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Development Of Optimized Chalcone-Based Small Molecules As JAK Inhibitors For Rheumatoid Arthritis Treatment

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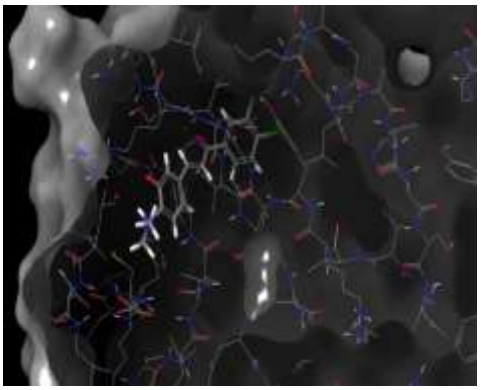
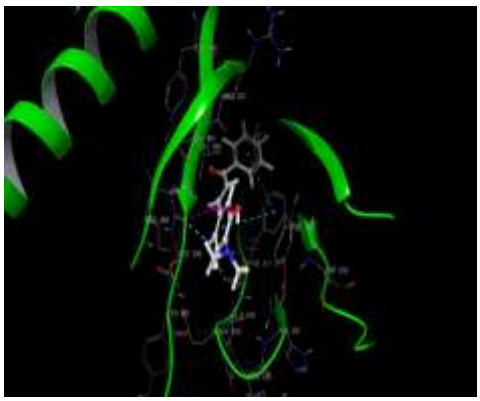
Abstract
Rheumatoid arthritis (RA) is a persistent autoimmune disorder that affects around 0.5-1% of the global population. The design and optimization of small molecule chalcone derivatives as JAK inhibitors encompass a series of essential stages. The design of chalcone derivatives was facilitated using the Chemdraw and ADMET Lab 3.0 studies conducted by Swiss ADME. Molecular docking, acute prediction, and derivatives analysis were conducted on several compounds related to chalcone derivatives using the Schrodinger Glide 30.8 software with proteins ligands JAK 3 Kinase (PDB-6NY4).





Quantification of docking score, ADMET analysis, prediction of acute toxicity, and determination of structural position of ligands in the active JAKs site enzymes. The C compounds have a binding affinity (Docking score: -9.158), VAL901, LEU822, PHE817, ILE816, LEU822, VAL889, & TYR891 showed hydrophobic interaction with protein, charge (positive) ARG899, ARG820 & ARG887, charge (negative) GLU818, GLU819, polar nature is the HIS821, SER890 & THR815, and water GLY892, GLY888 bind to each other and show of protein affinity and the M compounds has a binding affinity **(**Docking score: -9.084), LEU875, ILE872, TYR886, VAL889, PHE817, MET902, VAL901, LEU900, and LEU898.

Keywords: Chalcone, Rheumatoid arthritis, ADMET, JAK, Molecular docking.

1. Introduction:

A persistent autoimmune disorder, rheumatoid arthritis is marked by joint inflammation resulting in pain, swelling, and stiffness. One crucial factor in the development of rheumatoid arthritis is the Janus kinas (JAK) signaling system, which regulates several cellular processes. Therefore, the JAK inhibitor has become a highly promising treatment for the control of rheumatoid arthritis. Recent years have seen a surge in interest in the design and optimization of small molecule chalcone derivatives as JAK inhibitors for the treatment of rheumatoid arthritis, drawn to their distinctive chemical structures and varied pharmacological activities. [1-3]

The design and optimization of small molecule chalcone derivatives as JAK inhibitors encompass a series of essential stages. To discover prospective lead compounds with the most promising inhibitory effect, the researchers employ computer aided drug design tools to predict the binding affinity and selectivity of chalcone derivative to the JAK enzyme. Medicinal chemists seek to optimize the chemical structures of lead compounds by conducting structure-activity relationship (SAR) studies. [4,5]

This process involves systemically modifying various functional groups and substituents on the chalcone scaffold to improve potency, selectivity, and pharmacokinetics properties.[6] non-steroidal anti-inflammatory drugs (NSAIDS) and disease-modifying antirheumatic drugs (DMARDs) are frequently used drugs in the treatment of rheumatoid arthritis. Effective management of inflammatory diseases such as rheumatoid arthritis has posed a significant problem for scientists due to the absence of safe medications for treatment.[7] In animal models of inflammatory arthritis and in individuals with active rheumatoid arthritis (RA), tumor necrosis factor (TNF-alpha) and interleukin 1 (IL-1) play prominent roles as mediators of inflammation and tissue destruction.[8]

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The RA definition is based on phenotypic characteristics and was refined using a consensus process involving clinical experts, leading to the classification criteria for Rheumatoid arthritis in 1987 and 2010.[9] The diverse nature of the disease response contributes to varying outcomes with different treatments, including anti-tumor necrosis factor (anti-TNF) therapy.[10]

1.1 Chalcone

The term "chalcone" was used by Kostanecki and Tambor to describe a group of naturally occurring chemicals that are precursors of flavonoids, iso-flavonoids, and important components of natural products such as fruits, vegetables, spices, tea, and soy-based food commodities.[11, 12] The fundamental core of a variety of essential biological molecules is formed by 1,3-diphenyl-2-propene-1-one, which is chemically composed of two aromatic rings connected by a highly electrophilic three-carbon, beta-unsaturated carbonyl system.[13] The investigation of the biological activities and therapeutic effects of chalcones began in the 1980s. The compounds chalcone and its derivatives exhibit a wide range of biological and pharmacological activities, including antimicrobial, antimalarial, anti-tumor, antioxidant, antibiotic, anti-tuberculosis, antifungal, antiplatelet, antilipidemic, antiprotozoal (antileishmanial and antitrypanosomally), antibacterial, antioxidant, antimicrobial, antiviral, larvicidal, anti-inflammatory, and anti-cancer potentials. [14, 15] .

