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Full Length Research Paper

Highlighting of a New Flavonol Derivatives as a Potent Antihypertensive Compound using Molecular Docking

El Hassen Mokrani*, Yasmine Krid, Abderrahmane Bensegueni, Abdelouahab Chikhi, Soumia Teniou, Hanane Boucherit, Amina Merzoug, Khadidja Hioual
Applied Biology and Health Laboratory, Department of Biochemistry and Molecular and Cellular Biology, Faculty of Natural and Life Science, University Constantine 1 (Algeria)

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ABSTRACT

Angiotensin converting enzyme (ACE) is a key component in the renin angiotensin aldosterone system (RAAS) which regulates blood pressure. As the over expression of RAAS is associated with vascular hypertension, ACE inhibition has become a major target control for hypertension. The research on potential ACE inhibitors is expanding broadly and most are focused on natural product derivatives such as peptides, polyphenolics, and terpenes. Plant polyphenolics are antioxidant molecules with various beneficial pharmacological properties. This paper reports *in silico* molecular docking study of Angiotensin Converting enzyme inhibition by several flavonols. The aim of this study was to analyze the interactions between ACE and Flavonols with a view to identify new potent antihypertensive drugs. Binding modes have been studied using molecular docking AutoDock 4.2.5.1. By poly-substitution of a referred flavonol «compound 3». Binding energy was increased from -8.67 Kcal / mol to -13.37 Kcal / mol by introduction of the sulfone function on B2 position, méthylamine ($-CH_2-NH_3^+$) on S7 position and Amine function on positions : A7 and S8. Finally, biological potentialities of this proposed compound were checked by their pharmacokinetic properties and they showed no toxicity.

Key words: Angiotensin Converting Enzyme, AutoDock, inhibitor, Molecular docking

1. INTRODUCTION

Hypertension is a common progressive disorder leading to several chronic diseases such as cardiovascular disease, stroke, renal disease and diabetes. One-quarter of the world's adult population is afflicted by hypertension, and this is likely to increase to 29% by 2025 [1].
The pathogenesis of hypertension could be due to many reasons. For example, increased activity of renin angiotensin aldosterone system (RAAS), kalikerenin kinin system and sympathetic nervous system, and genetic influence are specified [2].
Among them over activation of RAAS (Fig. 1) is significant [3]. Angiotensin converting enzyme (ACE) plays a significant role in RAAS by converting the precursor angiotensin I into angiotensin II which is the peptide responsible in triggering blood pressure increasing mechanisms. Therefore, inhibition of ACE is a promising way of controlling over expression of RAAS.

*Corresponding author: mohsenmokrani@hotmail.fr

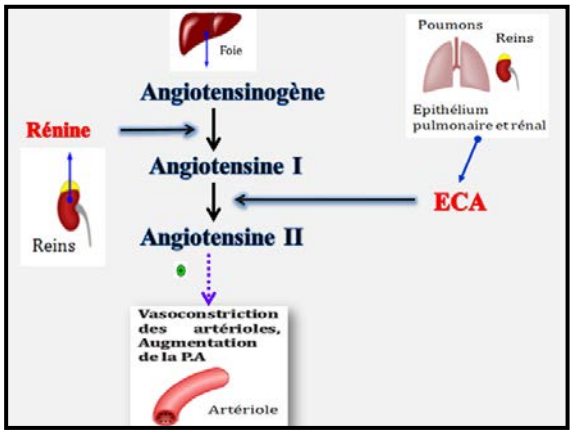


Fig 1: Renin angiotensin aldosterone system

ACE inhibitory drugs are first class therapeutics since decades. Captopril®, Lisinopril®, Enalpiril®, and Rampiril® are some examples for drugs targeted as ACE inhibitors. However, the prolonged use of these drugs could initiate adverse side effects like dizziness, coughing, and angioneuretic edema [4].

