Synopsis

Of

The Thesis Entitled

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

To be submitted to

The Maharaja Sayajirao University of Baroda

For the Degree

Of

DOCTOR OF PHILOSOPHY

In Chemistry

By

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Synopsis
Of
The Thesis

To be submitted for the degree of

Ph.D. in Chemistry, Faculty of Science
To

The Maharaja Sayajirao University of Baroda.

Name of the Student	**:**	***Suresh Pola***
Title of the Thesis	**:**	Design, Synthesis and Biological Evaluation of Novel GPR 119 Agonist for the Treatment of Type-2 Diabetes Mellitus.
Name of the Guide	**:**	Prof. Shailesh R. Shah & Dr. Mukul R Jain
Place of Work	**:**	Zydus Research Centre, Ahmedabad, India
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Research Student	**Research Guide**	**Research Guide**

CHAPTER 1: Introduction & Designing Strategy

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. 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.

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

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.

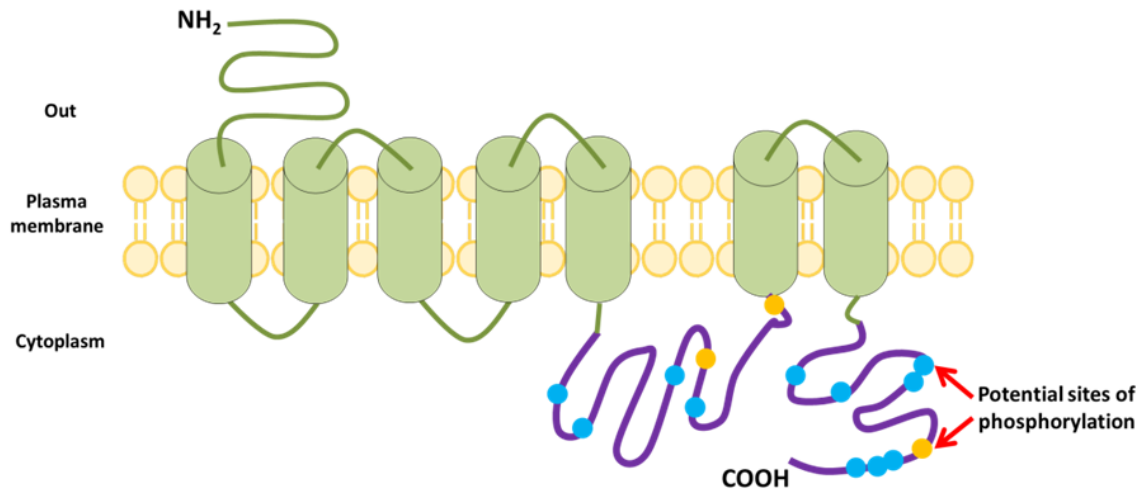


Figure 1. Schematic summary of Human GPR119 membrane topology model.

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]

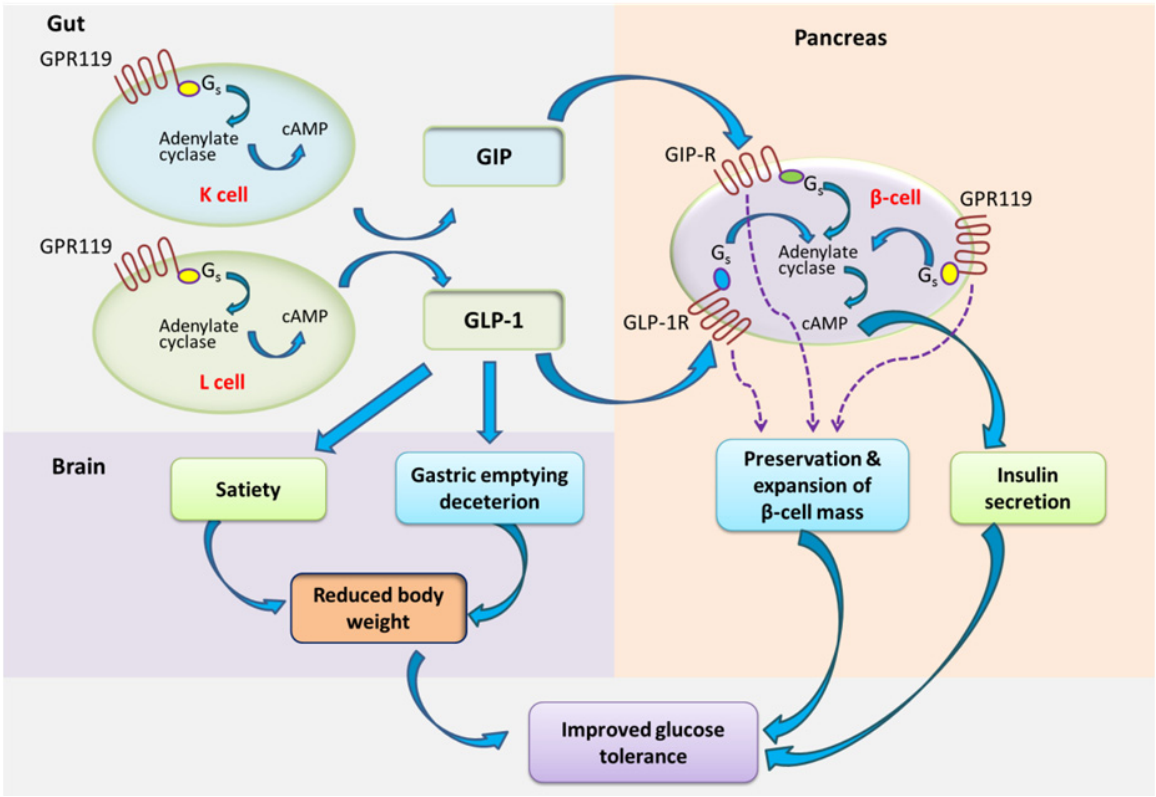


Figure 2. Schematic diagram illustrating the possible actions of GPR119 agonists.[28]

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**).

