Journal of Research in Pharmacy

Research Article

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Inhibition of pancreatic cancer via LPAR4 receptor with a *de novo* drug complex design using theoretical organic chemistry: Comprehensive molecular docking, molecular dynamics

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Received: 24 January 2024 / Revised: 19 February 2024 / Accepted: 22 February 2024

ABSTRACT: The present work relates to a *de novo* organic chemistry involved drug design and repurposing discovery of a Quercetin and Ascorbic Acid complex formation with the IUPAC nomenclature of ''3-((2S)-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethoxy)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-one'' to suppress pancreatic cancer via the inhibition of LPAR4 receptor. This was achieved with molecular docking and molecular dynamics studies and found that Ascorbic Acid is docking manoeuvre assistant for Quercetin to form Hydrogen bonds and Covalent bonds to shut down LPAR4 receptor with excellent inhibition constant. This study may very well lead to further *in vitro* organic synthesis, characterization and cell line results and *in vivo/ex ovo* animal testing for etherical bound Quercetin and Ascorbic Acid complex.

KEYWORDS: Pancreatic Cancer; LPAR4; Quercetin; Ascorbic Acid; Vitamin C; Molecular Docking; Molecular Dynamics; *in silico* drug design and repurposing.

1. INTRODUCTION

Pancreatic Cancer is responsible for 3.3% of newly reported cancers and 8.3% of cancer-related deaths by 2023, according to National Cancer Institute data [1]. The 5-year relative survival rate in Pancreatic Cancer patients is 12.5% and the course of the disease progresses asymptomatically until it reaches high grades [2]. Flavonoids are secondary plant metabolite phytochemicals and are classified according to their various chemical properties and structures; flavanols, flavones, flavanones, flavanols, flavanonols, isoflavones, and chalcones [3]. During the process, studies on cancer were carried out based on the fact that flavonoids are of plant origin and that there are various types of them. As a result of the studies, it was concluded that flavonoids may have anti-cancer, anti-metastatic, and apoptotic effects [4,5]. Quercetins (3,3^0,4^0,5,7-pentahydroxyflavones) are natural antioxidants found in many foods and plants as anti-oxidative flavonoids (Figure 1) [6,7]. In studies on Quercetins, the ways in which they have an anti-cancer effect have been examined and it has been shown to be effective in areas such as downregulation of mutant p53 protein, inhibition of various proteins expressed at high levels in cancer, and suppression of cell proliferation [8,9]. Ascorbic acid (AA, AscH2) is a ketolactone with two ionizable hydroxyl groups and known as vitamin C (Figure 2) [10]. Literature studies have shown that high doses of vitamin C have the capability to target cancer cells by regulating HIF1α and have other abilities that may suppress cancer growth like ROS generation, epigenetic modulation, and most importantly, immunomodulation [11,12].

How to cite this article: Agar S, Akkurt B, Arasan Y, Ulukaya E. Inhibition of pancreatic cancer via lpar4 receptor with a de novo drug complex design using organic chemistry: Comprehensive molecular docking, molecular dynamics. J Res Pharm. 2024; 28(4): 1033-1040.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.785

