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IN-SILICO INVESTIGATIONS FOR MANAGEMENT OF INFLAMMATORY DISORDERS BY TARGETING HUMAN P38A MAP KINASE: AN APPROACH TO TREAT *LEPTOSPIRA INTERROGANS* INFECTION

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ABSTRACT

Leptospira's species cause Leptospirosis, zoonotic disease. People in poor nations are more vulnerable to leptospirosis. Tubular damage and inflammation may be induced by Leptospira outer membrane proteins (OMPs) via a Toll-like receptor-dependent pathway, which includes mitogen-activated protein kinases (MAPK) and activation of nuclear transcription factor kappa B (NF-kB). Our goal is to find synthetic and natural compounds as novel leads in pharmaceutical research by employing a variety of commercial tools, including AutoDock 1.5.6, Accelrys Discovery Studio 4.1 client, auto dock vina, PyMOL, and python-3.5.0a4, for molecular docking investigations. Many natural and chemicals with anti-cancer, anti-inflammatory, and anti-microbial properties have been generated from research papers and databases for this purpose. The docking data were then used to conduct comparative assessments of natural and synthetic molecules in order to determine which was the best.

KEYWORDS: Leptospirosis, Mitogen activated protein kinases (MAPK), Hydroxy citronellal, Hydrocortisone.

INTRODUCTION

Adolf Weil originally defined leptospirosis in 1886, when he described an acute infectious condition characterized by spleen enlargement, nephritis and jaundice. This disease increasing in both developing and developed countries. Leptospira was discovered in 1907 after a post mortem examination of a renal tissue slice. It was initially identified as the causal organism by Inada and Ito in 1908, and its presence in rats was first observed in 1916 [1]. Leptospirosis is an uncommon and dangerous bacterial infection that develops when humans are exposed to polluted food, drink, or soil. The Leptospira system having complex, which includes more than 20 serogroups and 200 serovars, causes leptospirosis, an inflammatory disease caused by the Leptospira interrogans bacteria. The source of Leptospira infection in humans is the urine of infected animals. Skunks, Rats, foxes, opossums, raccoons and other vermin are all capable of transmitting leptospirosis. Leptospires infiltrate host tissues and fluids via mucous membranes of the nose, eyes, and throat, as well as wounds and abrasions on the skin [2]. Because Leptospira can thrive in hot and humid environments, leptospirosis is widespread all around the world. Leptospirosis may strike anybody at any age, but a variety of variables raise the risk of infection. After gaining access, the bacterium multiplies in tissue and blood. Leptospiremia can damage any region of the body, but it is most harmful to the liver and kidneys, producing tubular necrosis and interstitial nephritis, which can lead to renal failure. Clinical laboratory testing on urine and blood are used to confirm leptospiral infection. Antibiotics in large dosages are part of the therapy. However, the antibiotics mentioned above have a variety of negative effects. Natural medications are being utilised to treat several of the negative effects. One strategy is to utilize molecular docking, which involves using molecular modelling to predict how a protein binds with small ligands [3]. Docking is useful in the investigation of several aspects related with protein-ligand interactions, such as binding affinity, geometric compatibility, hydrogen bond donor acceptor qualities, polarizability and hydrophobicity [4]. Leptospirosis is mainly symptomized by inflammation. Inhibition of human p38a MAP kinase is a potential approach for the treatment of inflammatory disorders. Based on the *in-silico* investigation, a comparison of synthetic and natural chemicals is performed to determine which is the best for Leptospirosis.

MATERIAL AND METHODS

Software Requirements

Used for the production of proteins and ligands for docking Discovery Studio Client 4.1 for docking and discover acceleration is also used for changes in ligands. Other software such as PyMOL, python-3.5.0a4, and auto dock 1.5.6, auto dock vina and MedChem was used for docking.

Protein Selection and Preparation

Select the crystalline components of the human p38aMAP kinase as a new drug discovery target. RCSB protein data bank

(PDB ID: 3HVC) has been used to obtain its structure. The ligand already attached with protein downloaded from protein data bank. So first removed the ligand from protein through Accelrys discovery. The all-water molecules were removed and on the final stage and hydrogen atoms were added to the target protein molecule. Then the protein structure was converted into pdbqt file from auto dock 1.5.6 for docking. And protein grid also has been made which is used for docking.

