

Austroinulin from *Stevia rebaudiana* a Potential Agonist of the Peroxisome Proliferator-activated Receptor (PPAR γ): an *in-silico* Study.

Metibemu DS^{1*}, Akinloye OA³, Akinbamiwa BA¹, Adeniran OY², Eniafe OG¹, Akamo AJ³, Omotuyi IO¹

¹Centre for Biocomputing and Drug Development, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria

²Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko.

³Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria.

Abstract

Activators (agonists) of the nuclear receptor, peroxisome proliferator-activated receptor (PPAR γ) are therapeutically used to tackle hyperglycemia associated with type 2 diabetes. Current therapeutic compounds, thiazolidinedione used to sensitize cells to insulin have been reported to adversely affect the body. In view of this, the discovery of novel ligands is highly pertinent.

Plant products have been documented, to be a promising pool of structures for drug discovery, and the PPAR γ -activating potential of a wide range of natural products have been reported. In the present study, Computer-aided drug design techniques were employed in the screening of pharmacologically relevant compounds of medicinal plant. Seventy (70) phyto-ligands (phytochemicals) characterized from *Stevia rebaudiana* and obtained from literatures were screened for their agonistic effects on peroxisome proliferator-activated receptor (PPAR γ) belonging to the nuclear receptor family. Austroinulin was the lead compound with a binding energy of -8.1kcal/mol. The pharmacological kinetic analysis of austroinulin was determined. The results of this study demonstrated that the phyto-ligand (austroinulin) exhibit agonistic activity on PPAR γ and could be a potential insulin sensitizer.

Keywords: Peroxisome proliferator-activated receptor (PPAR γ); Thiazolidinediones; *Stevia rebaudiana*; Molecular docking; Austroinulin; Type 2 diabetes.

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Introduction

Type II diabetes (T2D) is a chronic disorder that constitutes about 90–95% of diabetes mellitus (DM). It is a heterogeneous group of metabolic disorders characterized by an elevated blood glucose level. Type II diabetes [1] is recognized as one of the dominant executors of disability and casualty in the modern world. T2D is characterized by preliminary loss of cells' insulin-sensitivity or a complementary deficiency in insulin secretion or both [2], resulting from the combination of both environmental factors and genetic composition [3].

WHO reported the global of diabetes mellitus (DM) [4] as of 1980 to be 108 million people, and over 110 million people in 1994 [5] and increased to 285 million people in 2010 [6], with a conceived future increment to 761 million by 2030 [7]. DM has been one of the top killing diseases for some decades, with an estimated value of 1.5-5.0 million deaths in 2012-2015 (IDF, 2006) owing to inefficient medical treatment.

Current therapeutic compounds used to sensitize cells to insulin, majorly the Thiazolidinediones, which when bind to PPAR γ activate the transcription of genes (e.g., adipocyte fatty acid-binding protein, lipoprotein lipase, and phosphoenol pyruvate carboxykinase in adipose tissue) involved in glucose metabolism and maturation of adipocytes, and the up-regulation of acyl-Co synthetase, fatty acid transporters, and uncoupling protein-2 are recently reported to exacerbate other life-threatening side effects which lead to their limited

usage [8,9]. Hence, the need for alternative therapeutic agents with little or no side effects [10].

In the present study, Seventy (70) phytoligands (phytochemicals) characterized from *Stevia rebaudiana* and obtained from literatures were screened for their agonistic effects on peroxisome proliferator-activated receptor (PPAR γ) belonging to the nuclear receptor family. *Stevia rebaudian* is a perennial shrub of the family Asteraceae. This plant is reported to produce a diverse range of bioactive molecules that possess antibacterial, antifungal, antiprotozoal, and anti-diabetic properties [11]. The drug likeness of the lead phytochemical compounds were determine through the calculation of their molecular properties; calculated logP, polar surface area, number of hydrogen bond donor, number of hydrogen bond acceptors and molecular weight (Lipinski's rule) [12].

