



## Binding fingerprint of rubiadin derived from *Morinda lucida* with lysophosphatidic acid receptors.

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### Abstract

#### Keywords:

Fingerprint, antagonist, bioinformatics, receptors, inhibiting, synthetic.

*Molecular binding fingerprint of Morinda lucida derived compound - rubiadin, with lysophosphatidic acid receptor one to six, to proffer solution to various diseases caused by lysophosphatidic acid receptors. Activities of the M. lucida derivative with lysophosphatidic acid receptors were determined using bioinformatics analysis. The results gotten were compared with the standard antagonists currently in use according to binding affinity to receptors. Rubiadin showed higher binding free energy with the receptors than the standard antagonists. This finding will proffer a new way of inhibiting the activities of lysophosphatidic acid receptors by using derived compound from M. lucida rather than synthetic compounds that are often characterized with side effects.*

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### INTRODUCTION

In Africa, people use natural resources for medical purposes. *Morinda lucida* is a medicinal plant found in Tropical Africa to treat many disorders. *Morinda lucida* belonging to the family *Rubiaceae* is a tropical rainforest tree with the English name “Brimstone tree”. It is also known as Sangogo or Bondoukoualongua (in Cote d’Ivoire), Twi, Konkroma or Ewe amake (in Ghana), Ewe amake or Atakake (in Togo) and Oruwo or Ruwo amongst the Yoruba tribe (South-West, Nigeria) and Huka or Ezeogu amongst the Igbo speaking tribe of South-East Nigeria (Adeneye, 2013). It is a medium size tree about 15-meter tall with scaly grey bark, short crooked branches and shining foliage. In West Africa, *Morinda lucida* is an important plant in traditional

medicine. In Nigeria, *Morinda lucida* is one of the commonly used plants in the preparation of traditional medicines against fever. The leaves are used as “oral teas”, which are usually taken orally for the traditional treatment of malaria, and as a general febrifuge, analgesic, laxative and anti-infections. The major constituents of *M. lucida* extract are the various types of alkaloids, anthraquinones and anthraquinols. Two compounds (oruwalol and oruwal) and anthraquinone have been isolated and characterized from the stem of the plant (Adewumi and Adesogan, 1984). In South-West Nigeria, fresh leaves of the plant are macerated in fresh palm wine and the filtrate taken orally for blood sugar control in suspected diabetic patients (Adeneye, 2013). The leaves have also been reported to possess strong hypoglycemia, trypanocidal and

aortic vasorelaxant activities (Asuzu and Chineme, 1990).

Further studies have shown that leaf and stem bark of *M. lucida* possess anticancer (Sowemimo *et al.*, 2007), hepatoprotective (Oduola *et al.*, 2010), antispermatogenic (Raji *et al.*, 2005), and antidiabetic activity (Daziel, 1973).

Chemical compounds include ursolic acid (Rao and Rao, 1983), polyphenolic compounds such as quercetin, kaempferol-3-O-rutinoside (3), acacetin-7-O- $\beta$ -D glucopyranoside and apigenin-5,7-dimethylether, asperuloside acid, rubiadin, damnacanthal, 9,10-anthraquinone, asperuloside tetraacetate and 4'-galactoside have been reported to be isolated from the leaves of *Morinda lucida* (Subramanian and Nair, 1971). The Larvicidal activity has been studied by (Bagavan *et al.*, 2008). Crude protein and crude fibre contents of the leaves ranged from 5.07 to 23.93% and from 6.37 to 30.24%, respectively. Leaves are generally rich in Ca, respective ranges for Fe, Cu, Mn, and Zn contents were 20.20 - 79.28%, 2.67 - 12.67, 0.15 - 7.20 and 6.0 - 45.0 ppm (Desai *et al.*, 1980). Lysophosphatidic acid (LPA), once thought to be an inert metabolite in the biosynthesis of membrane phospholipids, is now well-recognized as an important signaling molecule. Acting through G protein-coupled receptors (GPCRs), LPA alters many different cellular responses, such as proliferation, survival of cytoskeletal changes, calcium influx and much more (Fukushima *et al.*, 2001). In the past decades, numerous studies indicated that LPA could serve as a signaling molecule. The primary molecular mechanism was reported in 1996 with the cloning of the first cognate receptor for LPA (Hecht *et al.*, 1996). The receptor, now called LPA1, is a GPCR that couples to heterotrimeric G proteins (Gi, Gq, G12/13 alpha subunits) and can elicit multiple cellular responses upon LPA stimulation (Fukushima *et al.*, 2001). Based on sequence similarity, two other LPA receptors were soon