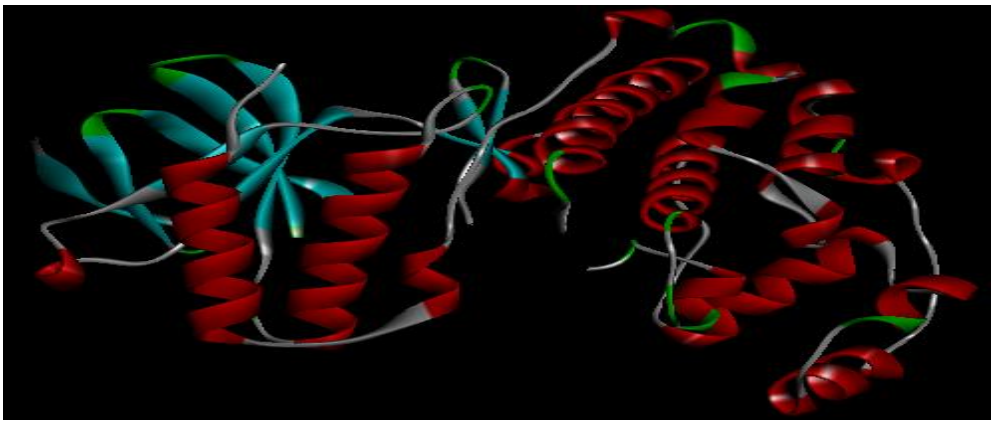


Figure No 1: p38αMAP kinase

Ligand

The following is a list of natural and manufactured anti-cancer, anti-inflammatory, and anti-microbial chemicals. The PubChem databases were utilised to find the ligand molecules used in this investigation. I have downloaded 2 natural and 2 synthetic compounds. Natural compounds are Quercetin and Biotin. Synthetic compounds are Hydroxy citronellal and hydrocortisone. These were used for docking.

ADME Investigation

ADME tests were carried out in MedChem designer using ADME descriptors. The Absorption, Distribution, Metabolism and Elimination (ADME) research offer perception into the Absorption, Distribution, Metabolism, and Elimination (ADME) studies provide insight into the pharmacodynamics of everyone examined herbal and synthetic substances. The significance of computational ADMET forecasts has risen in recent years. It's because, rather than a lack of effectiveness, the number of clinical trial rejections have been attributable to ADME concerns. The problem of predicting ADME qualities is quite challenging.

Molecular Docking Studies

Docking studies experiments were carried out. First downloaded the protein pdb files and then Ligands SDF files. The Ligand is already attached to the protein first removed the Ligands from protein through Accelrys Discovery Studio Client 4.1. Then open this pdb file of protein in auto dock 1.5.6 and removed the water and added the hydrogens then saved this protein file in pdbqt file and then made the grid of protein in auto dock 1.5.6. Then modified the Ligands both synthetic and natural in Accelrys Discovery Studio Client 4.1. Then open this modified Ligand in auto dock 1.5.6 and then selected the torsions then

save this in pdbqt file. Then these files placed in auto dock vina folder and noted the grid (while making the grid of protein) values and also protein and Ligand name putted in txt file and then this txt file also placed in vina folder then the docking done through vina which is operated in dos through commands. So, the 4 Ligands used in docking against this protein2 natural and 2 synthetics. I have used these Ligands with or without modification.

RESULTS

The docking results of both natural and synthetic compounds are as follows:

1) **Synthetic Ligands** **2) Natural Ligands**

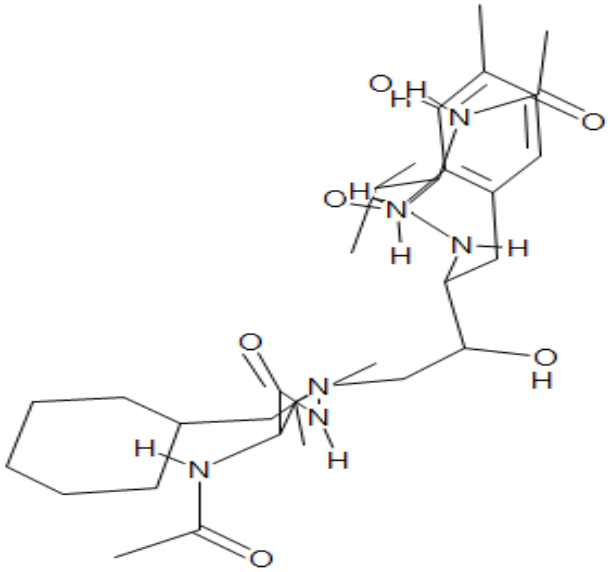
Hydrocortisone

Biotin.

