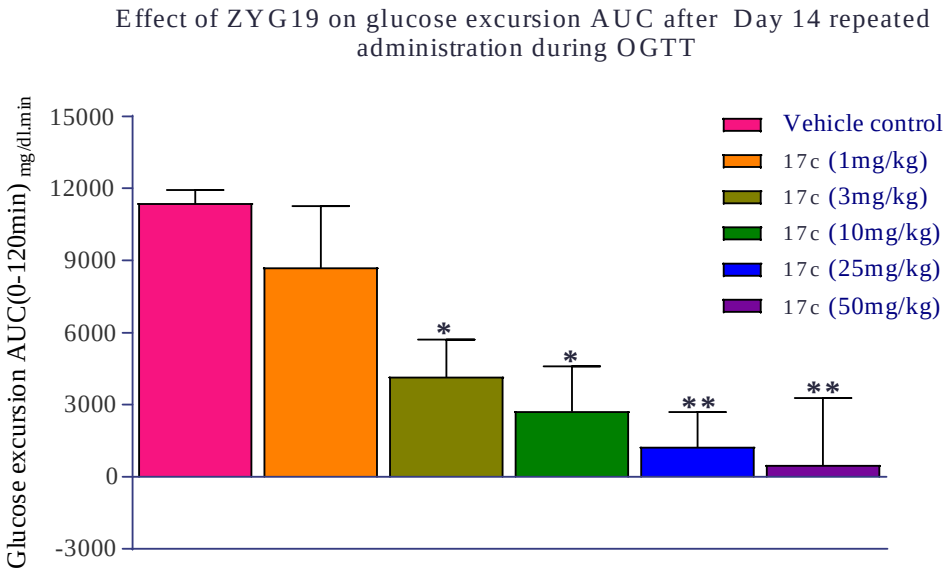
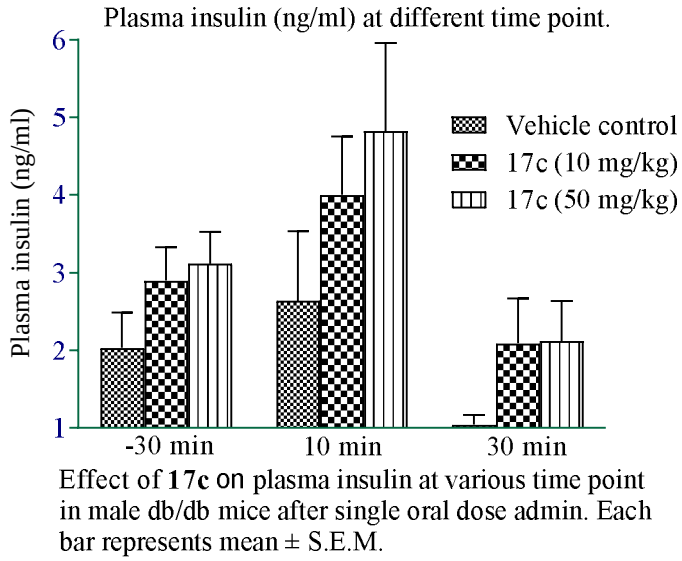


Figure 11: The effect of compound **17c** on AUC glucose excursion (mg/dl.min) in male db/db.

Evaluation of insulin secretion activity:

The effect of compound **17c** on glucose dependent insulin secretion was also evaluated where 50 mg/kg/day dose of **17c** showed significant increase in secretion of insulin at 10 and 30 minutes after glucose administration (**Figure 12**).

