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In silico analysis of quercetin as potential anti-cancer agents

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ABSTRACT

Molecular modelling and design are valued and vital tools in the pharmaceutical research for defining, developing and analyzing active biological and chemical molecules. It is based on the evolution of computational theories and methods to study molecules behavior enabling scientists to hypothesize potent drugs for a particular disease. Quercetin is one of the flavonoid family that possesses pharmacological properties due to its interface with cellular targets including, anti-inflammatory, anti-oxidant, anti-cytotoxicity and anti-cancer activities. Molecular docking analyses were performed to predicted quercetin possible binding action along with absorption, distribution, metabolism and excretion (ADME) study. The molecular docking results revealed the bind of quercetin to the active site of Serine/threonine mammalian sterile-20 (MST3) and peroxiredoxin 5 pockets. Moreover, ADME results show its vital properties absorption and drug mimic where the ability to enter blood brain barrier (BBB) was not high, low permeable and strongly bound. Pre-metabolism analyze results show that 2D9 liver microsomal enzyme has more effect on quercetin than CYP2D6 enzymes. In conclusion, molecular docking study documented some important mechanisms of quercetin as a promising anticancer and antioxidant compound.

Selection and peer-review under responsibility of the scientific committee of the 3rd International Conference on Materials Engineering & Science.

1. Introduction

Medicinal plants have been utilized for centuries as source of natural bioactive compounds for medication and treatment of several ailment. Several studies revealed medicinal properties of these phytochemical as antitoxic, antioxidant, antimicrobial and antitumor agents [1,2]. Flavonoids are part of the polyphenol family and include more than 8000 different compounds. These compounds are present in foods as glycosides with sugars at C3 [3].

Quercetin is one of the flavonoid family that can be present in many vegetable and fruits mostly in red onion, green tea and berries. The pharmacological properties of quercetin are due to its interface with cellular targets included, anti-inflammatory, anti-oxidant, anti-cytotoxicity and anti-cancer activity [4–6]. Literatures reported the use of natural and synthetic compounds as an alternative beneficialcancer therapy because of several mechanisms including blocking, transformation and suppression [7,8]. Flavonoids have been reported as a potential inhibitor of androgen-regulated protein [9]. Reactive oxygen species (ROS) play important roles in many physiological actions such as in innate immunity through encountered invading pathogens by its production from phagocytic cells. Also ROS mediate many redox signaling. However, overexpression of ROS can cause oxidative stress, so antioxidant can balance redox hemostasis

Based on these findings, researches were attracted to investigate new drug from plants or its derivative. Molecular docking studies have been done to analyze quercetin interaction either individual or as a complex to visually molecular affinity interaction [10,11]. Serine/threonine mammalian sterile-20 protein like kinases (MSTs-Ste20) was found to be involved in many cellular activities including cell cycle, ion transport, actin regulation and apoptosis [12]. All types of MST (1–4) have been implicated in reducing a variety of cancer. The present study was conducted to indicate molecular docking study in order to improve our understanding of the interactions between quercetin and a selected enzyme (MSTs-Ste, 20, peroxiredoxin 5). The main objective of this

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study is to obtain binding parameters of quercetin by docking analysis and prediction of its absorption and distribution properties.

2. Material and method

Chemicals were obtained from Sigma-Aldrich (St. Louis, Mo, USA) and all the reagents are analytic and laboratory grade. Molecular docking study, Absorption, distribution, metabolism, toxicity and carcinogenicity (ADMET) study along with the C Log P, molecular polar surface area and molecular volume correlation study were performed as described according to Al-Shmgani et al. [13] as the following: molecular docking was carry out to indicate the molecular binding types of quercetin to the active site of MST3 protein target site (mammalian STE20-like kinase3) belongs to the Ste20 serine/threonine protein kinase family) and peroxiredoxin 5 by using MOE 2015 Software. The binding sites were generated from the co-crystallized ligand within crystal protein (PDB codes: 4QMT). Also, C Log P value was used to measure the connection between the MST3 enzyme inhibitors and lipophilicity of molecules. The C Log P value used to calculate the degree of lipophilicity of molecule; the increase in this value indicates an increase in the lipophilic properties of the molecules. The C log P, TPSA and M.V values were calculated using Chem-informatics on free web.

