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"Unlocking Lyase Binding: A Computational Exploration of 2-(2-(5-Chloro-3-Methyl-1-Phenyl-1H-Pyrazol-4-Yl)Vinyl)-6-Methylpyrimidin-4-Ol Derivatives"

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Abstract

An entirely with pyrazole Nonsteroidal anti-inflammatory substances (NSAIDs), which are frequently employed in the treatment of cancer.Pyrazole, a crucial heterocyclic scaffold in medicinal chemistry, has a wide range of biological actions including antiviral, anticonvulsant, anticancer, antibacterial, and antifungal activity were the inspiration for the creation of pyrazole bearing pyrimidine derivative.[1,2] In order to evaluate pyrazole carrying Pyrimidine analogues as anti-inflammatory, anticancer, and COX inhibitors, successful marketed medications with pyrazole as the central core (Celecoxib, Deracoxib) were used as a foundation.By enhancing specificity towards COX2 as opposed to COX1, it could possibly be feasible to create Analgesics with greater medical value as well as reduced imperilment.[3]In the current investigation, the selective COX2 inhibitor celecoxib's central pyrazole core, which is coupled to the phenylsulfonamide group, has been employed. Through chemical modification novel pyrazole bearing Pyrimidine analogues were created. To explain the derivatives' binding affinities and their mechanisms for interaction with the active site of the lyase enzyme, a molecular interaction investigation was also conducted.[4]

Keywords: *Cyclooxygenase-1, cyclooxygenase-2, 4,5-dihydro-1H-pyrazole, inflammation, molecular docking*

Introduction

Until more than 20 years ago, celecoxib, a COX-2 selective inhibitor, has predominantly been implemented as an analgesic, antipyretic, and anti-inflammatory medication. NSAIDs function pharmacologically by inhibiting COX enzymes, which catalyse the synthesis of prostaglandins, prostacyclins, and thromboxanes from arachidonic acid.[5] The two different COX isoforms, COX1 [constitutive] and COX2 [inducible], are assumed to be equally significant for the synthesis of pathological and physiological prostaglandins. Celecoxib mainly regulates the proliferation, migration, and invasion of tumor cells by inhibiting the cyclooxygenases-2/prostaglandin E2 signal axis and thereby inhibiting the phosphorylation of nuclear factor-κ-gene binding, Akt, signal transducer and activator of transcription and the expression of matrix.[6]

Both preclinical and clinical studies on the use of Celecoxib in the treatment and prevention of cancer has showed promise; the best results were observed in cases of colon, breast, prostate,head and neck cancers. However, more clinical studies that provide real therapeutic breakthroughs based on evidence for celecoxib use are still needed.[7]The enzyme ATP citrate lyase (ACLY), which plays a key role in the synthesis of lipids connected to glucose metabolism, catalyses the conversion of citrate to oxaloacetic acid (OAA) and acetyl-CoA.The produced acetyl-CoA is essential for lipid synthesis during membrane biogenesis in cancer cells during acetylation procedures that regulate the expression of particular proteins. Studies show that ACLY contributes to the development of cancer by boosting metabolic activity by stimulating the Akt signalling system.[8]It is well recognised that inflammatory cells influence the development of cancer by promoting cell survival, migration, and proliferation in the tumour microenvironment. Thus, the use of anti-inflammatory drugs in cancer therapy seems appropriate.[9] One potential solution for the aforementioned issue is the suppression of cyclooxygenase-2, especially in light of the fact that overexpression of this enzyme has been linked to cancer tissues and a poor prognosis in a variety of different types of human malignancies.[10]The use of celecoxib for its anti-cancer effects has significantly increased in recent years.However, not enough research has been done to date on its anti-cancer properties.

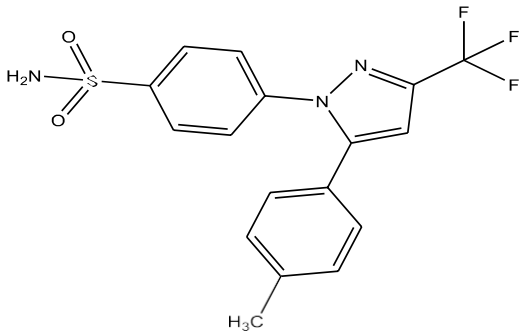


Figure 1: Structure of celecoxib

The pyrazole ring, a 5-membered ring system containing two nitrogen atoms, and its monounsaturated derivative, the 4,5-dihydro-1H-pyrazole ring, are in the structure of many nonsteroidal antiinflammatory drugs (NSAIDs) such as antipyrine, phenazone, metamizole and phenylbutazone [2,10].