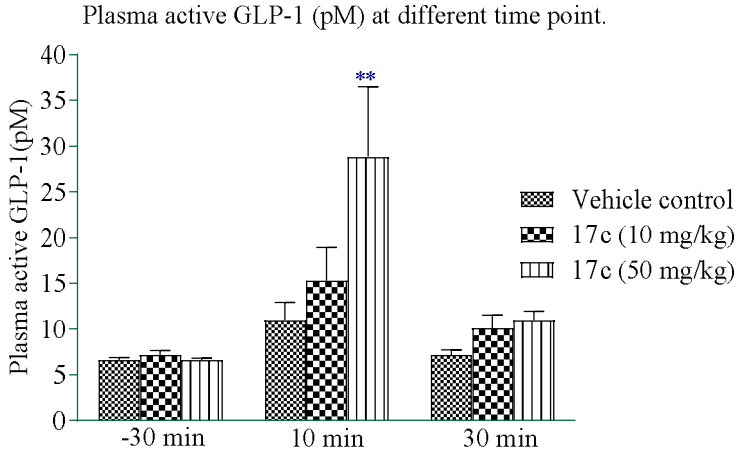


Effect of **17c** on plasma insulin at various time point in male db/db mice after single oral dose admin. Each bar represents mean ± S.E.M.

Figure 12: Effect on glucose dependent insulin secretion of compound **17c** using db/db model.

Evaluation of GLP-1 secretion

The effect of compound **17c** on plasma active GLP-1secretion at various time points in male *db/db* mice after single dose oral administration was also evaluated. Tretament with **17c** at 50 mg/kg has increased active GLP-1 levels by an impressive 2.6 fold at 10 min after glucose administration (**Figure 13**).



Effect of **17c** on plasma active GLP-1 at various time point in male db/db mice after single oral dose admin. Each bar represents mean ± S.E.M. **significant different from vehicle control group($p<0.01$)

Figure 13: Effect on plasma active GLP-1 of compound **17c** using db/db model.

Combinational study with DPP-IV Inhibitor:

Further when co-administered with **sitagliptin,** a DPP-4 Inhibitor, **17c** showed a synergistic and significant elevation in the active GLP-1 levels compared to that caused by either **17c** or sitagliptin alone as shown in **Figure 14.**

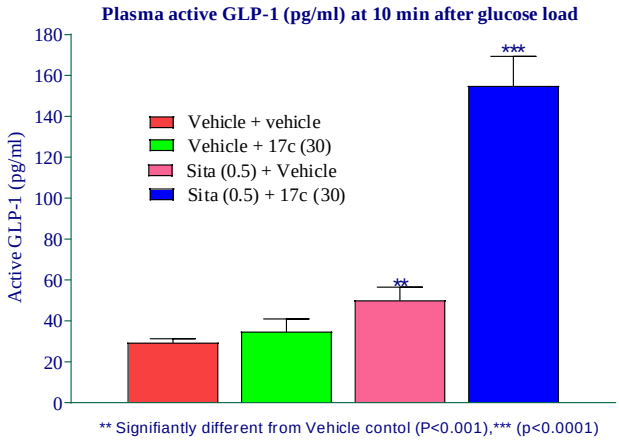


Figure 14: Plasma active GLP-1 (pg. /ml) at 10 min after glucose load.

Repeat dose Toxicity studies:

Having achieved the primary goal of identifying potent and efficacious GPR119 agonist, our next end point of this endeavour was to study the toxicity profile of the lead compound **17c** with repeated dose administration by oral gavage over the period of 28 days in *Wistar* rats. Groups of 10 animals of each male female rats were dosed orally with 100, 200 and 400 mg/kg compound **17c** once a day for 28 days. These doses corresponds to 50x, 100x and 200x of the ED_{50} (considering ED_{50} as ~2.0 mg) found from the efficacy study in db/db mice. There were no significant treatment related clinical manifestations noted in any of the treated group animals and there was no treatment mortality occurred in this study up to the dose level of 400 mg/kg.

No significant change in body weights of animals of both the sex was observed. Analysis of organ to body weight ratios (**Table 11**) did not show evidence of toxicity attributed to compound treatment at least at 100 mg/kg dose, which is 50x of ED_{50}. There was no changes in relative organ weight noted up to 400 mg/kg in male rats at the end of treatment periods.

Similarly, no significant alterations were observed in biochemical parameters (**Table 12**) except the decrease in urea levels in both sex animals and marginal decrease in glucose levels in male animals. No significant changes were observed in liver enzymes

(ALP, AST, and ALT), hemoglobin, albumin and creatinine at any doses in both sex animals.