identified: LPA2 and LPA3 (Bandoh *et al.*, 1999). Recently, two more distantly LPA4 and LPA5/GPR92 were identified (Lee *et al.*, 2006). LPA4 is more closely related to purinergic receptors while sharing only 20–24% amino acid identity with LPA1-3 (Noguchi *et al.*, 2003) related GPCRs have been shown to respond specifically to LPA, LPA4/P2Y9/GPR23.

LPA5 was identified using reverse transfection screening and shares about 35% identity with LPA4 (Kotarsky *et al.*, 2006). These receptors are encoded by distinct genes that are referred to as *LPAR1-5* (in humans) and *Lpar1-5* (MGI-Mouse Genome information). Two additional receptors, GPR87 and P2Y5, have been proposed to be new LPA receptors (Tabata *et al.*, 2007), however, further validation of these identities is required.

## MATERIALS AND METHOD

### Selection and Preparation of Macromolecule

The primary amino acid sequence of each of the five proteins were downloaded from the PubChem database and uploaded to the Swiss Model Server (<http://swissmodel.expasy.org>) where the homology modeling of the five proteins was done. The accession numbers of each protein were noted. The modeled proteins were downloaded and saved in pdb format. The 3D structure of the proteins was modeled using the co-ordinate file of template from protein data bank (PDB ID: 4GQR).

### Selection and Preparation of Rubiadin

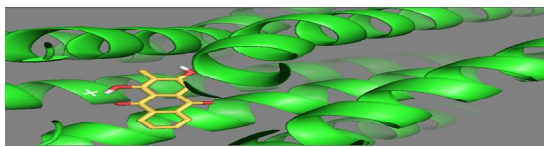
The three-dimensional (3D) structures of rubiadin was obtained from the pubchem database (Sayers *et al.*, 2011). The optimized ligand molecules were docked into the lysophosphatidic acid receptor proteins model using “Ligand Fit” in the AutoDock 4.2.

### Molecular Docking

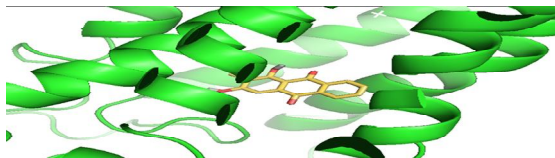
The docking of rubiadin to the binding site of lysophosphatidic acid receptor proteins was done using the autodock vina 4.2 (Morris *et al.*, 2009). The protein was treated as a rigid body (Chitranshi *et al.*, 2013), while the rotatable bonds of the ligands were set to be free. The grid box size was set at 13.44, 15.14 and 39.44 Å<sup>o</sup> (x, y, and z) to include all the amino acid residues. The spacing between grid points was 0.375 angstroms.

### RESULTS AND DISCUSSION

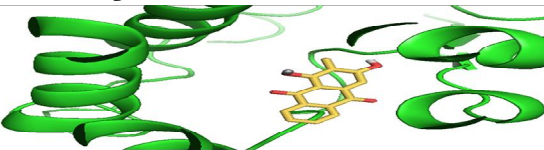
The figures below showed the binding signatures of rubiadin from *Morida lucida* and standard drugs with the pockets of the lysophosphatidic acids receptor protein.



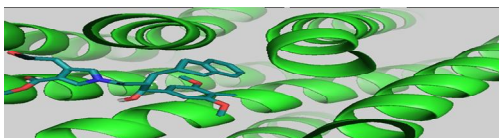
: Binding site of receptor 1 with Rubiadin image



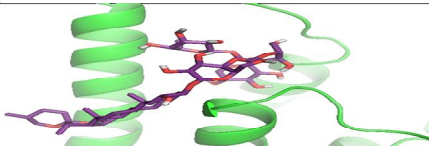
Binding site of lysophosphatidic acid receptor 3 with Rubiadin docked image.