These ring systems inhibit cyclooxygenase enzymes, particularly the COX-2 enzyme.This article discuss the design, synthesis, and pharmacological action of a novel class of hybrid pyrazole and pyrimidine analogues, known as (1–10). The1JCZwas provided from Protein Data Bank . Chain A was selected for symmetric chains and active sites of the protein present in all chains . Chain A selected, and other chains were deleted.[11]

Materials and methods

ChemDraw Ultra 12.0 was utilised to draw the 2D formula of the compounds. The 2-dimensional structures (2D) of the analogues, were retrieved from the databaseCAS Scifinder^{n}in formataccessed on November 12, 2022, 8:29AM.[13]For preparing the protein and ligands and determining the Grid map, Autodock 1.5.6 was used. Chain A was selected. Then, the water was deleted, and polar hydrogens and Gasteiger charges were added. A grid map was determined**(Table1).** Energy minimisation was applied with Avogadro software. The docking process wasrealised with Autodock Vina and visualisation of interactions was made by Discovery Studio 3.5.Docking studies were also conducted to investigate potential interactions between the strong compounds.[14] When docked into the COX2 active site, these selective COX2 inhibitors displayed the same orientation and method of binding as celecoxib .The SO_2NH_2 is tightly incorporated into the selective pocket of COX2-active site.[15]

To optimise the docking process, crystal structure of celecoxib (CEL) was compared with the predicted conformations of docking results. 2,3 and7,9 shows best celecoxib's considerable COX2-inhibitory effect (selectivity index = 78.06). Celecoxib’s docking value was -4.6 while the scores for the powerful compounds (AV9 and AV2,AV3) were -7.0 and -6.7,-6.7 respectively. Molecular Docking was used to determine the potential optimum binding pose of the compounds (1-10) by which they may be sorted for discovering promising leads. The primary steps in molecular docking research are choosing and preparing the right protein, creating the grid and the ligand, and then analysing the results of the docking and how they interact.

Grid box dimension		Pdb Id
X	35.112	1JCZ
Y	0.502	
Z	25.518	

Table 1: Grid box dimension

Protein Homology

The 1JCZ protein structure was prepared for docking using BIOVIA discovery studio visualizer (Discovery Studio Visualizer 2023-12-01T4;30:32z) [16]. The discrepancies in the structure (lacking hydrogens, removal of water molecules and ligands, orientation of the various functional groups) were examined and corrected.[17]

Molecular Docking

Docking was performed by converting both receptor and ligands file to the pdbqt format using AutoDock Tools (v1.5.6, https://autodock.scripps.edu, accessed on 06November 2023). Molecular docking and calculation of binding affinity were performed using AutoDock Vina (https://vina.scripps.edu, accessed 12 November 2023) [14]. The results of molecular docking were visualized using a BIOVIA discovery studio visualizer [16].

3D Structure Validation

To check the compatibility of atomic models (3D) with their primary amino acid sequences (1D), The VERIFY3D server was used [19-20]. The Verify 3D evaluated that the predicted protein has 90.91%% residues with an average 3D-1D score ≥0.1 which signifies the consistency and reliability of the 3D model because the ideal score values for Verify-3D should be 80% as shown in (Figure 1).[18]