3. Results and discussion

3.1. ADMET and carcinogenicity studies

The results of ADMET test with probability scores are summarized in Table 1. The ability of quercetin to enter BBB is not high, therefore, it is predictable to be harmless to the central nervous system. The lipophilicity increased due to the hydrophobic moiety. The investigation of quercetin reported that level 3 is equal with the aqueous solubility logarithmic, indicating excellent to moderate aqueous solubility.

The plasma protein binding model expects good binding capability of a molecule to the plasma proteins. Cytochrome P450 2D6 model is used to indicate the suppression and non– suppression properties of chemical structure. It was found that quercetin is non-inhibitor for CYP2D6. Consequently, their dysfunction effect on liver is low predictable in administration. Finally, if the carcinogenicity scores close to one means, the probability of predicting a cancer is higher, while if the carcinogenicity scores close to zero, the probability of predict a cancer is low (Table 1; Fig. 1).

Table 1
Quercetin predicted ADMET Solubility level: [(4) high sol., (3) and (2) intermediate sol., (1) less sol., (0) poor sol] Table (2): and quercetin predicted carcinogenicity [Carcinogen = 1 may be; Non carcinogen = 0].

BBB level	0.172
HIA%	63.48
Cyt. P450 3A4	Inhibitor
CYP2D6	0
PPB%	93.23
Solubility level	3
Ames test	Mutagen
Carcinogen on mouse	Negative
Carcinogen on rat	Positive
Carcinogenicity	0
HERG inhibitor	0
TA100-NA	Positive

3.2. Permeability and pre-metabolism study

C log P, molecular polar surface area and molecular volume correlation analysis

In the present study, it is noticed that the C log P value in correlation with MST3 enzyme result which was 1.68. The variation in their biological activity compared to lipophilicity might be a good explanation. Lipophilicity is an important parameter in determine drug transport through membranes and to understand the mechanisms of their binding ability to receptors this give a good knowledge to pharmacokinetic properties and toxicity [14].

MST3 is involved in cell growth activity and has been reported to play a role on carcinogenicity [15]. In addition, the total polar surface area (TPSA) is another method to assess drug bioavailability. Quercetin showed acceptable values of TPSA as it expresses less than 140. Moreover, molecular volume (M.V) descriptor regulates transport physical appearance of quercetin compound, such as intestinal absorption. The permeability of molecules is size dependent by which small one enter the cell quickly. Moreover, the good lipid soluble molecule penetrate fast through cell membrane. Based on the finding of the present study and values presented in Table 2, it can be concluded that quercetin showed good M.V. Moreover, quercetin properties which gives an overall druglikeness analyzed using Chem-informatics on Web (http://www.molinspiration.com/) were applied by which hydrogen acceptors quantity, hydrogen donors quantity, M.V. and log P were calculated.

3.3. Pre-metabolism study

It is well established that any chemical compounds in the body must cross the liver microsomal enzymes and a development a metabolism which can proceed in different sites of the chemical molecules depending on the nature and way of enzyme metabolism. Results of the present of pre-metabolism analyze show that 2D9 liver microsomal enzyme has more effect on quercetin than CYP2D6 enzymes (where CYP2D6 is involved in the metabolism of drug containing amine functional group), the most metabolic reaction can occur is carboxylation that's easy to execrate it from human body (Table 3).