Materials and Methods

Preparation of phyto-ligands

Seventy (70) Phytochemicals that have been characterized from *S. rebaudiana* were obtained from literatures. The structures of some of the phytochemicals were drawn with the ChemAxon software; MarvinSketch v15.11.30 and saved in the sdf format, while the other structures of the phytochemicals were downloaded in the sdf format from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>). All phytochemicals were converted from their sdf format to the pdb format by Open Babel and finally to the pdbqt using ligprep command lines.

Note: %babel H.sdf H.pdb -h -r -m --gen3D
Where; H is the name of the file (that contains the phytochemicals).

To convert the ligands from pdb to pdbqt:
%for i in {1..70}; do ligprep -l H\$(i).pdb -o H\$(i).pdbqt
Where; 1..70 indicate the number of phytochemicals in the file

Preparation of standard

The standard drugs used in the present study, Pioglitazone and Rosiglitazone, are a group of synthetic drugs named Thiazolidinediones. Pioglitazone and Rosiglitazone are the two potent well-known drugs of the class Thiazolidinediones that have been successfully used in restoring cells' sensitivity to insulin, though banned in some quarters due to its reported life threatening side effects [13-14]. The structure of the standards (Pioglitazone and Rosiglitazone) were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>)

Preparation of target protein

The Crystal structure of the ligand-binding domain of the human PPAR-gamma in complex with an agonist with crystallographic resolution of 2.07Å with PDB ID: 2F4D was downloaded from the protein data bank (<http://www.rcsb.org>). PyMOL plugin in Autodock/Vina was employed to extract the receptor. The SWISS-MODEL software was used to rebuild the missing residues and atoms of the human PPAR-gamma.

Molecular docking and virtual high throughput screening

Virtual high throughput screening (vHTS) method was employed, to screen a pool of the compounds library to investigate the binding affinity cum agonistic properties of the phytochemicals from *Stevia rebaudiana* against the binding pocket of the human PPAR-gamma target receptor [15]. To ensure the phytochemicals occupy the exact binding pocket as obtained in the co-crystallized compound, the grid coordinates was set exactly as those of the co-crystallized compound; x= 9.24, y= -5.89, z= 39.09. Force fields were applied to the phytochemicals for subsequent conversion to 3D and later to pdbqt, using command lines in auto dock vina, and then, processed for docking.

Validation of docking results

The docking results were validated with the blasting of the fasta sequences of the crystal structure of the ligand-binding domain of the human PPAR-gamma (ID: 2F4D), obtained from the protein data bank unto the online available ChEMBL Database (www.ebi.ac.uk/chembl/). The bioactivity generated by the database, having an identity of 100%, EC50 value of 2599, and KI value of 312, was downloaded in txt format. The bioactivity was sorted out; missing or misplaced data were removed. Only 88 of the total 2599 drug-like compounds were recovered. The compiled compounds were split and converted to 2D (in sdf format) by Data Warrior software (version 2), converted to pdb format by Open Babel, and finally to pdbqt. The ligands were docked into the binding domain of PPAR γ (as indicated by the config file). The config file indicated the grid map of the active site of PPAR-gamma generated by AutoDock Vina. A correlation coefficient graph was plotted

between the docking scores of the 88 compounds generated and their corresponding pIC₅₀ (experimentally determined) values. Spearman Rank correlation co-efficient graph was plotted to obtain the correlation (R^2) between the dockings results of the ChEMBL's compounds and their corresponding experimentally generated results.

Result and Discussion

Other than the testing of extracts and bio-guided approaches, virtual screening remained an effective approach for the discovery of novel PPAR γ ligands from natural sources [16]. Numerous 2D and 3D virtual screening techniques have successfully provided structurally diverse natural product PPAR γ activators, hence, revealing natural products as a rich source for novel PPAR γ agonists [16].