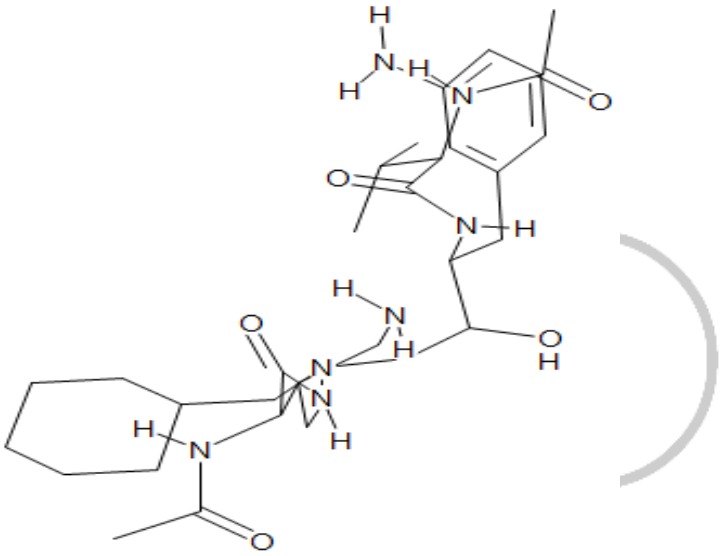
Hydroxy citronellal

Quercetin.

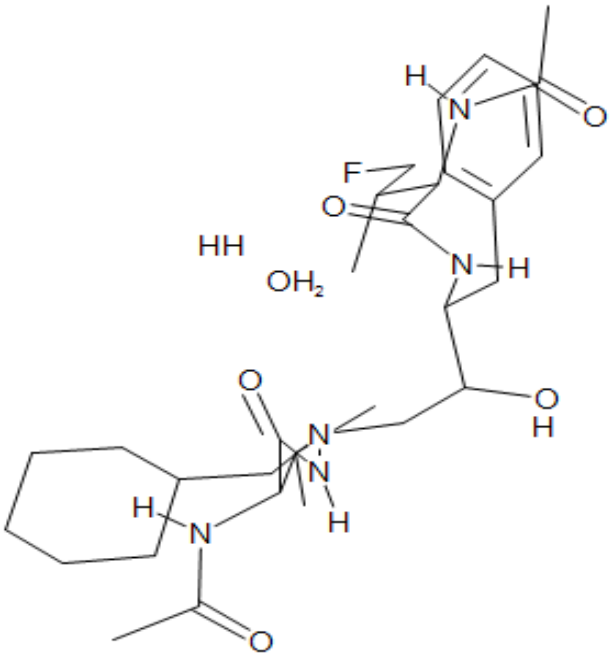
Structure of Ligands



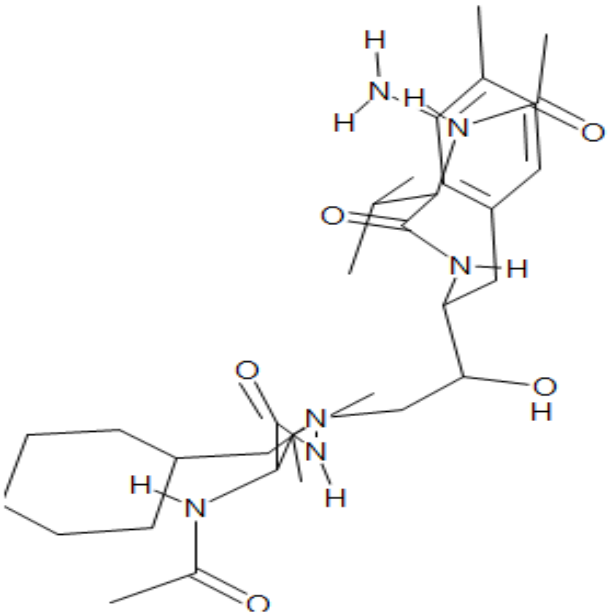
Ligand 1 (Hydrocortisone.)



Ligand 2 (Hydroxy citronellal)



Ligand 3 (Biotin.)



Ligand 4 (Quercetin)

Synthetic Ligands are:

Hydroxy citronellal

That is ligands are synthetic which I have been used against Mitogen activated protein kinases (MAPK), and the docking are shown in table with or without modification. The docking is also shown below.