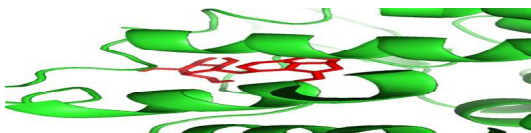
Docked image of Lysophosphatidic acid receptor 5 and Rubiadin



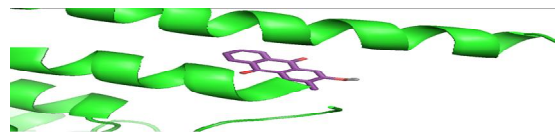
Binding site of receptor 1 and crystallized ligand docked image.



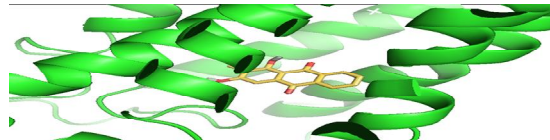
Binding site of receptor 3 with Diocetyl glycerol phosphate (standard) docked image



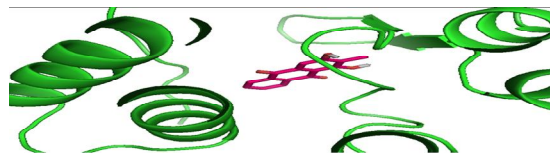
:Docked image of Lysophosphatidic acid receptor 5 and Farnesyl (Standard)



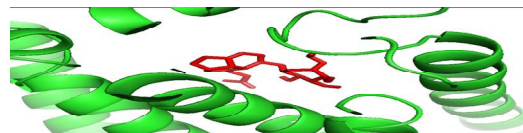
docked image showing binding affinity of Lysophosphatidic acid receptor 2 with Rubiadin.



Docked image of lysophosphatidic acid receptor 4 with rubiadin



Lysophosphatidic acid 6 receptor and Rubiadin docked image.



Lysophosphatidic acid receptor 2 with Ki16425 binding site image.

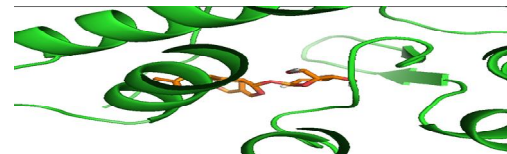


Image showing the binding site of Gintonin with LPA6.

Table 1: Showing maximum binding free energy of rubiadin with LPA 1 to 6

Ligand	Modes of conformations generated with an RMSD 0.000 binding affinity.					
	LPA1	LPA2	LPA3	LPA4	LPA5	LPA6
Rubiadin	-8.8	-6.5	-8.7	-9.1	-9.4	-7.9
	Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol

Table 2: Showing maximum binding free energy of each ligand with LPA 1 to 6

Ligands (Standard)	Receptor	RSMD
Co-crystallised	LPA1	-9.7Kcal/mol
Ki16423	LPA2	-6.4Kcal/mol
DiocetylGlycerolphosphate	LPA3	-7.7Kcal/mol
Alpha bromomethylene phosphate	LPA4	-6.7Kcal/mol
Farnesyl	LPA5	-8.6Kcal/mol
Gitonin	LPA6	-7.7Kcal/mol

## DISCUSSION

Phospholipid mediator, compound known to be lysophosphatidic acid receptor (LPA) elicits a host of biological effect including cell proliferation (Tigyi *et al.*, 2003). LPA causes series of these responses in extracellular space through G protein– coupled receptors that belong either to the endothelial differentiation gene family (EDG): EDG2/LPA 1, EDG4/LPA2, EDG7/LPA3 or to a subcluster in the purinergic GPCR family p2y9/LPA4, GPCR5/LPA5 and GPR6/LPA6 (Tabata *et al.*, 2007). Talking of the cardiovascular diseases and atherosclerosis LPA receptors are the culprits in these diseases because of their action on platelet, leucocytes and the different cell types of the arterial wall (Zhang *et al.*, 2004). LPA receptor has been shown to regulate cancer cell invasion, metastasis and resistance to both chemotherapeutic agents and radiation (Deng *et al.*, 2007). Due to these many therapeutically relevant effects to the LPA receptor, highly selective receptor antagonists which are important because of both

adverse and favorable effects of LPA receptors on the biological system.

Computational experiment was carried out on the receptors with *Morinda lucida* derived compound, because LPA receptors play specific role in the emanation of these diseases and their presence is difficult to assess because many cells express combination with LPA receptor (Mill and Moolenaar, 2003). The results above showed whether the derived compound of *Morinda lucida* is better than the chemicals currently being used as antagonist.

