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RESEARCH ARTICLE

GC-MS ANALYSIS AND *IN SILICO* DOCKING ANALYSIS OF EXTRACTS OF *ACACIA TORTA CRAIB*

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ABSTRACT

Acacia torta Craib. has many medicinal values and its drug like ability against skin infections caused by bacteria was done. Initially, the ethanolic extract of the plant was subjected to GC-MS analysis to identify the compounds present in the sample. Then, lead compounds were screened to be used for docking with iGEMDOCK. Docking was done for the lead compounds against the human penicillin binding protein to check their effectiveness in inhibiting the receptor. Ethanolic extract from powdered leaves and bark of the plant was prepared. GC-MS analysis showed there were 20 compounds present in the sample from which a total of 5 compounds were subjected to docking. Fitness scores of the ligands 1-Pentene, 1, 3-diphenyl-1-(trimethylsilyloxy;) Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester; 1, 3-Dioxolane, 2-(6-heptynyl) - and 2-heptyl 1, 3 Dioxolane with about -65 kcal/mol were more appreciable than tetramethylsilane having binding score -25kcal/mol. The ligands with appreciable fitness score can further be taken into preclinical studies to be used against pathogens involved in skin infection.

INTRODUCTION

Acacia torta Craib. is a perennial shrub known by its common name twisted acacia is distributed in major parts of India and the regional name of the plant is indu. The plant is known for its most of the medicinal uses. For example cough and dysentery is treated with the decoction of its stem bark (Ganesan and kesavan, 2003; Karthikeyan *et al*., 2015). Gas Chromatography and Mass Spectrometry (GC-MS) analysis method can be used to analyze the compounds present in a plant. It is generally based on separation of compounds based on their volatility and then detecting the individual compounds present in the sample based on the molecular weight (Karthikeyan *et al*., 2015). The applications of GC-MS analysis contributes major role in drug identification and also in other purposes such as forensic, security, monitoring the environment etc. Thus GC-MS is used to identify the compounds present in extract of *Acacia torta Craib* (Bai *et al*., 2014). Docking is a method used to analyze and predict the binding of the query molecule with one or more number of molecules in three dimensions resulting in the formation of stable complex. The function of protein is greatly dependent on the molecules which interact with them.

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A protein can be highly expressive or can be inhibited strongly when there is a stable complex formation (Gupta *et al*., 2016). Docking is more useful especially in drug discovery for the pharma companies as it helps to screen the drugs against a particular disease or pathological conditions. Docking done for screening of lead compounds in combination with other *in silico* methods helps greatly in reducing the time taken by *in vitro* approaches. There are many docking tools that are developed and being developed based on genetic algorithm and their own algorithm (Karavadi and Suresh, 2014). The efficiency of docking is measured with their docking scores that will be produced in terms of binding affinity and many other factors. iGEMDOCK, a structure based virtual screening docking tool which has graphic interfaces to prepare binding site and the library of the screening compounds. Then each compound is docked into protein subsequently generating various interaction profiles such as Vander Waals, hydrogen-bonding, and electrostatic profiles. Then it generates all the possible interactions and ranks them based on the fitness score (Sarojini and Krishnan, 2014).

MATERIALS AND METHODS

Preparation of plant material

The plant Acacia torta craib was collected from sivaganga Dt., Tamilnadu and its stem, leaves are subjected to shade drying.

Once they are dried they are powdered and stored for further analytical purposes (Hsu *et al*., 2011).

Preparation of plant extract

The powdered plant extract was set up for cold extraction with 80% ethanol employing hot percolation using Soxhelt apparatus. Then the extract was subjected to distillation to remove solvent and then the extract was used in GC-MS analysis (Karthikeyan *et al*., 2015; Bai *et al*., 2014).

Gas Chromatography and Mass Spectrometry Analysis

The plant extract was subjected to GC-MS analysis in order to identify active compounds present in the sample. Fatty Acids Methyl Acetate (FAME) library was used to identify the compounds from the mass spectra of the sample (Sarvalingam *et al*., 2011). The unknown compounds of mass spectrum were compared with the reference spectrum in the FAME library. By comparison, the name of the compound, molecular weight and molecular formula for the matching hits were identified (Saha *et al*., 2016).