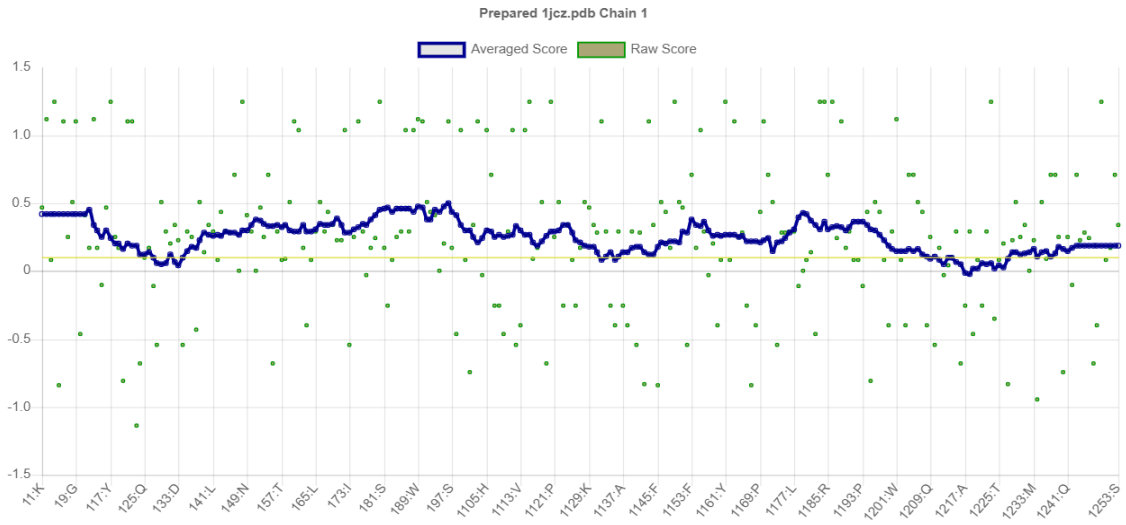


Figure 2: Stereochemical analysis of 1JCZ showing 90.91% of the residues have averaged 3D-1D score >= 0.1

Evaluation of predicted protein structure

Ramachandran plot can be used for evaluating the accuracy of predicted protein structure. Several tools and servers, such as PDBSUM SERVER, MOLPROBITY, STAN SERVER, and others, are used to generate Ramachandran plot. The saves v6.0 (https://saves.mbi.ucla.edu/) is a comprehensive toolkit and has five tools (ERRAT, VERIFY3D, PROVE, PROCHECK, AND WHAT CHECK), which predict different types of stereochemical parameters of the protein

structure. the Ramachandran plot predicts the structural stereochemical property. The procheck analyzes the overall model geometry with the residue by residue geometry and provides the stereochemical quality of a predicted model. Procheck tool requires modeled protein file as an input and generates the Ramachandran plot.To check the structural integrity, i.e., The stereochemical quality of the 3d model of protein 1jcz, procheck [3-4], a program that relies on

Ramachandran plots for structure verification, was used (figure 3). Ramachandran plot of the prepared protein represents 87.2% (197 amino acids) of the total residues in the most favored region and 12.8% (29 amino acids)in the additionally allowed region, indicating a good quality model for study.