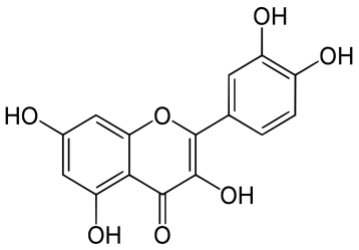

Figure 1. Quercetin

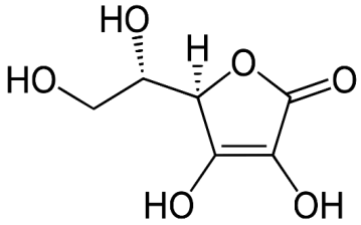

Figure 2. Ascorbic Acid

Lysophosphatidic acids (LPAs) are simple natural phospholipids and are formed by the combination of a fatty acyl chain, glycerol backbone, and free phosphate group [13,14]. The LPA family is effective in significant events such as cell cycle maintenance and cell proliferation. Lysophosphatidic acids are also responsible for cell differentiation, cell death inhibition, cell migration, and invasion [15]. Changes in LPA metabolism have been observed in various progressive studies related to cancer [16,17]. LPA interact with cells through LPARs, to exert their biological effects [18]. LPA can activate any of its specific receptors (LPAR1-6) on the plasma membrane, and these receptors interact with G proteins, β-arrestins, and different membrane receptors to transmit signals that increase cell proliferation and survival [19]. Being one of the LPA receptors, recent studies on LPAR4 have shown that this receptor has an ability to prepare the environment for tumor initiation in Pancreatic Cancer [20,21]. LPAR4 is an upregulated transmembrane receptor that functions as an adaptive response to stress that overcome solitary growth conditions come across during pancreatic tumor constitution [22].

Identifying the causes that trigger tumor formation may open ways to manage cancer progression and treatments. In this context, the increase in lysophosphatidic acid receptor 4 (LPAR4) expression exhibited by Pancreatic Cancer cells when exposed to environmental stress or chemotherapy needs to be addressed, as this high level of LPAR4 is associated with increased stress tolerance, resistance to drugs, self-renewal capacity and initiation of tumors in Pancreatic Cancer. With the drug design and repurposing approach, which is based on the principle of directing an existing drug to a different target to reduce time and cost in drug discovery; we aim to ensure that *de novo* designed complex of Ascorbic acid and Quercetin has inhibitory properties on LPAR4. An etheric complex consisting of Quercetin and Ascorbic acid (IUPAC nomenclature 3-((2S)-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethoxy))-2-(3,4-dihydroxy-phenyl)-3,5,7-trihydroxychroman-4-one) plays a pivotal role in the inhibition and shutdown of LPAR4 with minimal dosage. This work will shed light on authentic chemical analogs that can be derived from this point, where varying cofactors and organic functional groups can be studied both *in silico* and *in vitro* to collect more data and results for the development of drug discovery and production.

2. RESULTS and DISCUSSION

2.1 Molecular docking/dynamic analyses of the designed complex

As can be seen in Figure 3, the organic chemical structure plays a crucial role in the suppression of LPAR4 transmembrane receptor which plays a key role in Pancreatic Cancer. Ascorbic Acid, also known as Vitamin C, creates an increased affinity towards the groove between the α helical stacks of LPAR4 so that Quercetin can dock and shutdown LPAR4.

LPAR4 SUPPRESSOR:

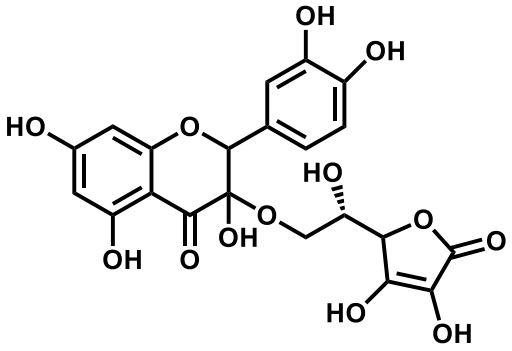

Figure 3. The chemical structure of LPAR4 Suppressor Drug: 3-((2S)-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethoxy)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-one.

Ascorbic Acid does the strong Hydrogen bonding and manoeuvres the rest of the etheric bound Quercetin into the groove of the receptor where it can form a covalent bond to irreversibly suppress LPAR4 and choke up the molecular entrance of this transmembrane protein. Such a reaction can be seen in Figure 4 with alternative poses taken from the Molecular Dynamics Simulations after the simulations reach and pass the equilibrium state of 50 nanoseconds.

Figure 5 illustrates an interesting aspect of the newly formed complex, the ligand drug (Q.A. LPAR4.Sup = Quercetin - Ascorbic Acid Complex LPAR4 Suppressor) with the nomenclature of 3-((2S)-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethoxy)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-one since, within its ^{1}H NMR Spectrum, for the purpose of wet-lab organic synthesis in the future, the molecule will be easy to be synthesized depending on its choice of design. It has a very specific Hydroxyl group in its furan ring reaching up to 16.77 ppm where after any organic wet-lab synthesis and purification, such characterization will be easier to be made. This is a cornerstone for such a molecule design, makes it unique for *in vitro* wet-lab analyses as well as its good results within *in silico* studies.