Molecular Docking studies

To study the interactions of the ligands with the human penicillin binding protein, molecular docking was carried out with iGEMDOCK which helps to estimate the protein-ligand interaction in terms of fitness. Fitness is the total energy of the predicted docking pose in the binding site (Sarojini and Krishnan, 2014).

Preparation of Receptor

The 3D structure of the human penicillin binding protein was retrieved from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/explore/explore.do?structureId=2zc3) (Vanitha *et al*., 2011).The preparation of receptor was done with the help of Discovery Studio Visualizer tool which involved the removal of bound ligands and water molecules present in the downloaded structure. Once these changes are done the receptor turns into a stable active structure (Yamada *et al*., 2008; Kumar *et al*., 2014).

Preparation of Ligand

The ligands, also the active components in the plant extract were retrieved from PubChem

LIBRARY SEARCH HIT LIST

#	Lib	Match	R.Match	Name	MW	Chemical Formula
1	mainlib	417	686	1,3-Dioxolane, 2-heptyl-	172.0	C10H20O2
2	mainlib	407	418	Bis(trimethylsilyl) 2,2-difluoro-1-(trifluoromethyl)ethenylphosphonate	356.0	C9H18F5O3PSi2
3	mainlib	404	672	1,3-Dioxolane-2-methanol	104.0	C4H8O3
4	mainlib	393	648	1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether	162.0	C8H18O3
5	mainlib	389	644	1,3-Dioxolane, 2-(6-heptynyl)-	168.0	C10H16O2
6	mainlib	388	597	1,3-Dioxolane, 2-(3-bromo-3-buten-1-yl)-	206.0	C7H11BrO2
7	replib	387	649	Silane, tetramethyl-	88.00	C4H12Si
8	mainlib	374	616	1,3-Dioxolane, 2-pentadecyl-	284.0	C18H36O2
9	mainlib	369	617	Silane, tetramethyl-	88.00	C4H12Si
10	mainlib	368	369	1,1,1,3,5,7,9,11,11,11-Decamethyl-5-(trimethylsiloxy)hexasiloxane	490.0	C13H42O6Si7

Fig. 1a. List of compounds identified from GC-MS analysis of ethanolic extract of *Acacia torta Craib.*

LIBRARY SEARCH HIT LIST

#	Lib	Match	R.Match	Name	MW	Chemical Formula
1	mainlib	511	511	Benzoic acid, 3-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	296.0	C14H24O3Si2
2	mainlib	507	512	Pyrocatechol, bis(tert-butyldimethylsilyl) ether	338.0	C18H34O2Si2
3	mainlib	487	488	1-Pentene, 1,3-diphenyl-1-(trimethylsilyloxy)-	310.0	C20H26OSi
4	mainlib	484	506	1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)-	338.0	C22H30OSi
5	mainlib	483	484	Benzoic acid, 5-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	296.0	C14H24O3Si2
6	mainlib	480	481	1-Heptene, 1,3-diphenyl-1-(trimethylsilyloxy)-	338.0	C22H30OSi
7	mainlib	479	479	Benzene, 1,2,3-tris[(tert-butyldimethylsilyl)oxy]-	468.0	C24H48O3Si3
8	mainlib	477	483	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	384.0	C12H36O4Si5
9	mainlib	473	473	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	296.0	C14H24O3Si2
10	mainlib	470	475	1,1,1,3,5,5,7,7,7-Nonamethyl-3-(trimethylsiloxy)tetrasiloxane	384.0	C12H36O4Si5

Fig. 1b. List of compounds identified from GC-MS analysis of ethanolic extract of *Acacia torta Craib.*

(https://pubchem.ncbi.nlm.nih. gov) in 2D format. With the help of Discovery Studio Visualizer tool .sdf format ligand files were converted to .pdb files (Miguet *et al*., 2009).