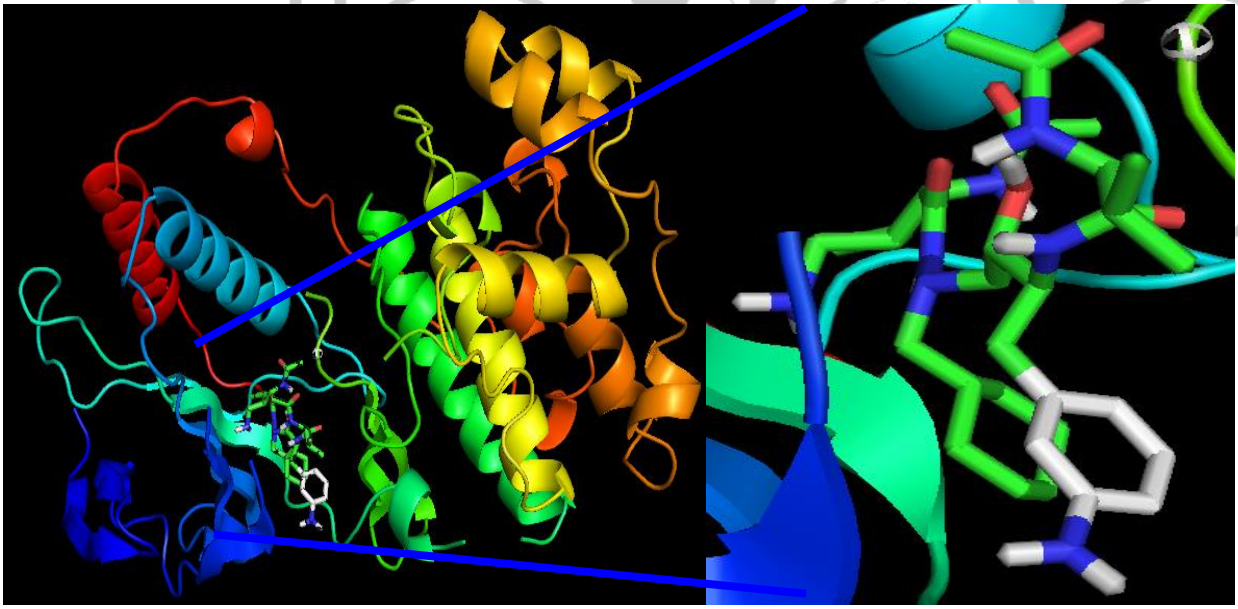


Figure 2: Ligand-protein docked structure

Then I modified the Ligand in Accelrys Discovery Studio Client 4.1. By adding N on H44 and hydrogens for completing the valency of Nitrogen, C on H47 and hydrogens for completing the valency of Carbon, Non H19 and hydrogens for completing the valency of Nitrogen. So, they converted into N82, C86, N39.Aftermodification results are also modified and Ligand becomes more effective

Hydrocortisone

That is ligand are synthetic which I have been used against Mitogen activated protein kinases (MAPK), and the docking are shown in table with or without modification. The docking is also shown below

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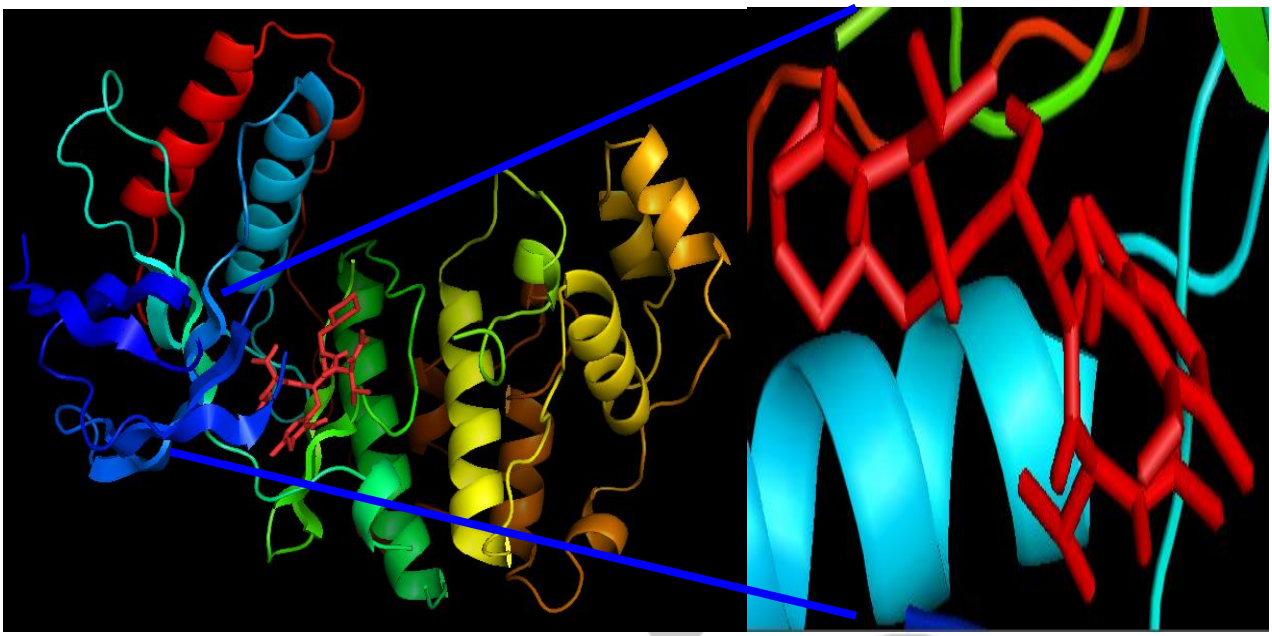