2.2 Covalent and Hydrogen bonding analysis

In Table 1, the docking energy of -17.11 kcal/mol Δ(ΔG) energy is significantly a good energy. However, the point that should be emphasized remarkably is the inhibition constant value of 1.18 μM and it is a tremendous value for defining the minimum dosage use for maximum effectivity. It can be estimated for the cell line, in vitro, and in vivo analyses that with quite tiny minimal dosages, such good docking results can occur with this chemical complex structure. Depending on this such findings, many improvements can be made to this molecule since it's a promising star among many other drug molecules.

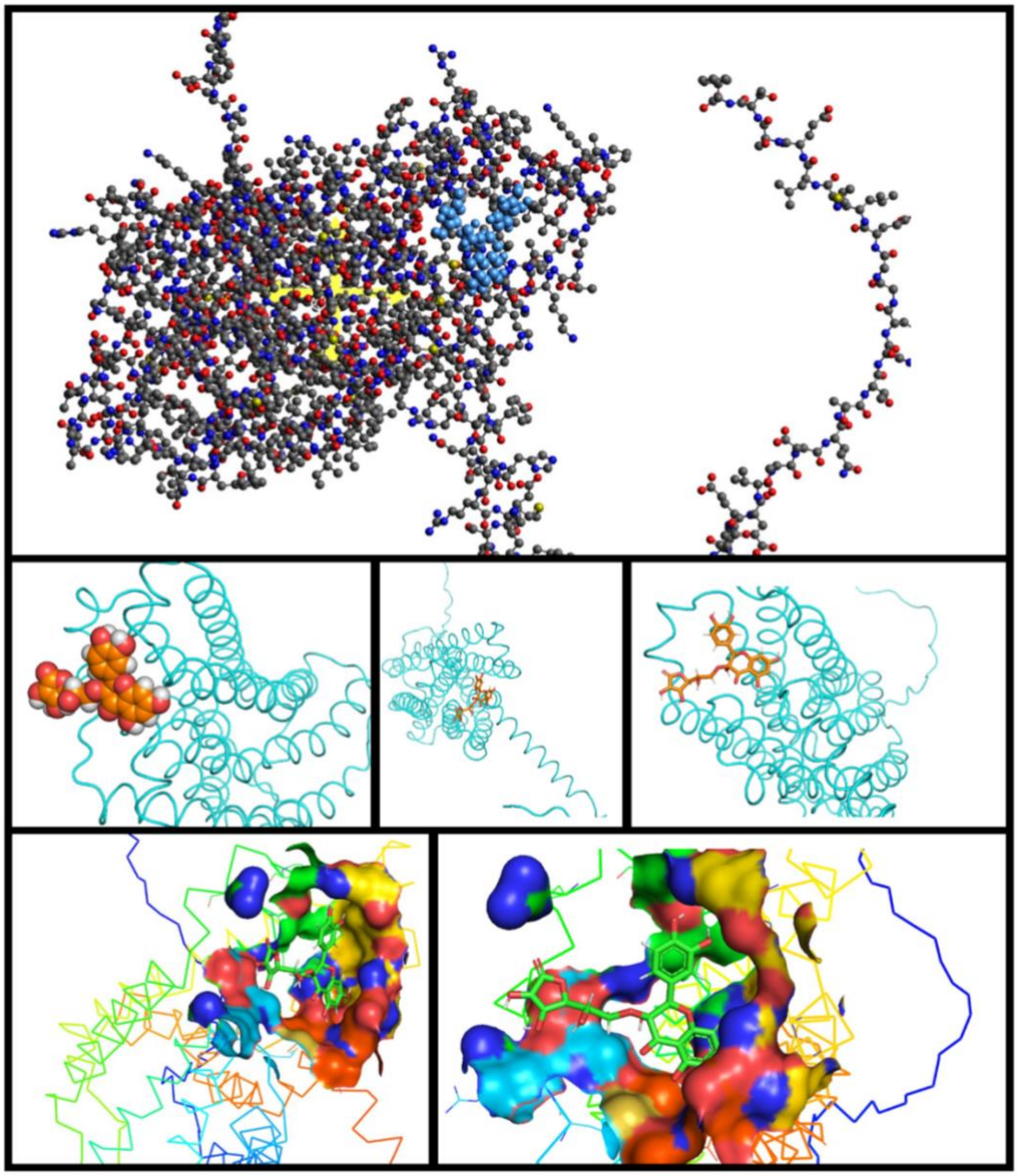

Figure 4. Suppressed poses of LPAR 4 by the drug, taken by the Molecular Dynamics Simulations under pH 5.5, OPLS 3.0 Forcefield.