Chemical synthesis of chalcones can be achieved by an aldol condensation reaction involving benzaldehyde and acetoaminophenone.[16] Chalcone and its variants have been extensively utilized in some traditional medical systems, such as homeopathy and Chinese medicine, where Ring A is constructed with prime numbers and the B ring is constructed with non-prime numbers.[17] Chalcones are conventionally synthesized by the Claisen Schemidt condensation,[18] which involves the reaction of benzaldehydes and active methylene ketones under homogeneous conditions. However, newer techniques for producing chalcones vary depending on the catalyst type, solvent, base, and reaction environments.[19]

2.Material and Methods

Software

The chemistry drawings were generated using ADMET 3.0, Glide 30.8, Pymol, and Hi Configuration System

Ligand

The JAK 3 Kinase **(**6NY4) is a single-chain framework derived from the sequence of Homo sapiens. Comprehensive crystallographic data can be obtained from PDB. Refer to First Glance for a guided tour of the structural components. Human JAK3 defects are responsible for severe combined immunodeficiency autosomal recessive T-cell-negative/B-cell-positive/NK-cell-negative syndrome (T (-) B (+) NK (-) SCID). A variant of severe combined immunodeficiency (SCID) is a genetically and clinically diverse collection of uncommon congenital diseases marked by compromised humoral and cell-mediated immunity, leukopenia, and reduced or nonexistent antibody levels. Patients in infancy exhibit recurring, chronic illnesses caused by opportunistic microorganisms. The defining feature of all forms of SCID is the lack of tT-cell-mediated cellular immunity caused by a disorder in T-cell maturation. [20, 21]

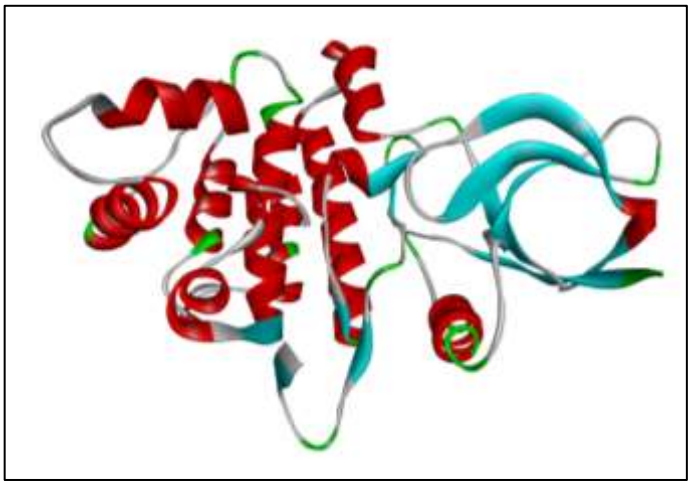

Figure 1: Structure of proteins ligands JAK 3 Kinase **(PDB**-6NY4)

Methods

Study physicochemical properties using ADMET Lab

It was found that many potential therapeutic agents fail to reach the clinic trials because of their unfavorable absorption, distribution, metabolism, elimination, and toxicity (ADMET). So, physicochemical properties were studied using the online tool (https://admetlab3.scbdd.com) It was checked the drug likeness, number of hydrogen bond acceptor, number

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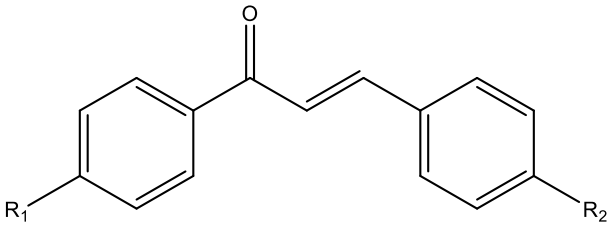
of hydrogen bond donors, flexibility, topological Polar Surface Area, aqueous stability value, distribution coefficient, acid base dissociation constant, natural Product kinas Score, if two properties are out of range, a poor, absorption or permeability is possible, one is acceptable, permeability, parallel artificial membrane permeability assay, the output value is the probability of being Pgp inhibitor, human intestinal absorption, 20% bioavailability, plasma Protein binding, volume Distribution, blood – brain barrier penetration, human Liver microsomal (HLM) stability, the unit of prediction CL plasma penetration, the unit of prediction T1/2 is hours.

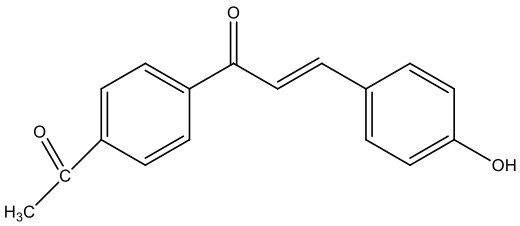
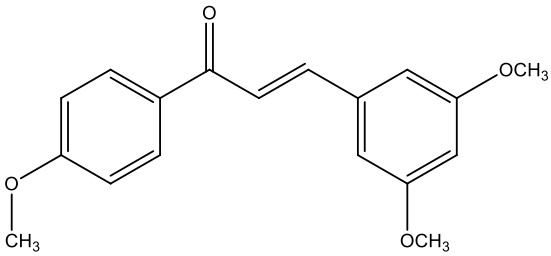
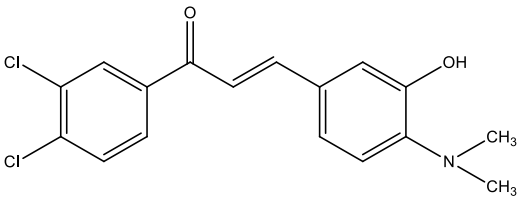
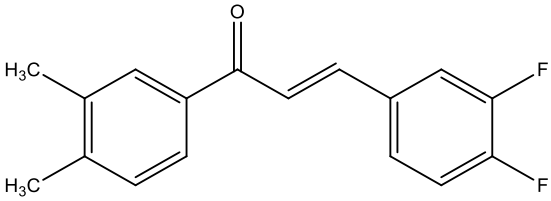
Docking Procedure