New alternatives have been explored extensively as replacements. Most of the researches have been targeted at bioactive compounds from natural resources. Peptides [5], anthocyanins [6], flavonols [7], triterpenes [8] are some examples. The objective of this review reports *in silico* molecular docking study of Angiotensin Converting enzyme inhibition by several flavonols in order to research new potent derivatives flavonols as ACE inhibitors.

2. MATERIAL

Molecular docking is a widely used computational tool for the study of molecular recognition. This new approach aims to simulate and predict the affinity of a very large number of ligands for the active site of a given therapeutic target, which is considerably easier to implement, cheaper, and faster than the use of experimental methods. Initiated in the beginning of 1980, this approach has developed to become an essential tool for bioactive molecules' research today [9].

2.1. Softwares and tools used

AutoDock 4.2.5.1

AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structures. It is a free software that has applications in:

- X-ray crystallography;
- structure-based drug design;
- lead optimization;
- virtual screening (HTS);
- combinatorial library design;
- protein-protein docking;
- chemical mechanism studies.

In our case, AutoDock was used for inhibitory mechanism studies of some flavonols in order to propose new potent derivatives flavonols as ACE inhibitors [10].

Mobyle@RPBS

Mobyle @ RPBS is a web portal for structural bioinformatics and chemoinformatics. This software is used to test the toxicity and other ADMET properties of inhibitors. It is maintained by University of Paris.

Molecular visualization

AutoDock tools 1.5.6 is a computer program for molecular graphics visualization. The 3D structure of ACE with their inhibitors was viewed with the help of molecular visualization software AutoDock tools [10].

Titan is the drawing tool of choice to create 3D structures of inhibitors in several formats such as *mol2, pdb, sdf, mol,* etc.

2.2. Databases

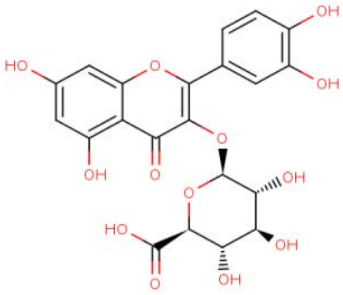
Protein Data Bank: The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by Xray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, can be accessed at no charge over the internet. The PDB is overseen by an organization called "Worldwide Protein Data Bank" (wwPDB)**.**

PubChem Compounds: PubChem is a database of chemical molecules. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely downloaded**.**

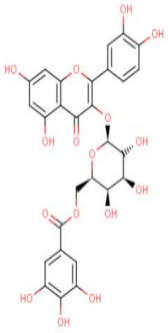
2.3. Data collection

Drug target: In this study, the Angiotensin Converting Enzyme is taken as a drug target. It is a dipeptidyl carboxypeptidase with a zinc atom that catalyzes conversion of the precursor angiotensin I into angiotensin II which is the peptide responsible in triggering blood pressure increasing mechanisms. The 3D structure of this enzyme was retrieved from Protein Data Bank (PDB ID of this enzyme is 3BKL).

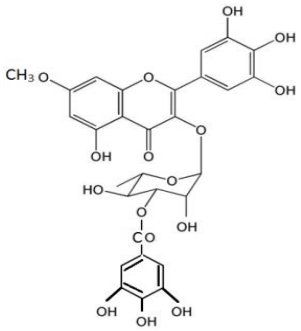
Inhibitors: Structures of 4 flavonols were constructed using Titan software and saved in *pdb* format. The structures of these compounds are presented in Fig. 2 [11].



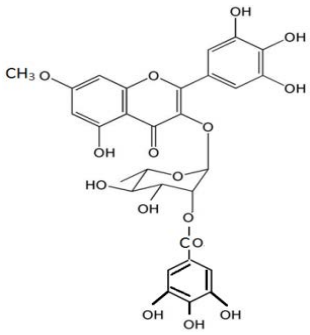
Compound 1



Compound 2



Compound 3



Compound 4

Fig. 2: Structures of 4 flavonols as ACE inhibitors.