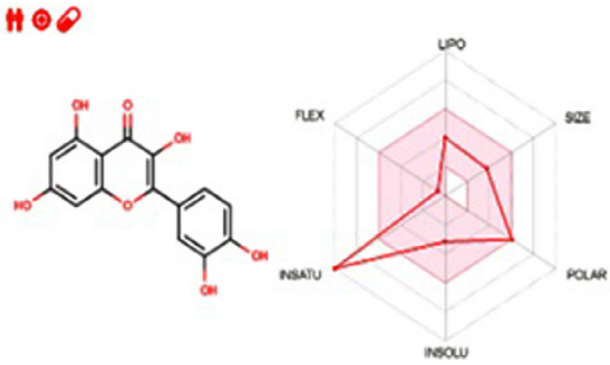
3.4. Docking study analysis

3.4.1. Preparation of receptor for virtual screening

Target site selection have been done by (https://www.rcsb.org/) protein data bank. After choosing protein of target site some processes should done to give insights of molecular binding modes of the tested compound to the pocket of MST3 protein target site (mammalian STE20-like kinase3) belongs to the Ste20 serine/threonine protein kinase family) and peroxiredoxin 5 by using MOE 2015 Software. The binding sites were generated from the co-crystallized ligand, within crystal protein (PDB codes: 4QMT).

Firstly, water molecules were removed from the complex, then, the crystallographic disorders and unfilled valence atoms were corrected using protein report and utility and clean protein options. Protein energy was minimized by applying CHARMM and MMFF94 force fields. The rigid of binding Site was structure of protein was obtained by applying fixed atom constraint.

The protein essential Amino acid defined and prepared for docking process. 2D structures of tested compounds were drawn using Chem-Bio Draw Ultra14.0 and saved in MDL-SD file format. From MOE 2015 software, the saved file was opened, 3D structures were protonated and energy was minimized by applying 0.05 RMSD kcal/mol CHARMM force field. Then, the minimized Structures were prepared for docking using prepares ligand protocol.



SMILES Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O

Physicochemical Properties	
Formula	C15H10O7
Molecular weight	302.24 g/mol
Num. heavy atoms	22
Num. arom. heavy atoms	16
Fraction Csp3	0.00
Num. rotatable bonds	1
Num. H-bond acceptors	7
Num. H-bond donors	5
Molar Refractivity	78.03
TPSA	131.36 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	1.63
Log $P_{o/w}$ (XLOGP3)	1.54
Log $P_{o/w}$ (WLOGP)	1.99
Log $P_{o/w}$ (MLOGP)	-0.56
Log $P_{o/w}$ (SILICOS-IT)	1.54
Consensus Log $P_{o/w}$	1.23

Water Solubility	
Log S (ESOL)	-3.16
Solubility	2.11e-01 mg/ml ; 6.98e-04 mol/l
Class	Soluble
Log S (Ali)	-3.91
Solubility	3.74e-02 mg/ml ; 1.24e-04 mol/l
Class	Soluble
Log S (SILICOS-IT)	-3.24
Solubility	1.73e-01 mg/ml ; 5.73e-04 mol/l
Class	Soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-7.05 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Medicinal Chemistry	
PAINS	1 alert: catechol_A
Brenk	1 alert: catechol
Leadlikeness	Yes
Synthetic accessibility	3.23