Docking with iGEMDOCK

The receptor, human penicillin binding protein was added to the iGEMDOCK using the 'Prepare Binding Site' option and ligands using the 'Prepare Compounds' option in Docking/Screening module. The algorithm chosen for docking was Standard Docking which involved a population size of 300 over 80 generations with 10 output modes of the interaction of the ligand (Sarojini and Krishnan, 2014; Kumar *et al*., 2014). Docking was initiated using the 'Start Docking' option in the same module. Once Docking is completed the best docking pose was subjected to analysis with the help of Docked Poses/Post-Screening Analysis module. In this module, fitness was calculated for the best docking pose in terms of energy values of the bonds involved in binding of receptor and ligand (Sasikala and Meena, 2016).

RESULTS AND DISCUSSION

The compounds present in the extract were identified with the help of the peaks and their retention time from the chromatogram produced from the GC-MS analysis. A total of 20 compounds were identified from the analysis as they were matching with the FAME library used in the GC-MS analysis and are given in Fig 1a and 1b. The following ligands were retrieved from the small molecule databases and the unbounded structures are seen in Fig 2 – 6. Similarly, the receptor human penicillin binding protein was retrieved under the ID 2ZC3 and Fig 7 shows the structure of the receptor after the ligands and water molecules were removed. The downloaded structure was in complex with biapenem, a carbapenem antibiotic against *Streptococcus pneumoniae*

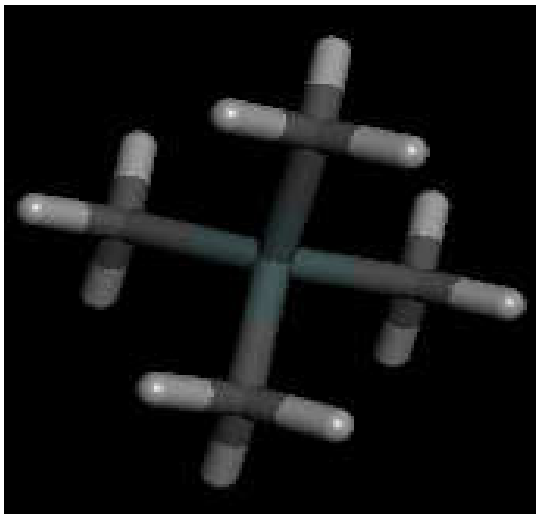

Fig. 3. Tetramethylsilane

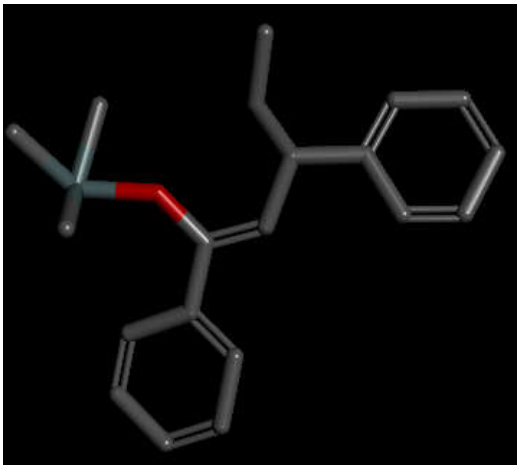

Fig. 4. 1-Pentene, 1, 3-diphenyl-1-(trimethylsilyloxy)

Table 1. Fitness scores for various ligands against the penicillin binding protein 2ZC3

Ligand	Fitness	VDW	HBond	Elec	AverConPair
2-heptyl 1, 3 Dioxolane	-61.9449	-55.9449	-6	0	29.25
Tetramethylsilane	-25.0733	-25.0733	0	0	28
1-Pentene, 1,3-diphenyl-1-(trimethylsilyloxy)	-79.4206	-79.4206	0	0	22.9545
Benzoic acid, 4-methyl-2-trimethylsilyloxy-,trimethylsilyl ester	-66.7366	-65.1887	-1.54793	0	22.3158
1,3-Dioxolane, 2-(6-heptynyl)-	-64.1737	-54.984	-9.18975	0	28.0833