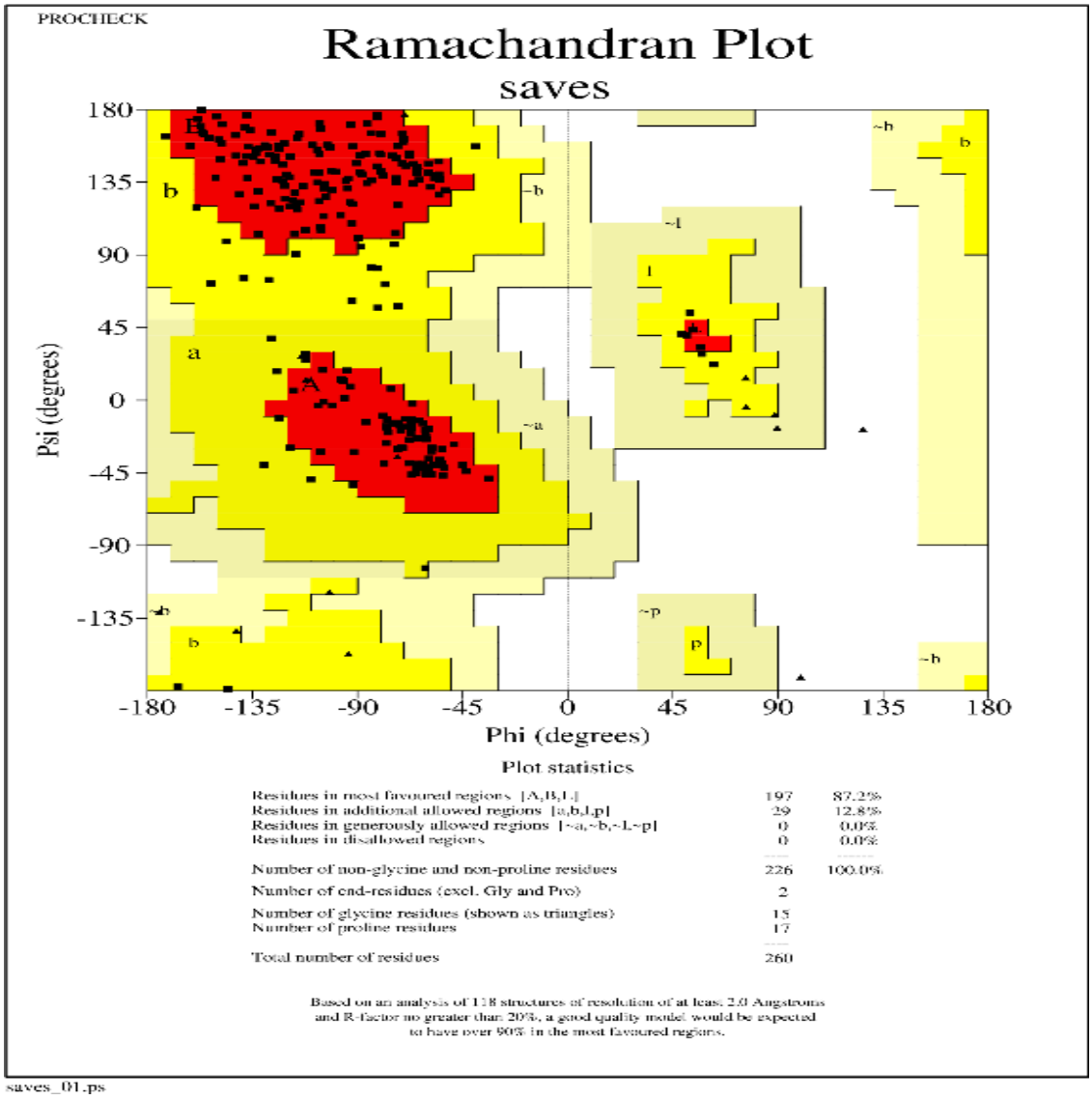
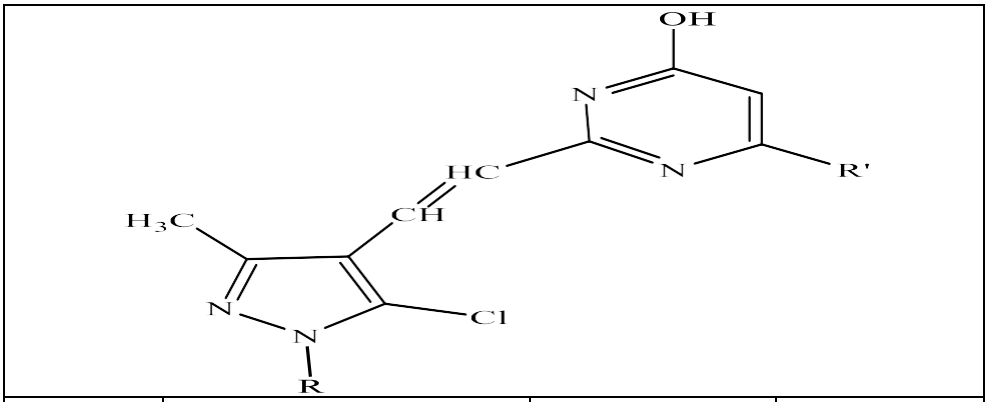


Figure 3: Stereochemical analysis of 1JCZ.

Result and discussion

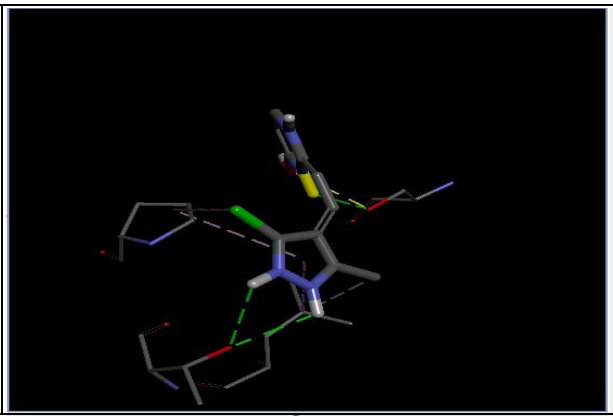
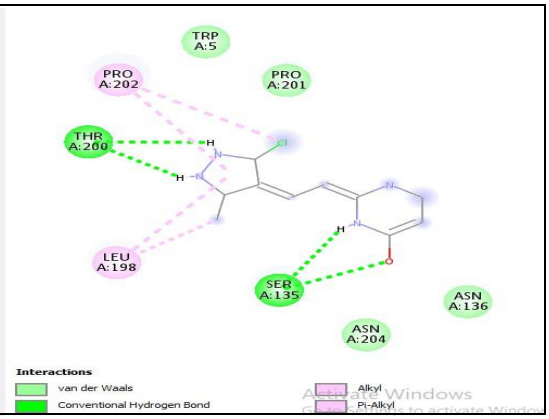