To identify potential active drugs against the 6NY4 protein, which was obtain from RSCB protein database 22, we performed docking using Schrodinger Glide 30.8 and calculated the docking score for each ligand molecule. Glide is a molecular modeling software for docking of small proteins and other biopolymers. The docking was done using Glide 30.8 in 6NY4. After the run, the out file stored in the user's folder where the path run was specified in the edit preference. These output files were stored in PDB files, each having fifteen files poses, the glide application file was launched with then showed the empty dashboard along with file "file" on the left-hand corner of the page. The out-file models were loaded using the "read molecule" application from the selected-out file folder. Different were analyzed in the Glide tools.



General structure of chalcone derivative

Table 1: List of Chalcone Derivative

A	 (E)-1-(4-acetylphenyl)-3-(4-hydroxyphenyl) prop-2-en-1-one	B	 (E)-3-(3,5-dimethoxyphenyl)-1-(4-methoxyphenyl) prop-2-en-1-one
C	 (E)-1-(3,4-dichlorophenyl)-3-(4-(dimethylamino)-3-hydroxyphenyl) prop-2-en-1-one	D	 (E)-3-(3,4-difluorophenyl)-1-(3,4-dimethylphenyl) prop-2-en-1-one

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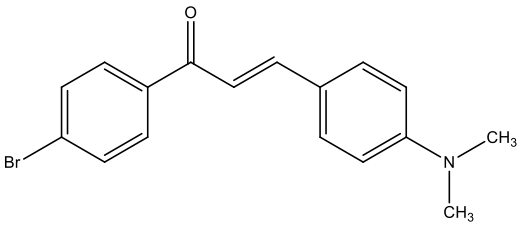
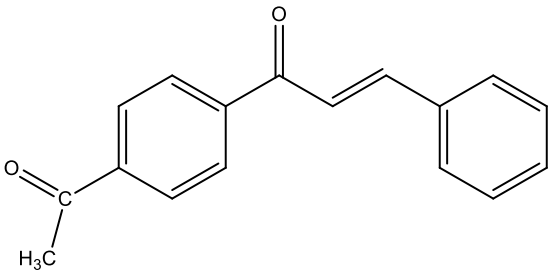
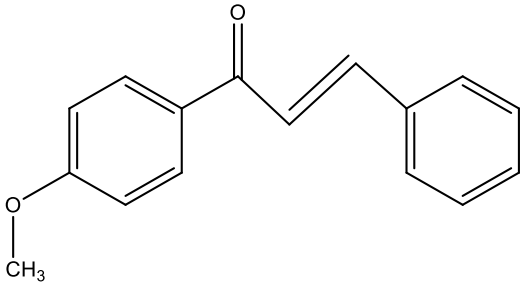
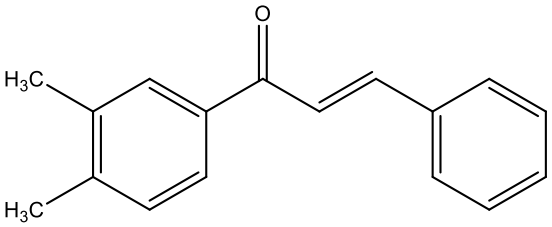
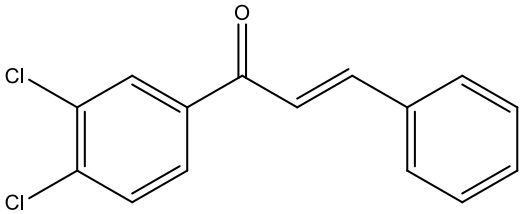
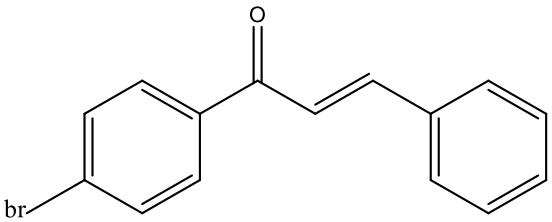
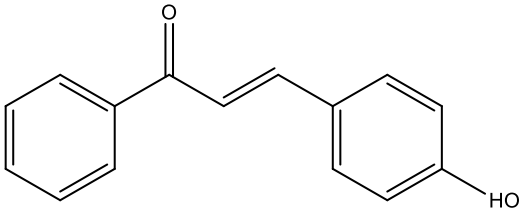
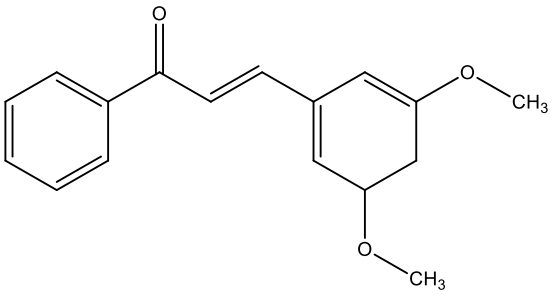
E	 (E)-1-(4-bromophenyl)-3-(4-(dimethylamino) phenyl)prop-2-en-1-one	F	 (E)-1-(4-acetylphenyl)-3-phenylprop-2-en-1-one
G	 (E)-1-(4-methoxyphenyl)-3-phenylprop-2-en-1-one	H	 (E)-1-(3,4-dimethylphenyl)-3-phenylprop-2-en-1-one
I	 (E)-1-(3,4-dichlorophenyl)-3-phenylprop-2-en-1-one	J	 (E)-3-phenyl-1-(p-tolyl)prop-2-en-1-one
K	 (E)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one	L	 (E)-3-(3,5-dimethoxycyclohexa-1,5-dien-1-yl)-1-phenylprop-2-en-1-one

