3 Results and Discussion

3.1 *para*-Benzylidene thiazolidinedione derivatives as novel GPR 119 agonists

3.1.1 Chemistry

As described in the previous section it was intended to synthesize the compounds represented by the general structure (**II) (Figure 14)**. Synthesis methodology was designed to targeting the compounds represented by structure **16, 17, 23** and **24** was based on the retrosynthesis analysis and the schemes is described below. Synthesis strategy was based on the reactions reported in literature for the synthesis of the common intermediates required.

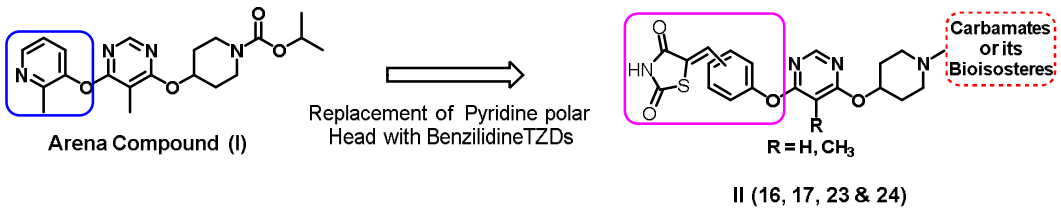


Figure 14: Designing of benzylidene thiazolidinedione derivatives

The objective here was to develop *para*-benzylidene thiazolidinedione derivatives as potent and efficacious GPR 119 agonists as were designed by replacing methyl pyridine ring (a polar head) of the previously reported Arena compound (**I**) with benzylidene thiazolidinedione (**Figure 15**).

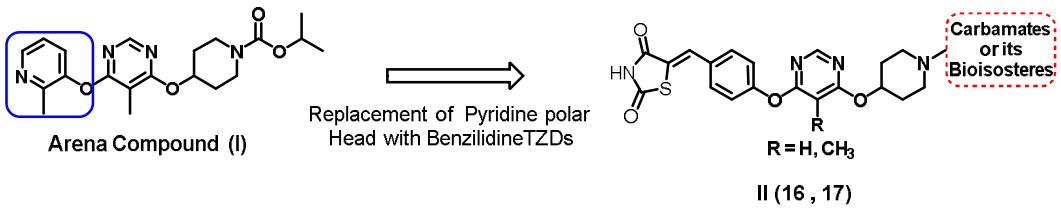
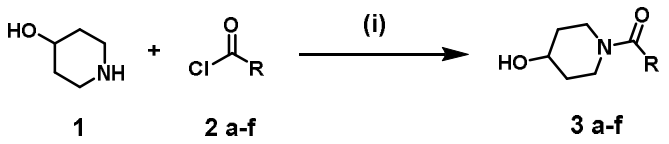


Figure 15: Designing of *p*-benzylidene thiazolidinedione derivatives

Synthesis of the compounds **16 & 17** started with the preparation of intermediates **3a-f** following the sequence outlined in **Scheme 1** using 4-hydroxy piperidine **1** and reacting it with the corresponding chloroformates or acetyl chloride **2a-f,** in the presence of triethylamine (TEA).

Scheme 1:



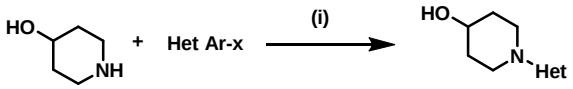
1 **2 a-f** **3 a-f**

	a	b	c	d	e	f
R	OMe	OEt	*t*-Butoxy	*i*-Butoxy	Benzyloxy	Me
Yield	73	84	82	81	65	92

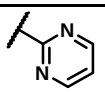
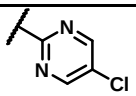
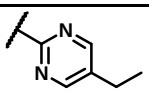
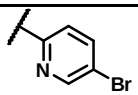
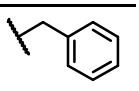
Reagents and Conditions: (i) TEA, Dichloromethane (DCM), 0-27 °C, 1 hr.

Synthesis of intermediates **3 g-k** (**Scheme 2)** was carried out by reacting 4-hydroxy piperidine **1** with aryl hetero halides **4 g-k** in the presence of Potassium carbonate (K_2CO_3) in N, N-dimethyl formamide (DMF) in quantitative yield.

Scheme 2:



1 **4 g-k** **3 g-k**

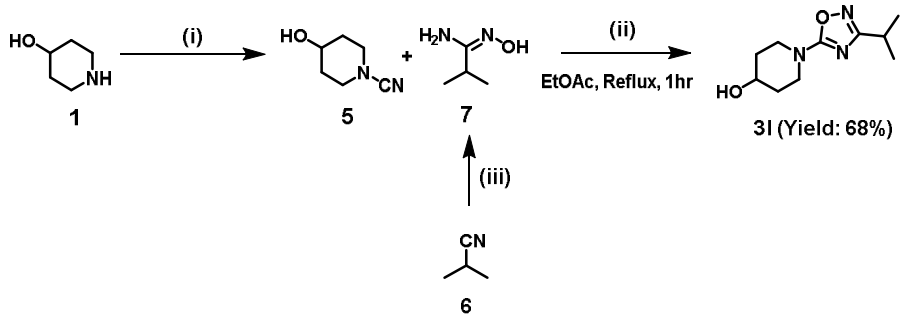
	g	h	i	j	k
Het Ar					
Yield	78	84	68	86	77

Reagents and Conditions: (i) K_2CO_3, DMF, 100 °C, 3 hrs

Synthesis of intermediate **3l** was carried out (**Scheme 3**) by reacting 4-hydroxy piperidine **1** with cyanogen bromide (CNBr) in the presence of aq $NaHCO_3$ and DCM giving the intermediate **5** which was reacted with **7** (prepared from isopropyl nitrile **6**

and hydroxyl amine hydrochloride) in the presence of 1N $ZnCl_2$ in Diethyl ether gave intermediate **3l**.

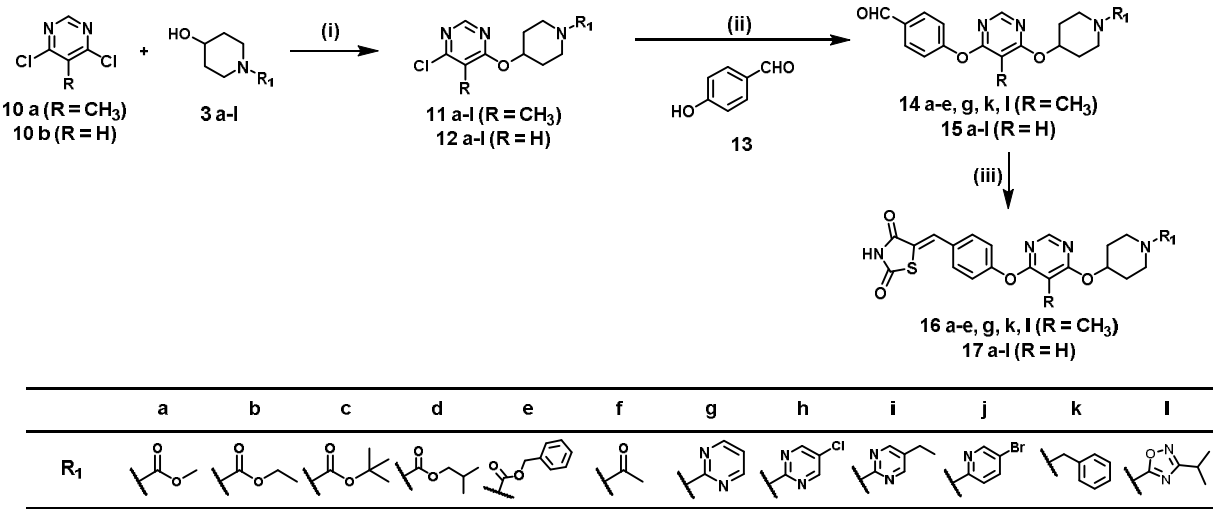
Scheme 3:



Reagents and Conditions: (i) CNBr, $NaHCO_3$, H_2O, DCM, 0-27 °C, 18 hrs, (ii) 1N $ZnCl_2$ in Et_2O, Ethyl acetate, Reflux, 1 hr, (iii) $NH_2OH.HCl$, NaOH, Ethanol, H_2O, Reflux, 2 hrs.

The synthesis of *para*-benzylidene thiazolidinedione analogues **16 a-e, g, l & 17 a-l** described below was achieved in a straightforward three-step route (**Scheme 4)**. Coupling reaction of commercially available substituted 4,6- dichloropyrimidines **10 a** and **10 b** with various N-substituted hydroxy piperidines **3 a-l** using potassium *tert*-Butoxide (*t*-BuOK) as a base in N, N-dimethylformamide (DMF) produced the intermediates **11 a-l & 12 a-l**. Treatment of the intermediates **11 a-e, g, l or 12 a-l** with 4-hydroxybenzaldehyde **13** in the presence of potassium carbonate (K_2CO_3) in N, N-dimethylacetamide (DMA) gave *para* substituted benzaldehydes **14 a-e, g, l or 15 a-l**. The Knoevenagel condensation of benzaldehyde intermediates **14 a-e, g, l or 15 a-l** with thiazolidine-2, 4-dione in the presence of piperidine and benzoic acid in toluene afforded the test compounds **16 a-e, g, l & 17 a-l** in good yields with high purity.[31] All the final compounds were well characterized with NMR (^1H, ^{13}C), IR, MASS, UPLC and melting points etc. and yields has been given in experimental section.

