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# NOVEL METABOLITES OF LOSARTAN AS HUMAN PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (PPAR Γ) AND HUMAN ANGIOTENSIN RECEPTOR (AT1R) BINDERS: AN *IN SILICO* STUDY

***G.Shyam Prasad[1], G. Shravan Kumar[2] and K.Srisailam[3]**

**[1]Department of Microbiology, Vaagdevi Degree and PG College, Ramnagar, Warangal-506009, Telangana State, India**

**[2]Bioinformtics Division, PGRRCDE, Osmania University, Hyderabad- 500007, Telangana State, India**

**[3]University College of Pharmaceutical Sciences, Satavahana University, Karimnagar-505001, Telangana State, India**

***Corresponding Author Email: shyamprasad1919@yahoo.com**

## ABSTRACT

*Losartan, a potent angiotensin II receptor antagonist used as anti-hypertensive agent and its novel biotransformed products were investigated for human peroxisome proliferator activated receptor gamma (PPAR γ) and human angiotensin receptor (AT1R) binding activity in silico. Novel metabolites of losartan viz. M3 and M5 exhibited strong interaction with both AT1R and PPAR γ receptors. The metabolites M3 was found to interact strongly followed by M5 with both the receptors compared to the standard reference drugs, losartan (for AT1R) and fenofibric acid (PPAR γ). In the present in silico study, novel metabolites of losartan established strong binding to both AT1R and PPAR γ receptors. Hence, it can be concluded that metabolite M3 and M5 of losartan may be developed as drugs to treat both hypertension and insulin resistance simultaneously with the same pharmaceutical agent. However, further in vitro and in vivo investigations are required to develop as final drug.*

## KEY WORDS

*Losartan, peroxisome proliferator activated receptor gamma (PPAR γ), human angiotensin receptor AT1R*

## 1. INTRODUCTION

Peroxysome proliferator activated receptors (PPARs) are members of the nuclear hormone receptor super family of ligand-activated transcription factors that are related to retinoid, steroid and thyroid hormone receptors (Murphy and Holder, 2000). Three PPAR isotypes viz. PPAR α, PPAR β/δ and PPAR γ were identified earlier (Guasch et al., 2012) and each of these subtypes has been recognized in a tissue-specific manner and plays a pivotal role in glucose and lipid homeostasis. The PPAR γ plays vital roles in regulating the storage and catabolism of dietary fats (Iwata et al., 2001). PPAR γ constitutes a primary target for the development of drug candidates to treat type II diabetes and PPAR γ full agonists may even induce cell growth arrest, apoptosis and terminal differentiation in various human malignant tumors (Guasch et al., 2012). Hypertension is a very important chronic disease affecting one third of adults worldwide and causing about half of the total mortalities, mainly due to stroke and heart problems. In fact, it accounts for 9.4 million deaths worldwide every year (Abdelhedi et al., 2017). Increase in blood pressure (BP) increases the risk of developing heart disease, Obesity, Kidney disease, eye damage, and stroke (Vamsidhar et al., 2010). Hypertension is treated by various anti-hypertensive agents which are classified as: Diuretics; Beta-blockers; ACE inhibitors; Angiotensin II receptor antagonists;

Renin inhibitors; Calcium channel blockers; Alpha blockers and other drugs (Teja, 2011).

Losartan (I, 2-n-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl]-imidazole-5 methanol monopotassium salt) is a potent, orally active, angiotensin II receptor antagonist used as antihypertensive agent (Prasad et al., 2018). Hypertension and insulin resistance are intimately linked and constitute two components of the metabolic syndrome or Syndrome X. Patients with metabolic syndrome are characterized as having three or more of the following comorbidities: impaired fasting glucose, hypertriglyceridemia, hypertension, low high-density lipoprotein cholesterol levels, and central obesity (Garcia et al., 2011).

Currently, there are no therapies whereby both hypertension and insulin resistance can be simultaneously treated with the same pharmaceutical agent. Angiotensin II receptor blockers (ARBs) are a highly regarded class of drugs used for the treatment of hypertension. They are safe and effective with few side effects. One particular ARB, telmisartan, a potent selective AT1 receptor antagonist, was reported to also have weak activity at PPAR γ (Benson et al, 2004). The main PPAR γ synthetic full agonist studied to date ere the thiazolidinedione (TZD) insulin-sensitizing drugs (eg. rosiglitazone and pioglitazone) which were withdrawn from the market due to their pharmacovigilance identified undesired adverse effects such as weight gain, oedema, bone loss and congestive heart failure (Ahmadian et al., 2013).