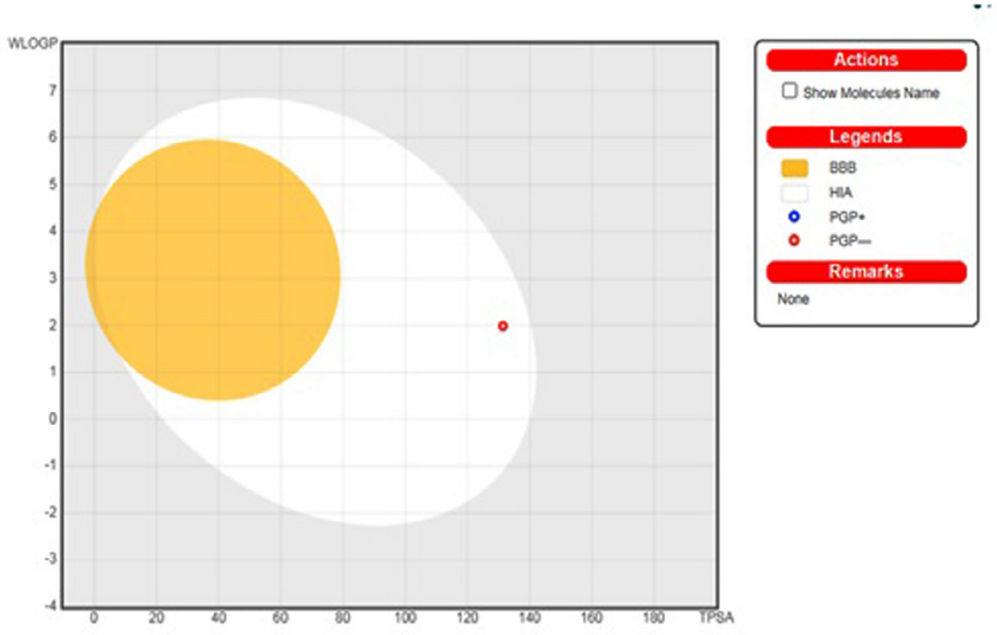


Fig. 1. quercetin physicochemical properties, druglikeness, pharmacokinetics, lipophilicity, water solubility, medicinal chemistry, blood brain barrier and human intestinal absorption (HIA).

Table 2
C log P, molecular polar surface area and molecular volume of quercetin.

TPSA	MV	Clog P	Comp.
131.35	240	1.68	Quercetin

3.4.2. Molecular docking processes

The receptor was held rigid while the ligands were allowed to be flexible during the refinement. Each molecule was allowed to produce seven different interaction poses with the protein. Then docking scores (-CDOCKER interaction energy) of the best-fitted poses were recorded (Tables 4 and 5).

Firstly, the main binding pocket of MST3 protein has been reported in previous literatures, containing amino acid Ser34A, Leu102 A, Glu28A, Glu100A, and other amino acid can form extra bonds as Lys32A, Leu151A, Gly103A, Asp109A, Gly105A and Tyr291A (Fig. 2).

The key binding site of quercetin showed affinity value of −6.61 kcal/mol with three hydrogen bonding with distance range 2.07, 2.08 and 2.00 Å between (OH) groups and residues of Ala148, Met99 and Glu100, respectively (Fig. 3, Table 4).

Secondly, the key binding site of Human peroxiredoxin 5 enzyme has been informed by the literatures, containing amino acid Arg127, Thr44, Gly46 and other amino acid can form extra bonds as Pro19 and Phe79 (Fig. 4) (See Fig. 5).

The key binding site of quercetin showed affinity value of −4.31 kcal/mol with two hydrogen bonding with distance range 1.86 and 2.13 Å between (OH) groups and residues of Asp145 and Gly46. (Fig. 4, Table 5).

Processes used in this study were to predict the proposed binding mode, affinity, preferred orientation of each docking pose and binding free energy (ΔG) of the tested compound with MST3 protein. The calculated interaction energies for the tested compound was in complete agreement with experimental result where the compound care strong suppression against MST3 protein when compared to the other compounds as anti-oxidative stress anti-cancer (apoptosis inducers). On the other hand, quercetin has anti-oxidative stress activity by the side it role as apoptosis inducers, by doing docking virtual screening on reactive oxygen species (ROS) regulating proteins (biological anti-oxidative proteins), interestingly results showed that quercetin has great activity and affinity on Human peroxiredoxin 5 (PDB ID:4mmm). Peroxiredoxins as an antioxidant enzymes regulate ROS level in tissue and cells so that prevent or reduced from oxidative stress and damage leading to cell death by ROS [16,17].

Table 4
The (DG) kcal/mol for quercetin against MST3 protein target site PDB ID: 4QMT.

Compounds No.	RMSD Value	binding free energies (DG) kcal/mol	No. of bonds	
Quercetin	1.50	−6.61	H.b 3	Pi 0
Crystal ligand	1.29	−8.88	3	0

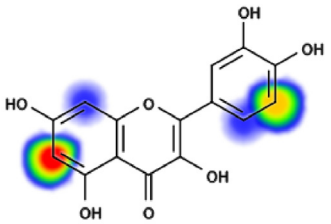
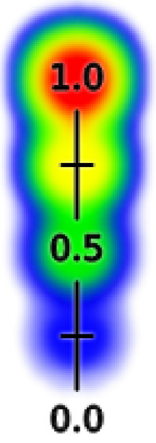
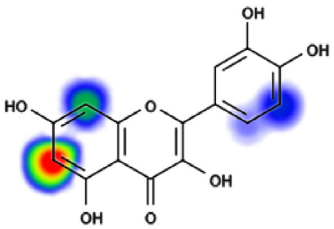
Table 5
Binding free energies (DG; kcal/mol) for quercetin against Human Pdrx5 enzyme target site PDB ID: 4 mm.