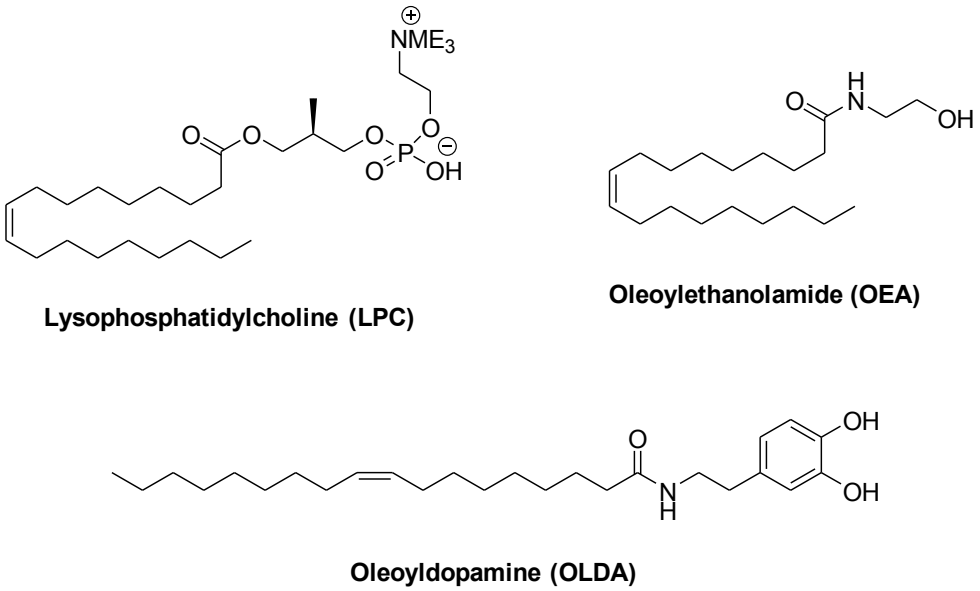


Figure 3: Endogenous ligands of GPR-119

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.

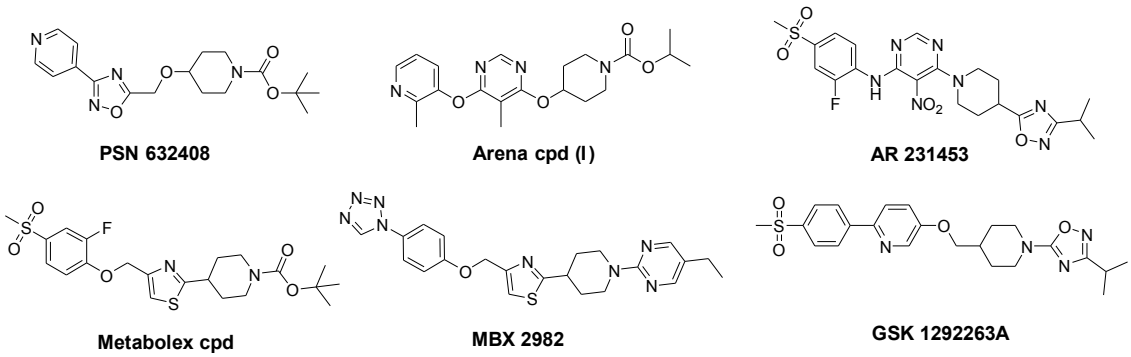


Figure 4: The synthetic ligands of GPR119 Agonists

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**	**Co-development**	**Highest Development Phase (Indication)**	**Status**
PSN821	Astellas (Prosidion)	AstraZeneca did not exercise option	Phase II (T2DM, obesity)	Discontinued (Nov. 2012)
GSK1292263	GlaxoSmith Kline	-	Phase II (T2DM, dyslipidemia)	Discontinued
MBX-2982	CymaBay Therapeutics (Metabolex)	Sanofi returned rights to Metabolex (Apr. 2011)	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	Code: JNJ-28630368 Johnson & Johnson terminated collaboration (Dec. 2010)	Phase I (T2DM)	Discontinued (Jan. 2008)
APD597	Arena	Code: JNJ-38431055 Johnson & Johnson terminated collaboration (Dec.	Phase I (T2DM)	Discontinued (Aug. 2011)

		2010)		
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.