Tables 1 and 2 compared the results of crystallized standards (-1-4[2, 3R)-2-2, 3-dihydroxy-1H-inden-2-ylloxy-3(-3,5-dimethoxy-4) and rubiadin interaction on to the binding pocket of the lysophosphatidic acid receptor 1. The highest binding energy was shown by crystallized standard -9.7Kcal and Rubiadin 8.8Kcal/mol. Though, the crystallized standard has

highest affinity for the receptor but the ligand from the *Morinda lucida* can also serve as antagonist for the lysophosphatidic acid receptor 1 as co-crystallized compound because they both have high binding energy.

In lysophosphatidic acid receptor 2, the results of the standard ligand, Ki16425 ki16426 (3-(-4-[-4-(1-2chlorophenyl)ethoxy]carbonyl amino)-3methyl-5-isoxazolyl]benzysulfanyl) interaction on the binding pocket of the lysophosphatidic acid receptor 2 with a binding energy of -6.4Kcal/mol. and the highest binding energy was shown by rubiadin -6.5Kcal/mol. Ki16426 (3-(-4-[-4-(1-2chlorophenyl)ethoxy]carbonyl amino)-3methyl-5-isoxazolyl]benzysulfanyl) is a derivative of isoxazole, it inhibits the LPA2 induced Ca<sup>++</sup> response in THP-1 cell and also inhibits long term DNA synthesis and cell migration as induced by LPA2 in fibroblast (Mill and Moolenar, 2003). The results showed that the rubiadin could be a suitable antagonist for the receptor than the standard because of the high free energy produced by the ligand with LPA receptor 2.

Results of the dioctyl glycerol phosphate (standard) interaction on the binding pocket of the lysophosphatidic acid receptor 3 showed a binding affinity of -7.7Kcal/mol to the receptor while rubiadin showed a binding affinity of 8.7Kcal/mol. From the results obtained, rubiadin could be a preferred inhibitor for the receptor than the standard because of the high free energy it produced with the receptor than the dioctyl glycerol phosphate.

The results of rubiadin and the alpha bromomethylene phosphate (standard) binding fingerprint on the binding pocket of the lysophosphatidic acid receptor 4 showed their maximum binding affinities to the receptor. The highest binding energy was shown by rubiadin (-9.1 Kcal/mol) compared to alpha bromomethylene phosphate (6.7Kcal/mol). The results gotten indicate that the ligand rubiadin could

be a preferred antagonist for the receptor than the standard because of the high free binding energy produced with the receptor more than alpha bromomethylene phosphate standard.

Binding free energy of the lysophosphatidic acid receptor 5 was analysed and shown on the tables above. The results of rubiadin and farnesyldiphosphate (standard) interactions on the binding pocket of the lysophosphatidic acid 5 receptor, and their maximum binding affinities to the receptor also shown that rubiadin (-9.4Kcal/mol) exhibited a higher binding energy more than the standard ligand-farnesyldiphosphate with a binding energy of -8.6 Kcal/mol. The results showed that the ligand- rubiadin is a suitable antagonist for the receptor 5 than the standard because of the high free energy it produced with the receptor more than farnesyldiphosphate.

The results of rubiadin and the gitonin (standard) interactions on the binding pocket of the lysophosphatidic acid 6 receptor was shown on the tables above with their maximum binding affinities to the receptor 6. Gitonin, the standard, contains protein with a high hydrophobic, acidic amino acids, fatty acid in ester form and glucose, these features allow gitonin to bind LPA6. Meanwhile, the highest binding energy of the ligand to the receptor 6 was shown by rubiadin (-7.9Kcal/mol) relative to gitonin (7.7Kcal/mol). Therefore, the results gotten showed that rubiadin could be a suitable inhibitor for the receptor 6 than the standard because of the high free energy it produced with the receptor 6 more than gitonin (standard).

## CONCLUSION

Based on the present in silico studies, phytochemical of *Morinda lucida* – rubiadin could be considered for clinical trials as antagonist of lysophosphatidic acid receptors 1-6. Further investigation to elucidate the

biosafety of the ligands is in progress by using in-silico means to determine the toxicity or carcinogenicity of the phytochemical.

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