Compound No.	RMSD Value	Binding free energies (DG) kcal/mol	No. of bonds	
Quercetin	1.36	−4.31	H.b 2	pi 0
Crystal ligand	1.98	−3.97	3	0

3.4.3. Validation of molecular docking algorithm

The molecular docking algorithm was initially validated by re-docking of the co-crystallized ligands into the active site of the respective receptor with calculation of root mean square deviation (RMSD) for reliability and reproducibility of the proposed docking algorithm. Crystal ligands of MST3 (4qmt) and peroxiredoxin 5 (4mmm) redocked and RMSD of 1.29, 1.95 A° respectively, indicat-

Table 3
Effect of liver microsomal enzymes on quercetin.

ENZYME	CYT. P450 enzymes effect on Quercetin	Reference scale bar
2D9		
2D6		1. = less effect2. = high effect

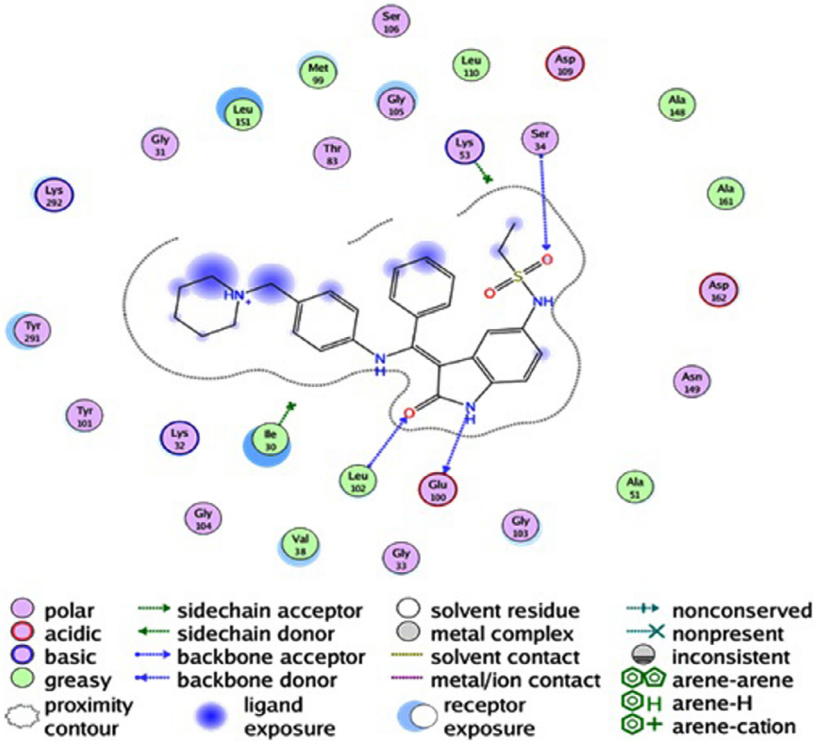


Fig. 2. Binding mode of crystal ligand of MST3 protein.

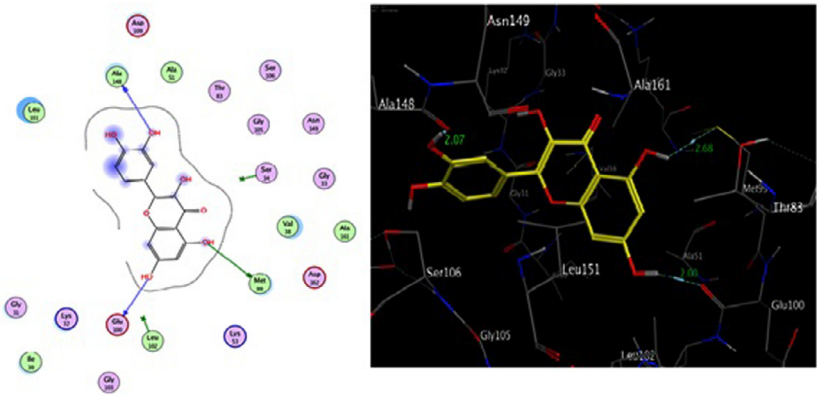


Fig. 3. Binding mode 2D and 3D structure of Quercetin against MST3 protein as potent anti-oxidative anti-cancer.