Hence, in the present investigation novel metabolites of anti-hypertension drug losartan produced by biotransformation process employing thermophilic fungus *Rhizomucor pusillus* NRRL 28626 (Prasad et al., 2018) was studied *in silico* to find a common drug which possess both PPAR γ and AT1 receptor binding activity.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of target Proteins:

Molecular docking of losartan, an anti-hypertension drug and two of its novel metabolites produced by thermophilic fungus *Rhizomucor pusillus* (Prasad et al., 2018) was performed with AT1R and PPAR γ receptor using Auto dock vina software, an interactive molecular graphics programme to understand the protein-ligand interactions (available from http://viba.scripps.edu/). The crystal structures of Human AT1R of 2.8 A° resolution (PDB I D: 4ZUD) with bound ligand inverse agonist Olmesartan (Zhang et al., 2015). Similarly, crystal structures of Human PPAR γ receptor of rsolution of 2.3°A (PDB I D: 2PRG) with bound ligand BRL49653 (Nolte et al., 1998) were obtained from PDB data base (http://www.rcsb.org./pdb). The bound ligands, hetero atoms, water molecules were removed, and polar hydrogen atoms were then added, Kollman charges and salvation parameters were assigned by default using Auto dock software.

### 2.2. Preparation of Ligands:

The novel metabolites of losartan M3 and M5 and the standard reference drugs viz. fenofibric acid and losartan which were used as ligands in the present investigation were drawn using Chem Draw software and converted to 3D PDB format from mol format by Accelrys Discovery Studio 2.3.

### 2.3. Validation of Software:

The software Autodock was validated by downloading the X-ray crystal structure of the receptors AT1R (PDB I D: 4ZUD) and PPAR γ receptor (PDB I D: 2PRG) from protein data bank and redocking the co-crystallized ligand reproducing the original interactions of the reference protein-ligand complexes comparing the root-mean square distance of the experimentally determined pose with the docked pose.

## 3. *In silico* studies: Virtual screening for interaction of 2 novel metabolites of losartan with AT1R receptor (PDB I D: 4ZUD) and PPAR γ receptor (PDB I D: 2PRG) by molecular docking.

After preparing the ligands as well as AT1R, and PPAR γ receptors, both were converted into the pdbqt format using the automated docking tool Auto Dock which was later used for docking. A grid box was prepared to cover the pocket with the main residues of protein binding site by maintaining the grid size of X = 40, Y = 40, and Z = 40. Coordinates used for docking the ligands with 4ZUD werex=-33.550; y=64.363; z=22.901and the coordinated for 2PRG used were x= 55.172; y = -27.914; z = 19.647. An advanced molecular docking program Auto Dock vina, version 1.1.2 available from http://vina.scripps.edu/download.html was used for docking against the receptors to estimate the binding affinities (kcal mol-1). The ligands were evaluated *in silico* against AT1R (PDB ID: 4ZUD) and PPAR γ receptor (PDB I D: 2PRG) in triplicates and the average of the best conformation was chosen with the

lowest docked energy, based on complete docking search (ten runs). The interaction of AT1R and PPAR γ receptors with the ligands, hydrogen bonds, bond lengths and Root Mean Square Difference (RMSD) was analyzed using PyMOL software (http://pymol.sourceforge.net/).

## 4. RESULTS AND DISCUSSION:

In our previous study, losartan an anti-hypertension drug was biotransformed employing thermophilic fungus *Rhizomucr pusillus* and a total of five metabolites (Fig.1.) were reported (Prasad et al., 2018). Among the metabolites detected two of them viz. M3 and M5 were found to be novel. In the present investigation, a preliminary study was undertaken to find the interaction of novel metabolites of losartan M3 and M5 with AT1R and PPAR γ receptors to find dual activity of losartan metabolites so as to treat both hypertension and insulin resistance with the same pharmaceutical agent. The structures of the novel metabolites of losartan were presented in Table 1.