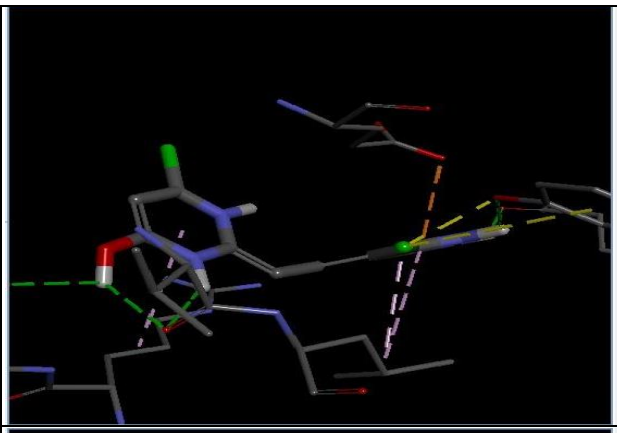
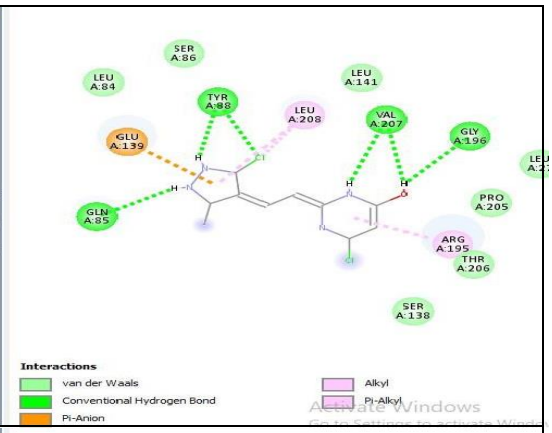
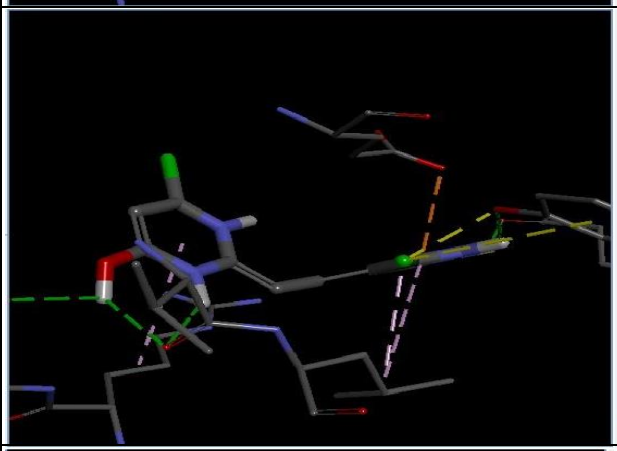
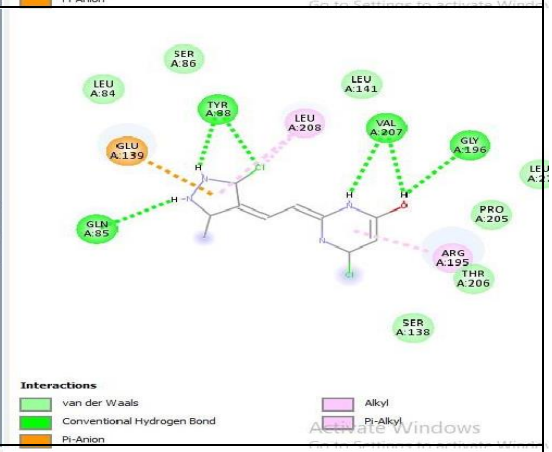
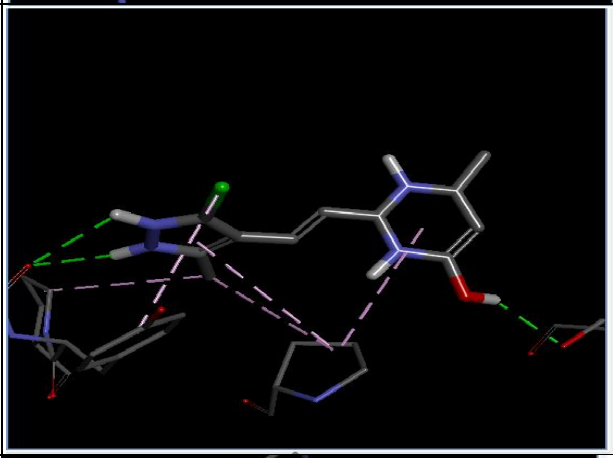
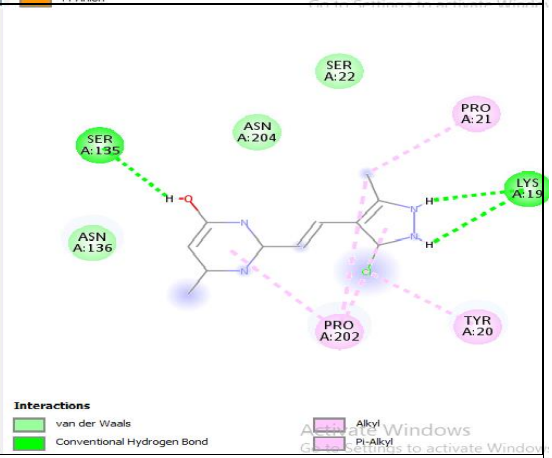