1.5 Designing strategy

Based on the seminal work by Arena and Prosidion on GPR119 agonists and subsequent efforts of many pharmaceutical companies, a well-defined structural architecture has emerged that displays high agonistic activity. Most reported GPR119 agonists contain (a) a (hetero)aryl moiety (“head group”) acting as, or being substituted with a hydrogen bond acceptor, and (b) a piperidine (or piperazine) ring *N*-substituted with a moiety containing a hydrogen bond accepting group in a lipophilic environment (“tail group”), such as carbamates, 5-substituted pyrimidines or substituted oxadiazoles (Figure 5). The spacer between the two pharmacophores ensures the right distance and optimal orientation relative to each other for interaction with the receptor.

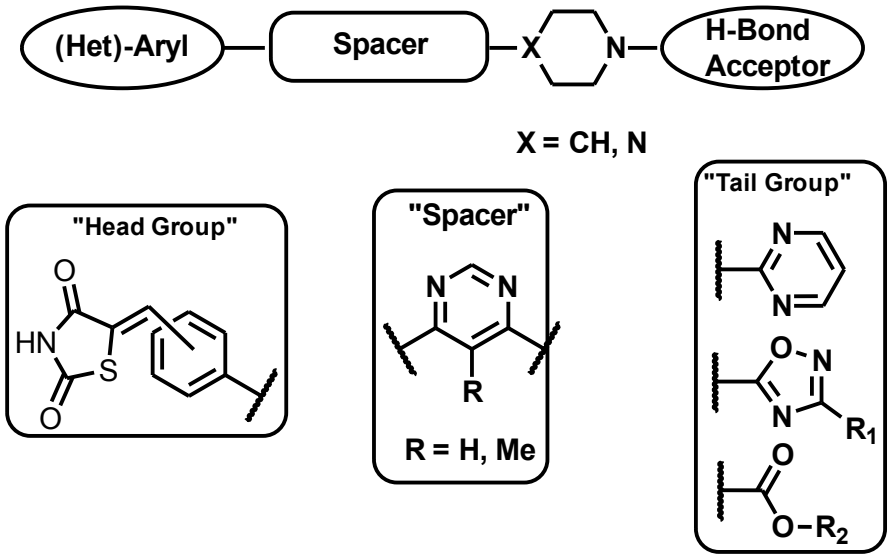


Figure 5. Designing of Novel GPR-119 agonists.

CHAPTER 2: Design, synthesis and biological evaluation of compounds based on p-benzilidine thiazolidine dione derivatives.

2.1 Designing strategy

Arena compound (I) (**Figure 4**) is reported to be a selective and potent agonist of the GPR119 receptor across several species (EC_{50}, melanophore: 2 nM, human; 1 nM, dog; 35 nM, cynomolgus monkey; 41 nM, mouse; 44 nM, rat) and possesses aqueous solubility with no appreciable inhibition of at least five cytochrome P450 enzymes (CYP2C9, 15 uM; 1A2 2D6 3A4, > 40 uM; 2C19, 10 uM). Compound (I) also demonstrated a dose-dependent inhibition of glucose excursion in the 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.). However, further development of this compound has been suspended for reasons not disclosed. In our efforts to discover promising and safe GPR119 agonists, we replaced 2-methyl pyridine, a polar head of Arena compound (I) (**Figure 4**) with clinically proven pharmacophore benzylidenethiazolidinedione, as a novel heterocyclic head and identified this moiety as a pyridine surrogate (**Figure 6)**. Thiazolidinediones (TZDs) are insulin sensitizers having a pleotropic pharmacology including reduction of insulin resistance, a root cause of diabetes. Importantly these agents also preserve pancreatic beta cell function or mass better than insulin secretagogues such as sulfonylureas. The main adverse effect of TZD is weight gain.[34] Herein, we report the synthesis and biological evaluation of novel thiazolidinedione derivatives as potent GPR119 agonists devoid of adverse side effects.

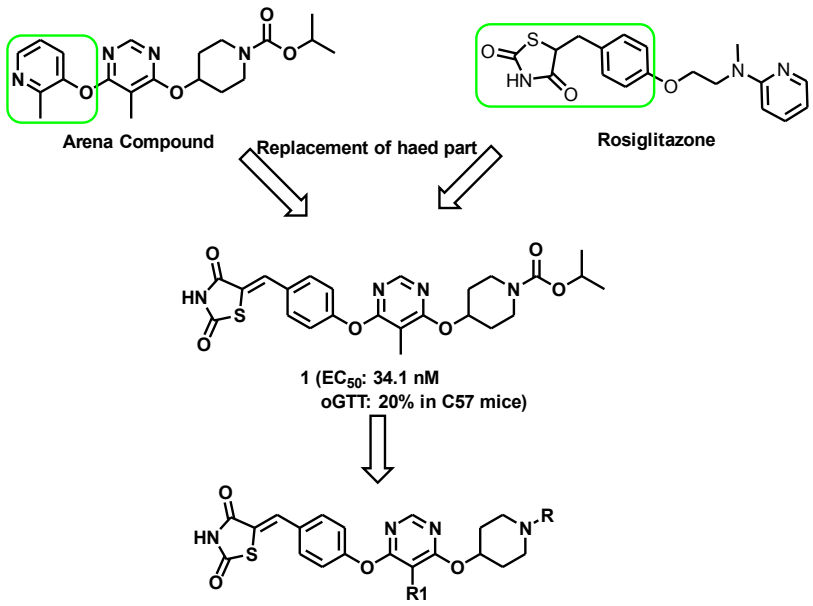
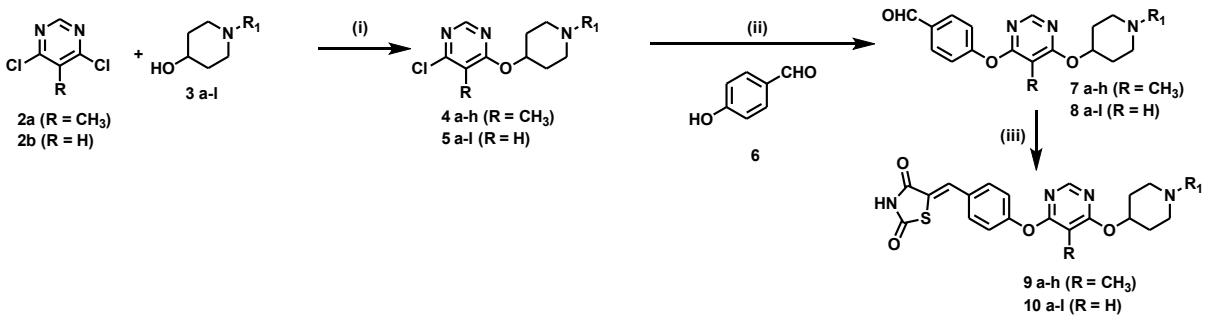