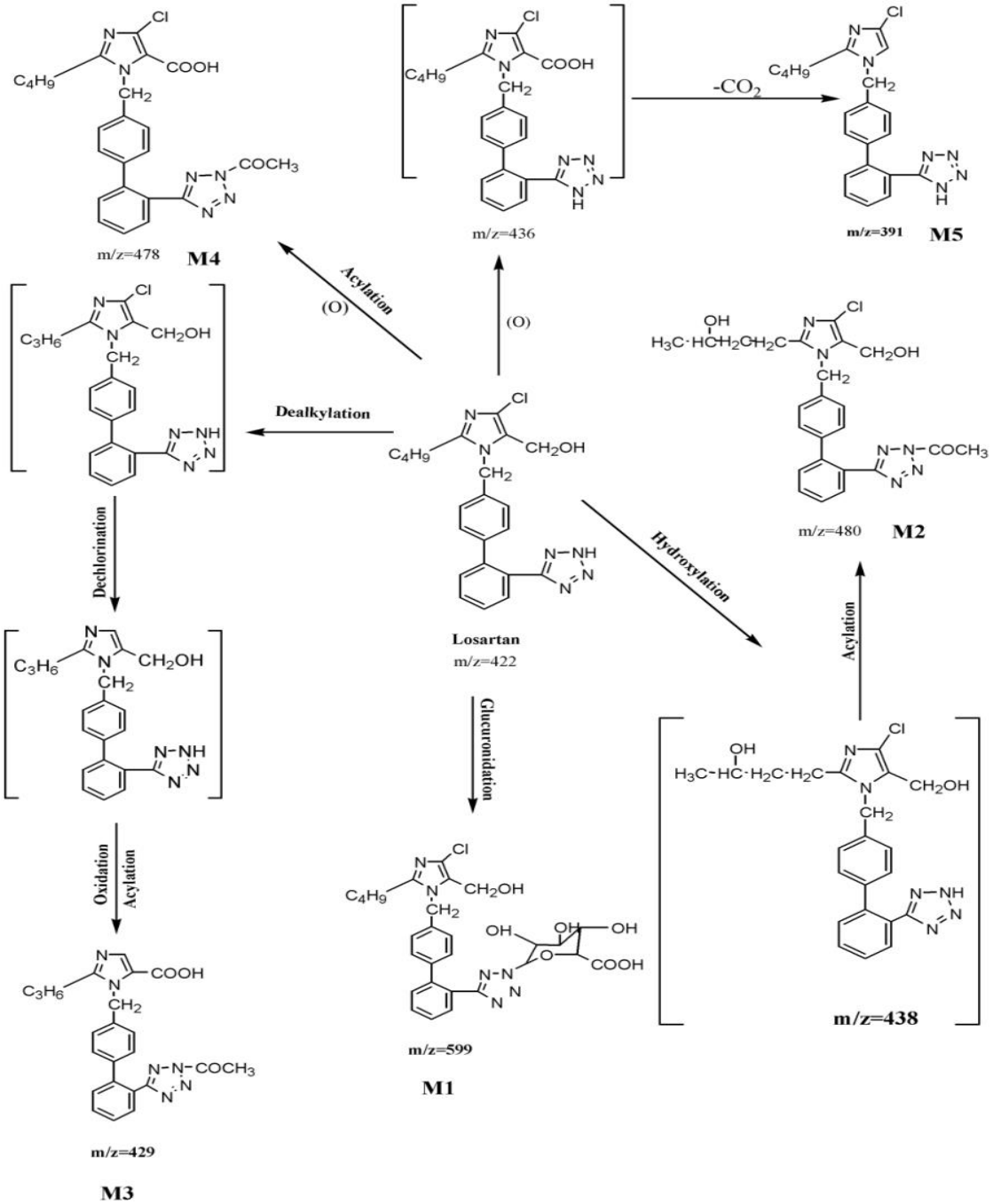
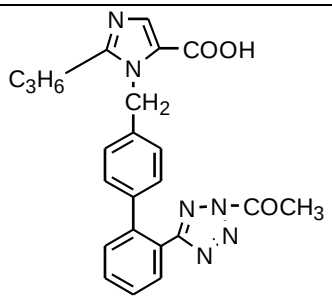
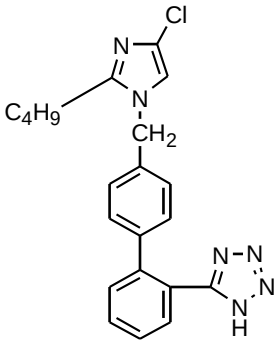
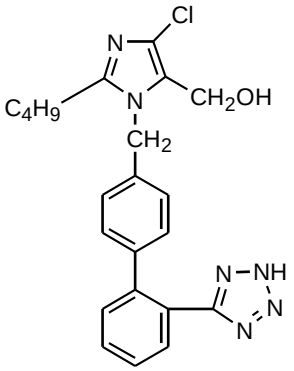
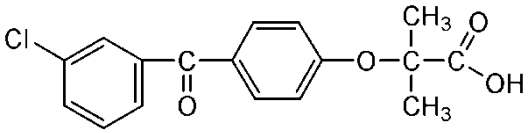