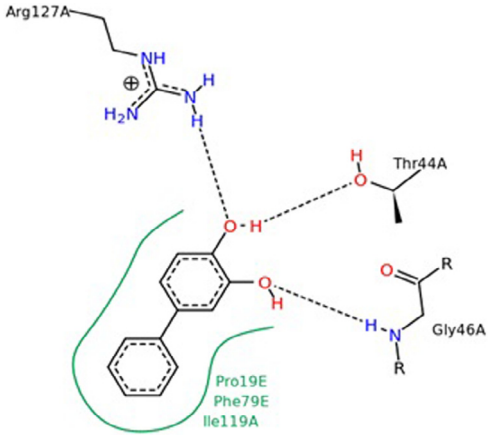


Fig. 4. Binding mode of crystal ligand of peroxiredoxin 5 enzyme.

ing a validated algorithm compared to the crystallographic structure. RMSD value of quercetin is lower than 1.60 A°, that indicate to good binding position for tested compounds.

4. Conclusion

In this study, the molecular docking analyze was performed to elucidate the main binding site for better understanding of the underlying mechanisms of quercetin bioactivity as anticancer agent. Docking results suggested quercetin as a good possible anti-cancer drug candidates with natural requirements properties achieved. Quercetin plasma protein binding model expects good binding ability to the receptor, good absorptivity, low BBB and low toxicity. Further *in vivo* study are required for development and verification of its potential proper function.

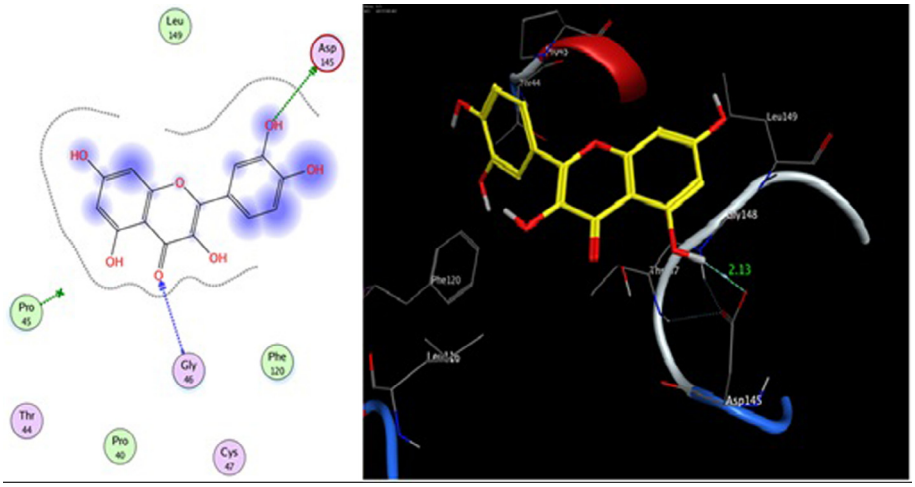


Fig. 5. Binding mode 2D and 3D structure of quercetin (against Human Pdrx5) as potent anti-oxidative anti-cancer.

CRediT authorship contribution statement

Sahar S. Anwar: investigation, methodology. writing-original draft and visualization. **Hanady S. Al-Shmgani:** conceptualization, Data curation, Ressources, formal analysis, funding acquisition, investigation, validation, Writing - review and editing and validation. **Amer T. Tawfeeq**: Project administration, supervision and validation. **Ghassan M. Sulaiman:** conceptualization, Data curation, Project administration, supervision, formal analysis, Writing - review and editingand validation. **Yasmin H. Al-Mousawi:** software, Writing - review and editing and resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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