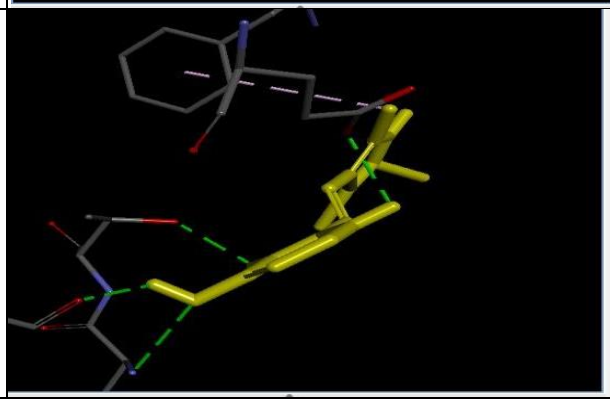
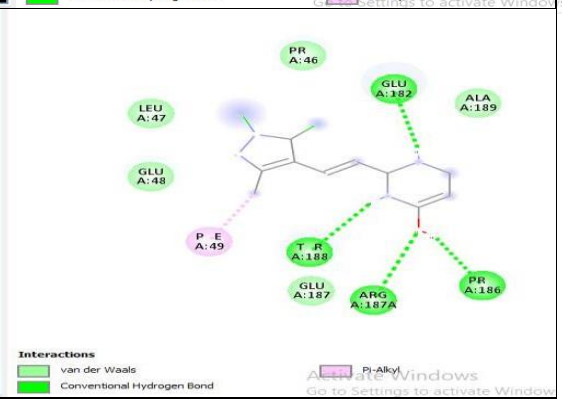
S.No.	COMPOUNDS	R	R'
1	**AV1**	H	H
2	**AV2**	H	Cl
3	**AV3**	H	NO_2
4	**AV4**	H	CH3
5	**AV5**	Cl	H
6	**AV6**	OCH3	H
7	**AV7**	F	CH3
8	**AV8**	NO_2	H
9	**AV9**	NH2	H
10	**AV10**	F	H