Table 1. The binding energy (kcal/mol) and inhibition constant (millimolar) values of the best docked pose.

Feature	Value
Δ(ΔG) Covalent bond	-190.85 kcal/mol
Δ(ΔG) Hydrogen bond	-17.11 kcal/mol
Ligand efficiency	-0.12 kcal/mol/heavy atom
Inhibition constant (K_I)	1.18 μM
Electrostatic energy	-0.18 Joule
Total internal energy	-3.31 Joule

After the molecular dynamics simulations, it was observed that the Pyranone group derivative of Quercetin side of this such whole complex made also an irreversible covalent binding of -190.85 kcal/mol (C=O binding) with LPAR4 on top of Quercetin's Hydrogen bonding of -5.52 kcal/mol.

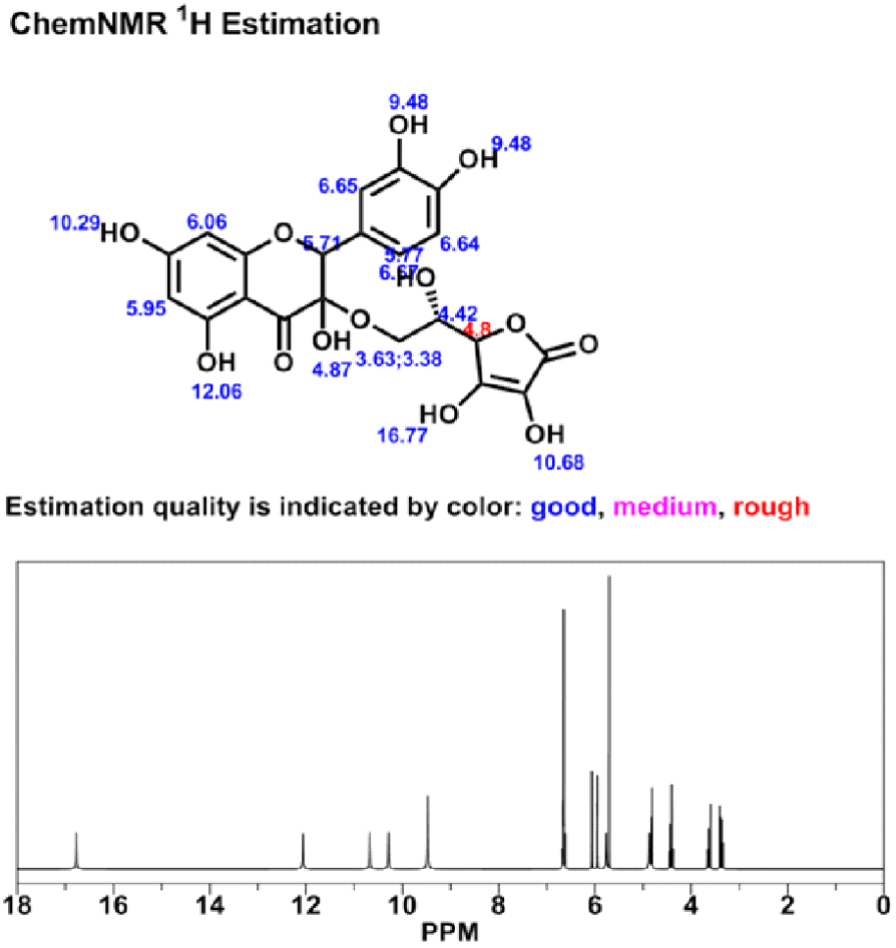

Figure 5. ¹H NMR Spectrum of LPAR4 Suppressor Complex.