3. METHODOLOGY

3.1. Docking

Docking is a term used for computational schemes that attempt to find the best matching between two molecules: a receptor and ligand. Angiotensin Converting Enzyme is docked with all the 4 flavonols inhibitors using AutoDock software. During the docking study, interactions between the active site of ACE and flavonols were simulated. The output file gave the energy values in Kcal/mol. This value was documented for every docked molecule.

3.2. ADME/Tox Screening

ADME/Tox is an acronym in pharmacokinetics and pharmacology for absorption, distribution, metabolism, excretion and toxicity which describes the disposition of a pharmaceutical compound within an organism. The five criteria all influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drug. The SMILES string for inhibitors were prepared using **Titan** software and were submitted to the Mobyle@rpbs server in order to test their ADME/Tox properties [12].

4. RESULTS AND DISCUSSIONS

Binding energies between ligand and receptor play the most crucial role in drug designing. In this work, the Angiotensin Converting Enzyme was selected as drug target and binding energies with 4 flavonols (inhibitors) were evaluated using AutoDock:

Compound	Binding energy (Kcal/mol)	IC_{50} (uM)	predicted Ki (uM)
1	-6.05	200	37.03
2	-6.50	160	17.19
3	-8.67	60.32	0.44
4	-7.34	151.32	4.14

Table 1: The energy value obtained by docking ACE with 4 flavonols.

In this study, it was observed that these compounds have greater affinity towards ACE to successfully inhibit this enzyme. Among these flavonols, compound 3 showed the highest docking score of -8.67 Kcal/mol.

4.1. Interactions between ACE's active site and compound 3.

Interesting interactions were observed between ACE's active site and compound 3 with a high binding energy (-8.67 Kcal/mol). Hydrophobic and hydrogen bonds play very important roles in inhibitory activity of this compound toward ACE, which is confirmed by molecular docking using AutoDock. As shown in Figure 3, compound 3 forms six hydrogen bonds with amino acids residues of ACE'S active site. The first is formed between oxygen of C7 of the inhibitor and amine function of Ala356. The second is formed between oxygen of inhibitor's cycle C and amine function of His385. The third is formed between amine function of His353 and oxygen of C3's inhibitor. The forth hydrogen bond is observed between carbon 4-bonded hydroxyl and amine function of Gln281. Finally, the carboxylic function of Glu376 forms 2 hydrogen bonds with carbons C4-C5-bonded hydroxyls. Compound 3 is also stabilized by hydrophobic interactions in the binding pocket, specifically with Val380, Asp456, Phe457, Thr282, Tyr523, His513 amino-acids residues.

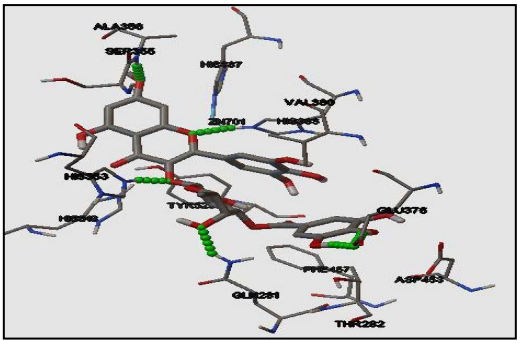


Fig. 3: Binding modes of compound 3 with the active site of ACE.

In the light of these results, compound 3 represents the best flavonol-inhibiting ACE. In order to research others compounds more potent towards ACE, we choose compound 3 as starting structure for the development of new potent flavonols derivatives inhibitors.

4.2. Design of new potent inhibitors

In the perspective of developing new potent ACE inhibitors, the structure of compound 3 was taken and several types of substitutions were carried. Specially, we have introduced new groups having ability to form hydrogen bonds such as hydroxyl (OH), carboxyl (COOH), amine (NH_2), Amide (CON), Carbonyl (C=O), Nitro (NHO_2), Nitroso (NHO), Phosphin oxyd (PH_3O), sulfon (SH_2O_2), sulfoxid (SH_2O). These groups were introduced on several positions (Fig 4).