**Fig.1 Proposed metabolic pathway of Losartan by *Rhizomucor pusillus* (Prasad et al., 2018)**

**Table.1 showing Losartan and proposed structures of two of its novel metabolites**

| S.No. | Novel metabolites | Structure of the metabolite | Molecular weight |
|---|---|---|---|
| 1 | M3 |  | 429 |
| 2 | M5 |  | 391 |
| 3 | Losartan (Parent Compound) |  | 422 |
| 4 | Fenofibric acid (Standarad drug) |  | 318 |

**4.1. *In silico* studies with AT1R and metabolites M3 and M5.**

Molecular docking of test compounds into the active site of AT1R was found to be successful based on the formation of complexes of AT1R with ligands (M3 and M5). The hydrogen bond interactions, binding energy, bond length, RMSD, active site residues and orientation of the docked compound within the active site were visualized. All the test compounds screened showed best fit RMSD value of 0.000, indicating statistically significant interaction. The negative and low value of ΔG indicated a strong and favourable bonding between 4ZUD and the ligands in their most favourable conformations. The binding energies of metabolites M3, M5 and the standard drug losartan were found to be, -9.6, -8.7, -8.6 kcal mol-1respectively, indicating a relatively higher interaction of test compounds with AT1R. The details of binding energies, the number of hydrogen bonds formed, and catalytic site residues involved in the protein-ligand complex of AT1R with different ligands is depicted in Table 2 and Fig 2.

### 4.2. *In silico* studies with PPAR γ receptor and metabolites M3 and M5.

Molecular docking of the ligands into the active site of PPAR γ receptor was found to be victorious based on the development of complexes of PPAR γ receptor with ligands (M3 and M5). The binding energy, hydrogen bond interactions, bond length, RMSD, active site residues and orientation of the docked ligands within the active site were visualized. All the compounds tested showed best fit RMSD value of 0.000, indicating statistically significant interface. The negative and low value of ΔG indicated a strong and favourable bonding between 2PRG and the ligands in their most favourable conformations. The binding energies of metabolites M3, M5 and the standard drug fenofibric acid were found to be, -10.3, -8.9, -8.3, kcal mol-1 respectively, indicating a relatively higher interaction of test compounds with PPAR γ receptor. The details of binding energies, the number of hydrogen bonds formed, and catalytic site residues involved in the protein-ligand complex of PPAR γ receptor with different ligands is depicted in Table 3 and Fig 3.

**Table.2 showing binding energy, hydrogen bonds, interacting residues of protein (PDB ID: 4ZUD) and ligands**

| S.No | Name of the Ligand | Binding Energy Kcal $mol^{-1}$ | Hydrogen bonds | Distance (°A) | Interacting Amino acids |
|---|---|---|---|---|---|
| 1 | Metabolite M3 | -9.6 | 04 | 3.1<br>3.1<br>2.9<br>2.3 | Arg-167;<br>Arg-23;<br>Tyr-87<br>Cys-180 |
| 2 | Metabolite M5 | -8.7 | 03 | 3.2<br>3.0<br>1.9 | Arg-23,<br>Arg-23<br>Pro-19 |
| 3 | Losartan | -8.6 | 06 | 2.9<br>3.4<br>2.2 | Arg-167;<br>Arg-167<br>Tyr-184; |