Scheme 4:



Reagents and Conditions: (i) *t*-BuOK, THF, 0 °C, 30 min, (ii) K_2CO_3, DMA, 138 °C, 3 hrs, (iii) Thioazolidine-2,4-dione (TZD), Piperidine, benzoic acid, Toluene, Reflux, 7 hrs.

3.1.2 Biology

3.1.2.1 GPR 119 agonistic activity: *in-vitro* evaluation

GPR119 agonistic activity of all the synthesized compounds was measured using a cAMP assay in the human GPR119 cell line. A standard GPR119 agonist AR231453 compound was used as the positive control and the results are shown in **Table 6**. cAMP stimulation was measured for all the synthesized compounds and the activity is reported as EC_{50} values, which is the concentration of test compound that exhibits half-maximum activity and % max of stimulation compared to maximal effect at 1 µM of **AR231453**. The detailed procedure of *in-vitro* experiments has been described in experimental section.

As described earlier, a typical structural design of GPR 119 agonists comprises of a (Het-Aryl) as “head group” and “Carbamates or heterocycles” as tail group with a pyrimidine linker in-between. Keeping this motive in mind, initially to start with the synthesis of novel compounds, *para*-benzylidene thiazolidinedione was chosen as “head group” and N-substituted piperidines as “tail group” with a pyrimidine as a “linker” in-between. As hypothesized, compounds **16 a-e, g, l & 17 a-l** were synthesized by replacing pyridine ring of **Arena compound (I)** with benzylidene thiazolidinedione. To start with compounds **16 a-e, g, l** possessing methyl pyrimidine

as central ring were synthesized and evaluated for GPR-119 agonistic activity. The test results are presented in **Table 6**.

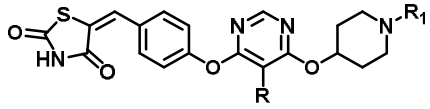
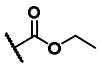
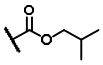
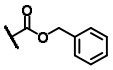
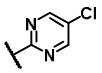
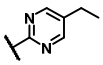
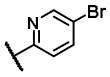
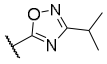


Table 6: GPR119 agonistic activities of compounds 16a-e, g, k, l and 17 a-l.

Compound	R	R_1	hGPR119 agonist Activity	
			EC_{50} (nM)	% E_{max}
16a	CH_3		1414	120.4
17a	H		IA	ND
16b	CH_3		157	170.1
17b	H		790	75.0
16c	CH_3		88	68.8
17c	H		130	121.6
16d	CH_3		75	90.2
17d	H		428	63.5
16e	CH_3		805	73.0
17e	H		107	98.1
17f	H		916	64.0
16g	CH_3		595	97.6
17g	H		IA	ND
17h	H		990.6	67.5
17i	H		274	84.6
17j	H		>1 µM	ND
16k	CH_3		IA	ND
17k	H		IA	ND
16l	CH_3		124	101.8
17l	H		113.5	92.2
AR231453			6.0	100

IA denotes Inactive where compounds did not show any activity above the basal lavel shown by vehicle control and ND denotes not determined; hGPR 119 denotes human GPR 119.

Compound **16a**, the methyl carbamate derivative showed a weak GPR119 agonistic activity with EC_{50} = 1.4 µM, while its homologue, the ethyl carbamate **16b** showed improvement in the activity (EC_{50} = 157 nM; E_{max} = 170%). This 9-fold increase in the potency encouraged me to study the effect of various substitutions on the piperidine nitrogen. Subsequently the compounds **16c** and **16d** were prepared with *t*-butyl and *I*-butyl carbamates respectively. Both compounds demonstrated a significant

improvement in the potency compared to their lower homologues **16a** and **16b** with EC_{50} of 88 nM (**16c**) and 75 nM (**16d**). Further increase in bulk at this position with benzyl carbamate found detrimental as evident from EC_{50} of 805 nM and E_{max} of 73% for compound **16e**. Now it was essential to know the role of carbamates on pyridine ring and hence some hereto aryl groups such as pyrimidine and oxadiazole and even benzyl substitutions were introduced on piperidine ring in the place of carbamates to yield the compounds **16g, 16k** and **16l**. Compound **16g** (with pyrimidine) exhibited moderate potency EC_{50} of 595 nM with E_{max} of 95.6% and compound **16l** (having oxadiazole) shows good potency as EC_{50} of 124 nM with E_{max} of 101.8%. However, **16k** (with benzyl gr.) failed to show the agonistic activity towards GPR 119.

In the next stage, it was required to study the role of methyl group on central pyrimidine ring and hence a series of the compounds **17 a-l** without the methyl group on pyrimidine ring were synthesized. The compound **17a** with methyl carbamate was found to be inactive, whereas the ethyl carbamate **17b** showed moderate potency with EC_{50} = 790 nM. As expected, compound **17c** with *t*-butyl carbamate showed a greater potent GPR119 agonistic activity with EC_{50} of 130 nM and E_{max} of 121%. Surprisingly compound **17d** with *i*-butyl carbamate group was found 10-fold inferior to its methylated analogue **16d**. Interestingly a contrasting trend was observed with benzyl carbamates. Compound **17e** with EC_{50} of 107 nM and E_{max} of 98% was found 8-fold more potent than its methylated analogue **16e**. When acetyl group was attached on the piperidine ring nitrogen instead of carbamate giving the compound **17f,** it showed a weak activity with EC_{50} of 916 nM and E_{max} = 64%. Interestingly compound **17g** with hydrogen failed to show the agonistic activity contrary to its methylated counterpart **16g**.

Next, it was decided to expand the SAR study by introducing various heteroaryl groups such as 5-chloro pyrimidine (**17h**), 5-ethyl pyrimidine (**17i**) and 3-bromo pyridine (**17j**) on piperidine ring. Surprisingly, compounds **17h** and **17j** were found to be inactive with no GPR 119 agonistic activity, indicating that hetero aryl halides substituted on piperidine ring failed to show the activity. However, compound **17i** with 5-ethyl pyrimidine possessed a moderate potency with EC_{50} = 224 nM and E_{max} = 84.6%. Compound **17k** with benzyl group was found inactive as was observed for

its methylated counterpart **16k** also**.** Compound **17*l*** with oxadiazole exhibited a good potency (EC_{50} = 113.5 nM and E_{max} = 92.2%) comparable to its methylated counterpart **16l**. The agonistic activity results of the compounds from **16 & 17** series were in line with the hypothesis. From the above *in-vitro* structure activity relationship (SAR) of GPR119 agonist with different carbamate and hetero aryl substitutions on piperidine ring, the compounds **16c**, **17c** and **16l** were selected for the evaluation of pharmacokinetic parameters and efficacy in the relevant animal models.

3.1.2.2 Pharmacokinetic Parameters

Encouraged with the above *in-vitro* results, the pharmacokinetic parameters of **16c, 17c** and **16l** were studied in *Sprague Dawley* rats at 25 mg/kg/day oral dose and the results are included in **Table 7.** Compound **17c** showed excellent plasma exposure in fasted SD rats with Cmax of 5961 ng/ml and AUC of 34293 hr.ng/ml when orally administered. Compounds **16c** and **16l** also exhibited favorable pharmacokinetic parameters.

Table 7: Pharmacokinetic parameters[a] in fasted SD Rats at 25 mg/kg dose

Compound	C_{max} (ng/ml)	$T_{1/2}$ hr)	AUC $_{(0\text{-}t)}$ (hr.ng/ml)
16c	834	4.37	8108
17c	5961	4.91	34293
16l	1452	6.81	11522

[a] Values indicated are the mean of n=6 animals and $p < 0.05$ vs vehicle control.

3.1.2.3 Glucose lowering activity: *in-vivo* studies

Based on the impressive *in-vitro* and pharmacokinetic results, compounds **16c**, **17c** and **16l** were subjected to primary *in-vivo* screening using oral glucose tolerance test (oGTT) in C57 BL/N 6 and in diabetic model (db/db mice). During oral glucose tolerance test (oGTT) an acute screening assay, the blood glucose levels were monitored at 120 min after oral administration of glucose in C57 BL/N6 mice, which were dosed with 50 mg/kg of compounds **16c**, **17c** and **16l** along with Sitagliptin 30 min prior to the glucose challenge and the data is presented in **Table 8**. Compound 1**6c** significantly reduced plasma glucose levels, showing 34.6% improvement in

glucose excursion, the AUC was comparable with sitagliptin, while compounds **17c** and **16l** were found to show modest 23.4% and 29.3% improvement in glucose excursion AUC respectively. Subsequently in an oGTT experiment in db/db mice, a diabetic disease model, compounds 1**6c** and **17c** showed a significant 56.5% and 52.4% improvement of in glucose excursion AUC when dosed orally at 50 mg/kg (**Table 8**).