Figure 6: Designing Strategy

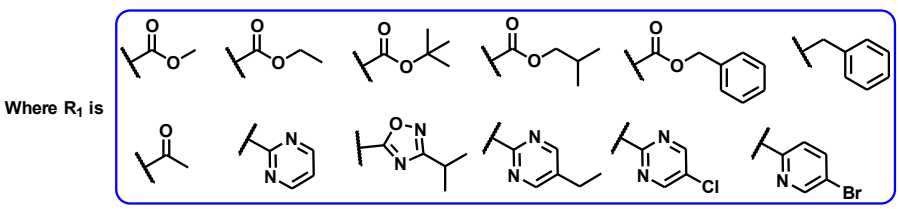
2.2 Synthesis Scheme for preparation of *p*-benzilidine thiazolidine diones derivatives

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 °C, 30 min, (ii) K_2CO_3, DMA, 138 °C, 3 hrs, (iii) TZD, Piperdine, benzoic acid, Toluene, Reflux, 155 °C, 3hrs.

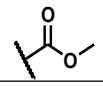
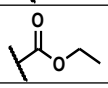
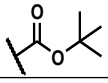
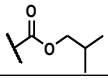
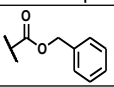
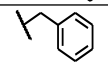
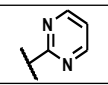
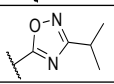
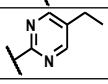
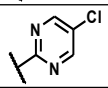
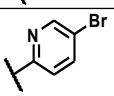


2.3 Biological evaluation of p-benzilidine thiazolidine diones derivatives

2.3.1 *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

2.3.2 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

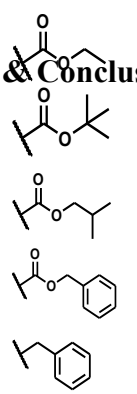
2.3.3 *in-vivo* studies (Glucose lowering activity)

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

2.4 Summary & Conclusion

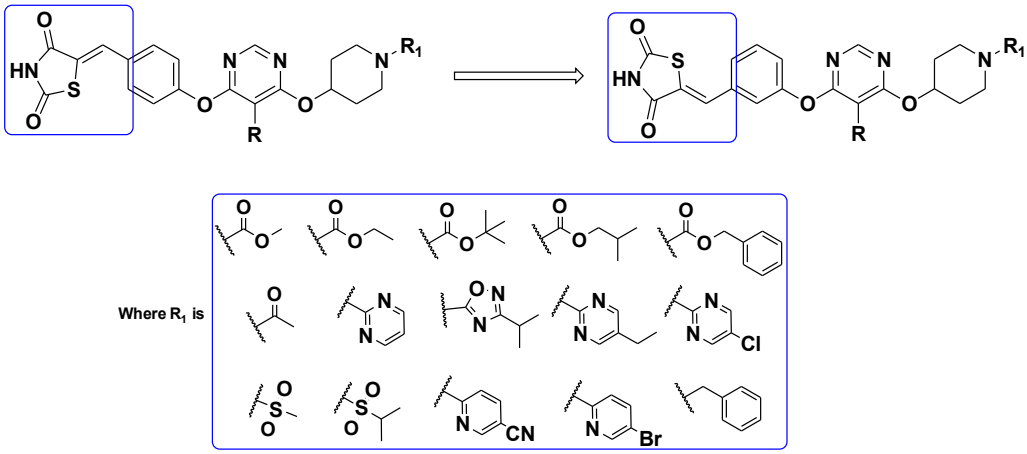


A novel series of p-benzilidine thiazolidine Dione derivatives were discovered and synthesized as GPR 119 agonists. All synthesized final compounds have highest purity (>95%) and characterized by using ^{1}HNMR, ^{13}CMR, ESI-MS, IR. Few compounds in this series showed potent an agonistic activity in *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.