In the present study, virtual high throughput screening (vHTS) technique was employed to screen a pool of seventy (70) phyto-compounds from *Stevia rebaudiana* plant for their PPAR γ agonistic properties. Austroinulin was discovered as the lead compound with the binding energy of -8.1 kcal/mol (appendix). The drug-likeness of austroinulin was confirmed by subjecting it to the Lipinski's rule of five, interestingly the lead compound, austroinulin violated none of the rules and with three (3) number of rotatable bonds, it possesses good bioavailability (number of rotatable bonds influences the bioavailability and the binding potency) (Tables 1 and 2).

We validated the accuracy of our docking protocol by re-docking the co-crystallized ligand (Indol-1-yl Acetic acid) back into the binding pocket of the PPAR γ (2F4B1) [17], (Figure 1). As revealed above, the re-docked pose overlapped almost totally with the experimental orientation, indicating that autodock vina

Table 1: Showing the number of rotatable bonds in Austroinulin and in the standard drugs (Pioglitazone and Rosiglitazone). Compounds that meet the criteria of (1) 10 or fewer rotatable bonds and (2) polar surface area $\leq 140 \text{ \AA}^2$ (or 12 or fewer H-bond donors and acceptors) have high probability of good oral bioavailability. The number of rotatable bonds influences bioavailability and binding potency.

Compound Name		Number of rotatable bonds	Binding Energy (Kcal/mol)
Austroinulin		3	-8.1
Thiazolidinediones	Pioglitazone	7	-8.5
	Rosiglitazone	7	-7.1

Table 2: Lipinski's drug-like properties of austroinulin: The rule describes drug candidate's pharmacokinetics in the human body which also including their absorption, distribution, metabolism, and excretion ("ADME"). Austroinulin, violated none of the rules. This table was generated using the ChemAxon suite.

Molecular Properties	Lipinski's Rule Of Five	Austroinulin Drug-Like Properties
Molecular Mass	<500 Dalton	322.48216
Hydrogen Bond Acceptors	<10	3
Hydrogen Bond Donors	<5	3
High Lipophilicity (Log P)	< 5	4.5
Molar Refractivity	Between 40-130	94.87
Polar Surface area	$\leq 140 \text{ \AA}^2$	60.69

with a very high accuracy re-docked the co-crystallized Indol-1-yl Acetic acid back into the binding pocket of the PPAR γ , this implies that our docking methodology was reliable and the docking scores obtained are correct. The accuracy of our docking scores was further validated using the online available ChEMBL Database, the Fasta sequence of the crystal structure of the Human PPAR-gamma (ID: 2F4B) was blasted on www.ebi.ac.uk/chembl/. The compounds obtained were docked into the ligand-binding domain of the Human PPAR-gamma, a correlation coefficient graph plotted between the docking scores of the compounds generated and their corresponding ChEMBL's Pchem values (experimentally determined IC₅₀), Figure 2. The strong correlation coefficient between the docking scores and the experimentally derived data in the present study, lend credence to the fact that computational experiment can replicate experimental data at least in this present study and that our docking scores (appendix) using AutoDock Vina algorithm is dependable (Figure 3).

Austroinulin, the lead compound has a binding energy of -8.1 kcal/mol, while the standard drugs pioglitazone and rosiglitazone have binding energies of -8.5 kcal/mol and -7.1 kcal/mol respectively (Table 3). The highest binding energy (-8.5kcal/mol) accrued to pioglitazone in this regard is believed to be due to the extensive high number of hydrophobic interactions (sixteen) of pioglitazone with key residues at the active site of

the PPAR γ ; Met – 329, Ala-2923, Ile-326, Leu-336, Phe-282, His-323, Leu-453, Leu-469, His-449, Leu-465, Gln-286, Cys-285, Leu-333 (Figure 4) and coupled with the high number of hydrogen bonds (Arg-288, Tyr-474 and Ser-289). However, Austroinulin and rosiglitazone though possess different binding energies (-8.1 kcal/mol, -7.1 kcal/mol respectively) shared some common amino acids interactions (six) at the binding pocket of PPAR γ (Figure 5 and 6); Leu-270, ser-342, Ile-262, Gln-345, Gly-258 and Glu-259.