Table 8: The oGTT effect [a] of compounds 16c, 17c & 16l in C57 and db/db mice**.**

Compound	% Improvement in glucose excursion AUC at 50 mg/kg	
	C57 mice	db/db mice
16c	34.6 ± 3.7	56.5 ± 9.2
17c	23.4 ± 5.4	52.4 ± 8.5
16l	29.3 ± 6.1	22.4 ± 7.3
Sitagliptine	39.7±5.0	82±11

[a] Values indicated are the mean of n=6 animals and $p < 0.05$ vs vehicle control.

3.1.3 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 **16c**, **17c** and **16l**. 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 **16c** forms a π-π interactions with the side chain of PHE 168 and PHE 249 and in addition, thiazolidinedione of compounds **16c** forms H-bonding interactions with the amino acid THR 85. These interactions are shown by dashed lines **(Figure 16)**. Pyrimidine and phenyl rings of compounds **16l** and **17c** showed π-π interactions with PHE 249 and in addition to these interactions, the compounds **16l** and **17c** 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 **16c, 16l** and **17c.**

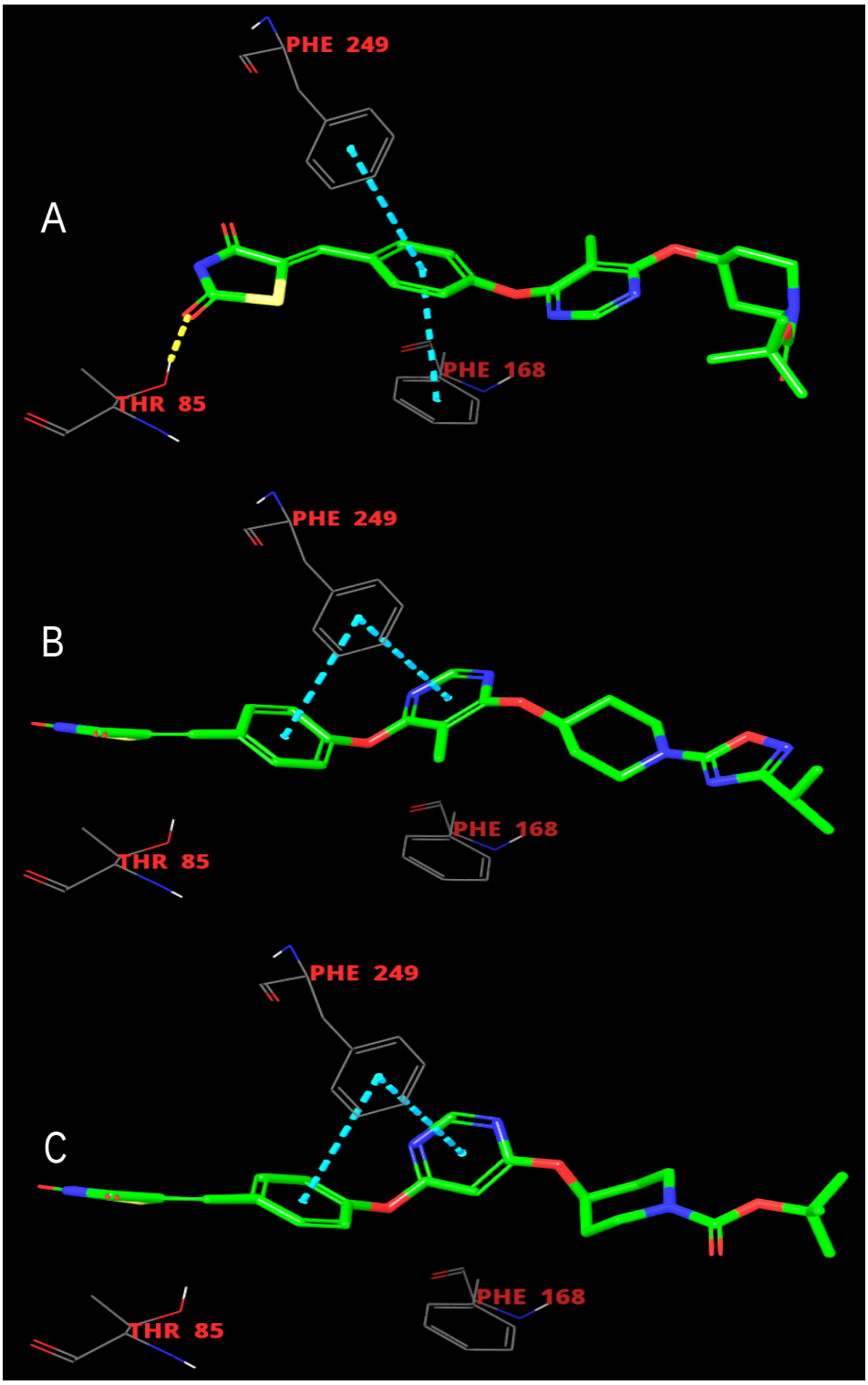


Figure 16: Compounds 16c (A), 16l (B) and 17c (C) docked into GPR 119 binding site**.**

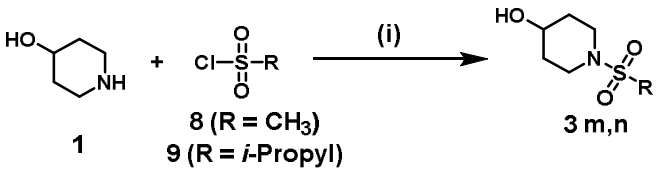
H-bonding and π-π interactions with amino acids are shown as dashed lines.

3.2 *meta*-Benzylidene thiazolidinedione derivatives as novel GPR 119 agonists.

3.2.1 Chemistry

The next task in this endeavour was to optimize the position of thiazolidinedione on phenyl ring. Thus, *meta*-benzylidene thiazolidinedione derivatives **23 (a-n)** and **24 (a-m)** were synthesized with a similar substitution pattern as in *para*-benzylidene thiazolidinedione derivatives **16** and **17.** The general synthesis procedure leading to these compounds is outlined in **Scheme 5 and Scheme 6.** As illustrated in **Scheme 5**, the synthesis of intermediates **3 m-n** started with commercially available 4-hydroxy piperidine **1** which was reacted with alkyl sulfonyl chlorides **8** and **9** in the presence of 2M Aq NaOH solution giving the intermediates **3 m, 3n** as colourless liquids.

Scheme 5:



1

8 (R = CH_3)
9 (R = *i*-Propyl)

3 m,n

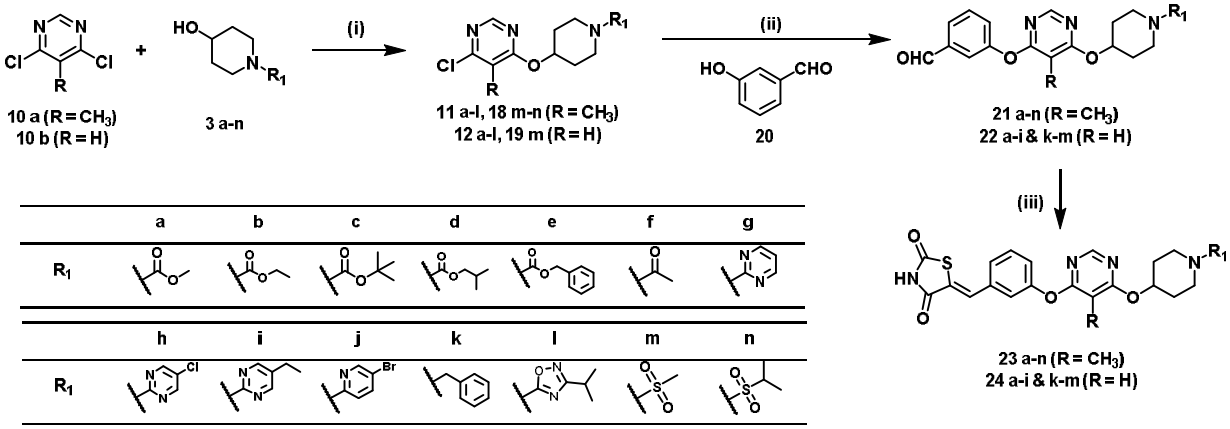
	m	n
R	Me	i-Pr
Yield	25	20

Reagents and Conditions: (i) 2M aq NaOH, 27 °C, 1 hr.

The synthesis sequence leading to *m*-benzylidene thiazolidinedione derivatives **23 a-n** and **24 a-m** involved a straightforward three-step route. The coupling of commercially available substituted 4,6-dichloropyrimidines **10 a** and **10 b** was carried out with various protected 4-hydroxy piperidines **3m and 3n** using *t*-BuOK as a base in N, N-dimethylformamide (DMF) leading to the intermediates **18 m-n** and **19 m**. Treatment of **11 (a-l), 12 (a-l), 18 (m-n)** and **19m** with 3-hydroxybenzaldehyde **20** in the presence of K_2CO_3 in N, N-dimethylacetamide (DMA) gave the *para*-substituted benzaldehydes **21 (a-n)** and **22 (a-m)**. The Knoevenagel condensation of

benzaldehyde intermediates **21 (a-n)** and **22 (a-m)** with thiazolidine-2, 4-dione in the presence of piperidine and benzoic acid in toluene afforded the desired compounds **23 (a-n)** and **24 (a-m)** in good yield with high purity.[31] All the final compounds were well characterized with NMR, IR, MASS, UPLC and melting points etc. and yields has been given in experimental section.