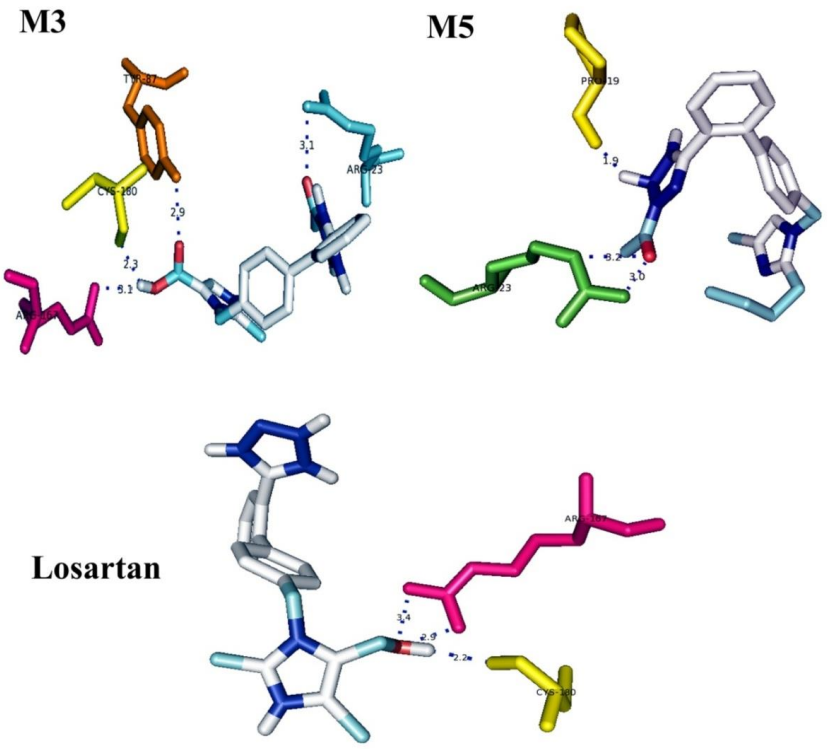



**Fig.2. Protein-ligand complex of Angiotensin receptor (PDB.ID:4ZUD) with different ligands**

**Table.3 showing binding energy, hydrogen bonds, interacting residues of protein PDB ID: 2PRG and ligands**

| S.No | Name of the Ligand | Binding Energy Kcal $mol^{-1}$ | Number of hydrogen bonds formed | Distance (°A) | Interacting Amino acids |
|---|---|---|---|---|---|
| 1 | Metabolite M3 | -10.3 | 04 | 2.9<br>2.8<br>2.8<br>2.1 | His-323<br>Ser-289<br>Arg-288<br>Ile-326 |
| 2 | Metabolite M5 | -8.9 | 03 | 2.2<br>3.4 | Gly-284<br>Ser-342 |
| 3 | Fenofibric acid (Standard drug) | -8.3 | 03 | 3.3<br>3.0<br>2.1 | His-323<br>Ser-289<br>Tyr-473 |