Austroinulin has a higher binding energy (-8.1 kcal/mol) when compared with rosiglitazone (-7.1 kcal/mol) (Table 1), there are a total of eleven amino acids interaction of austroinulin with the binding pocket of PPAR γ (Figure. 4); Leu-270, Ser- 342, Cys-261, Gly-346, Phe-247, Gln-345, Gly-258, Glu-259, Thr-268 (Ten (10) hydrophobic interactions and two (2) hydrogen bonds interaction; Thr-268 and Lys-265) (Figure 4), also, rosiglitazone formed a total of eleven (11) interactions within the 4Å of the PPAR γ binding pocket (Figure. 6) ; Lys-265, Gly-347, Ile- 341, Ser- 342, Ile-249, Ile-281, Arg-280, Glu-259, Leu-270, Ile-262, Gly-258 (Ten (10) hydrophobic interactions and one (1) hydrogen bond interaction – Gly-347). The differences in the binding energies of pioglitazone, austroinulin (the lead compound) and rosiglitazone is believed to be as a result of the variations in the amino acids interactions most likely the hydrogen bond

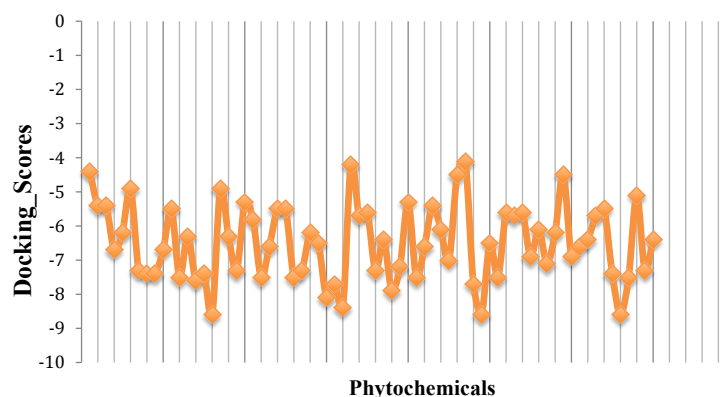


Figure 1. Chart displaying the virtual high throughput screening's docking scores of phytochemicals from *Stevia rebaudiana*.

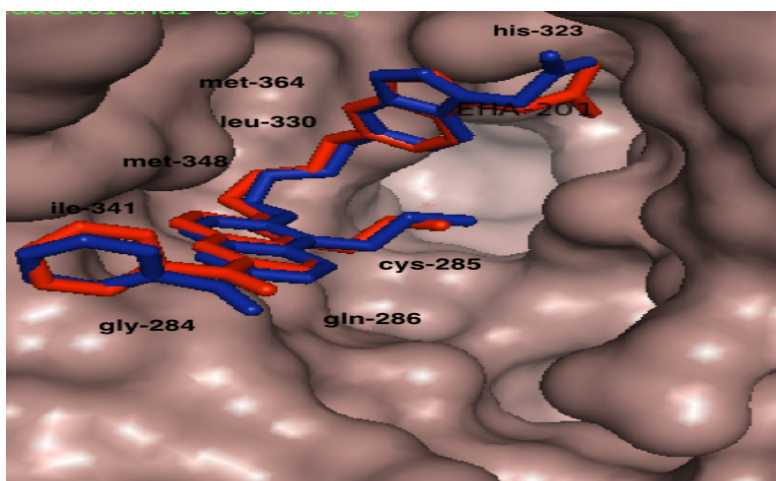


Figure 2. Validation of docking: Comparability of the re-docked binding mode (red) and the co-crystallized pose (blue) of Indol-1-yl Acetic acid with the accompany residues of PPAR-gamma binding pocket.