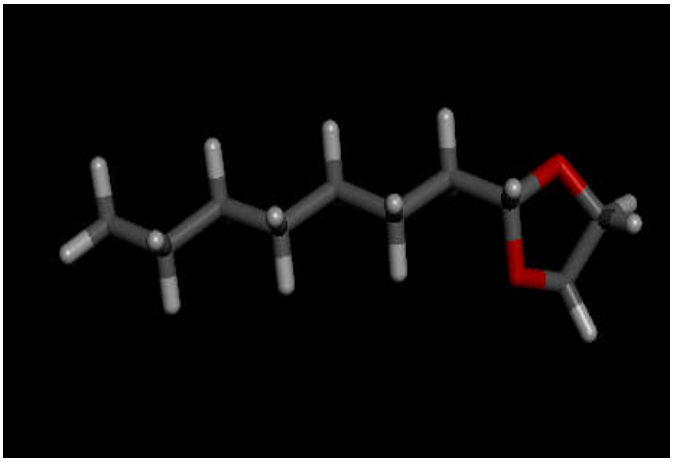

Fig. 2. Heptyl-1,3-dioxolane

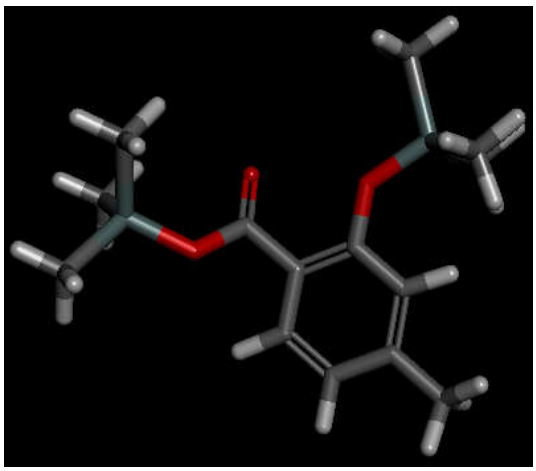

Fig. 5. Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester

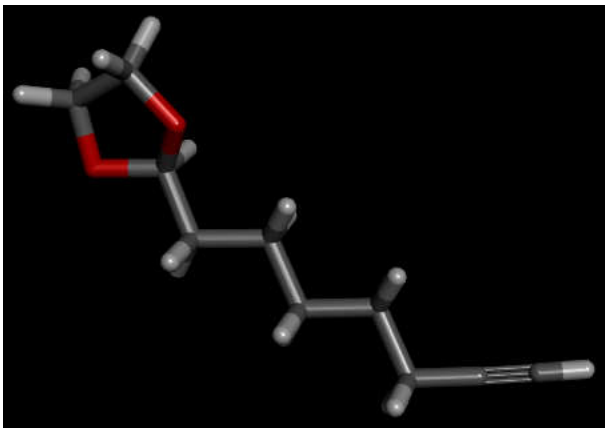

Fig. 6. 1,3-Dioxolane, 2-(6-heptynyl)

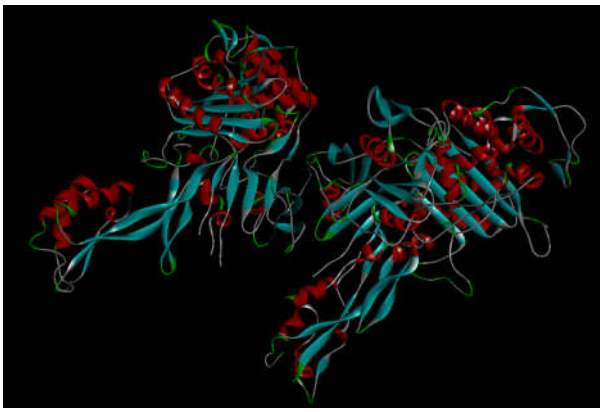

Fig. 7. 2ZC3 (Penicillin Binding Protein 2x)