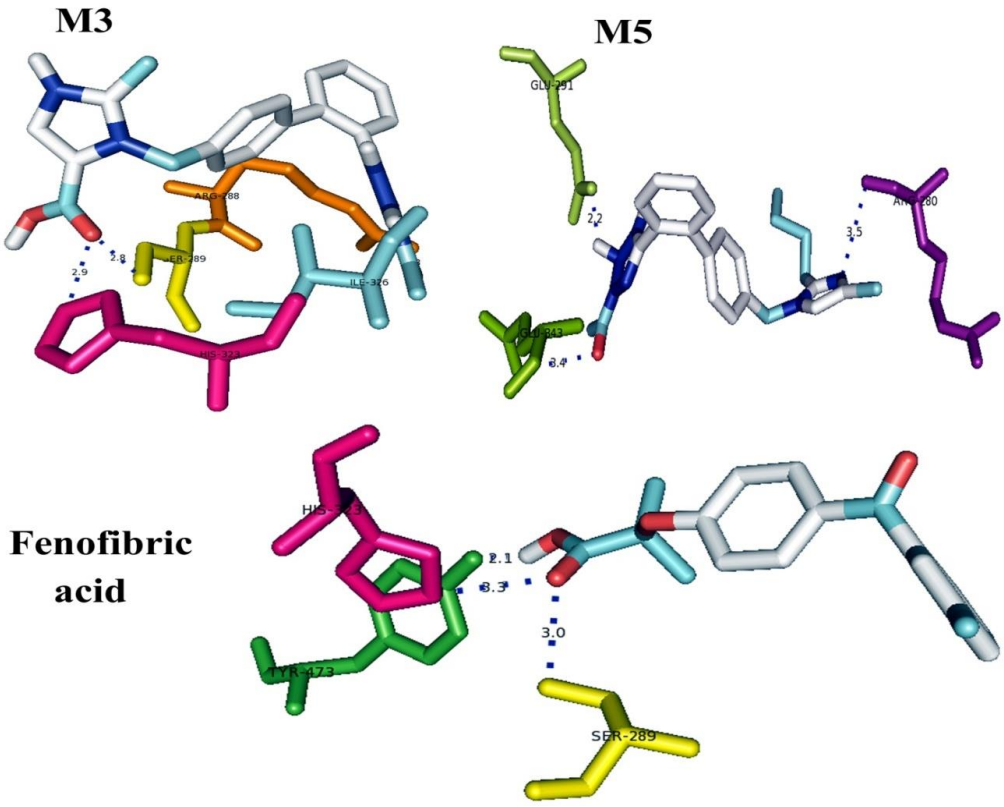



**Fig.3. Protein-ligand complex of PPAR γ receptor with different ligands**

In the present investigation, docking simulations were performed for interaction of novel metabolites of losartan viz. M2 and M5 with AT1R and PPAR γ receptors. The interaction with AT1R receptor show that metabolite M3 anchored the receptor through Arg167 as the key residue while the standard drug losartan also developed interaction with Arg167 forming hydrogen bonding. Our results are in agreement with Kellici et al. (2016) who recorded strong interaction with Arg167. The other key residues recorded by Kellici et al. (2016) with AT1R receptor interactions were Cys180, Tyr184, Tyr92 and Tyr87 which are in accord with our results with metabolite M3. Unlike metabolite M3, the interaction of M5 with AT1R was not noted with Arg167 or Cys180, Tyr184, Tyr92 and Tyr87 but recorded with Arg23 and Pro19 which could be due to variation in

structure of M5 compared to standard drug losartan and metabolite M3. The standard drug losartan exhibited strong binding with Arg167 and Tyr143. This clearly states that Arg167 to be the main residue involved in binding the ligand for both M3 and losartan. Similarly, virtual screening for interaction of the ligand M3 with the target protein PPAR γ receptor provided strong binding with the residues His323, Ser289, Arg 288 and Ile326. The binding site residues of PPAR-γ (PDB ID: 2PRG) which were found to interact with the anti-diabetic drug rosiglitazone were Gln286, Ser289, His323 and Tyr473 (Nolte et al., 1998). Similar interactions were visualized for the novel metabolite of losartan M3 which were found to be embedded in the binding pocket of the target similar to rosiglitazone, stating similarity of structures of M3 and rosiglitazone. The standard drug fenofibric acid also showed strong interactions with His323, Ser289 and Tyr473. Similarly, Irudayaraj et al. (2014) in his *in silico* studies also recorded binding with Arg288 which clearly states that His323, Ser289, Arg288 and Tyr473 to be the key residues involved in bonding the ligand. The metabolite M5 unlike M3 and standard drug fenofibric acid has not shown any interaction with same amino acids but has shown with Gly284 and Ser342. This may be due to structural variation of M5 compared to M3 and fenofibric acid.

In the present study, metabolite M3 showed binding score of -10.3 for 2PRG and -9.6 for 4ZUD followed by M5 with scores of -8.9 for 2PRG and -8.7 for 4ZUD followed by metabolite M5 which showed -8.9 score for 2PRG and -8.7 score for 4ZUD. This clearly states that metabolite M3 is more superior to bind both the receptors 4ZUD and 2PRG compared to metabolite M5 and standard reference drugs losartan for 4ZUD and fenofibric acid for 2PRG receptors.

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**CONCLUSION:**

Over stimulation of angiotensin receptor leads to hypertension, cardiovascular hypertrophy and fibrosis. Similarly, PPAR γ constitutes a primary target for the development of drug candidates for the treatment of type II diabetes. In the present *in silico* study the novel metabolites of losartan M3 and M5 exhibited strong interaction with both angiotensin receptor (AT1R) and Peroxisome proliferator activator receptor gamma (PPAR γ). Hence, it can be concluded that both hypertension and insulin resistance can be simultaneously treated with the novel metabolites of losartan. However, further investigation using *in vitro* and *in vivo* methods are needed to develop these metabolites as final drugs.

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***Corresponding Author:**
***G.Shyam Prasad****
*Email:* **shyamprasad1919@yahoo.com**