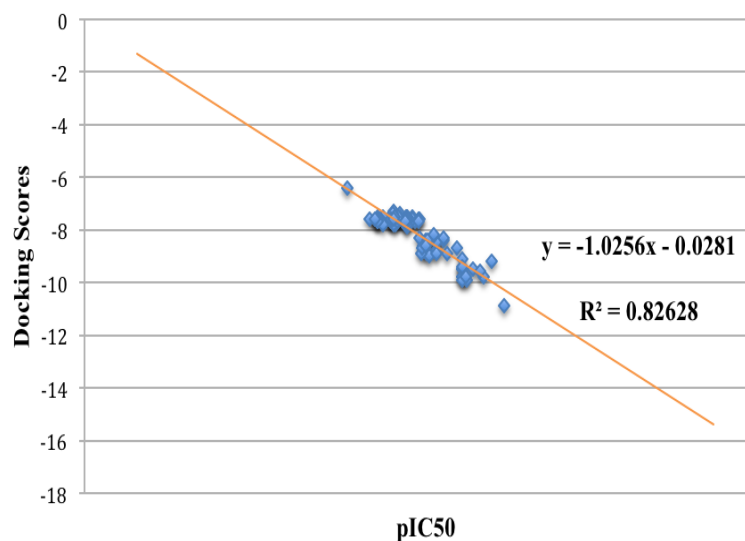


Figure 3. Correlation coefficient graph of docking scores of various agonists of the peroxisome proliferator-activated receptor (PPAR γ) and their corresponding experimental pIC_{50} values. The agonists (compounds) and their corresponding pIC_{50} (experimentally derived IC_{50}) were downloaded from the ChemBL database, the strong correlation (0.83) between the docking scores and pIC_{50} shows that computer can reproduce experimental values and this gives credence to the docking scores generated, in the present study.

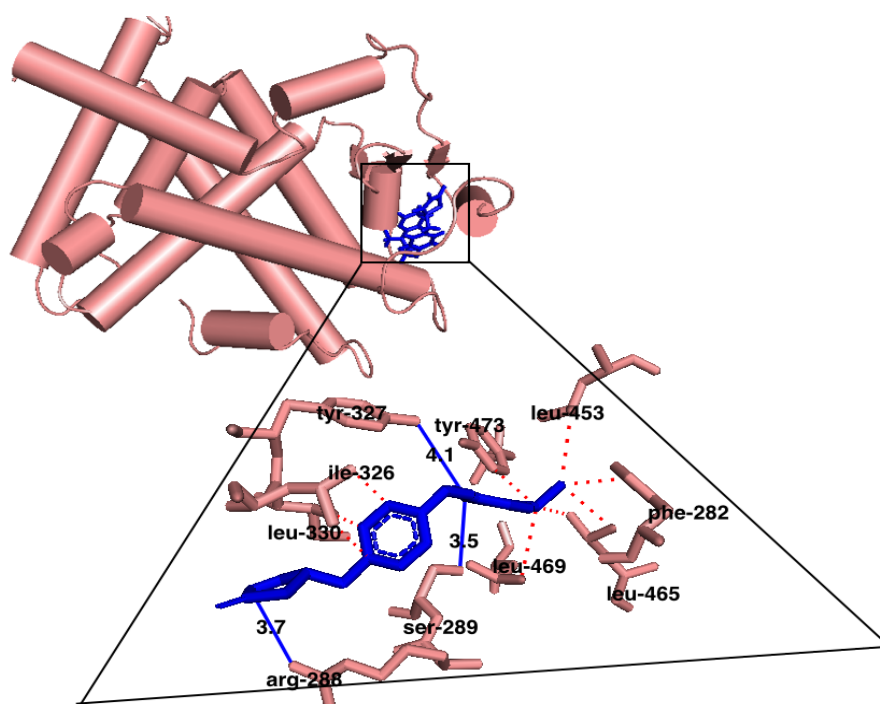


Figure 4. 3D dimensional structure of the interactions and binding pose of pioglitazone (blue stick) with key residues (salmon red) within the 4Å of the PPAR γ binding pocket; the blue lines between the residues and pioglitazone indicate hydrogen bonds while the dotted lines (red) represent hydrophobic interactions.

Table 3: Showing the docking scores of the six (6) lead phytochemicals from *Stevia rebaudiana*.