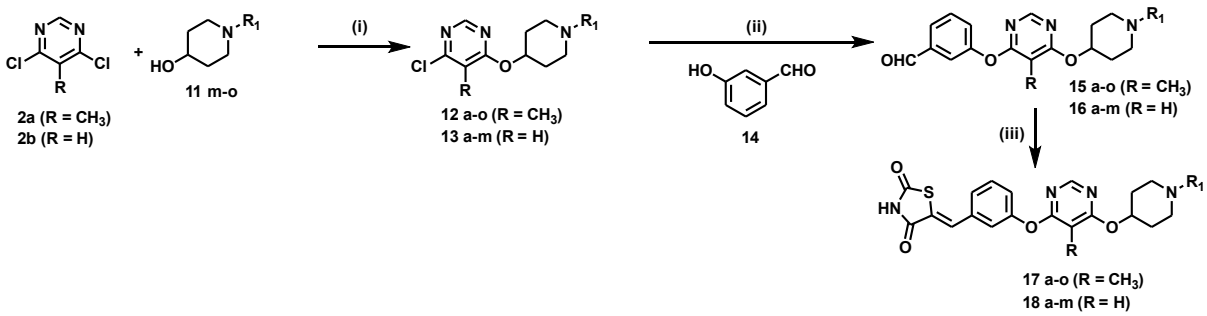
CHAPTER 3: Design, synthesis and biological evaluation of compounds based on *m*-benzilidine thiazolidine dione derivatives.

3.1 Designing strategy

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



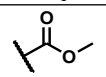
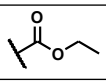
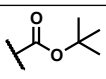
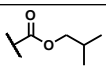
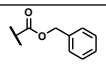
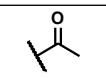
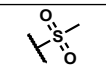
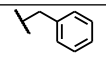
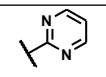
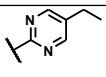
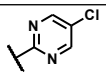
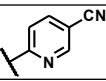
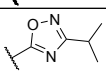
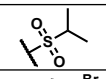
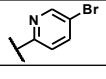
Scheme 1. Reagents and Conditions: (i) t-BuOK, THF, 0 °C, 30 min, (ii) K_2CO_3, DMA, 138 °C, 3 hrs, (iii) TZD, Piperdine, benzoic acid, Toluene, Reflux, 155 °C, 3hrs.

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

3.2.1 *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

3.2.2 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

3.2.3 *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

3.3 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 7**. 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**.

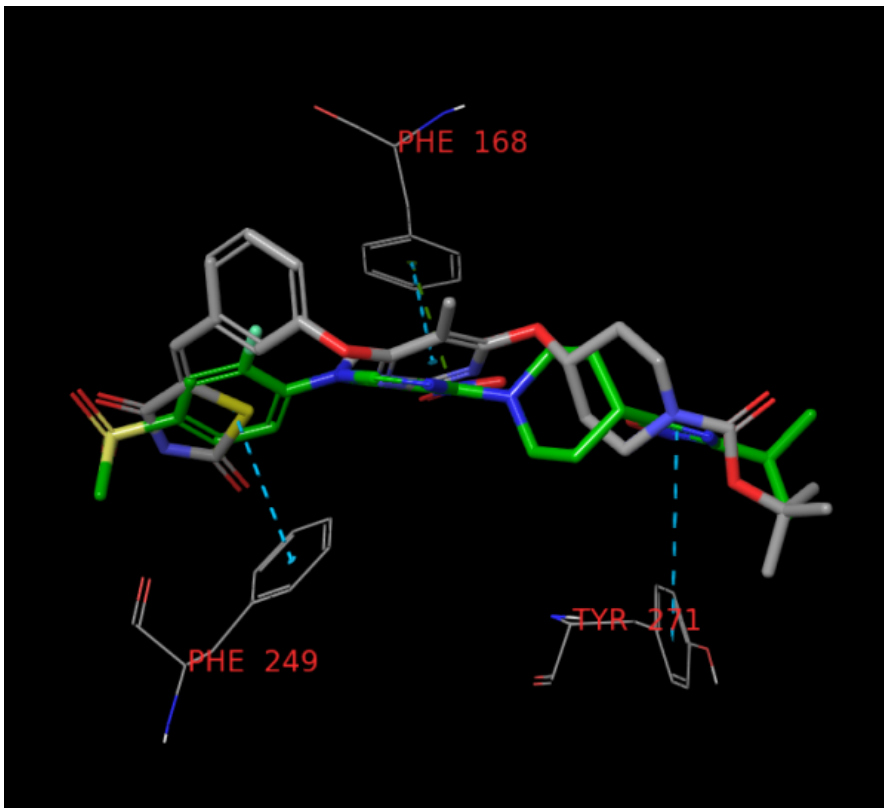


Figure 7: AR 231453 (green) superposed with 17c (grey) in the GPR119 binding site.