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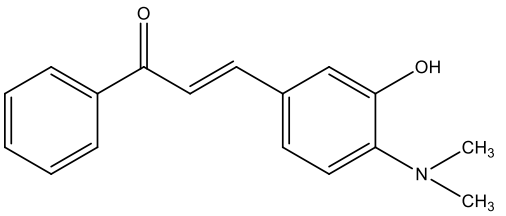
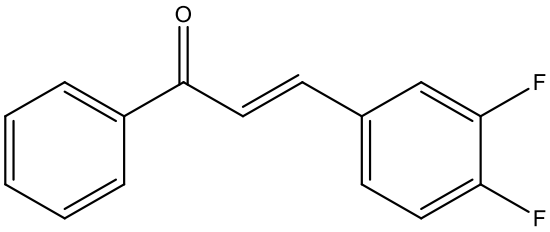
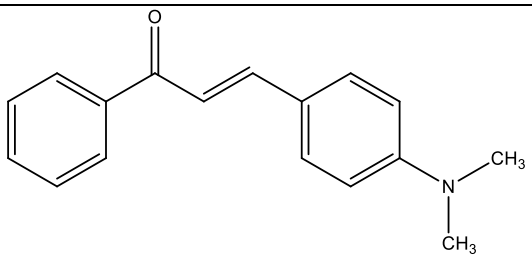
M	 (E)-3-(4-(dimethylamino)-3-hydroxyphenyl)-1-phenylprop-2-en-1-one	N	 (E)-3-(3,4-difluorophenyl)-1-phenylprop-2-en-1-one
O	 (E)-3-(4-(dimethylamino) phenyl)-1-phenylprop-2-en-1-one		

2. Results

2.1. Studied using ADMET Lab

This line all data mention here by using studied by ADMET Lab 3.0, all these things were studied in physicochemical characteristics like, the unit of prediction CL plasma penetration, the unit of prediction $T_{1/2}$ is hours, number of hydrogen bond acceptor, number of hydrogen bond donors, flexibility, topological Polar Surface Area, Optimal: 0-140, aqeous stability value, distribution coefficient, distribution coefficient, acid base disociation constant (Table.no.2), in absorption characteristics like, permeability-Optimal, parallel artificial membrane permeability assay, value is the probability of being Pgp inhibitor, 20% bioavailability (Table.no.3).

Table 2: Physicochemical Characteristics of Chalcones Derivatives

S.no.	compounds	Physicochemical characteristics							
		nHA	nHD	Flexibility	TPSA	logSE	logP	logD	pka
1	A	3.0	1.0	0.267	54.37	-4.108	2.808	2.959	9.036
2	B	4.0	0.0	0.429	44.76	-4.473	3.418	3.374	9.926
3	**C**	**3.0**	**1.0**	**0.286**	**40.54**	**-5.24**	**4.467**	**3.792**	**9.48**
4	D	1.0	0.0	0.214	17.07	-5.052	4.084	3.814	9.166
5	E	2.0	0.0	0.286	20.31	-4.969	3.825	3.674	9.66
6	F	2.0	0.0	0.267	34.14	-3.932	3.19	3.355	8.676
7	G	2.0	0.0	0.286	26.3	-3.535	3.504	3.617	9.382
8	H	1.0	0.0	0.214	17.07	-4.315	3.852	3.812	9.216
9	I	1.0	0.0	0.214	17.7	-5.227	4.455	4.036	9.14
10	J	1.0	0.0	0.214	17.07	-3.84	3.63	3.615	9.376
11	K	2.0	1.0	0.214	37.3	-3.614	3.082	3.183	5.176
12	L	3.0	0.0	0.357	35.53	-3.314	2.626	2.791	9.986
13	**M**	**3.0**	**1.0**	**0.286**	**40.54**	**-4.154**	**3.342**	**3.407**	**9.995**
14	N	1.0	0.0	0.214	17.07	-4.055	3.496	3.542	8.737
15	O	2.0	0.0	0.286	20.31	-4.044	3.315	3.472	10.189

nHA = Number of hydrogen bond acceptor, nHD = Number of hydrogen bond donors, Flexibility = Flexibility = nRot/nRig, TPSA = Topological Polar Surface Area, Optimal: 0-140, Logs = Aqeous stability value, logP = Distribution coefficient, logD = Distribution coefficient, Pka (acid) = Acid base dissociation constant

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In distribution characteristics like, plasma Protein binding, volume distribution, blood – brain barrier penetration (Table.no.4), in metabolism characteristics like, human liver microsomal (HLM) stability (Table.no.5), in excretion characteristics like, the unit of prediction CL plasma penetration, the unit of prediction T1/2 is hours (Table.no.6).

