# SYNTHESIS AND ORAL HYPOGLYCEMIC ACTIVITY OF PYRAZOLOXAZOLIDINEDIONES

**Tathagata Dey[*1], Jincy Jacob[1], Sudeep Sahu[2], Mayukh Baidya[3],**

East Point College of Pharmacy, Bidarahalli, Virgonagar post, Bangalore-560 049, India.
SAF Fermion Ltd. Millennium City, #62, Salt Lake City, Kolkata-700 091, India.
Krupanidhi College of Pharmacy, Chikkabelandur, Carmelaram Post, Varthur Hobli, Bangalore-560035, India.

**\*Corresponding author**
Tathagata Dey
East Point College of Pharmacy
# 147, Bidarahalli, Virgonagar Post,
Bangalore- 560049.
E-mail: tathagatad@gmail.com
Mobile No: +91-9886291249
 Fax No:     +91-8028472999

## Summary

Among a wide variety of nitrogen heterocycles that have been exploited to develop pharmaceutically important molecules, out of which pyrazole and oxazolidinediones are clinically used potential drug candidates showing oral hypoglycemic and antidiabetic activities. Here we achieved the synthesis of some pyrazole derivatives of oxazolidinedione **9** for exploring their oral hypoglycemic activity by condensing pyrazole **4** with 5-(4′-flurobenzyl)-2, 4-oxazolidinedione **8** in tetrahydrofuran (THF). Structures of the synthesized compounds were characterized by IR and NMR studies, and were screened for oral hypoglycemic activity in animal model. The statistical significance was carried by one-way ANOVA test.   Some of the novel compounds with lipophilic side chains containing strong electronegative centers were found to possess significant oral hypoglycemic activity in rats. A probable mechanistic explanation of the variation in activity is also attempted.

**Keywords:** *Oral hypoglycemic; Oxazolidinedion; Pyrazole.*

## Introduction

Diabetes mellitus is a chronic disorder of carbohydrate, fat, and protein metabolism[1]. A defective or deficient insulin secretory response, which translates into impaired carbohydrate (glucose) use, is a characteristic feature of diabetes mellitus, as is the resultant hyperglycemia. It is the fourth leading cause of death in developed countries and afflicts more than 5% of world's population; suffer from diabetes, making this one of the most common Non communicable diseases[2]. There are more than 220 million persons with diabetes in the world today, and by 2025, this number is expected to approach 300 million[3]. A standard treatment regimen has considered the use of a number of chemical classes of drugs such as sulphonyl ureas, biguanides, thiazolidinediones that reduce the hyperglycemia by inducing the β-cells to release more insulin. However, undesired consequence of prolonged use of sulphonylureas and others include hypoglycemic episodes, ultimate exertion of β- cell as well as long term angiogenic side effects that are results of chronic day long exposure to increased insulin level[2]. From literature survey, it was found that thiazolidine & oxazolidine derivatives exhibited 10 fold more potent antidiabetic activity ($ED_{25}$=0.05mg/kg/d) than pioglitazone ($ED_{25}$=0.05mg/kg/d) in Wistar fatty rats[4]. Even it was found that 3,5-dimethyl pyrazoles possess hypoglycemic activity as greater as 100 times that of tolbutamide in glucose prime in rats[5].
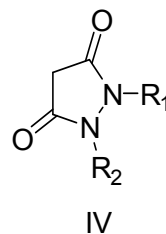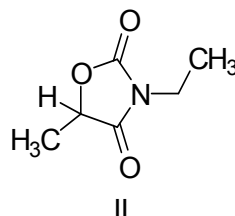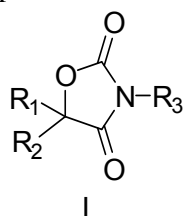


Both 2,4 oxazolidinedione and pyrazole heterocycles have been found to possess either clinically proven or experimentally observed hypoglycemic or antidiabetic activities. Hence, an attempt was made to explore the possibility of developing potent novel oral hypoglycemics by fusing oxazolidinedione with various pyrazole.