Figure 3: Ligand-protein docked structure

Then I modified the Ligand in Accelrys Discovery Studio Client 4.1. By adding the **Con H18 and hydrogens for completing the valency of Carbon, O on H19, N on H20 and hydrogens completing the valency of Nitrogen.** So, the converted into **C38, O39, N40.** After modification results are also modified and Ligand becomes more effective. **Hydrocortisone**

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2) Natural Ligands are:

Quercetin

That is ligand are natural which I have been used against Mitogen activated protein kinases (MAPK), and the docking are shown in table with or without modification. The docking is also shown below

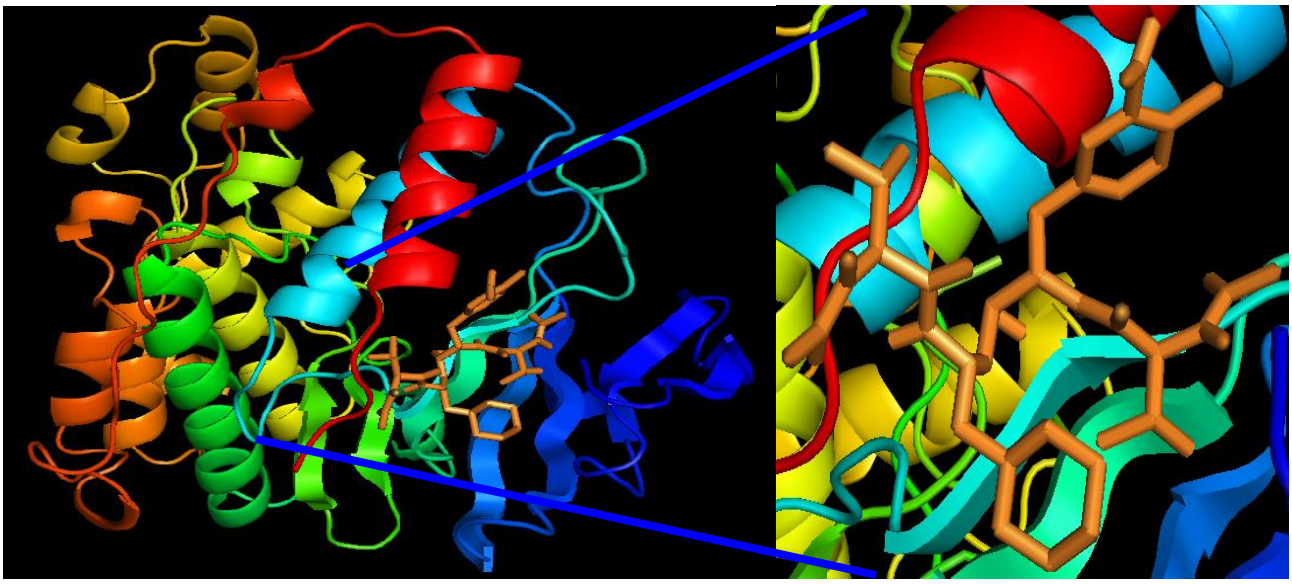

Figure 4: Ligand-protein docked structure

Then I modified the Ligand in Accelrys Discovery Studio Client 4.1. By adding the N on H19 and hydrogens for completing the valency of Nitrogen, C on H18 and hydrogens for completing the valency of Carbon. So, they converted intoN39, C38.After modification in natural compounds the results didn't modify. The modification in natural compounds didn't increase the ligand effectiveness.

Biotin

That is ligand are natural which I have been used against Mitogen activated protein kinases (MAPK), and the docking are shown in table with or without modification. The docking is also shown below.