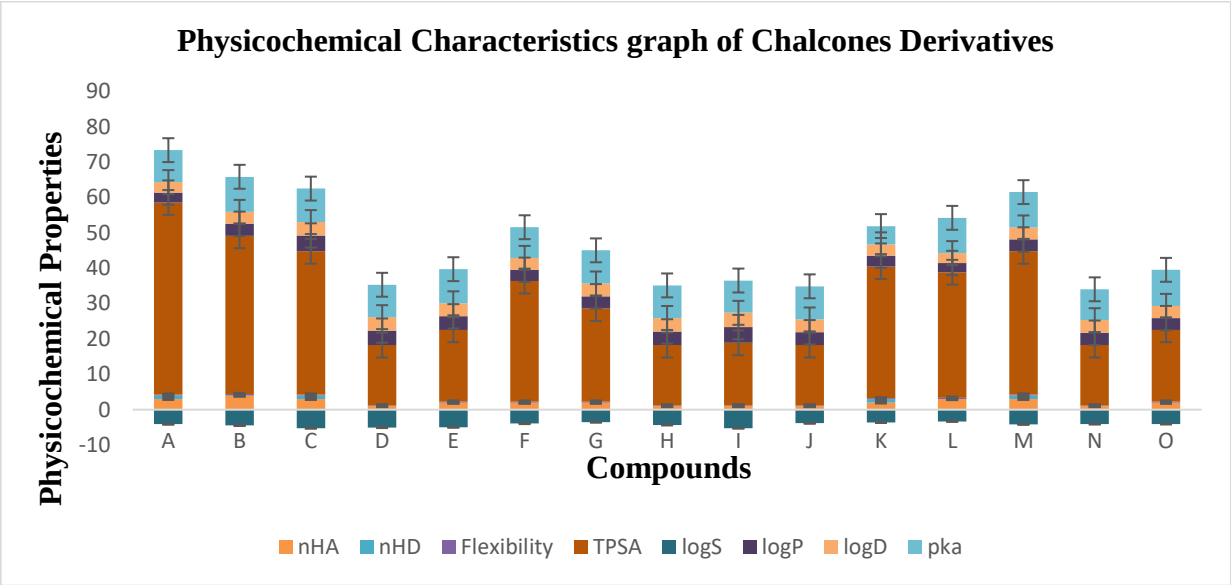

Figure: 2 Physicochemical Characteristics graph of Chalcones Derivative

Table 3: Absorption Characteristics of Chalcone Derivative Compounds

S.no	compounds	Permeability	PAMPA	Pgp-inhibitor	F20%
1	A	-4.837	0.073	0.887	0.166
2	B	-4.707	0.001	0.975	0.078
3	C	**-5.197**	**0.06**	**0.978**	**0.007**
4	D	-4.698	0.011	1.0	0.015
5	E	-4.99	0.006	1.0	0024
6	F	-4.741	0.025	0.997	0.017
7	G	-4.709	0.02	0.989	0.087
8	H	-4.524	0.029	0.999	0.045
9	I	-4.995	0.032	0.098	0.001
10	J	-4.719	0.045	0.999	0.026
11	K	-4.817	0.08	0.921	0.178
12	L	-4.736	0.041	0.226	0.057
13	M	**-4.883**	**0.076**	**0.975**	**0.198**
14	N	0.653	0.014	0.06	0.007
15	O	-4.8	0.019	0.019	0.105

Permeability = Permeability – Optimal : higher than – 5.15 log unit, PAMPA = Parallel artificial membrane permeability assay, Pgp inhibitors = The output value is the probability of being Pgp inhibitor, F20% = 20% bioavailability

Table 4: Distribution properties of chalcone derivatives

S.no.	compounds	Distribution		
		PPB	VDss	BBB
1	A	93.802	0.061	0.061
2	B	86.592	-0.066	0.002
3	**C**	**98.797**	**0.021**	**0.927**
4	D	97.803	0.279	0.87
5	E	98.05	0.518	0.176

6	F	97.058	0.064	0.58
7	G	97.622	0.12	0.307
8	H	98.445	0.264	0.839
9	I	98.5	0.183	0.998
10	J	98.309	0.098	0.784
11	K	97.164	-0.146	0.116
12	L	90.997	0.152	0.398
13	**M**	**98.157**	**-0.311**	**0.249**
14	N	97.8	0.068	0.932
15	O	98.107	0.161	0.028

PPB = Plasma Protein binding, VDss = Volume Distribution, BBB = Blood – brain barrier penetration

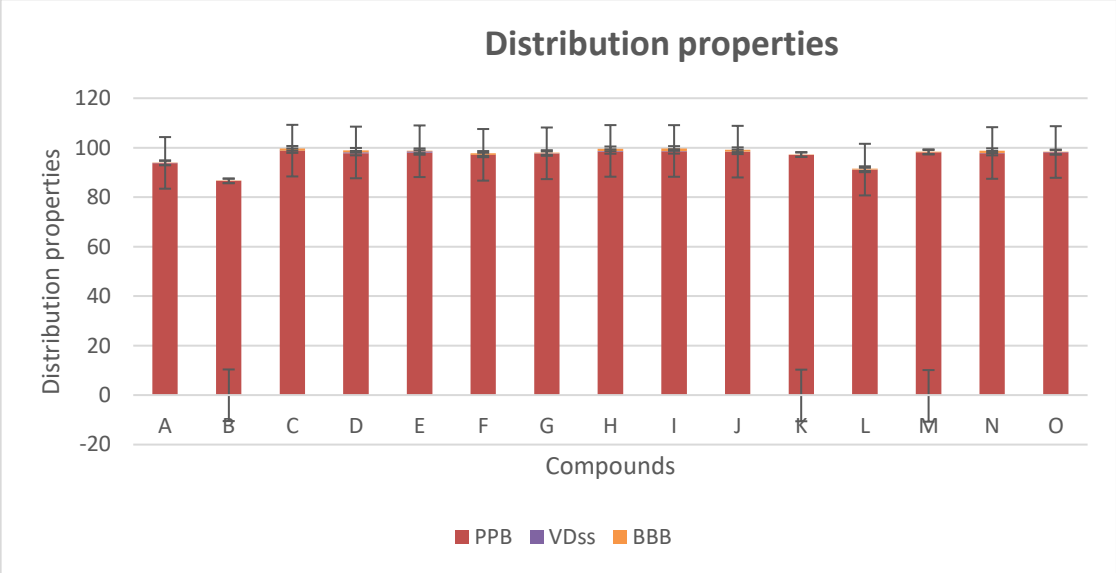

Figure 3: Distribution properties of chalcone derivative

Table 5: Metabolism Properties of Chalcone Derivative Compounds

S. No.	Compounds	Metabolism	S. No.	Compounds	Metabolism
		HLM stability			HLM stability
1	A	0.991	9	I	0.038
2	B	0.138	10	J	0.368
3	**C**	**0.079**	11	K	0.422
4	D	0.368	12	L	0.963
5	E	0.191	13	**M**	**0.079**
6	F	0.896	14	N	0.161
7	G	0.427	15	O	0.201
8	H	0.157			