The scheme of synthesis is summarized in the **Scheme-1**. The physical data of synthesized compounds (**9a-j**) were given in **Table-1** and the IR, NMR spectral data were summarized in **Table-2.**

**Scheme-1**
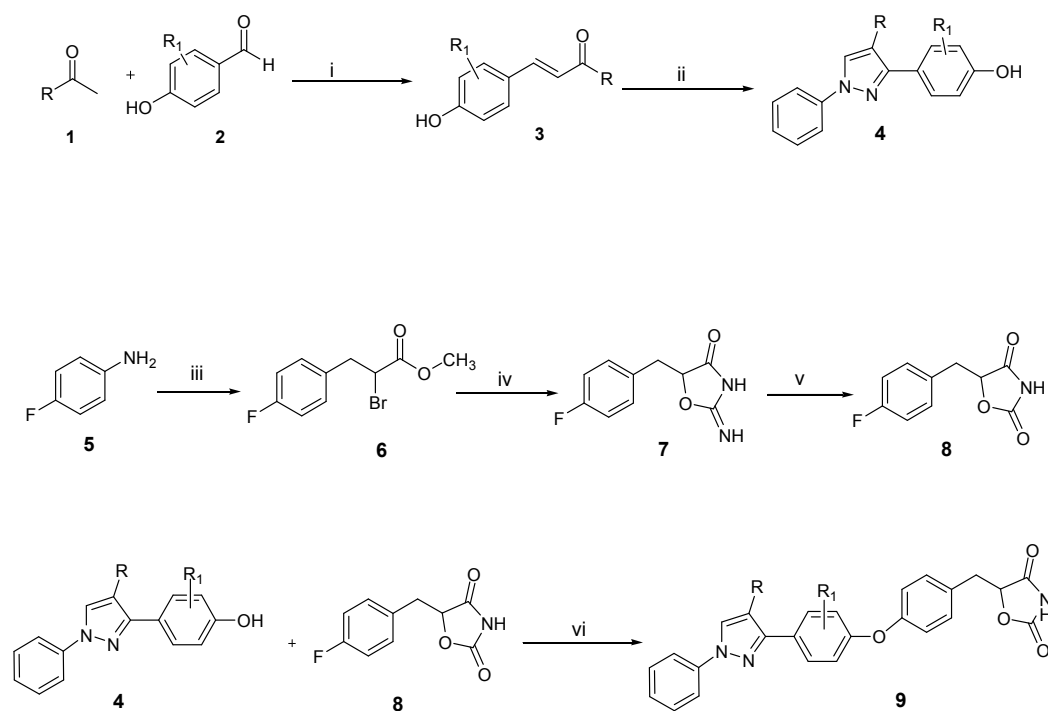


R= H, Cl, $NH_2$, $CH_3$, $C_6H_5$

$R_1$=H, OH, $CH_3$, $OC_2H_5$

*Reagents and Condition:* i )NaOH, EtOH, 10°C, ii)Phenyl hydrazine hydrochloride, EtOH, reflux, iii) $NaNO_2$, HBr,methyl acrylate, 0°C-rt. stirr, iv) Urea, sodium aceate reflux, v) HCl, EtOH stirr, vi)NaH,THF, Stirr.

## EXPERIMENTAL

All the chemicals were purchased from Aldrich, Fluka, and S.D.Fine Chemical. Melting points were determined by open capillaries and are uncorrected. TLC was carried out by using silica gel. $^1$H NMR spectra were recorded on Bruker model DRX 400 NMR spectrometer in acetone–$d_6$, $CDCl_3$ or DMSO-$d_6$ using tetramethylsilane (TMS) as an internal standard. IR spectra were recorded on Perkin Elmer FT-IR spectrometer using KBr pellet.