Scheme 6:



Reagents and Conditions: (i) *t*-BuOK, THF, 0 °C, 30 min, (ii) K_2CO_3, DMA, 138 °C, 3 hrs, (iii) Thioazolidine-2,4-dione (TZD), Piperidine, benzoic acid, Toluene, Reflux, 7 hrs.

3.2.2 Biology

3.2.2.1 GPR 119 agonistic activity: *in-vitro* evaluation

Analogues **23 (a-n)** and **24 (a-m)** were evaluated for their ability to activate the human GPR 119 receptor in cell-based cAMP assay. EC_{50} and % max values were determined. The EC_{50} values are presented in the concentration of the tested compounds for 50% cAMP of AR231453, while the % max values are presented as the relative response (%) of the tested compounds compared to the maximal effect of AR231453 and the results are summarized in **Table 9**.

Methyl carbamates **23a** and **24a** were found to be inactive similar to their corresponding *para*-substituted analogues. Compound **23b** with ethyl carbamate not showing activity, contrary to its *para* substituted analogue **16b,** was surprising. However, the compound **24b** with EC_{50} of 819 nM was found to be a weak agonist like its para substituted analogue **17b**. Interestingly, the compound with *t*-butyl

carbamate **23c** was found highly potent with EC_{50} of 43 nM and E_{max} of 160% and emerged out as the most potent compound in this series.

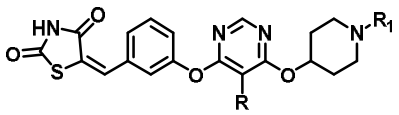
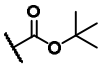
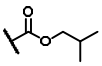
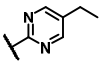
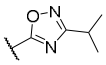


Table 9: GPR119 agonistic activities of compounds 23 a-n and 24 a-m

Compound	R	R_1	hGPR119 agonist Activity	
			EC_{50} (nM)	% E_{max}
23a	CH_3		IA	ND
24a	H		187	90.7
23b	CH_3		IA	ND
24b	H		819	70.0
23c	CH_3		43	160
24c	H		125.3	92.5
23d	CH_3		IA	ND
24d	H		720	75.0
23e	CH_3		105.7	99.2
24e	H		153.4	119.7
23f	CH_3		IA	ND
24f	H		IA	ND
23g	CH_3		1005	68.2
24g	H		702	77.6
23h	CH_3		63.4	51.0
24h	H		343	ND
23i	CH_3		92.2	112.0
24i	H		104.2	102.8
23j	CH_3		122.1	101.8
24j	H		419	ND
23k	CH_3		184.4	81.1
24k	H		1102	70.7
23l	CH_3		71.09	130.0
24l	H		134.7	144.6
23m	CH_3		IA	ND
24m	H		IA	ND
23n	CH_3		139.6	93.8
AR231453			6.0	100

IA denotes Inactive where compounds did not show any activity above the basal level shown by vehicle control and ND denotes not determined; hGPR 119 denotes human GPR 119.

Compound **24c** was also found to be active but was inferior to **23c** in the terms of potency as well as efficacy. Surprisingly *i*-butyl carbamate **23d** failed to show activity. However, compound **24d** showed moderate potency comparable to its *para* substituted analogue **17d**. Among benzyl carbamates **23e** was found to be 8-fold

potent than its corresponding *para*-analogue (**16e**) with EC_{50} of 105 nM and E_{max} = 137% whereas, compound **24e** was found to be equipotent to **17e**. It was not surprising that the acetamide derivatives **23f** and **24f** were inactive as their corresponding *para*- substituted analogue **17f** also did not exhibit a potent agonistic activity. The SAR was further extended by substituting with sulfonamides such as methane sulfonamide (**23m** & **24m**) and *i*-propyl sulfonamide (**23n**) on the piperidine ring. Compounds **23m** and **24m** failed to activate human GPR 119 receptors, however, the compound **23n** exhibited stronger GPR 119 agonistic activity with EC_{50} of 139 nM and % max of 93.8%. Further potency optimization at the terminal groups produced varying results. By replacing carbamates with hetero aryl groups such as substituted pyrimidines, pyridine, oxazole or with benzyl group on piperidine ring, some more new compounds were synthesized. Interestingly the contrasting trends were observed in pyrimidine derivatives. Compound **23g** failed to show a strong agonistic activity (EC_{50} = 1.0 µM) but its para substitute analogue (**16g**) showed a moderate potency and the compound **24g** exhibited a partial agonistic activity with EC_{50} = 702 nM and %max = 77.6% where as its *para* substituted analogue (**17g**) was found to be inactive. Surprisingly, compound **23h** showed a stronger agonistic activity with EC_{50} = 63.4 nM and %max = 51% while compound **24h** (EC_{50} = 343 nM) had 3-fold increased activity towards GPR 119 than its *para* analogue (**17h**). 5-Ethyl pyrimidine derivatives (**23i** and **24i**) exhibited equi-potency with EC_{50} of 92.2 nM, 104.2 nm and % max of 112, 102.8% respectively. These data indicate ~3-fold increased activity than its *para* substituted counterpart (**17i**). The substitution pattern of the pyrimidine ring was vital for retention of *in-vitro* activity, as can be seen with the compounds **23h**, **24h**, **23i** and **24i**. Interestingly, substituted pyridine analogues (**23j** and **24j**) on the piperidine ring displayed a moderate agonistic activity with EC_{50} of 122.1 nM, %max of 101.8% and EC_{50} of 419 nM respectively than its *para* substituted analogue (**17j**). Benzyl piperidine derivatives (**16k**, **17k** and **24k**) failed to show agonistic activity but interestingly, compound **23k** displayed a partial agonism towards GPR 119 receptor. As expected, the oxazole derivatives (**23l** & **24l**) showed a stronger agonistic activity with EC_{50} = 71.09 nM, %max = 130% and EC_{50} = 134.7 nM, % max = 144.6% respectively like their *para* substituted derivatives (**16l** & **17l**). Finally, meta-benzylidene thiazolidinedione derivatives increased their agonistic activities towards GPR 119 receptors compared to the *para*-benzylidene derivatives.

Based on the above *in-vitro* structure-activity relationship (SAR) with different substitutions on piperidine ring as GPR 119 agonists, compounds **23c**, **23i** and **23l** were selected for further biological evaluation (*in-vivo* experiments) in the relevant animal models.

3.2.2.2 Pharmacokinetic Parameters

In-vitro results encouraged us to study the pharmacokinetic parameters of the compounds **23c**, **23i** and **23l** in Sprague Dawley (SD) rats at 25 mg/kg /day as oral dose, and the results are presented in **Table 10**. Compound **23c** showed a remarkable plasma exposure in fasted SD rats with C_{max} = 4955 ng/ml and $AUC_{(0-t)}$ = 19055 hr.ng/ml.

Table 10: Pharmacokinetic Parameters[a] of compounds 23c, 23i and 23l**.**

Compound	C_{max} (ng/ml)	$T_{1/2}$ (hr)	$AUC_{(0-t)}$ (hr.ng/ml)
23c	4955	3.09	19055
23i	2379	5.27	22852
23l	875	3.27	3909

[a] Values indicated are the mean of n=6 animals and $p < 0.05$ vs vehicle control.

3.2.2.3 Glucose lowering activity: *in-vivo* experiments.

With an impressive *in-vitro* potency and bioavailability goals met, *in-vivo* study of the compound **23c** for oral glucose tolerance test (oGTT) in C57 BL/N 6 and in diabetic model (db/db mice) was undertaken. During oral glucose tolerance test (oGTT) an acute screening assay, the blood glucose levels were monitored at 120 min after oral administration of glucose in C57 BL/N6 mice which were dosed with 50 mg/kg of the compound **23c** along with Sitagliptin 30 min prior to the glucose challenge. Compounds **23c** significantly reduced the plasma glucose levels with 32.4% improvement in the glucose excursion AUC comparable sitagliptin. Subsequently in an oGTT experiment in db/db mice, a diabetic disease model, compound **23c** showed a significant 69% improvement in the glucose excursion AUC when dosed orally at 50 mg/kg along with sitagliptin as a positive control. The results are summarized in **Table 11**.

Table 11: The oGTT effect a of compounds 23c in C57 and db/db mice

Compound	% Improvement in glucose excursion AUC at 50 mg/kg	
	C57 mice	db/db mice
23c	32.4 ± 5.9	69 ± 10.3
Sitagliptine	39.7±5.0	82±11

[a] Values indicated are the mean of n=6 animals and $p < 0.05$ vs vehicle control.

3.2.3 Molecular Docking study of compound 23c

To explain the impressive *in-vitro* potency and *in-vivo* results of the compound **23c**, docking studies were carried out using Glide, an automated docking program implemented in the Schrodinger package. A homology model of GPR119 receptor was constructed based on the template protein crystal structure (PDB ID: 2R4R) with the sequence identity 26.94 % using the Prime module of Schrodinger. 5-methyl pyrimidine of Compound **23c** forms a π-π interactions with the side chain of Phe 168. As shown in F**igure 17**.