HLM Stability = Human Liver microsomal (HLM) stability

Table 6: Excretion Characteristics of Compounds Derived from Chalcones

S. no.	Compounds	Excretion	
		CL plasma	$T_{1/2}$
1	A	10.399	0.88
2	B	11.442	0.529

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3	**C**	**7.052**	**0.774**
4	D	10.466	0.455
5	E	9.287	0.561
6	F	9.668	0.662
7	G	11.659	0.557
8	H	10.967	0.458
9	I	9.221	0.68
10	J	10.696	0.68
11	K	11.639	0.919
12	L	12.526	1.192
13	**M**	**8.579**	**0.879**
14	N	10.508	0.905
15	O	10.583	0.507

CL Plasma = The unit of prediction CL plasma penetration, T1/2 = The unit of prediction T1/2 is hours

2.2 Docking results of derivatives compounds

Reported data as the docking score of chalcone derivatives all compounds docking score, glide lipo, glide hydrogen bonding, and glide energy of all total fifteen compounds score mention here, and data docking score is calculated by using the schrodinger glide 0.3. And two most better score of docking (C and M) derivatives compounds, docking score of **(C = -9.158 & M = -9.084)** is better score, Table 7 & Figure 4.

Table 7: Glide docking assessment of compounds

Docking Score data				
Ligands	**Docking score**	**Glide lipo**	**Glide hydrogen binding**	**Glide energy**
A	-8.363	-3.303	-0.343	-39.982
B	-8.415	-3.726	-0.32	-40.607
C	**-9.158**	**-3.89**	**-0.583**	**-44.507**
D	-8.504	-3.606	-0.32	-33.372
E	-8.187	-3.494	-0.32	-40.404
F	-8.666	-3.324	-0.343	-39.558
G	-8.595	-3.643	-0.32	-37.488
H	-8.602	-3.622	-0.32	-36.923
I	-8.737	-3.671	-0.32	-39.598
J	-8.506	-3.493	-0.32	-36.318
K	-8.056	-3.134	-0.32	-34.23
L	-8.531	-3.132	-0.341	-35.341
M	**-9.084**	**-3.547**	**-0.601**	**-39.083**
N	-8.547	-3.548	-0.324	-36.348
O	-8.191	-3.331	-0.32	-35.558