The docking was carried out with iGEMDOCK and docked poses of the lead compounds from the GC-MS analysis can be seen in Fig from 8 to 12. Fitness of the docked pose for the individual ligands is given in the Table 1. Among the five ligands 1-Pentene, 1,3-diphenyl-1-(trimethylsilyloxy) showed the highest fitness energy of -79.4206(kcal/mol) followed by Benzoic acid, 4-methyl-2-trimethylsilyloxy-,trimethylsilyl ester with –66.7366(kcal/mol), 1,3-Dioxolane, 2-(6-heptynyl)- with -64.1737(kcal/mol). Second least being 2-heptyl 1, 3 Dioxolane with -61.9449(kcal/mol) and the last being Tetramethylsilane. The following binding energy contributed to fitness of ligands with receptor were Vander Waal's force and Hydrogen bonding and electrostatic interactions does not contribute to any fitness between the ligand and receptor. The average connection pair was good for 2-heptyl 1, 3 Dioxolane had the best average connection pair of 29.25 (kcal/mol)

DISCUSSION

A total of five lead compounds from GC-MS analysis were checked for their inhibiting property against the penicillin binding protein using iGEMDOCK and these compounds may be reported for the first time in this study. The fitness scores of docking results indicate that the following ligands 1-Pentene, 1,3-diphenyl-1-(trimethylsilyloxy); Benzoic acid, 4-methyl-2-trimethylsilyloxy-,trimethylsilyl ester; 1,3-Dioxolane, 2-(6-heptynyl)- and 2-heptyl 1, 3 Dioxolane are effective in their binding with the penicillin binding protein present in the *Streptococcus pneumoniae* (PBP 2X) which was also used by (Kumar *et al*., 2014; Sasikala and Meena, 2016). The other lead compound tetramethylsilane has poor binding with the receptor; hence, it cannot be taken next stage for example, in vitro validation using animal cell line. The fitness scores of the potential ligands were more than that of the fitness scores for the 3, 5, 6-Trichloro-2-pyridinol docked against the model protein reported in (Sasikala and Meena, 2016) and can be comparable to those antibiotics reported in (Yamada *et al*., 2008).