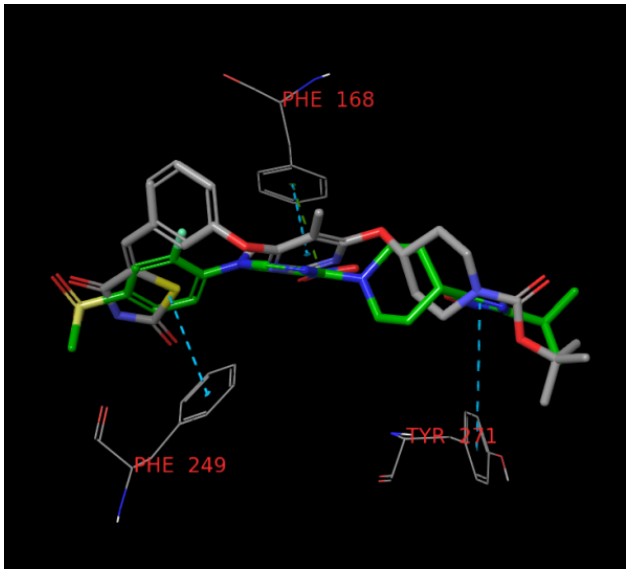


Figure 17: AR 231453 (green) superposed with 23c (grey) in the GPR119 binding site

In addition to these interactions, compound **23c** forms van der Waals interactions with Phe 172, Phe 249, Arg 270, Ty2 271 and Gln 66. These interactions and similar binding modes with respect to AR231453 molecule should be contributing to the

potent agonistic activity of the compound **23c**. The glide score and binding energies of compound **23c** and Arena compound are shown in **Table 12.**

Table 12: Glide score and binding energies of compound 23c.

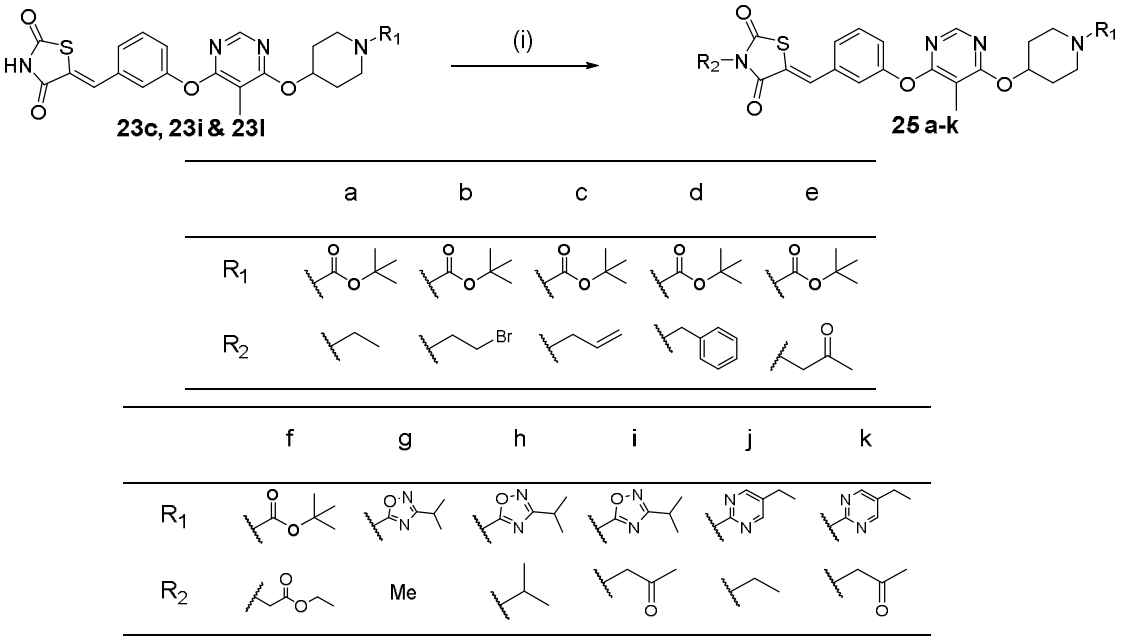
Compound No	Glide Score	MMGBSA dG Bind (Energy in kcal/mol)
AR231453	-7.72	-113.3
23c	-6.65	-105.30

3.3 N-substituted *meta*-benzylidene thiazolidinedione derivatives as novel GPR 119 Agonists

3.3.1 Chemistry

Based on the significant and encouraging results on the glucose lowering activity of **23c**, it was planned to investigate the effects of substitutions on *meta*-benzylidene thiazolidinediones in the search of a compound with an improved glucose lowering activity and further optimize the lead compound **23c**. Thus, N-substituted *meta*-benzylidene thiazolidinedione derivatives **25 a-k** were synthesized as outlined in **Scheme 7.**

Scheme 7:



Reagents and Conditions: (i) R_1-X, NaH (55%), DMF, 0-27 °C, 3 hrs

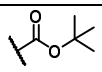
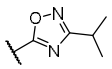
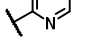
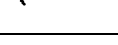
A straightforward reaction of *m*-benzylidene thiazolidinedione derivatives (**23c**, **23i** and **23l**) with different alkyl halides in the presence of sodium hydride (NaH, 50%) in N, N-dimethylformamide (DMF) gave the compounds **25 a-k** with a high purity and in nearly quantitate yields.

3.3.2 Biology

3.3.2.1 GPR 119 agonistic activity: *in-vitro* evaluation

The GPR119 agonist potency of the synthesized compounds **25 (a-k)** was measured using as cAMP assay on the human GPR119 cell lines. GPR119 agonist, AR231453 compound was used as the reference standard and the results are shown in **Table 13** below. cAMP stimulation is measured for all the compounds **25 (a-k)** and the activity is represented as EC_{50} values and % max of stimulation compared to maximal effect at 1µM AR231453.

Table 13: GPR119 agonistic activities of compounds 25 a-k

Compound	R_1	R_2	EC_{50} (nM)
25a			100
25b			94
25c			107.4
25d			1025
25e			79
25f			586
25g		CH_3	120.6
25h			821
25i			160.9
25j			102.5
25k			240.3

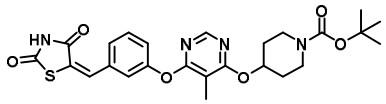
Compounds **25a**, **25b, 25c, 25e** and **25j** exhibited a potent agonistic activity in *in-vitro* study. However, compound **23c** showed a greater agonistic activity than N-substituted *m*-benzylidene thiazolidinedione compounds. Based on the overall activity profile, compound **23c** was subjected to further detailed biological studies.

3.4 Developmental studies of the lead compound 23c

Owing to the promising biological activity in preliminary *in-vitro* and *in-vivo* assays compared to all the other compounds prepared in this study, compound **23c** was selected for the pre-clinical development wherein the compound was evaluated in multiple models of efficacy followed by assessment of its ADME parameters like permeability, metabolic stability, pharmacokinetics in multiple species including non-human primates and CYP inhibition etc. Finally, **23c** was evaluated for its safety index in 28 days repeat dose toxicity at the doses many folds higher than efficacy dose. The results of these studies are discussed in the following sections.

The detailed physicochemical parameters are given in the following section 3.4.1.

3.4.1 Structure & Physico chemical characters:

Structure	
Molecular Formula	$C_{25}H_{28}N_4O_6S$
Molecular Weight	512.58 g/mole
Colour	Pale-yellow coloured powder
Solubility	Soluble in DMF, DMSO, THF, sparingly soluble in Dichloromethane (DCM) and insoluble in water
Partition Coefficient:	
Log P (50µM conc.)	4.41
Log D (50µM conc.)	4.45
Ionization Constant pKa (s)	7.4
pH of the compound	8.79 (1% in water suspension)
Melting Point	190.0 °C
Thermal analysis-DSC	onset at 188.00 °C and peak at 189.92 °C
Crystallographic Properties	crystalline (by XRD)

3.4.2 Target engagement & off target interaction studies

3.4.2.1 *in-vitro* GPR119 activation Dose Response Curve

Compound **23c** was tested at various concentrations ranging from 1 pM to 10 µM against the human GPR 119 receptors expressing CHO-K1 cells in cAMP Assay. The detailed method of the experiment is described in the experimental section.

Stimulation of cAMP was quantified using commercially available Enzyme-Linked Immunoassay (ELISA) kit for direct cAMP measurement. EC_{50} of the compound was calculated using Graph Pad Prism and result has been shown in **Figure 18**. The *in-vitro* potency of compound **23c** was found to be EC_{50} = 46.76 nM.

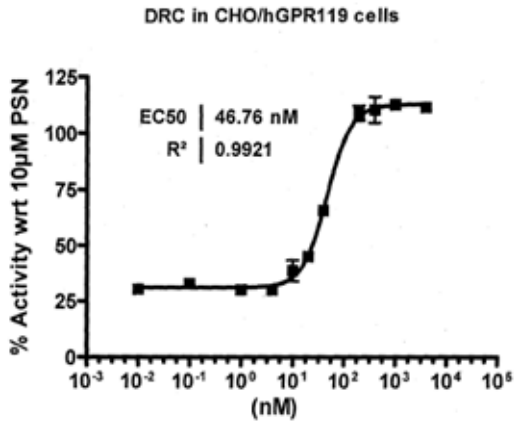


Figure 18: The effect of Compound 23c in CHO-K1 cell based Assay

*PSN compound used in this assay is a standard GPR119 agonist (PSN632408), developed by Prosidion, Inc.