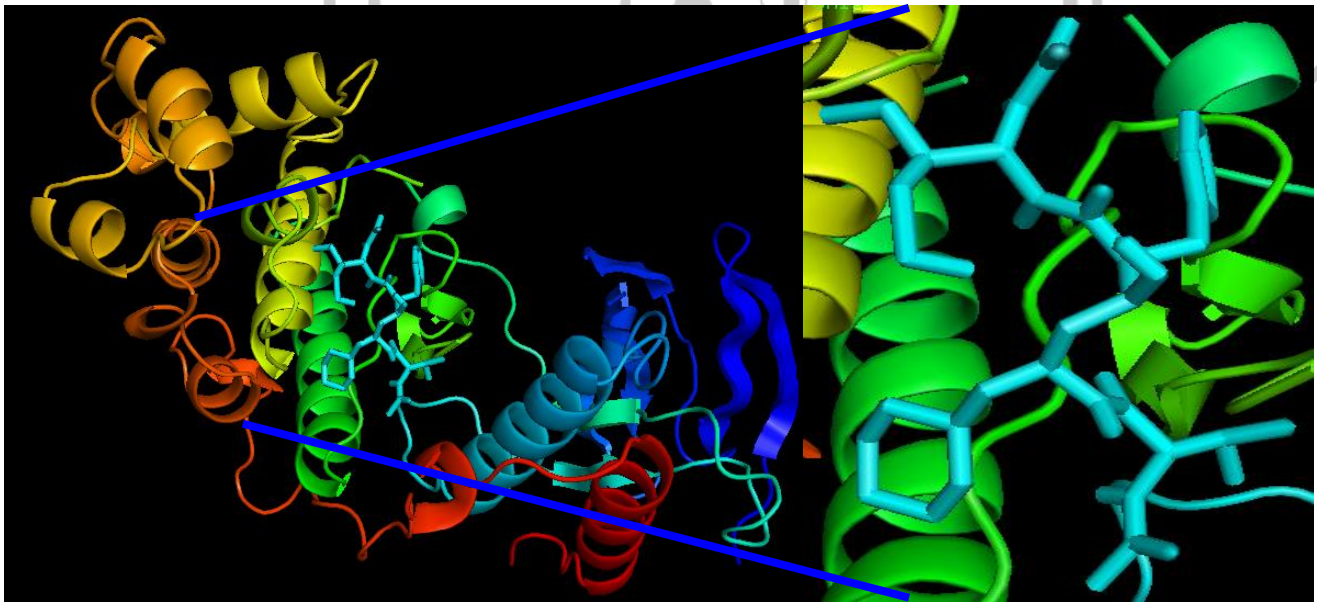

Figure 5: Ligand-protein docked structure

Then I modified the Ligand in Accelrys Discovery Studio Client 4.1. By adding the Oon H8, F on H11.So they converted intoO22, F26.After modification in natural compounds the results didn't modify. The modification in natural compounds didn't increase the ligand effectiveness.

Table No 1: Modification of original ligands

Ligand Name	Original Ligand	Modifications
Ligand 1	Hydrocortisone.	CH3, O, NH3
Ligand 2	Hydroxy citronellal.	NH3, CH3, NH3
Ligand 3	Biotin	O, F
Ligand 4	Quercetin	NH3, CH3