S/N	Phytochemicals	Docking scores
1	Austroinulin	-8.1
2	Caryophyllene_Oxide	-8
3	Jhanol	-8
4	Steviol	-8
5	Catechin	7.9
6	Cyclohexadecane	-7.9

interactions of the three compounds with critical residues within the binding pocket of the PPAR γ ; Pioglitazone with three (3) hydrogen bonds, austroinulin with two (2) hydrogen bonds and pioglitazone with only one (1) hydrogen bond.

Natural PPAR γ agonists have different binding modes (Figure 7) when compared with the thiazolidinedione agonists, and these may be associated with the reduced side effects of natural agonists of PPAR γ (Wang *et al.*, 2014).

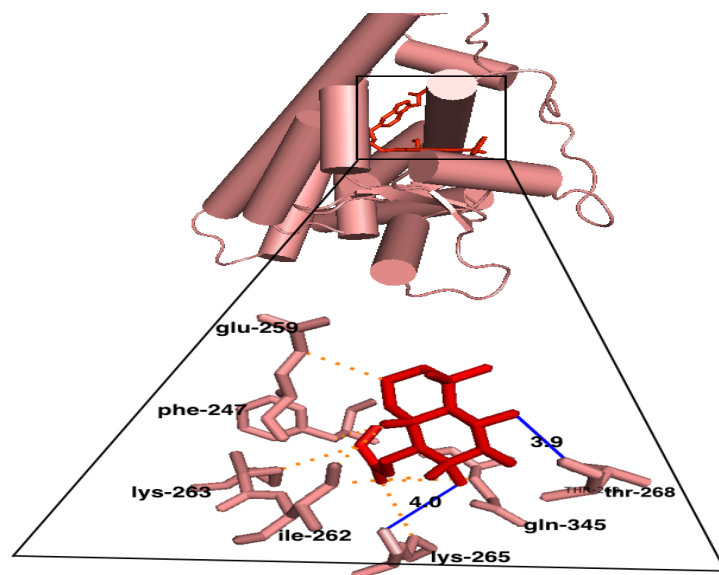


Figure 5. 3D dimensional structure of the interactions and binding pose of austroinulin (red stick) with key residues (salmon red) within the 4Å of the PPAR γ binding pocket; the blue lines between the residues and austroinulin indicate hydrogen bonds while the dotted lines (magenta pink) represent hydrophobic interactions. 3D dimensional structure of the interactions and binding pose of pioglitazone (blue stick) with key residues (salmon red) within the 4Å of the PPAR γ binding pocket; the blue lines between the residues and pioglitazone indicate hydrogen bonds while the dotted lines (red) represent hydrophobic interactions.