3.4.2.2 *in-vitro* Off-target interactions

Having established the target engagements, the compound **23c** was then screened against several other targets in order to rule out the off-target engagement (GPCR, non-GPCRs and CYPs) where it showed weak/no interactions (IC_{50} >30 fold higher than GPR119 EC_{50}) at 10 µM (free drug) concentration. The results are summarized in **Table 14**. Further, these results are in accordance with the results of safety pharmacology toxicity studies where no indication of off-target related clinical signs are seen. Hence, they appear to be clinically non-relevant.

Table 14: Primary Biochemical Assay results at 10 µM concentration.

Sr.No.	Biochemical Assay	% Inhibition	Sr.No.	Biochemical Assay	% Inhibition
1	Adrenergic β_1	52	18	Cannabinoid CB_2	-12
2	Dopamine D_1	52	19	Estrogen ERα	6
3	Dopamine D_3	68	20	G Protein-coupled Receptor GPR 103	16
4	Dopamine $D_{4.2}$	52	21	$GABA_A$, Flunitrazepam, Central	-3
5	Glucocorticoid	56	22	$GABA_A$, Muscimol, Central	10

6	Histamine H_1	67	23	Glucagon	-11
7	Thyroid hormone	60	24	Glucocorticoid	56
8	Adenosine A_1	37	25	Glutamate, NMDA, Agonism	25
9	Adrenergic α_{1D}	11	26	Growth Hormone Secretagogue (GHS, Ghrelin)	7
10	Serotonin (5-Hydroxytryptamine) 5-HT_3	7	27	Melanin-Concentrating Hormone MCH_1 (SLC_1)	6
11	Androgen (Testosterone) AR	15	28	Neuropeptide Y Y_2	14
12	Bradykinin B_1	-17	29	Nicotinic Acetylcholine	-9
13	Bradykinin B_2	6	30	Nicotinic Acetylcholine α_1, Bungarotoxin	4
14	Calcium channel L-type, Benzothiazepine	-26	31	Opiate δ (OP1, DOP)	9
15	Calcium channel L-type, Dihydropyridine	8	32	Platelet Activating Factor (PAF)	6
16	Calc1ium channel N-type	4	33	Potassium Channel [K_{ATP}]	6
17	Cannabinoid CB_1		34	Potassium Channel hERG	6

3.4.3 Absorption, distribution, metabolism and excretion (ADME) Profile of Compound 23c

Encouraged with the *in-vitro* potency against GPR 119 and clean off-target screening results, 23c was then evaluated for its ADME profile and the detailed results are as follows.

3.4.3.1 Caco-2 permeability:

Compound **23c** was moderately permeable with around 80% mass balance.

3.4.3.2 Metabolic stability:

in-vitro metabolic stability of the compound **23c** in pooled liver microsomes of mouse, rats, dog and human at 120 min after incubation with 2.5 µM concentration showed moderate metabolic stability in rats and mice as compared to a good stability in dogs and human pooled liver microsomes as shown in **Table 15**.

Table 15: *in-vitro* metabolic stability of compound 23c.

Pooled Liver microsomes	% metabolized
Human	17.10
Dog	6.41
Rat	55.56
Mouse	56.68

3.4.3.3 CYP inhibition potential:

CYP450 inhibition study of the compound **23c**, did not show any clinically relevant interaction with CYP450 (1A2, 2C19, 2C9, 2D6 and 3A4) at 10μM concentration.

3.4.3.4 Plasma protein binding:

Compound **23c** has 99% *in-vitro* plasma protein binding in all tested species i.e. mice, rat, dog and human by equilibrium dialysis method at 2.5 μM concentration using rapid equilibrium devices (RED).

3.4.3.5 Pharmacokinetics in multiple species:

Single dose pharmacokinetics in male ICR Mice, Wistar Rats and Beagle dogs showed 12, 22 and 6% bioavailability with 3, 12 and 20 hours half-life respectively (**Table 16**). Single dose pharmacokinetics in Female ICR Mice, Wistar Rats and Beagle dogs showed 16, 17 and 7% bioavailability with 5, 6 and 21 hours half-life respectively. (**Table 17**).

Table 16: Pharmacokinetic parameters[a] of compound 23c in male species**.**

PK Parameters	ICR mice Oral (8.2 mg/kg)	I.V (0.82 mg/kg)	Wistar Rats Oral (10mg/kg)	I.V (1 mg/kg)	Beagle Dogs Oral (10mg/kg)	I.V (1 mg/kg)
$C_{max\ (ng/ml)}$	231.78±38.28	667.16±139.81	256.41±106.37	1549.8±203	1047±46.28	4006±906
T_{max} (hr)	0.88±0.75	NA	1.50±0.55	NA	3.00±2.00	NA
$T_{1/2}$ (hr)	3.06±1.59	2.78±1.91	12.30±4.41	0.80±0.18	19.31±5.66	13.04±1.86
$ACU_{0\text{-}inf}$ (ng.hr/ml))	1250±239	1065±248	1898±352	875±129	15195±6311	29378±15393
Vz (ml/kg)	28405±10578	2945±1358	95649±39130	1320±198	20716±8539	849±580
Cl (ml/hr/kg)	6729±1222	801±180	5420±1008	1161±161	756±333	42.96±24.13
%F	11.75±2.25		21.67±4.02		5.17±2.15	

[a] Values indicated are the mean of n=4 animals; NA denotes Not Applicable

Table 17: Pharmacokinetic parametersa of compound 23c in Female species

PK Parameters	ICR mice Oral (8.2 mg/kg)	I.V (0.82 mg/kg)	Wistar Rats Oral (10mg/kg)	I.V (1 mg/kg)	Beagle Dogs Oral (10mg/kg)	I.V (1 mg/kg)
$C_{max\ (ng/ml)}$	230±38	551±72	359±145	1550±203	973±78.8	3494±2043
T_{max} (hr)	1.25±0.50	NA	0.92±0.20	NA	3.50±1.00	NA
$T_{1/2}$ (hr)	4.56±2.24	1.44±0.21	6.19±4.04	0.82±0.15	21.48±5.34	13.46±1.91
$ACU_{0\text{-}inf}$ (ng.hr/ml)	1403±525	882±73	1569±226	957±245	22399±12451	32271±11597
Vz (ml/kg)	37419±5226	1947.72±320.5	57814±39895	1286.16±296	15186±3244	655±219
Cl (ml/hr/kg)	6341±1778	934±79	6491±981	1106±296	525±189	34.05±11.66
%F	16±6		16±2		7±4	

[a] Values indicated are the mean of n=4 animals; NA denotes Not Applicable

3.4.3.6 Tissue Distribution:

Single dose oral tissue distribution (mean tissue to plasma ratio) of compound **23c** was evaluated at T_{max} (1 hr) and at 24 hr in male and female Wistar rats at 10 mg/kg (**Table 18**). Tissue Distribution in rats showed highest level in liver (organ to plasma ratio was 7)> Kidney >Heart >Testes>Spleen> thymus > brain (Least distribution).

Table 18: Tissue distribution[a] of compound 23c in Wistar rats

Tissue	Mean tissue/organ to plasma ration of compound **23c**			
	Male		Female	
	T_{max} (1 hr)	24 hr	T_{max} (1 hr)	24 hr
Brain	0.06	0.00	0.26	0.00
Heart with aorta	0.47	0.00	0.40	0.00
Kindney	0.79	0.00	0.67	0.00
Liver	7.59	0.00	5.92	0.00
Spleen	0.22	0.00	0.18	0.00
Thymus	0.13	0.00	0.14	0.00
Testes	0.29	0.00	NA	NA
Ovary	NA	NA	0.56	0.00

[a] Values indicated are the mean of n=5 animals; NA denotes Not Applicable

3.4.3.7 Distribution in whole blood:

Distribution of compound **23c** between plasma and RBCs in whole blood of rat and human is shown in **Table 19**. Most of the compound was present in the plasma fraction.

Table 19: Distribution of compound 23c in whole blood.

Matrix	% Distribution of compound **23c**	
	Plasma	RBCs
Rats whole blood	83	17
Human whole blood	87	13

3.4.3.8 Excretion pattern:

Compound **23c** was excreted in feces (49% and 74%), and bile (0.42% and 0.43%) after oral administration of 10 mg/kg dose in male and female rats respectively. A negligible amount of the unchanged compound **23c** was found to be excreted in urine after oral administration of 10 mg/kg dose in both the male and female rats.

3.4.4 *in-vivo* efficacy evaluation:

In continuation with the pre-clinical assessment of the compound **23c**, its efficacy was to be evaluated in the relevant animal models and improvement in various other parameters like glucose lowering activity, active GLP-1 secretion, insulin secretion and effects on body weight were to be studied.