Modification sites of ligands

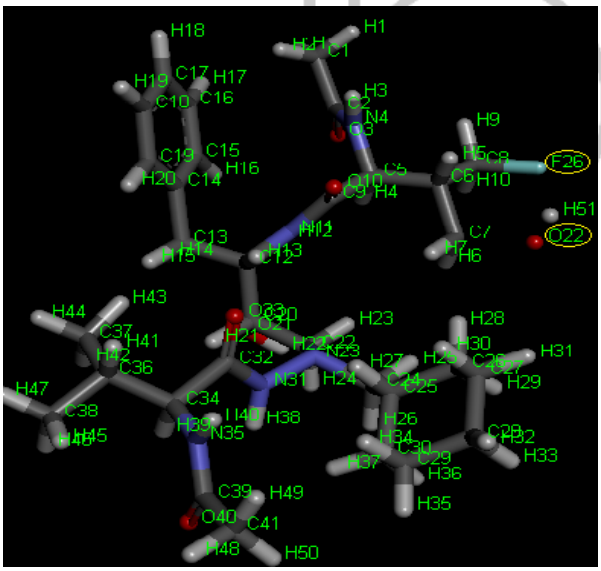

Figure 6: Ligand 1modified from Biotin

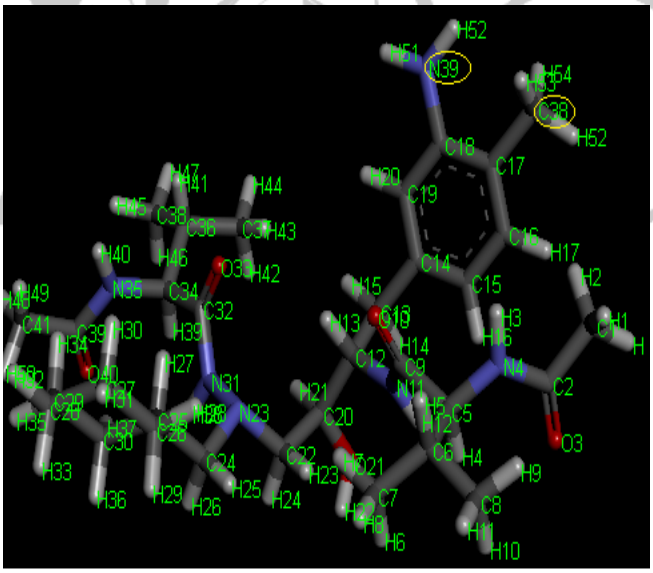

Figure 7: Ligand 1 modified from Quercetin

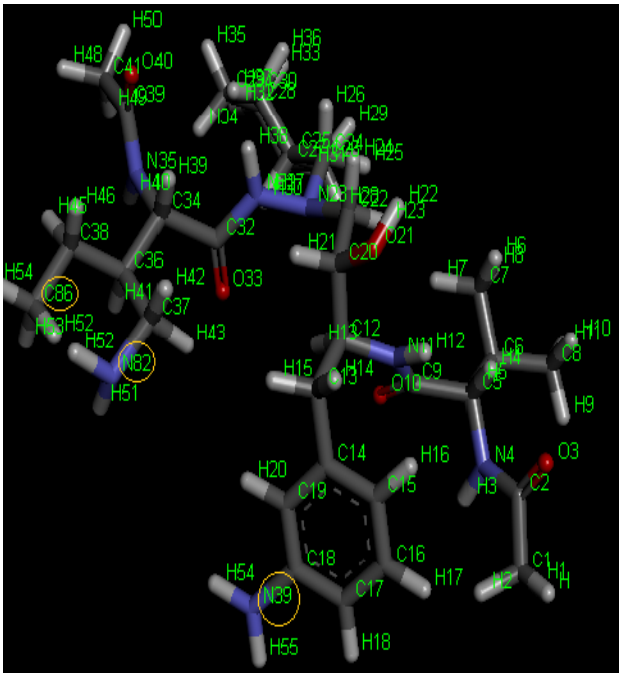

Figure 8: Ligand 1modified from Hydroxy citronellal

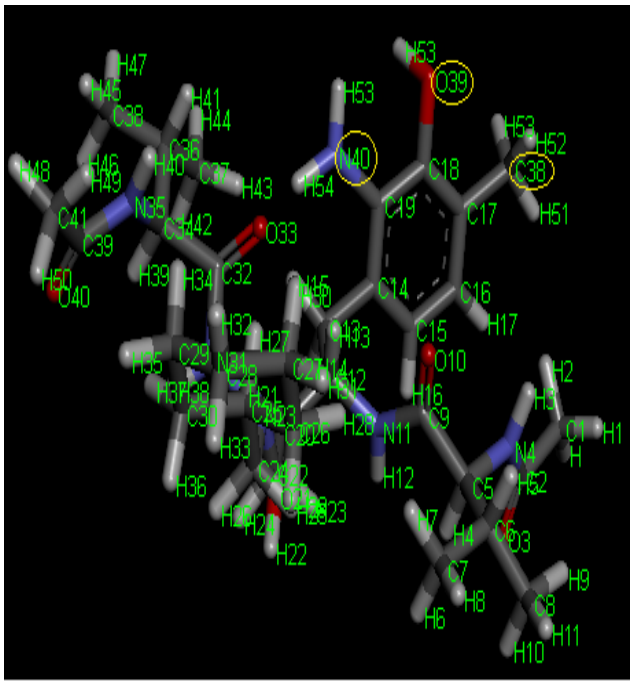

Figure 9: Ligand 1 modified from Hydrocortisone

DOCKING OF LIGANDS

Table No 2: Docking results of my ligands

Ligands	1	2	3	4	5	6	7	8	9
Ligand 1 (synthetic)	-7.0	-6.9	-6.7	-6.5	-6.4	-6.4	-6.3	-6.2	-6.2
Hydrocortisone.	-6.9	-6.8	-6.8	-6.7	-6.6	-6.6	-6.6	-6.6	-6.4
Ligand 2 (synthetic)	-7.6	-7.3	-7.1	-6.9	-6.5	-6.5	-6.4	-6.4	-6.4
Hydroxy citronellal	-7.2	-7.1	-6.3	-6.3	-6.3	-6.1	-6.1	-6.1	-6.1
Ligand 3 (Natural)	-6.6	-6.4	-6.2	-6.2	-6.2	-6.1	-6.0	-6.0	-5.9
Biotin	-7.4	-7.3	-7.1	-6.5	-6.3	-6.3	-6.3	-6.3	-6.2
Ligand 4 (Natural)	-7.3	-6.9	-6.8	-6.4	-6.3	-6.2	-6.0	-5.9	-5.9
Quercetin	-7.4	-7.0	-7.0	-7.0	-6.8	-6.8	-6.7	-6.7	-6.7

ACTIVE SITE PREDICTION

Auto dock vina is built on the receptor cavity approach. For the target protein, we discovered nine active sites.