Table 11: Relative organ weights[a] of Wistar rats administered orally with **17c** for 28 days

Dose (mg/kg)	Heart	Liver	Kidneys	Spleen	Adrenals	Brain	Testes	Epididymides	Thymus
Male									
Control	0.333±0.036	3.142±0.231	0.787±0.078	0.206±0.026	0.020±0.003	0.766±0.038	1.248±0.073	0.361±0.028	0.148±0.023
100	0.347±0.021	3.237±0.160	0.831±0.057	0.222±0.010	0.023±0.002	0.830*±0.074	1.228±0.322	0.371±0.056	0.167±0.028
200	0.363*±0.024	3.304±0.160	0.847±0.067	0.220±0.024	0.023±0.002	0.824*±0.038	1.289±0.105	0.406±0.058	0.175±0.037
400	0.338±0.017	3.220±0.253	0.845±0.064	0.211±0.010	0.023*±0.004	0.809±0.038	1.230±0.060	0.365±0.027	0.196**±0.045
Female							Ovaries	Uterus	
Control	0.380±0.037	3.219±0.221	0.801±0.071	0.244±0.032	0.045±0.007	1.100±0.078	0.091±0.009	0.335±0.108	0.260±0.025
100	0.410±0.030	3.243±0.268	0.875±0.125	0.241±0.027	0.046±0.007	1.103±0.066	0.089±0.013	0.345±0.103	0.241±0.022
200	0.394±0.016	3.285±0.262	0.840±0.057	0.242±0.033	0.044±0.010	1.114±0.036	0.089±0.011	0.330±0.142	0.257±0.048
400	0.367±0.044	3.172±0.301	0.806±0.093	0.228±0.029	0.042±0.006	1.069±0.051	0.081±0.007	0.429±0.142	0.244±0.043

[a] Presented as organ-to-body weight percent ratio

* = Significant from control group at 5% level ($p<0.05$), ** = Significant from control group at 1% level ($p<0.01$)

Table 12: Biochemical Parameters of Wistar rats administered orally with **17c** for 28 days

Dose (mg/kg)	Globulin (g/dl)	Glucose (mg/dl)	Creatinine (mg/dl)	ALP (U/L)	AST (U/L)	ALT (U/L)	Albumin (g.dl)	Urea (mg/dl)
Male								
Control	2.62±0.12	89.02±13.41	0.61±0.04	174.77±25.42	119.40±9.30	30.47±2.63	3.56±0.08	58.14±9.13
100	2.50±0.11	87.56±12.75	0.57±0.03	152.22±24.91	114.13±24.86	28.90±3.98	3.47±0.08	55.36±10.27
200	2.64±0.11	75.19*±9.02	0.57±0.06	164.68±37.51	116.98±15.65	30.15±2.96	3.61±0.07	55.62±10.44
400	2.53±0.15	82.17±10.39	0.57±0.05	177.44±46.84	116.36±16.65	29.60±4.46	3.54±0.07	51.15±6.00
Female								
Control	2.44±0.18	78.40±8.36	0.60±0.04	100.14±21.98	128.84±21.42	21.28±2.31	3.57±0.14	48.09±5.30
100	2.39±0.11	79.83±8.27	0.63±0.05	103.72±28.12	120.42±23.17	22.37±1.82	3.59±0.11	47.84±4.89
200	2.42±0.11	82.10±7.81	0.66±0.07	91.73±23.20	128.19±23.17	22.20±2.31	3.65±0.08	46.42±5.37
400	2.42±0.13	76.54±8.67	0.60±0.07	100.97±26.55	123.93±14.28	23.30±3.22	3.57±0.14	43.20±7.38