### Step I (a) Synthesis of p-Hydroxy Styarylphenyl Ketones 3

Aqueous NaOH (50%, 20ml) was added in small increments to a solution of 4-Hydroxybenzaldehyde (0.02mol) and acetone (0.01mol) in EtOH (50ml) over a period of 30 minutes with stirring. The contents of flask were stirred for 3 hr at 10° C. After completion of the reaction the reaction mixture was neutralized with 1% dilute HCl and cooled over night. The greenish yellow solid **3** was precipitated and then it was filtered under vacuum and dried[6,7].

### (b) Synthesis of pyrazoles 4

A mixture of p-Hydroxy Styarylphenyl Ketones (0.01mol) and phenyl hydrazine hydrochloride (0.40 ml, 0.01mol) in ethanol (30 ml) was refluxed for about 10 hours. The reaction mixture was then poured in ice-cold water containing dilute HCl, product separated; it was filtered, washed and recrystallized from ethanol[6,7] **4.**

**Step II:** The synthesis of following compounds were done according to literature methods

**(a) Synthesis of methyl-2bromo-3- (4-flurophenyl) propionate[8,9] 6.**

**(b) Synthesis of 5-(4-flurobenzyl)-2-iminooxazolidin-4-one[8,9] 7.**

**(c) Synthesis of 5-(4-flurobenzyl)-2, 4-oxazolidinedione[6] 8.**

***StepIII: Synthesis of pyrazole derivatives of oxazolidinedione*9**
Sodium hydride, 5-(4-flurobenzyl)-2,4-oxazolidinedione [**2c**] (60% oil, 29g) and pyrazole [**1b**] was added gradually to a stirred ice-cold solution of in tetrahydrofuran (THF, 600 ml). After stirring for 2 hrs the reaction mixture was poured into $H_2O$. The product crystallized out, filtered and recrystallized from hexane[6,8]. The Physical characterization data of the compounds is given in (**Table-1**).

**Table-1:** Physical data of synthesized compounds (9a-j)
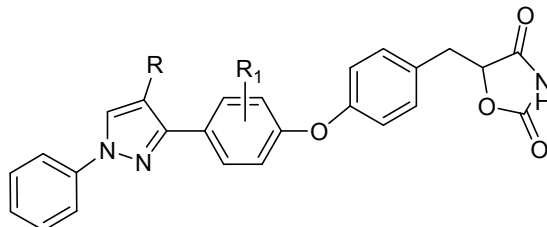


| Compd | R | R' | Mol formula | Mol weight | m.p. ($^0$C) | Yield (%) | Rf value |
|---|---|---|---|---|---|---|---|
| **9a** | $CH_3O$—⬡—$CH_2$- | H | $C_{33}H_{27}N_3O_5$ | 545.60 | 179ºC | 67.25 | 0.67 |
| **9b** | $H_2N$—⬡—$CH_2$- | -$OC_2H_5$ | $C_{34}H_{30}N_4O_5$ | 574.64 | 205ºC | 74.50 | 0.72 |
| **9c** | $H_2N$—⬡—$CH_2$- | H | $C_{32}H_{26}N_4O_4$ | 530.59 | 185ºC | 61.55 | 0.65 |
| **9d** | $O_2N$—⬡—$CH_2$- | H | $C_{32}H_{24}N_4O_6$ | 560.57 | 195ºC | 56.66 | 0.67 |
| **9e** | $H_2N$—⬡—$CH_2$- | H | $C_{32}H_{26}N_4O_4$ | 530.59 | 202ºC | 65.00 | 0.61 |
| **9f** | $O_2N$—⬡—$CH_2$- | -$OC_2H_5$ | $C_{34}H_{28}N_4O_7$ | 604.62 | 208ºC | 72.33 | 0.58 |
| **9g** | ⬡—$CH_2$- | H | $C_{32}H_{25}N_3O_4$ | 515.57 | 183ºC | 63.28 | 0.62 |
| **9h** | $H_3C$—⬡—$CH_2$- | H | $C_{33}H_{27}N_3O_4$ | 529.60 | 185ºC | 66.44 | 0.71 |
| **9i** | OH ⬡—$CH_2$- | H | $C_{32}H_{25}N_3O_5$ | 531.57 | 193ºC | 57.22 | 0.62 |
| **9j** | OH ⬡—$CH_2$- | -$OC_2H_5$ | $C_{34}H_{29}N_3O_6$ | 575.63 | 205ºC | 59.99 | 0.66 |