3.4.4.1 Glucose lowering Activity of compound 23c:

After oral glucose tolerance test (oGTT), the glucose lowering activity of the compound **23c** was evaluated in male C57 mice by single dose administration. An oGTT experiment was conducted to evaluate time and dose dependent effects on serum glucose levels in C57 mice with doses of 2.5, 5, 10, 20 and 40 mg/kg (**Figure 19**). The serum glucose levels were measured for 0-120 min and after 30 min, glucose (3mg/kg) was administered orally and AUC was calculated. Compound **23c** demonstrated a significant decrease in glucose levels at 5, 10, 20 and 40 mg/kg.

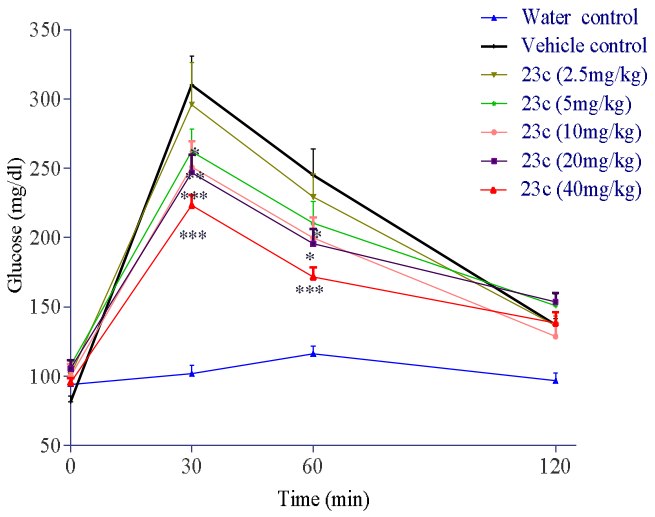


Figure 19: The effect of compound 23c on serum glucose at various time point in C57 mice.

Each line represents mean ± s.e.m. *** Significant different from vehicle control group ($p<0.001$), ** ($p<0.01$), *($p<0.05$).

The compound **23c** showed up to 43% decrease in glucose excursion and ED_{50} was 8.2 mg/kg (**Figure 20**).

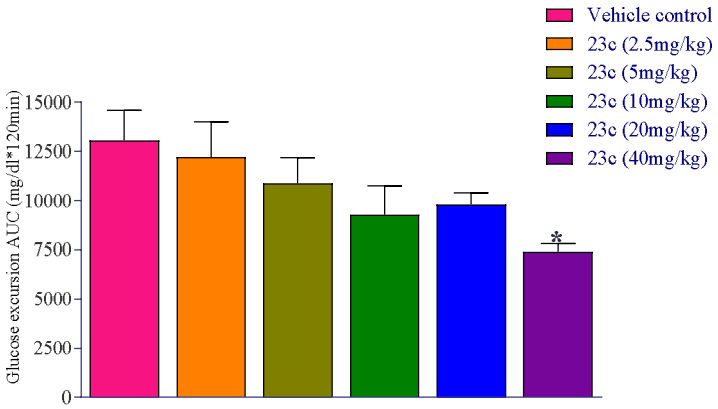


Figure 20: The effect of compound 23c on glucose excursion AUC (mg/dl.min.) in C57 mice

Each bar represents mean ± s.e.m.*Significant different from vehicle control group ($p<0.05$).

With these encouraging results, the oGTT experiments were conducted to study time and dose dependent effects of compound **23c** on glucose excursion in male *db/db* mice. Compound **23c** in doses ranging from 1 to 50 mg/kg and sitagliptin at 50 mg/kg were administrated orally for 14 days and the blood glucose levels were measured during 0-120 min after 30 min of the compound administration and AUC was calculated for Day1 and Day14. Compound **23c** showed significant dose

dependent reduction of glucose excursion compared to day 1 *vs* day 14 at all the tested doses as shown in **Figure 21.**

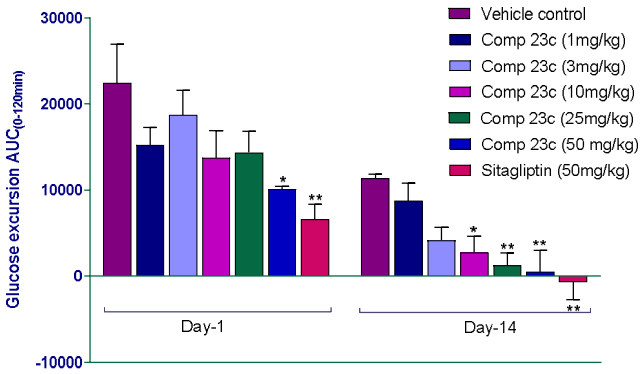


Figure 21: Effect of compound 23c on AUC glucose (mg/dl.min) in db/db mice.

Single oral dose administration (glucose load was 2gm/kg/10ml, p.o.) Each bar represents mean ± s.e.m. * Significantly different from Vehicle control group ($p<0.05$), ** ($p<0.01$)

The effects of compound **23c** on glucose dependent insulin secretion were evaluated at 10 and 50 mg/kg/day single oral dose administration and at different intervals in male *db/db* mice. A significant increase in secretion of insulin at 10 and 30 minutes after glucose administration was observed (**Figure 22**). Compound **23c** exhibited dose dependent insulin secretion activity which incresed 2 folds at 10 min after glucose administration.

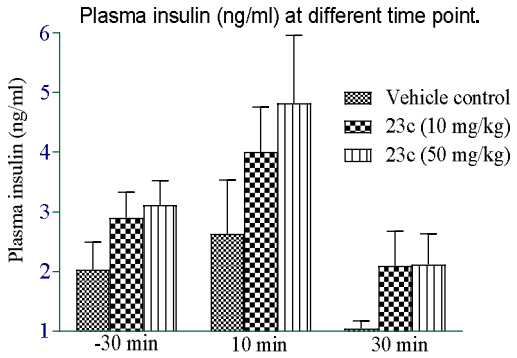


Figure 22: Effect of 23c on plasma insulin at various time points in db/db mice

Each bar represents mean ± S.E.M

The effects of compound **23c** on plasma active GLP-1secretion at various time points in male *db/db* mice after single dose oral administration were evaluated with

10 and 50 mg/kg doses. It increased active GLP-1 levels for 50 mg/kg dose by an impressive 2.6 folds at 10 min after glucose administration (**Figure 23**)

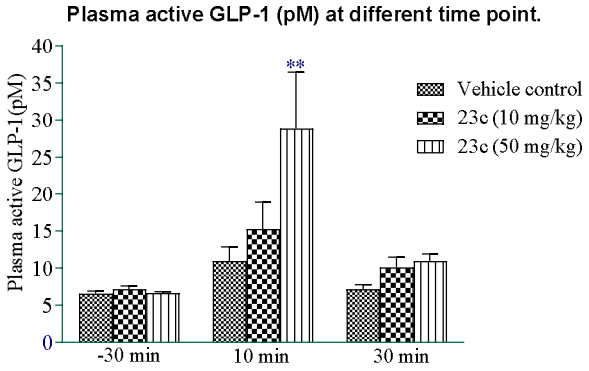


Figure 23: Effect of compound 23c on plasma active GLP-1 in db/db mice

Each bar represents mean ± S.E.M. **significant different from vehicle control group ($p<0.01$)

Further when co-administered with **sitagliptin,** a DPP-4 Inhibitor, compound **23c** showed a synergistic and significant elevation in the active GLP-1 levels compared to that caused by either **23c** or sitagliptin alone (**Figure 24).**

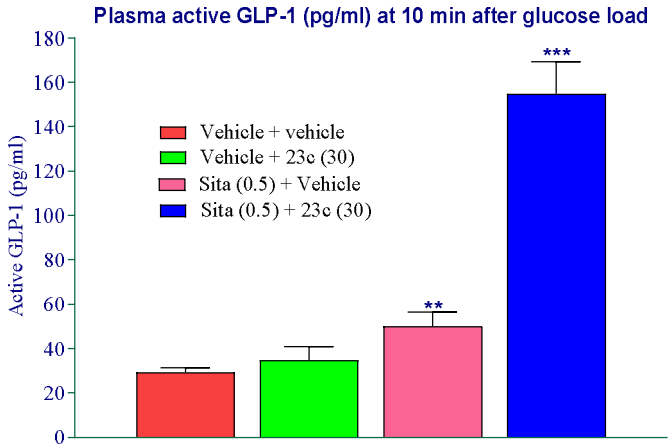


Figure 24: Plasma active GLP-1 (pg/ml) at 10 min after glucose load.

** Significantly different from Vehicle control group ($p<0.001$), *** ($p<0.0001$)

In order to investigate the potential to reduce body weight gain, **23c** was dosed to male diet induced obese *Sprague Dawley* (DIO SD) rats for 28 days. No significant effect on body weight gain was observed in the animals treated at 25 and 50

mg/kg/day (**Figure 25)**. However, the graph showed a trend of lowering in body weight towards the end of the treatment (50mg/kg/day dose) which indicated that the treatment if prolonged further may result in further reduction in body weight gain.