* = Significant from control group at 5% level ($p<0.05$),

CHAPTER 4: Summary and conclusion

- In order to achieve the goal of identifying novel GPR 119 agonists, the design and synthesis of three different series of compounds was undertaken. All the final compounds were characterized and were tested for *in-vitro* GPR 119 agonistic activity. The *in-vivo* studies, pharmacokinetic studies as well as molecular modelling studies were carried out for the compounds having promising *in-vitro* activity.
- A novel series of *p*-benzilidine thiazolidine Dione derivatives were discovered and synthesized as GPR 119 agonists. All synthesized final compounds have highest purity (>95%) and characterized by using ^{1}HNMR, ^{13}CMR, ESI-MS, IR. Few compounds in this series showed potent an agonistic activity in *in-vitro*. **9c** and **10c** exhibited very good glucose lowering activity in primary animal models (C57 mice) as well as disease models (db/db mice). Compound **10c** has excellent pharmacokinetic parameters i.e auc: 34293 hr.ng/ml & Cmax: 6.0 µg/ml.
- A novel series of *m*-benzilidine thiazolidine dione derivatives were discovered and synthesized as GPR 119 agonists. All synthesized final compounds have highest purity (>95%) and characterized by using ^{1}HNMR, ^{13}CMR, ESI-MS, IR. **17c**, **17j** & **17m** were showed potent agonistic activity in *in-vitro* study. Compound **17c** has showed excellent oral bioavailability in rats i.e. auc: 19055 hr.ng/ml & Cmax: 5.0 µg/ml. **17c** exhibited excellent good glucose lowering activity in primary animal models (C57 mice) as well as disease models (db/db mice). The molecular docking study of compound **17c** also demonstrated that the agonistic activity towards GPR-119 agonist.
- Having identified a potent GPR 119 agonist, optimization of *meta*-benzylidene thiazolidinedione derivatives (**17c**, **17j** and **17m**) was undertaken. Thus, N-substituted benzylidene thiazolidinedione analogs were designed, synthesized and evaluated for their GPR 119 agonistic activity. The compounds from this series exhibited partial potent GPR 119 agonistic activity.
- Based on the overall profile, the compound **17c** was subjected to further detailed biological studies.
- *in-vitro* study of compound **17c** showed a potent GPR119 agonistic activity with EC_{50} = 46.76 nM

- *in-vivo* studies of compound **17c**, showed a potent anti-diabetic activity with ED_{50} = 8.2 mg/kg in C57 mice single dose oral glucose tolerance test (oGTT) and 1.9 mg/kg after 14 days repeated dose treatment in db/db mice.
- Incretin-mediated (GLP-1 release) mechanism for antidiabetic effects of compound **17c** was confirmed since it increased active GLP-1 and insulin levels in diabetic mice plasma (db/db mice).
- It showed a desirable ADME profile.
- Compound **17c** demonstrated that it has a good preclinical safety profile with no adverse effect level (NOAEL) observed in Rats (>400 mg/kg) and in Dogs (50 mg/kg).

Overall, pre-clinical profile (**Figure 15**) revealed that a novel potent, effective and safe GPR -119 agonist was successfully designed and synthesized for the treatment of type 2 diabetes mellitus (T2DM).

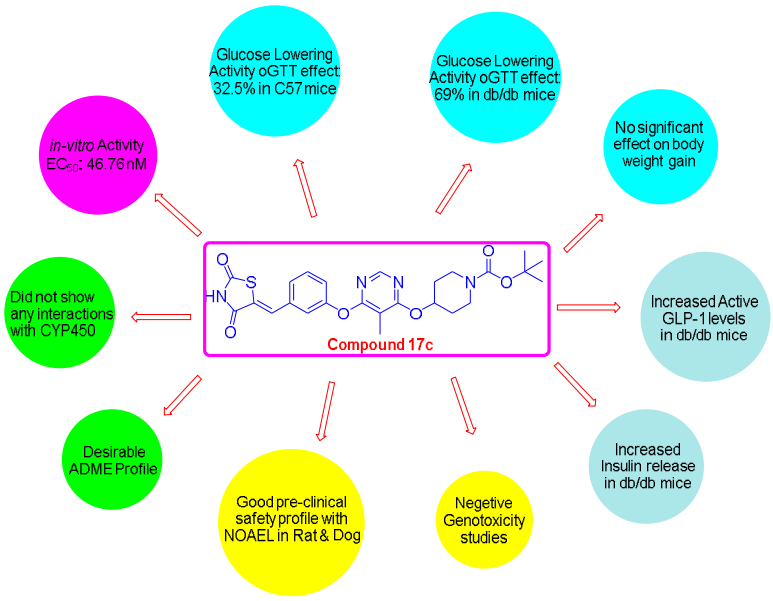


Figure 15: Overall profile of the lead Compound **17c**

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