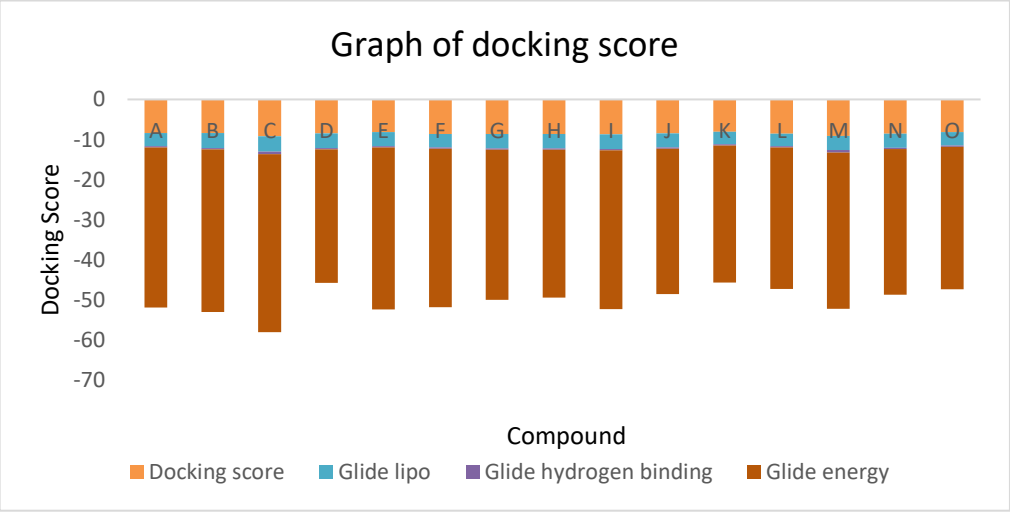

Figure 4: Docking score properties of chalcone derivative

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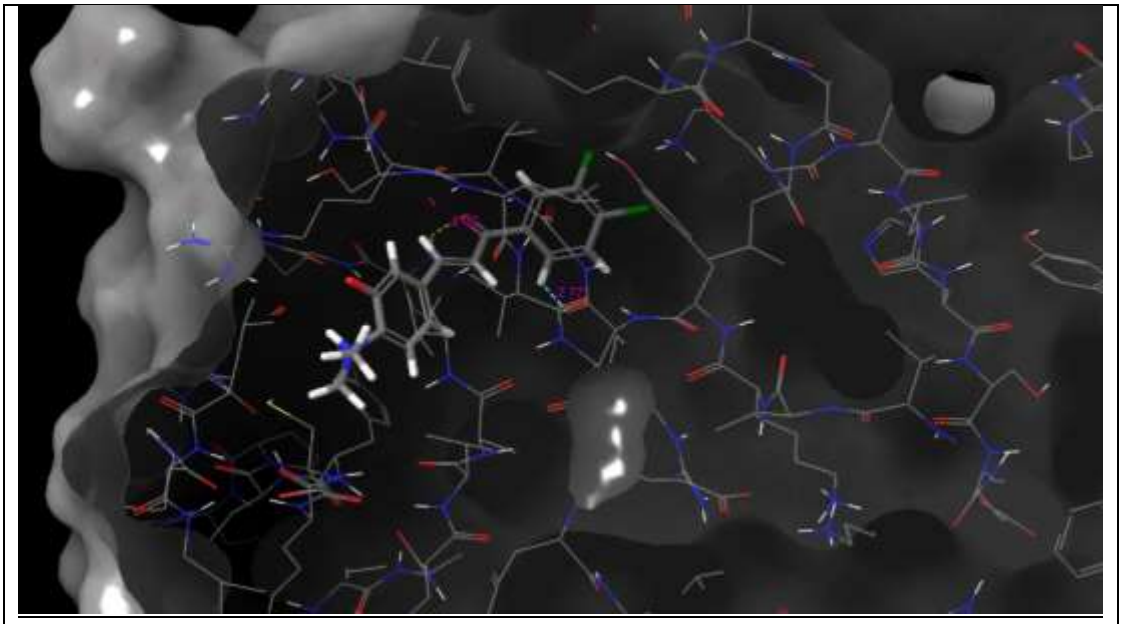
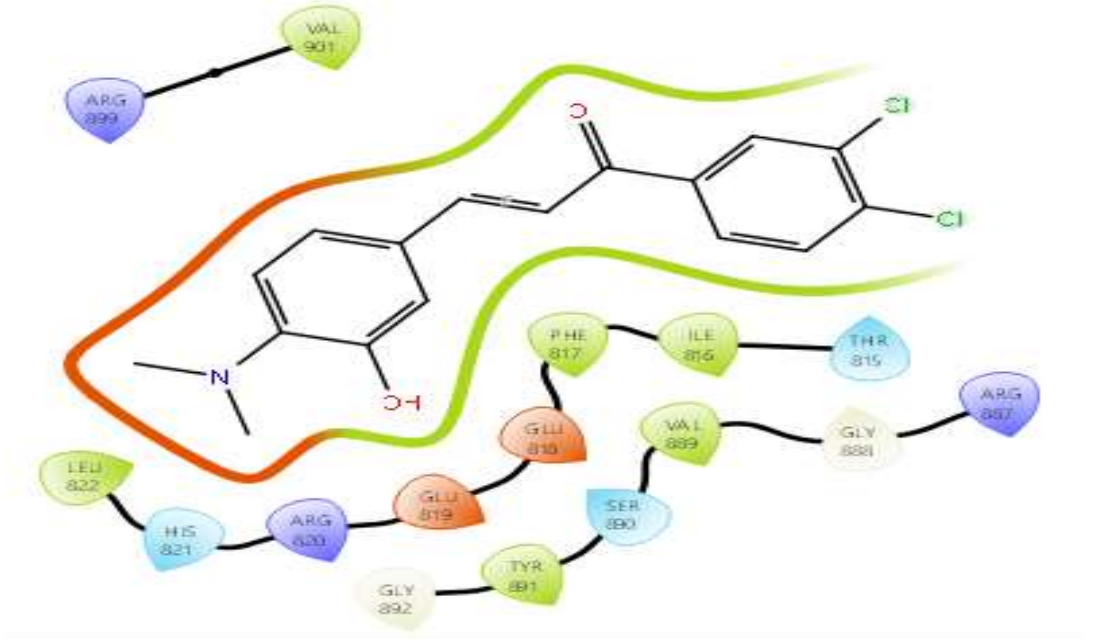