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

3.4 Summary and conclusion

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

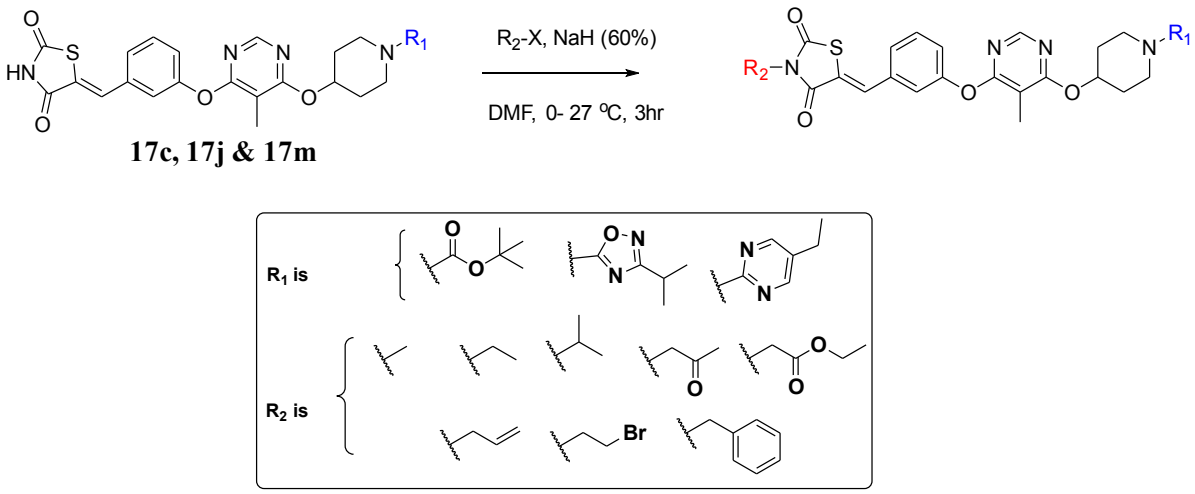
bioavailability in rats i.e. auc: 19055 hr.ng/ml & Cmax: 5.0 µg/ml. **17c** exhibited excellent good glucose lowering activity in primary animal models (C57 mice) as well as disease models (db/db mice). The molecular docking study of compound **17c** also demonstrated that the agonistic activity towards GPR-119 agonist.

CHAPTER 4: Design, synthesis and biological evaluation of compounds based on N-substituted *m*-benzilidine thiazolidine dione derivatives.

4.1 Designing strategy

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

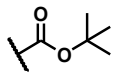
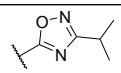
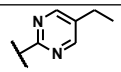


4.2 Biological evaluation of Compounds 19-29:

4.2.1 *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 **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.

CHAPTER 5: Developmental studies of lead compound 17c

5.1 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 8.**

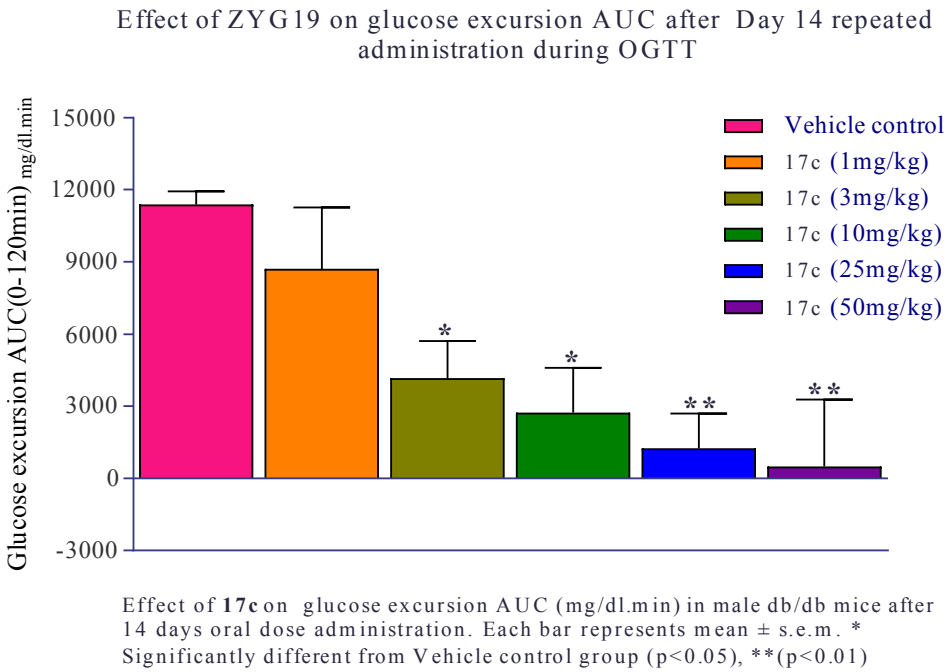


Figure 8: The effect of compound **17c** on AUC glucose excursion (mg/dl.min) in male db/db.

5.2 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 9**).

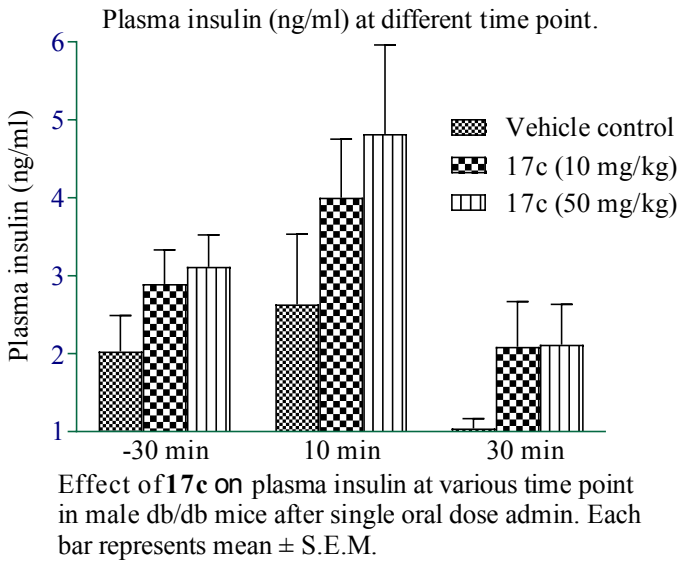


Figure 9: Effect on glucose dependent insulin secretion of compound **17c** using db/db model.