**Table-2:** Spectral data of various synthesized compounds (9a-j).

| Sl No | Compound No. | Spectral data (IR, NMR) |
|---|---|---|
| 1 | 9a | IR(KBr cm$^{-1}$): 3210(NH), 3030(C-H -Ar), 2854.5(CH-Ali), 1651(N=O), 1282.6($CH_2$-O)<br>NMR (400MHz, Acetone-$d_6$): $\delta$8.9(1H, s, NH), $\delta$8.08(1H, S, CH), $\delta$7.748-$\delta$6.883 (17H, m, Ar-H), $\delta$2.0(4H, m, $CH_2$). |
| 2 | 9b | IR(KBrcm$^{-1}$): 3436.53(NH), 3046(C-H-Ar), 2920.66(C-H-Ali),1605(C=O), 1489(C=N), 1267.97(-$CH_2$ -O).<br>NMR (400MHz, Acetone-$d_6$): $\delta$8.25(3H,s,NH), $\delta$7.952-6.203(17H, m, Ar-H), $\delta$4.610- 4.542(1H, t, CH), $\delta$1.448(4H, s, $CH_2$). |
| 3 | 9c | IR(KBrcm$^{-1}$): 3250.8(NH), 3037.7$^1$(C-H Ar), 2828.4(C-H Ali),1658(C=O), 1598.9(C=N), 1238(-$CH_2$-O)<br>NMR (400MHz,Acetone-$d_6$): $\delta$7.8(1H, s, NH), $\delta$7.5(2H, s, $NH_2$), $\delta$7.2(1H, s, CH), $\delta$7.3-6.57(17H, m, Ar-H), $\delta$4.55-4.519(1H, t, CH), $\delta$2.0(4H, m, $CH_2$). |
| 4 | 9d | NMR(400MHz, DMSO-$d_6$): $\delta$8.882(1H, s, NH), $\delta$7.605-$\delta$6.086(17H, m, Ar-H), $\delta$4.595- 4.550(1H, t, CH), $\delta$3.601(2H, s, Ali H), $\delta$1.158-$\delta$1.126(2H, d, CH). |
| 5. | 9e | NMR(400MHz,DMSO-$d_6$): $\delta$8.9 (1H, s, NH), $\delta$8.68 (1H, s, $NH_2$), $\delta$7.80 (1H), $\delta$7.90- 6.36 (17H, m, Ar-H), $\delta$4.95- 4.55 (1H, t, CH), $\delta$2.95- 2.92 (2H, d, Ali H), $\delta$0.79 (2H, s, Ali H). |
| 6 | 9f | NMR(400MHz, $CDCl_3$): $\delta$8.2 1H (s), $\delta$8.01-7.0(17H, m, Ar-H), $\delta$3.057-2.998(1H, t, CH), $\delta$2.15(2H, s, Ali $CH_2$), $\delta$1.5(2H, d, $CH_2$). |
| 7 | 9g | NMR(400MHz, $CDCl_3$): $\delta$7.9(1H, s, NH), $\delta$7.8(1H, s), $\delta$8.1-6.9(18H, m, Ar H), $\delta$4.6(1H, CH), $\delta$3.6(2H, d, $CH_2$), $\delta$1.3(2H, s, $CH_2$). |
| 8 | 9h | I.R.(KBr cm$^{-1}$): 3432.67(-OH), 3050(CH-Ar), 2902.66(CH), 1674(C=O), 1556.27(C=N), 1246.86(-$CH_2$ -O) |
| 9 | 9i | NMR(400MHz,$CDCl_3$): $\delta$8.9-8.2(1H,s,OH), $\delta$7.8 (1H,s), $\delta$ 7.5-6.3(17H, m, Ar-H), $\delta$3.7(1H, CH), $\delta$2.12- 2.106 (2H, d, $CH_2$), $\delta$1.2 (2H,d, $CH_2$). |
| 10 | 9j | NMR(400MHz, $CDCl_3$): $\delta$9.8(1H, s, OH), $\delta$7.8(1H, s), $\delta$7.6(1H, s, NH), $\delta$8.8-6.8(17H, m, Ar-H), $\delta$4.5(2H, s, $CH_2$), $\delta$3.5(3H, t, $CH_3$), $\delta$2.623-$\delta$2.608(1H, t, CH), $\delta$2.1(2H, q, $CH_2$). |