3D-docking poses C



2D-docking poses

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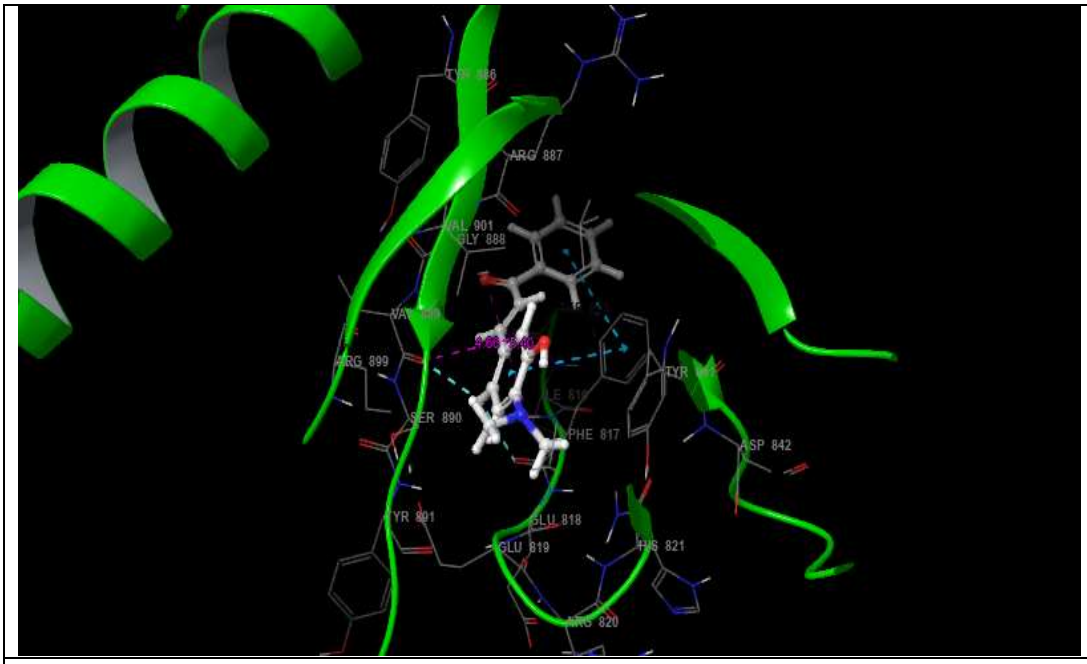
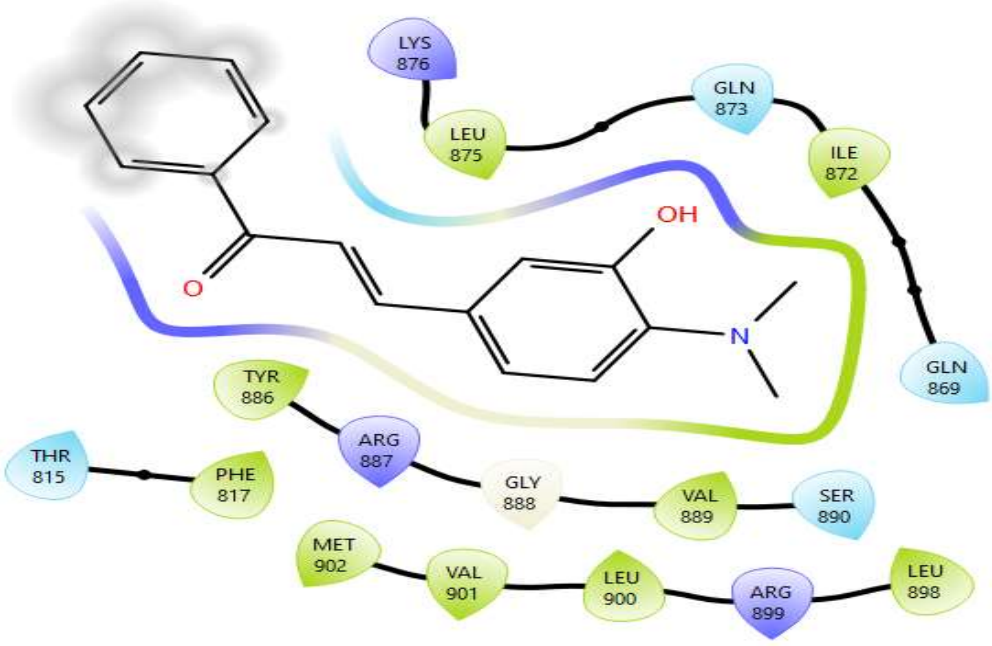


3D- docking poses of compound M



2D-docking poses of compound M

Figure 5: Docking pose of optimized compound

The **C** compounds have a binding affinity **(-9.158),** VAL901, LEU822, PHE817, ILE816, LEU822, VAL889, & TYR891 showed hydrophoic interaction with protein, charge (positive) ARG899, ARG820 & ARG887, charge (negative) GLU818, GLU819, polar nature is the HIS821, SER890 & THR815, and water GLY892, GLY888 bind to each other and show of protein affinity. The **M** compounds has a binding affinity **(-9.084),** LEU875, ILE872, TYR886, VAL889, PHE817, MET902, VAL901, LEU900, and LEU898, charge (positive) LYS876, polar nature is the GLN873, GLN869, ARG887, SER890, THR815, ARG899, and water GLY815 bind to each other and show of protein affinity Figure 5.

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4. Discussion:

Analysis of ADME

All compounds C to M (Table.no.7) were conclusively identified as orally active drugs in this study. Evaluation of the ADME properties of compounds was conducted using Swiss ADME software. The rule of fifteen proposed by Lipinski asserts that substances with a molecular weight below 500 have sufficient oral bioavailability. Each chemical met the established criterion. The gastrointestinal safety profile was also conducted using Swiss ADME software. The online web servers were provided with the list of SMILES and subsequently generated the required functions of the specific compounds.

Predicted acute toxicity of molecules

The goal of acute toxicity is to identify the amount that, whether administered once or over a few administrations, will result in death or major toxicological consequences. They also function as a source of knowledge on dosages that ought to be used in later research. These investigations offer an additional chance to ascertain compound-induced effects as shown by clinical chemistry, morphology, or other assessments. Additionally, acute investigations may provide an early indicator of acute toxicity studies.

Docking Analysis

It was the purpose of the docking investigation to ascertain the degree of affinity that the new Chalcone derivatives possessed for the binding site of the 6NY4 JAK Kinase protein. Presented in Tables 8 are the docking scores of the compounds that were created. Compounds C and M, which are the most powerful derivatives, were chosen based on their docking positions. Additionally, all the substances exhibited hydrogen bond interactions with amino acid residues, including specific amino acids. These interactions were comparable to the chemical interactions that were seen with the active region of JAK kinase. Figure 4 displays the relevant docking poses in both two and three dimensions.

5. Conclusion

In summary, several 6NY4 compounds were synthesized to offer improved JAK kinase inhibitors with significant anti-inflammatory characteristics. Among the compounds in the series, compounds C to M (Table.no.8) have shown the highest level of JAK kinase inhibitory action. Furthermore, the docking study of compounds and all their derivatives showed a favorable orientation within the active bond region of the JAK kinase. The docking score of these compounds was comparable to that of a kinase inhibitor. Each of the synthesized compounds was determined to be oral active pharmaceuticals by ADME assays, which were conducted according to Lipinski's rule of fifteen. Moreover, the compounds exhibited exceptional gastrointestinal absorption within the permissible limits.

6. Conflicting research findings

All authors assert that they have no conflicts of interests.

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