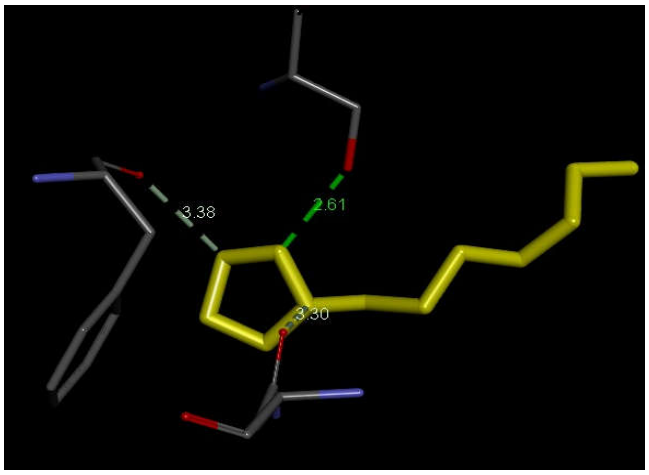

Fig. 8. Interaction of 2-heptyl 1, 3 Dioxolane with Penicillin binding protein

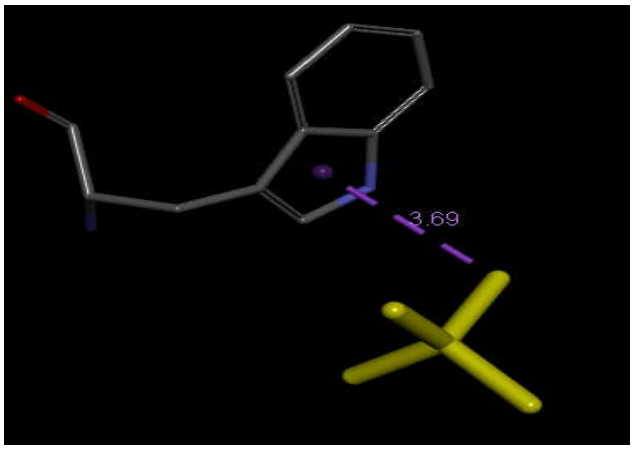

Fig. 9. Interaction of Tetramethylsilane with Penicillin binding protein

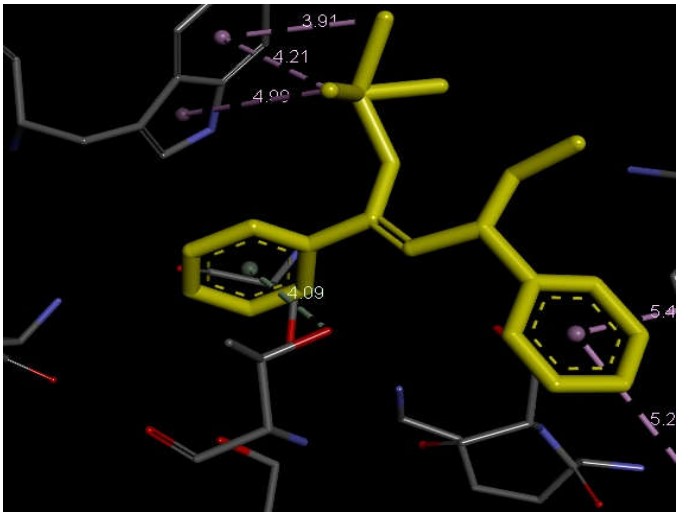

Fig. 10. Interaction of 1-Pentene, 1,3-diphenyl-1-(trimethylsilyloxy) with Penicillin binding protein

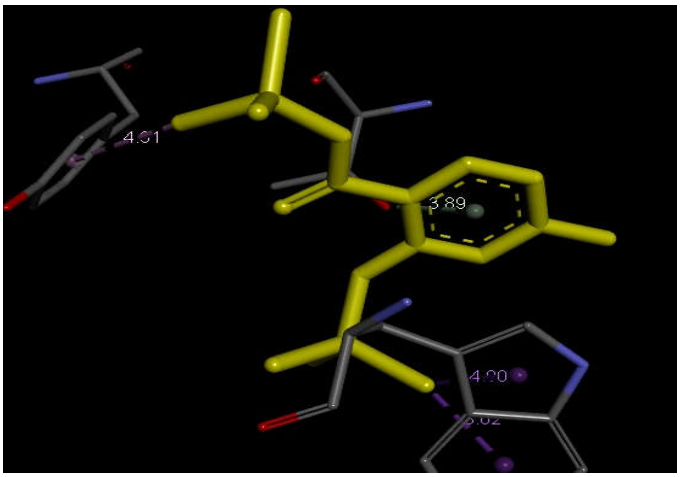

Fig. 11. Interaction of Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester with Penicillin binding protein

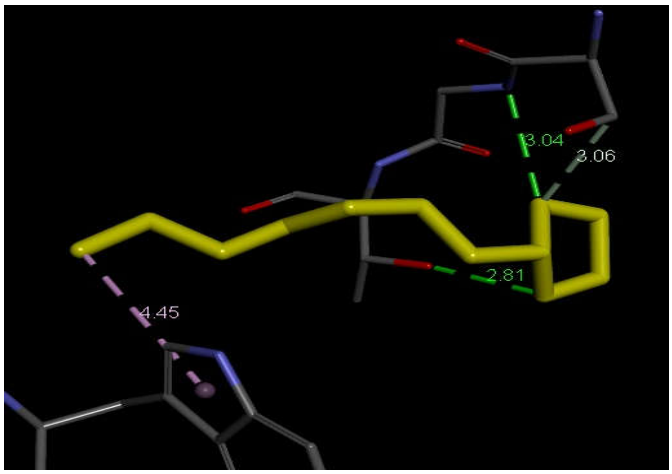

Fig. 12. Interaction of 1,3-Dioxolane, 2-(6-heptynyl)- with Penicillin binding protein

Conclusion

The ethanolic extract of *Acacia torta Craib.* was subjected to GC-MS analysis to identify the lead compounds in the sample. A total of 20 compounds were found in the extract from which 5 were selected as lead compounds and subjected to docking studies with penicillin binding protein 2x and 4 compounds were found to be more effective. Further, these compounds can then be validated using either in vitro studies using cell lines and in vivo validation using animal testing followed into preclinical trials for these ligands to be used as a drug in skin infections.

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