3. CONCLUSION

The current research focuses on the *de novo* design of organic compounds for drug development and repurposing, specifically discovering a complex formed by Quercetin and Ascorbic Acid. The objective was to suppress Pancreatic Cancer by inhibiting the LPAR4 receptor. Molecular docking and molecular dynamics studies were employed, revealing that Ascorbic Acid assists in the docking maneuvers of Quercetin, forming hydrogen bonds and covalent bonds. This interaction effectively shuts down the LPAR4 receptor, demonstrating an excellent inhibition constant. The findings suggest the potential for further in vitro exploration, including organic synthesis, characterization, cell line results, and subsequent in vivo/ex ovo animal testing for the Quercetin and Ascorbic Acid complex with promising therapeutic implications.

4. MATERIALS and METHODS

4.1. Geometric Oprtimization

The chosen molecules, along with the LPAR4 structure and their respective stable molecular configurations, underwent processing using density functional theory (DFT)/B3LYP functionality through the Gaussian 09 Program, employing the 6-31G(d,p) basis set (23). For subsequent steps, files for molecular docking and dynamic studies were prepared using Gaussview 6.0 and Avogadro software tools [24,25]. The gathered data were then analyzed and visualized using the PyMol molecular graphics program [26].

4.2. Molecular Docking

All of the molecular docking simulations in this paper were executed by using AutoDock Vina 1.1.2 [27]. Ranging from 50 posed simulations to 100 posed simulations, totaling 400 poses, where the drug Quercetin and Ascorbic Acid were drawn in Avogadro and etherical bound with organic chemistry knowledge to form a geometrically optimized organic product complex (a *de novo* structure) using Gaussview and Avogadro. Then this optimized complex was run illustrating the interaction and binding of the drug and the receptor LPAR4 with the AF-A0A6P6NGJ0-F1 id from the AlphaFold Database. The docking scores of all simulations were in kcal/mol as units which is the Gibbs free binding energy. The most precise and advantageous docking configurations, determined from the well-clustered data, were selected as the starting structures for molecular dynamics (MD) simulations for each drug. Docking scores were assessed in terms of kilocalories per mole (kcal/mol), relying on the Gibbs free energy of binding.

4.3. Molecular Dynamics

Utilizing Schrödinger's Desmond Software (2023.4 latest version), all the ligands were run for molecular dynamics (MD) with 50 nanoseconds, each comprising 5000 poses at 10 ps time intervals [28]. Each MD simulation was repeated three times with using particular seed numbers to ensure certainty of simulation parameters and protein-bound ligand complex structures. The aim of the MD simulations was to examine the dynamic properties of the ligand-receptor complex over time. The grid box dimensions were set at 110 × 110 × 110 $Å^3$. TIP3P-type water molecules were emplaced in the box and 0.15 M NaCl ions were incorporated to neutralize the system. Temperature and pressure parameters included NPT at 310 K with Nose-Hoover temperature coupling [29] and the constant pressure of 1.01 bar via Martyna Tobias-Klein pressure coupling [30]. System had no constraints and the initial velocity values were employed for forcefield calculations fitting for OPLS 3.0 standards.

Acknowledgements: This study was funded by Kocaeli Health and Technology University infrastructure, supercomputers and Assistant Professor Soykan Agar at the faculty of Pharmacy.
Author contributions: Concept – S.A.; Design – S.A.; Supervision – S.A., E.U.; Resources – S.A.; Materials – S.A.; Data Collection and/or Processing – S.A., E.U.; Analysis and/or Interpretation – S.A.; Literature Search – S.A., B.A., Y.A., E.U.; Writing – S.A., B.A., Y.A., E.U.; Critical Reviews – S.A., B.A., E.U.
Conflict of interest statement: The authors declared no conflict of interest.

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