## PHARMACOLOGICAL ACTIVITY

Three months old Wistar Albino rats of either sex, weighing 200-250 gm were used. The animals were allowed food and water *ad libitum.* They were housed in room temperature at 25±2˚C for 24 hrs. The animals were randomly allocated into 12 groups, each group contained 6 animals. The blood glucose level was induced by Streptozotocin Model[10]. All the test compounds and standard drug were suspended in Tween- 80 and were administered orally. The standard drug used was rosiglitazone.

*Hypoglycemic activity*

The test groups were treated with different derivatives of pyrazole oxazolidinedione (**9a-9j**) with defined dose (200 mcg/kg) orally. Control and standard groups of animals were treated with normal saline and rosiglitazone (200mcg/kg) respectively. After 2 hrs blood samples were collected via retro orbital plexus. Blood glucose level was estimated by Semi-auto analyzer (Qualigens AR 601, GSK), using commercially available glucose estimation kit (Span diagnostics), as per manufacturers' brochure. The statistical analysis of data was carried out by one way ANOVA followed by Tukey-Kramer's Multiple Comparisons Test.

*Determination of $LD_{50}$*[11].

There was no mortality in control group of rats up to a dose of 5000 mg/kg body weight, all the pyrazole derivatives of oxazolidinediones, up to a dose of 1000 mcg/kg body weight did not produce mortality. Hence $1/5^{th}$ dose of $LD_{50}$ i.e. 200 mcg/kg body weight for the synthesized pyrazole derivates of oxazolidinedione was used for screening oral hypoglycemic activity. The results are shown in the (**Table-3**). All the values are expressed as mean blood glucose level (mg/dl) $\pm$ S.E.M, (n=6). (F**igure-1)** It was found from the pharmacological screening studies that derivatives **9d, 9b, 9e, 9f, and 9c** showed significant hypoglycemic activity compared to rosiglitazone standard in decreasing order. The other derivatives did not show any statistically significant activity in the animal model.

**Table-3:** Hypoglycemic activity of the test compounds (9a-j) were compared with respect to control.

| Sl. No | Compound No. | Mean glucose concentration mg/d ± SEM | Mean % change in hypoglycemic activity ± SEM |
|:------:|:------------:|:-------------------------------------:|:--------------------------------------------:|
| 1 | Control | $104.55 \pm 2.409$ | - |
| 2 | Standard | $65.58 \pm 1.031^{a}$ | $37.089 \pm 1.843$ |
| 3 | 9a | $97.63 \pm 1.455^{**}$ | $6.370 \pm 2.556$ |
| 4 | 9b | $75.58 \pm 1.375^{***}$ | $27.567 \pm 1.708$ |
| 5 | 9c | $75.63 \pm 1.197^{***}$ | $27.394 \pm 2.564$ |
| 6 | 9d | $84.26 \pm 1.49^{***}$ | $19.197 \pm 2.241$ |
| 7 | 9e | $78.38 \pm 1.193^{***}$ | $24.825 \pm 2.113$ |
| 8 | 9f | $82.53 \pm 1.019^{***}$ | $20.764 \pm 2.592$ |
| 9 | 9g | $99.18 \pm 1.575^{ns}$ | $4.880 \pm 2.653$ |
| 10 | 9h | $87.88 \pm 2.462^{***}$ | $15.569 \pm 3.919$ |
| 11 | 9i | $83.53 \pm 1.493^{***}$ | $19.885 \pm 1.862$ |
| 12 | 9j | $105.8 \pm 1.535^{ns}$ | $-1.495 \pm 3.021$ |