ADME STUDIES

Various features of natural and synthetic substances were investigated in this study, including aqueous solubility, human intestinal absorption, plasma protein binding (PPB), blood-brain barrier (BBB) penetration, cytochrome p450 inhibition, and hepatotoxicity levels.

Calculated through MedChem these are modified ligand' ADMET properties.

Table No 3: ADMET properties of ligands

Structure Name	MlogP	S+logP	S+logD	Ruleof5	Ruleof5_code	MWt	M_NO	T_PSA	HBDH
Hydrocortisone (Modified)	1.795	1.517	1.516	3.000	Hb, Mw, NO	618.822	12.000	186.120	8.000
Hydroxy-citronella (Modified)	1.016	0.657	0.259	3.000	Hb, Mw, NO	617.837	12.000	191.910	9.000
Hydrocortisone (Unmodified)	2.001	2.034	2.034	1.000	Mw	573.781	10.000	139.870	5.000
Hydroxy-citronella (Unmodified)	2.001	2.034	2.034	1.000	Mw	573.781	10.000	139.870	5.000

SCORING

Scoring was executed online (dsx-online) server.

Table No 4: Scoring values of ligands

Ligand Name	Rmsd	Rank(score)	Score
Hydrocortisone (Un Modified)	None	1	1842
Hydrocortisone (Modified)	None	1	-126
Hydroxy citronellal (Un Modified)	None	1	1842
Hydroxy citronellal (Modified)	None	1	-176

DISCUSSION

Natural substances have a larger role in the treatment and prevention of human illnesses than manufactured medications, which are more poisonous or have more negative effects. The major purpose of the study is to discover natural chemical compounds that, when compared to manufactured medications can be employed as a therapy for the treatment of Leptospirosis. The idea of docking is crucial for determining features such as, electron distribution, binding affinity, hydrogen bond donor acceptor qualities, and hydrophobicity related with protein-ligand interactions. Random screening is an expensive and time-consuming method, thus computational tools for structure-based drug development provide a potential alternative [5]. There is significant evidence that the pathogen implicated in leptospirosis is Leptospira

interrogans, according to Manjula Sritharan, Karen V Evangelista, and Jenifer Coburn [6]. CW Yang [7]. It's been proposed that Leptospira outer membrane proteins (OMPs) can cause tubular interstitial nephritis or necrosis by inducing tubular damage and inflammation via a Toll-like receptors (TLRs)-dependent mechanism. Durga Devi M et al [8]. According to CHS Venkataramana et al, [9] aqueous volatility aids in the prediction of a compound's solubility in water. The compounds are shown to have high solubility in this setting, allowing for full oral absorption and efficient dosing. Blood Brain Penetration rate demonstrates the infiltrating effectiveness of chemical towards the brain, according to Gade Deepak Reddy et al, [10]. High expression of p38 increases cytokine synthesis, which leads to cancer-causing inflammation. As a result, the protein p38 was chosen as a target for slowing the growth of inflammatory tumors. Human p38 was chosen as a therapeutic target for the manufacture of natural medicines for the condition based on the findings of the previous investigation. PDB was used to extract the target protein's 3D structure (PDB-ID: 3HVC). The study's ligands were gathered from a variety of academic publications and databases. (PubChem). Then modified in Accelrys Discovery Studio Client 4.1. After that, the ligand and protein are both changed to pdbqt using auto dock since we required a pdbqt file to dock using auto dock vina. Then, using auto dock vina, docking was completed. According to Durga devi M et al., the majority of human p38 inhibitory synthetic therapeutic compounds discovered to date are in preclinical phases. The drug molecules in these clinical investigations have revealed negative impacts such as liver damage and the development of lung malignancies. The docking data are analyzed and compared to see how effective natural bioactive substances are at controlling Leptospirosis compared to synthetic drugs. It will be critical to identify the crucial structural properties necessary to increase inhibitory chemicals based on the findings.

Our findings indicate that the majority of the chemicals have a high ability to permeate the membrane and attach to plasma proteins. The amount of hepatotoxicity forecasts the molecule's organ toxicity and is divided into two categories. Non-toxic equals 0; toxic equals 1. The results imply that the chemicals are non-toxic at these concentrations, therefore they can be employed in further research.

CONCLUSION

The protein-ligand association is crucial in the creation of structure-based medications. The current work docked the target protein p38 [PDB ID: 3HVC] with both natural and synthetic Ligands. The current study determined that the natural compounds Biotin and Quercetin are likely compounds for leptospirosis based on *in-silico* examination of natural compounds on the human p38 protein. When compared to manufactured drugs, natural substances have demonstrated to be effective. These chemicals can be used in further preclinical and clinical investigations since they have a positive effect on the Leptospirosis target.

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