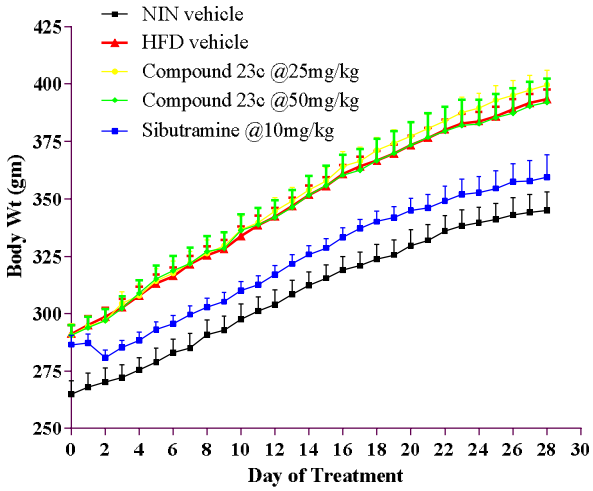


Figure 25: The effect of compound 23c on body weight (gm) in DIO male SD Rats.

Each line represents Mean ± SEM.

3.4.5 Toxicity Studies

3.4.5.1 Acute toxicity study of compound 23c

A preliminary *in-vivo* tolerability study of the compound **23c** was carried out in the Institute of Cancer Research (ICR) mice and Wister rats with a single dose at 2.0 g/kg. All the animals survived after oral administration followed by a 7-day observation. Notably, the result of bacterial reverse mutation assay in Salmonella typhimurium (AMES) test was negative at the doses of 3.33, 10, 30, 90 and 270 µg/plate and chromosomal aberration test in human lymphocytes was non-clastogenic, which suggests a low potential for genotoxicity. Compound **23c** showed moderate inhibition of the hERG channel in stably transfected HEK 293 cells with a manual patch clamp assay (IC_{50} = 16.38 µM). The results are summarized in **Table 20**.

Table 20: Preliminary toxicity study of compound 23c.

Compound	Acute Toxicity Study (LD_{50})		Genotoxicity Studies		hERG Inhibition IC_{50} (µM)
	ICR mice	Wistar Rat	AMES Test	Chromosomal Aberration Test	
23c	>2.0 g/kg	>2.0 g/kg	Negative	Non-clastogenic	16.38

3.4.5.2 Repeat dose Toxicity Studies of Compound 23c

Having achieved the primary goal of identifying a potent and efficacious GPR119 agonist with acceptable ADME profile and with clean off-target interactions, the next end point of this endeavour was to study the toxicity profile of the lead compound **23c** under a repeated dose toxicity study by oral gavage over the period of 28 days in *Wistar* rats. Groups of 10 animals of each male and female rats were dosed orally with 100, 200 and 400 mg/kg of compound **23c** once a day for 28 days. These doses correspond 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 treatment related adverse neurobehavioral changes were noticed at 400 mg/kg. Food consumption was comparable to that of the control groups throughout the study period in both the treated groups of both male and female animals. There were no findings of toxicological effects during ophthalmic examination at 400 mg/kg in both the sexes. Animals were sacrificed on day 29 and data analysis of blood biochemical parameters, organ weight ratios and histopathological findings was carried out.

The administration of compound **23c** was not associated with any adverse effects during the haematological estimations (**Table 24**) in this study up to 400 mg/kg in both the sexes. No significant change in body weights of animals of both the sexes was observed. Analysis of the organ to body weight ratios (**Table 23**) did not show any evidence of toxicity attributed to the compound treatment at least at 100 mg/kg dose, which is 50x of ED_{50}. There was no change in relative organ weights noted up to 400 mg/kg dose in the male rats at the end of the treatment periods. Although statistically significant changes such as higher weights of adrenals (at 400 mg/kg), heart (at 200 mg/kg), brain (at 100 & 200 mg/kg), thymus (at 400 mg/kg) were

noticed, none of these effects were clearly associated with any histomorphological changes during the microscopic examination. No treatment related changes in the relative organ weights were evident in the female rats either at the end of treatment period or at post recovery termination. Similarly, no significant alterations were observed in the biochemical parameters (**Table 21**) except the decrease in urea levels in both sex animals and a marginal decrease in the glucose levels in male animals.

Table 21: Biochemical Parameters of the compound 23c in *Wistar* rats 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 ($p<0.05$)

No significant changes were observed in the liver enzymes (ALP, AST and ALT), haemoglobin, albumin and creatinine at any doses in both sex animals. Histopathological examination did not reveal any treatment related adverse effects up to 400 mg/kg in both the sex animals. Although changes were noticed in liver, these were minimal and could not be attributed to adverse effects of the compound treatment. No evidence of local toxic effects were noticed after repeated oral treatment with Compound **23c** in the gastrointestinal tract of the animals. Oral administration of compound **23c** for 28 days at the dose levels of 100, 200 and 400 mg/kg did not affect the survival of *Wistar* rats. No adverse changes were noticed during haematological, biochemical estimations, organ weights and histopathological examinations (**Table 22**). No observed adverse effect level (NOAEL) of compound **23c** was found to be more than 400 mg/kg in rats. These results clearly indicate that

the treatment with **23c** on rodents did not exert any significant side effects even at 200 times higher dose than ED_{50} value.

Table 22: Histopathological Findings of compound 23c in *Wistar* rats for 28 days

Dose (mg/kg)		0	100	200	400
Liver					
Single cell necrosis- focal/multifocal	Minimal	1	0	0	3
Inflammatory foci	Minimal	1	1	1	2
Necrotic foci	Minimal	0	0	0	1
Hepatocelluar apoptosis-focal	Minimal	0	0	0	1
Pancreas					
Increased acinar cell apoptosis-focal/ multifocal	Minimal	3	-	-	2
Kidneys					
Dilatation of pelvis-unilateral	Mild	0	-	1	1
Adrenals					
Accessory cortical tissue		0	-	-	1
Testes					
Atrophy -bilateral	Moderate	0	1	-	0
Epididymides					
Atrophy & aspermia -bilateral	Moderate	0	1	-	0
No Abnormalities Detected					

Table 23: Relative organ weights[a] of *Wistar* rats administered orally with compound 23c 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 24: Haematological Estimations of *Wistar* rats administered orally with compound 23c for 28 days

Dose (mg/kg)	WBC (10^3/µL)	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/µL)
Male								
Control	8.54±1.69	8.31±0.33	15.34±0.49	48.75±1.88	58.66±1.88	18.49±0.55	31.52±0.42	738.00±67.58
100	7.62±1.71	8.12±0.29	15.24±0.53	48.18±1.61	59.38±1.84	18.75±0.55	31.59±0.28	740.60±58.84
200	8.33±2.32	8.06±0.32	15.07±0.35	47.69±1.19	59.22±1.36	18.71±0.55	31.62±0.44	711.90±59.01
400	8.77±3.51	8.22±0.20	15.33±0.29	48.44±1.04	58.94±1.43	18.65±0.45	31.66±0.22	717.30±55.12
	NEUT (10^3/µL)	LYMPH (10^3/µL)	MONO (10^3/µL)	EOSIN (10^3/µL)	BASO (10^3/µL)	RETI (10^3/µL)	PT (Sec)	APTT (Sec)
Control	0.859±0.210	7.452±1.561	0.082±0.059	0.064±0.024	0.078±0.030	202.30±38.32	15.57±2.97	26.14±4.54
100	0.863±0.163	6.527±1.697	0.085±0.088	0.064±0.015	0.087±0.028	206.30±42.72	15.25±2.94	26.65±4.11
200	0.906±0.245	7.187±2.208	0.064±0.060	0.080±0.026	0.098±0.044	201.80±46.02	14.32±3.23	26.27±3.66
400	0.962±0.281	7.526±3.327	0.093±0.094	0.083±0.026	0.102±0.077	197.20±56.51	14.34±2.42	24.67±3.24

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

Contd…

Dose (mg/kg)	WBC (10^3/µL)	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/µL)
Female								
Control	7.54±2.25	7.57±0.45	14.31±0.44	45.16±1.75	59.72±1.64	18.93±0.68	31.70±0.37	765.80±64.03
100	5.33*±0.97	7.79±0.28	14.48±0.56	45.52±1.60	58.48±1.67	18.60±0.54	31.80±0.32	739.50±48.50
200	7.30±2.60	7.55±0.20	14.17±0.34	44.81±1.05	59.32±1.05	18.78±0.37	31.65±0.36	677.80**±36.6
400	5.61±1.14	7.40±0.35	13.99±0.51	44.10±1.70	59.68±2.02	18.94±0.55	31.72±0.37	714.00±53.81
	NEUT (10^3/µL)	LYMPH (10^3/µL)	MONO (10^3/µL)	EOSIN (10^3/µL)	BASO (10^3/µL)	RETI (10^3/µL)	PT (Sec)	APTT (Sec)
Control	0.862±0.459	6.475±2.078	0.102±0.061	0.051±0.027	0.047±0.024	352.30±127.69	9.86±0.74	21.15±1.46
100	0.609±0.255	4.551*±0.877	0.078±0.063	0.053±0.021	0.035±0.023	337.60±125.79	9.38±0.38	22.54±2.10
200	0.780±0.250	6.301±2.385	0.102±0.081	0.068±0.027	0.042±0.028	368.70±108.31	9.44±0.57	23.95**±1.03
400	0.647±0.546	4.811±0.848	0.078±0.077	0.043±0.022	0.031±0.018	290.00±67.28	10.73*±0.78	23.88**±1.64

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