Data were analyzed by one way ANOVA followed by Tukey-Kramer's Multiple Comparisons Test n = 6, $^{a}p<0.001$, $^{***}p<0.001$, $^{**}p<0.01$, $^{ns}p>0.05$
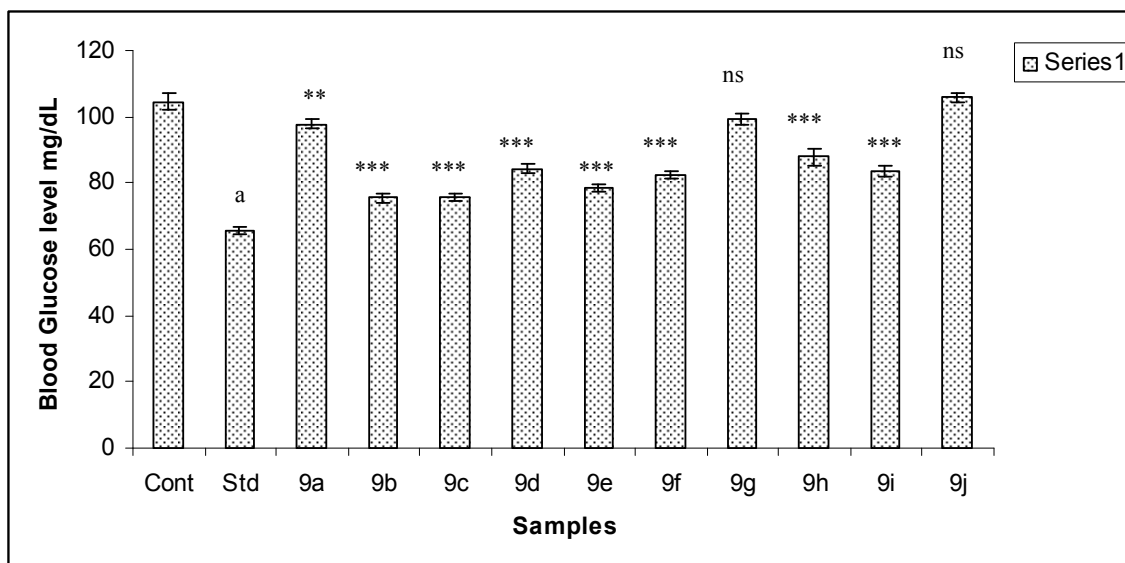
**Figure-1:** Comparative study of serum blood glucose level in standard (Rosiglitazone) and samples (9a-j). All the values are expressed as mean blood glucose level (mg/dl) $\pm$ S.E.M. p (vs control with one way ANOVA followed by Tukey-Kramer's Multiple Comparisons Test) n = 6, [a]p<0.001, [***] p<0.001, [**] p<0.01, [ns]p>0.05.