5.3 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 10**).

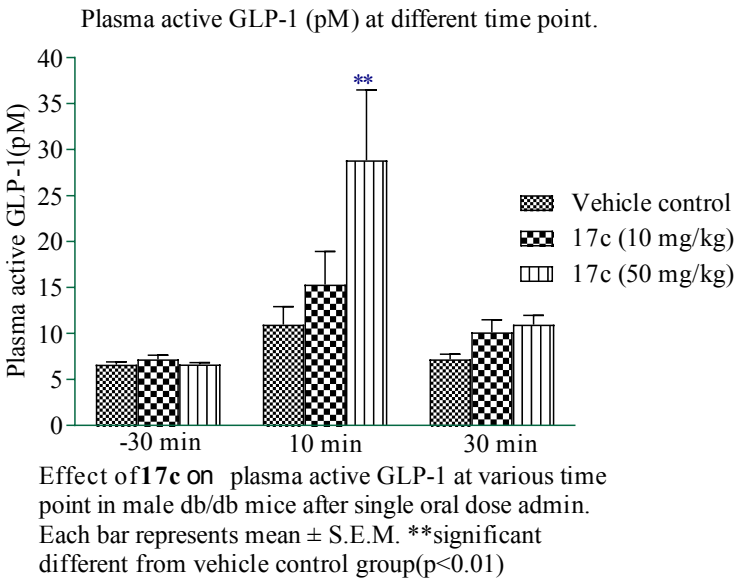


Figure 10: Effect on plasma active GLP-1 of compound **17c** using db/db model.

5.4 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 11.**

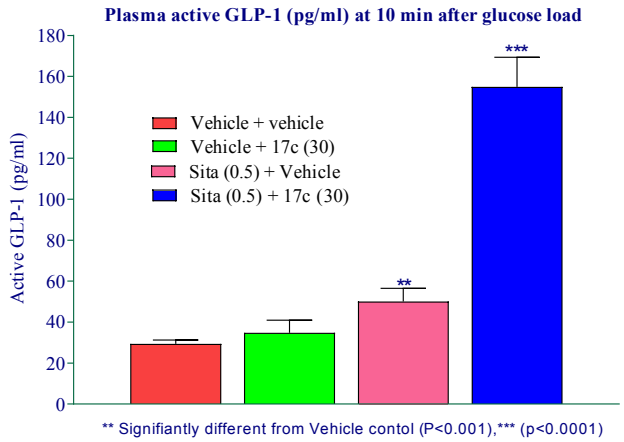


Figure 11: Plasma active GLP-1 (pg. /ml) at 10 min after glucose load.

5.5 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±0.0	3.172±0.30	0.806±0.09	0.228±0.02	0.042±0.00	1.069±0.05	0.081±0.00	0.429±0.14	0.244±0.04

44	1	3	9	6	1	7	2	3

[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 ($p<0.05$),

Summary and conclusion

In conclusion, we have identified benzylidenethiazolidinedione as a novel polar head for discovering a new series of GPR119 agonists. The efforts on SAR generation lead to the discovery of compound **17c** with potent *in vitro* activity, *in vivo* efficacy in various animal models and decent pharmacokinetic profile with no signs of toxicity at dose 200 times higher than efficacy dose. **17c** (**ZY-G19)** has been identified as a candidate for clinical development.

CHAPTER 6

Protocol for biological studies, Experimental procedure for intermediate and final compound along analytical data of selected intermediate and final compound will be written in this chapter.

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Discovery of a potent G-protein-coupled receptor 119 agonist for the treatment of type 2 diabetes

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REFERENCES

1. Singh, VP. *Sci. Technol. J.* **2016**, *4*, 113–123.
2. International Diabetes Federation, *IDF DIABETES ATLAS*, 9th ed. **2019**.
3. Bugger, H.; Abel, ED.; *Diabetologia.* **2014**, *57*, 660–671.
4. Kostev, K.; Rathmann, W. *Diabetologia*. **2013**, *56,* 109–111.
5. Gray, SP.; Cooper, ME; *Nat. Rev. Nephrol.* **2011**, *7*, 71–73.
6. Martin, CL.; Albers, JW. *Diabetes Care*. **2014,** *37,* 31–38.
7. Wukich, DK.; Armstrong, DG.; Attinger, CE.; Boulton, AJM.; Burns, PR.; Frykberg, RG.; Hellman, R.; Kim, PJ.; Lipsky, BA.; Pile, JC.; Pinzur, MS.; Siminerio L. *Diabetes Care*. **2013**, *36,* 2862–1871.
8. Metzger, BE.; Lowe, LP.; Dyer, A.R.; Trimble, ER.; Chaovarindr, U.; Coustan, DR.; Hadden, DR.; McCance, DR.; Hod, M.; McIntyre, HD.; Oats, JJ.; Persson, B.; Rogers, MS.; Sacks, DA. *N. Engl. J. Med*. **2008**, *358*, 1991–2002.
9. National Diabetes Fact Sheet, **2007**, *Department of Health and Human Services, Centres for disease control and prevention.*
10. J. Dowarah, V. Prakash Singh, *Bioorganic and Medicinal Chemistry* (**2019**).
11. (a) Tahrani AA, Bailey CJ, Prato SD, Barnett AH. Lancet. **2011**, 378, 182,
    (b) UMHS Management of Type 2 Diabetes Mellitus, May **2014**.
12. Wild S, Roglic G, Green A, Sicree R, King H. *Diabetes Care*. 2004, 27, 1047.