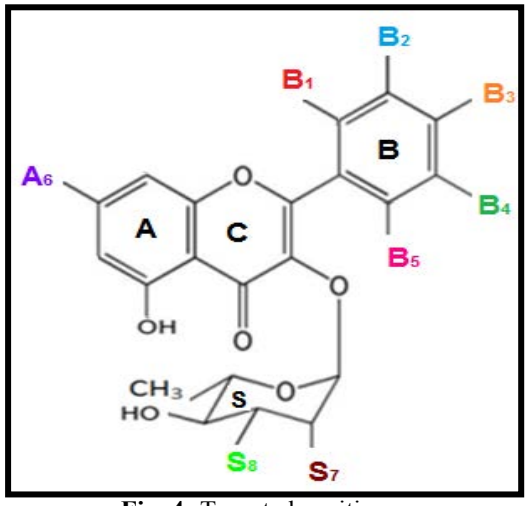
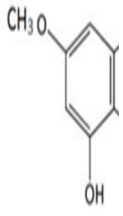
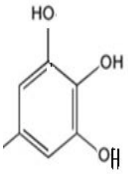
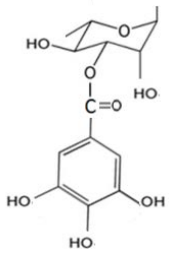
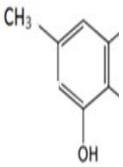
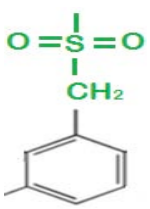
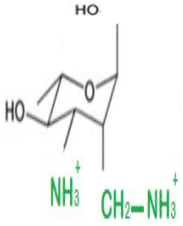
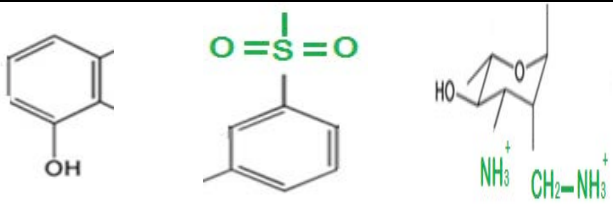
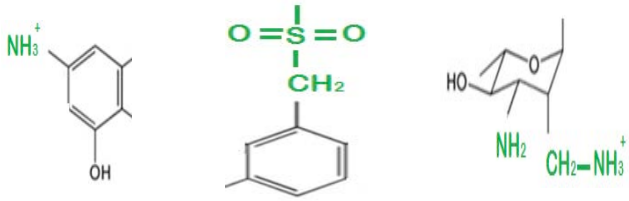
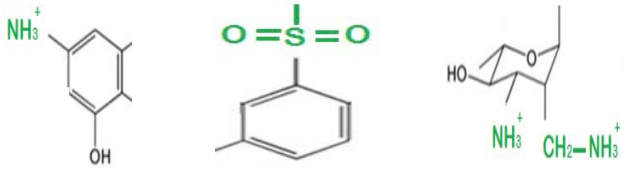


Fig. 4: Targeted positions

Interactions between flavonol derivatives and ACE were studied using AutoDock software. Results of poly-substitution are shown in Table 2. Docking calculations reveal that binding energy increase with all designed inhibitors. The best binding energy was obtained by compound P4.

Table 2: Binding energy between ACE and several flavonol derivatives.

Compound	Cycle A	Cycle B	Cycle S	Binding energy (Kcal/mol)
Compound 3 (started)				-8.67
P1				-10.86

P2				-11.38
P3				-12.74
P4				-13.37

In the case of compound P4, the binding energy was increased from -8.67 to -13.37 Kcal/mol by introduction of a sulfon group in B2 position, a methylamine group in S7 position, and an amine function in A7 and S8 positions.