## Discussion

### *Structural activity relationship*

A look at the structures of the active molecules may give a clue to variation in hypoglycemic activity. It is known from the QSAR studies of PPAR- γ agonists that three structural requirements must be fulfilled for a molecule of this class to successfully activate the PPAR-γ receptor –an acidic fragment which usually anchors itself at the binder site of the receptor, a central aromatic and heteroatomic linker fragment and a lipophilic side chain which will bury itself deep into the effector site of the receptor (**Figure-2**)[12]. Stronger the hydrogen bonding between the side chain and the effector site, greater is the hypoglycemic efficacy. The relative efficacy of the synthesized derivatives may be explained based on this hypothesis. **(Figure-1)** shows the structural requirements for a PPAR- γ agonist.
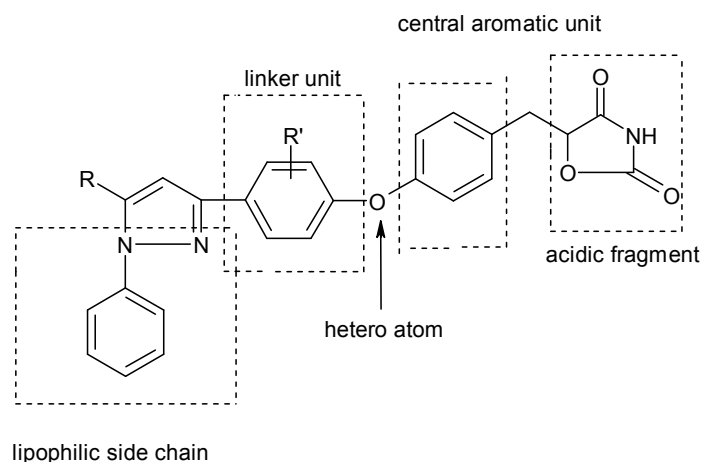


**Figure-2**: Structural requirements for a PPAR- γ agonist.

The acidic fragment in the synthesized derivatives is the oxazolidinedione moiety which binds strongly with the binder site and the central aromatic fragment and heteroatom constitutes the flat linker region which acts as the suitable spacer to orient the molecules favorably along the receptor. The lipophilicity of the side chain determines how deep the fragment would penetrate inside the effecter site cavity. The crystallographic studies have identified that glitazones constitute a 'U' shape with the receptor **(Figure-3)**. As the lipophilicity of the side chain increases, and more electronegative center is introduced in the side chain, it penetrates deeper inside the effecter site cavity due to higher lipophilicity and stronger hydrogen bonding with the amino acid residues of the cavity. This trend is observed in the hypoglycemic activity of the synthesized derivatives.
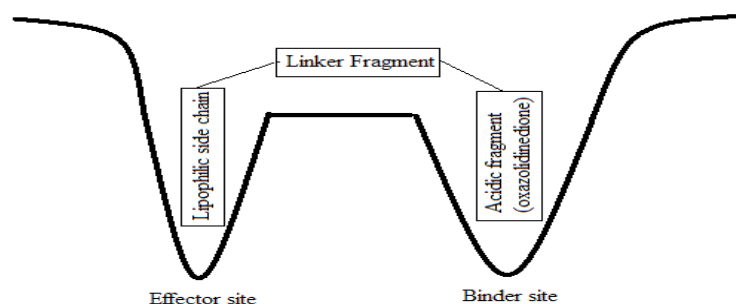


**Figure-3**: Shows the three point binding mode of PPAR-γ agonists with the receptor showing the 'U' shaped orientation of the agonist molecules.

The electronegativity decreases in the order $NO_2$ (**9d**) >p-$NH_2$ (**9b**) >m- $NH_2$ (**9e**) >o-OH (**9i**) which is also the order in which the H-bond strength with PPAR-γ effector site is presumed to decrease. Presence of –$OCH_3$ group may be leading to stearic hindrance in achieving favourable orientation of the pyrazole side chain penetration into the lipophilic cavity that might lead to the decrease in activity of **9a** and **9g** and loss of activity for 9j.

**Conclusion**

Several pyrazole derivatives of oxazolidinedione could be developed condensing various pyrazoles with oxazolidinediones and their structures ware validated through standard analytical techniques. Among the synthesized derivatives, the compounds **9b**, **9c**, **9d**, **9e**, **9f**, **9h** and **9i** were significantly active, where as others were inactive derivatives. The lipophilicity of the side chains and their electronegativity seemed to be responsible for variation of the hypoglycemic activity.

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