13. Fredriksson R, Hoglund PJ, Gloriam DE, Lagerstrom MC, Schioth HB (2003) Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives. FEBS Lett. j. 554:381-388.
14. Shah U, Kowalski TJ (2010) GPR119 agonists for the potential treatment of type 2 diabetes and related metabolic disorders. Vitam Horm. j. 84:415-448.
15. Fyfe (2008) GPR119 agonists as potential new oral agents for the treatment of type 2 diabetes and obesity. Expert Opin Drug Discov. j. 3:403-413.
16. Bonini JA, Borowsky BE (2001) DNA encoding SNORF25 receptor. p. US6221660.
17. Bonini JA, Borowsky BE (2002) Methods of identifying compounds that bind to SNORF25 receptors. p. US6468756.
18. Jones RM (2004) 1,2,3-trisubstituted aryl and heteroaryl derivatives as modulators of metabolism and the prophylaxis and treatment of disorders related thereto such as diabetes and hyperglycaemia. p. WO2004065380.
19. Takeda S, Kadowaki S, Haga T, Takaesu H, Mitaku S (2002) Identification of G protein-coupled receptor genes from the human genome sequence. FEBS Lett. j. 520:97-101.
20. Davey J (2004) G-protein-coupled receptors: new approaches to maximise the impact of GPCRS in drug discovery. Expert Opin Ther Targets. j. 8:165-170.
21. Griffin G (2006) Methods for identifi cation of modulators of OSGPR116 activity. p. US7083933.
22. Fu J (2003) Oleoylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. Nature. j. 425:90-93.
23. Ohishi T (2003) Method of screening remedy for diabetes. p. EP1338651. [13] Soga T, Ohishi T, Matsui T, Saito T, Matsumoto M, Takasaki J, Matsumoto S, Kamohara M, Hiyama H, Yoshida S, Momose K, Ueda Y, Matsushime H, Kobori M, Furuichi K (2005) Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan G-protein-coupled receptor. Biochem Biophys Res Commun. j. 326:744-751.
24. Chu ZL, Jones RM, He H, Carroll C (2007) A role for beta-cell-expressed G proteincoupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. Endocrinology. j. 148:2601-2609.
25. Ohishi T, Yoshida S (2012) The therapeutic potential of GPR119 agonists for type 2 diabetes. Expert Opin Investig Drugs. j. 21:321-328.
26. Chu Z-L (2006) Combination therapy for the treatment of diabetes and conditions related thereto and for the treatment of conditions ameliorated by increasing a blood GLP-1 level. p. WO2006076231.

27. Drucker DJ, Jin T, Asa SL, Young TA, Brubaker PL (1994) Activation of proglucagon gene transcription by protein kinase-A in a novel mouse enteroendocrine cell line. Mol Endocrinol. j. 8:1646-1655.

28. S. Dhayal,N. G. Morgan, *Drug News and Perspectives*. **2010**, *23*, 418.

29. H. A. Overton, A. J. Babbs, S. M. Doel, M. C. Fyfe, L. S. Gardner, G. Griffin, H. C. Jackson, M. J. Procter, C. M. Rasamison, M. Tang-Christensen, P. S. Widdowson, G. M. Williams,C. Reynet, *Cell Metab*. **2006**, *3*, 167.

30. M. M. Yore, I. Syed, P. M. Moraes-Vieira, T. Zhang, M. A. Herman, E. A. Homan, R. T. Patel, J. Lee, S. Chen, O. D. Peroni, A. S. Dhaneshwar, A. Hammarstedt, U. Smith, T. E. McGraw, A. Saghatelian,B. B. Kahn, *Cell*. **2014**, *159*, 318.

31. Ritter K, Buning C, Halland N, et al. *J. Med. Chem*. 2015, 59 (8), 3579-3592.

32. S. U. Kang, *Drug Discov Today*. **2013**, *18*, 1309.

33. J. S. Scott, K. J. Brocklehurst, H. S. Brown, D. S. Clarke, H. Coe, S. D. Groombridge, D. Laber, P. A. MacFaul, D. McKerrecher,P. Schofield, *Bioorg Med Chem Lett*. **2013**, *23*, 3175.

34. M.J. Nanjan, Manal Mohammed, B.R. Prashantha Kumar, M.J.N. Chandrasekar, *B ioorganic Chemistry*. 2018, 77, 548-567.