The inhibition mechanism of this new compound (P4) toward ACE was studied using AutoDock Tools. Visual analysis shows that compound P4 is well positioned on the active site of ACE **(**Fig 5). It forms three hydrogen bonds with ACE amino acids residues. The first is formed between its newly introduced sulfon groups and the amine function of Ala354. The second is formed between inhibitor's C4 bonded hydroxyl and the imidazol cycle of His353. The last hydrogen bond is formed between the new amine function which is introduced on S7 position and carboxylic function of Glu384 (Fig 6). Compound P4 is also stabilized by hydrophobic interactions in the binding pocket, specifically with His353, Glu376, Val380, His513, Trp520 and Tyr523.

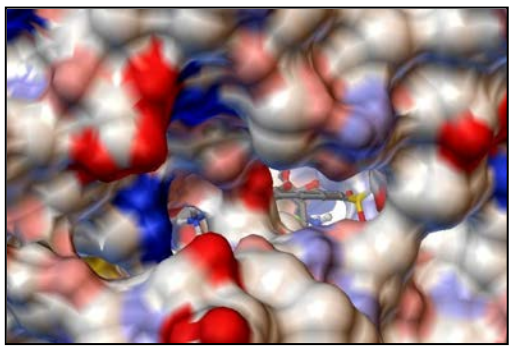


Fig. 5: Positioning of P4 on the active site of ACE.

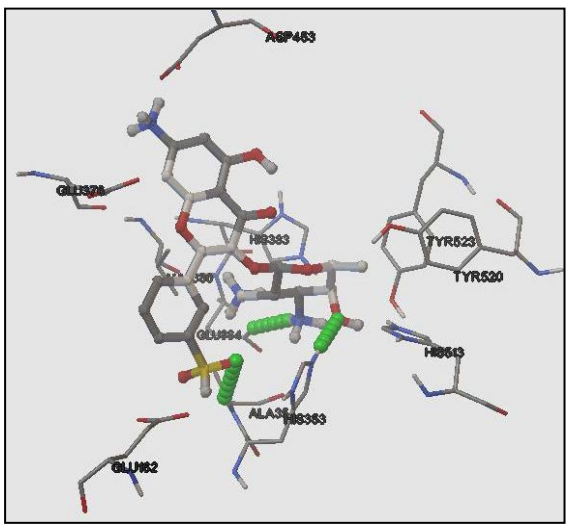


Fig. 6: binding modes of the new compound P4 with the active site of ACE.

4.3. Pharmacokinetic study

Lipinski's Rule of Five is a rule of thumb to evaluate drug-likeness or to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Lipinski *et al* [12]. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, excretion and toxicity (ADME/Tox).

The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule.

Lipinski's Rule of Five states that an orally active drug must complete three of these fives proprieties:

- No more than 5 hydrogen bond donors
- No more than 10 hydrogen bond acceptors
- No more than 15 rotatable bonds
- Molecular weight under 500 g/mol^{-1}
- Partition coefficient logP less than 5

Compound	Molecular weight (g/mol)	LogP	H donors	H acceptors	Flexibles bonds
P4	490.51	1.19	8	11	5

Table 3: Results of ADME/Tox screening

The ADME/Tox screening of proposed compound (P4) shows that it completes three of five Lipinski's parameters, which indicate the potentiality of this compound to become a drug. It is accepted to be orally bioavailable.

5. CONCLUSION

The inhibitory activity against ACE of flavonols and its derivatives was tested using molecular docking with AutoDock.

Among the compounds studied, compound P4 is the best inhibitor of ACE. Its binding energy was increased from -8.67 to -13.37 Kcal/mol by introduction of sulfon group in the B2 position, methylamine group in the S7 position and amine function in the A7 and S8 positions.

This new compound successfully passed ADME/Tox screening.

The results showed greater potentiality of Compound P4 to become antihypertensive drugs. This work demands a thorough study *in vitro* and *in vivo* so that this molecule can come to the market as antihypertensive drugs successfully.

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