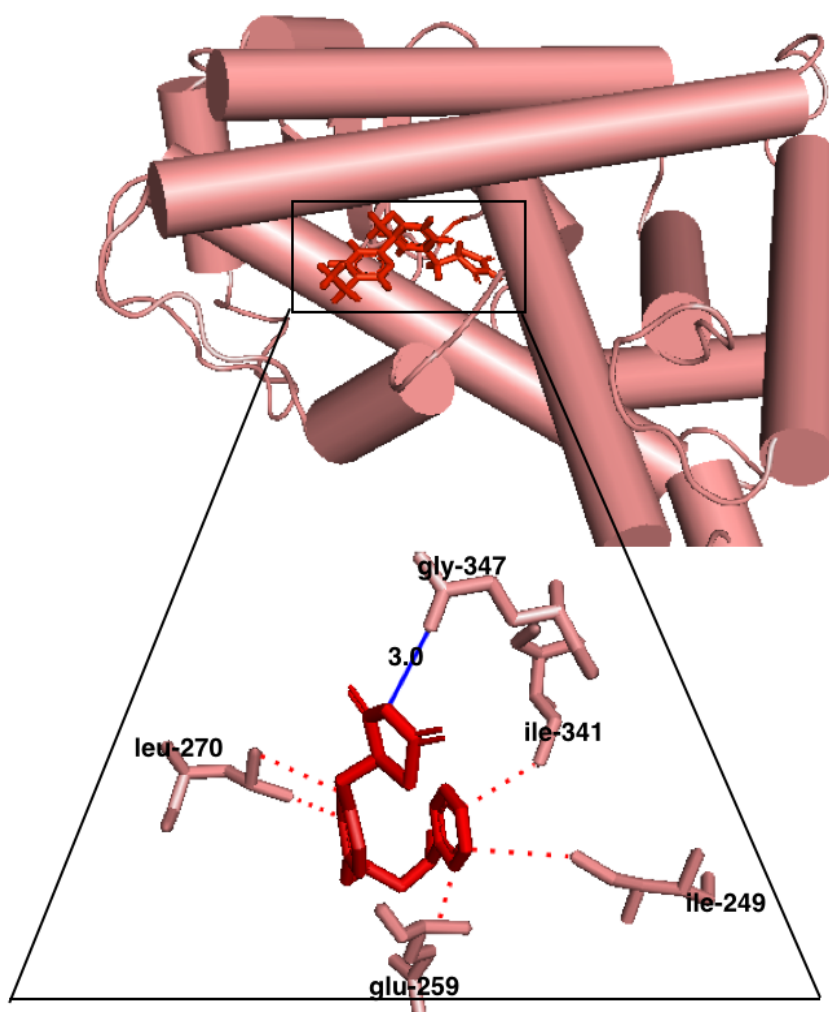


Figure 6. 3D dimensional structure of the interactions and binding pose of rosiglitazone (red stick) with key residues (salmon red) within the 4Å of the PPAR γ binding pocket; the blue line between the residue and rosiglitazone indicate hydrogen bond while the dotted lines (red) represent hydrophobic interactions.

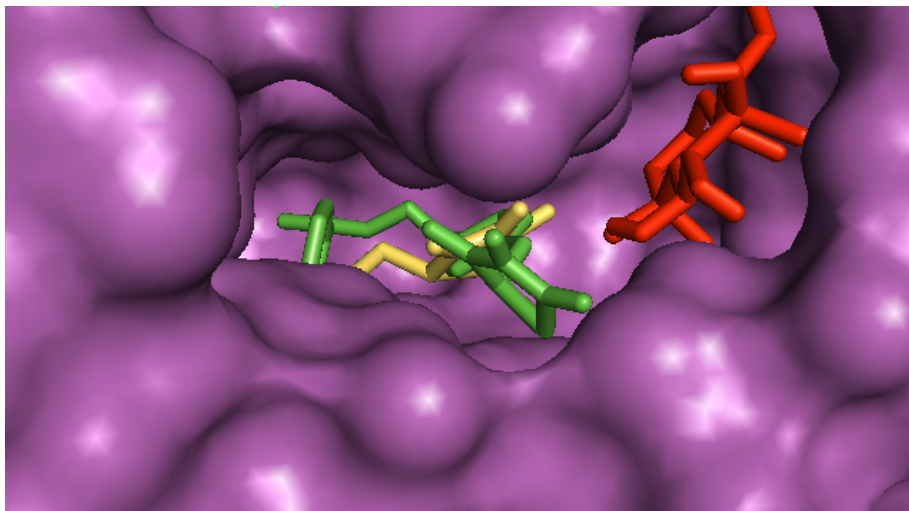


Figure 7. Binding mode (Orientations) of austroinulin (red stick), pioglitazone (yellow stick) and rosiglitazone (green) within the PPAR γ binding pocket. Natural PPAR γ ligands have been reported to have different binding modes to the receptor in comparison to the full thiazolidinedione agonists, as shown above.

Conclusion

There have been few or little reports in the literatures, on the active constituents of numerous medicinal plants extracts indicated to be activators of the PPAR γ . Austroinulin from *Stevia rebaudiana*, bear a good potential as a possible agonist of the PPAR γ , hence, it needs to be further investigated both *in-vitro* and *in-vivo*.

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*Correspondence to:

Metibemu DS
Centre for Biocomputing and Drug Development
Adekunle Ajasin University
Akungba-Akoko
Ondo State
Nigeria
E-mail: damilohun.metibemu@aaua.edu.ng.