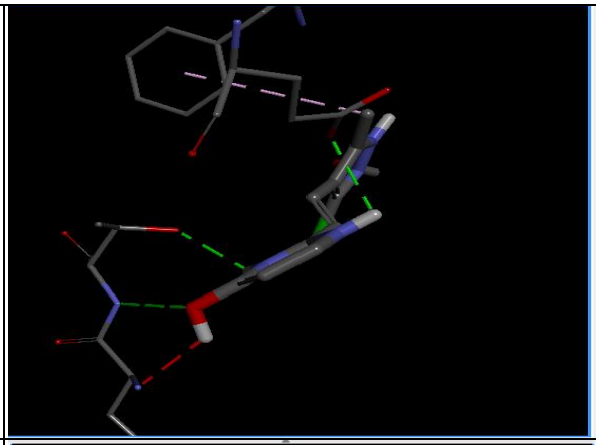
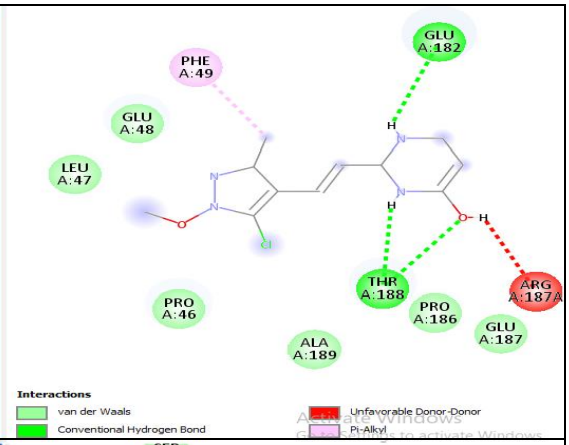
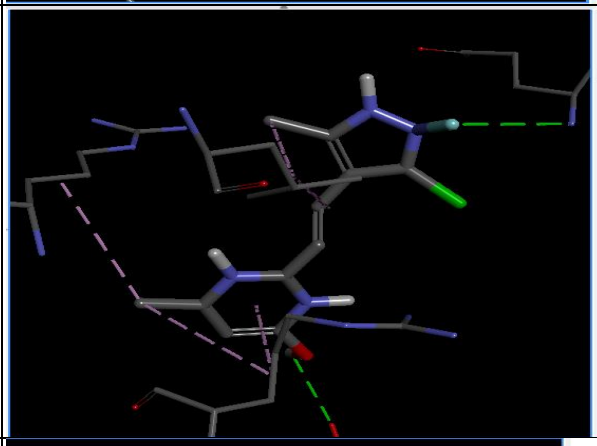
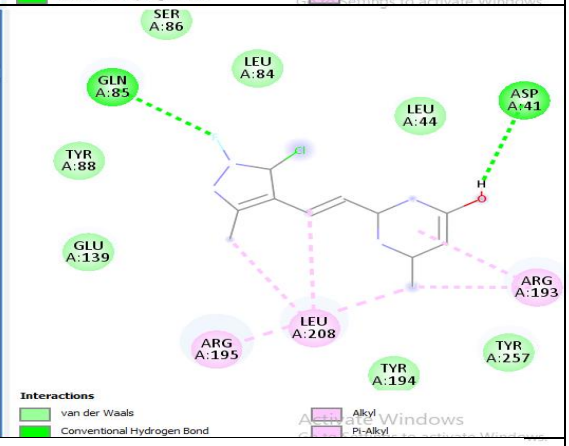
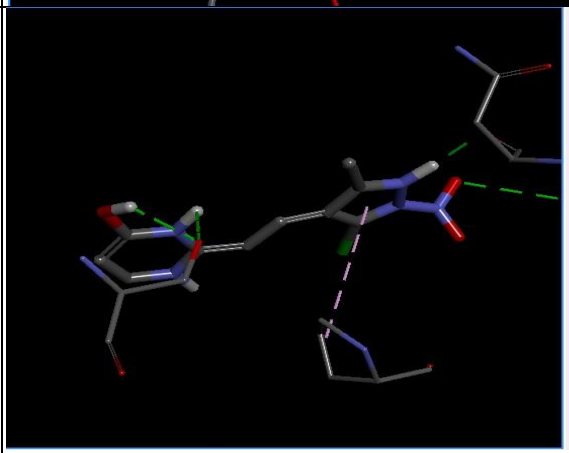
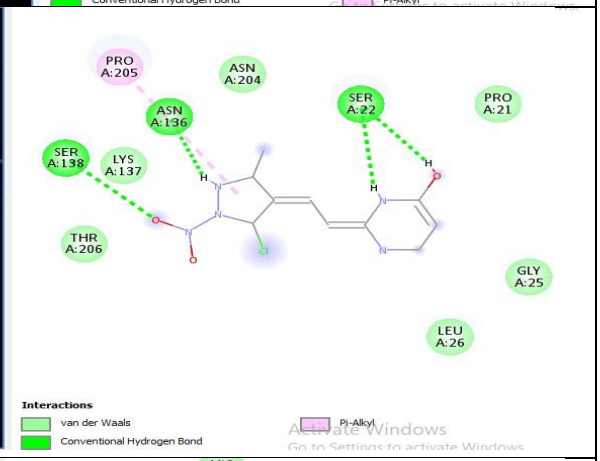
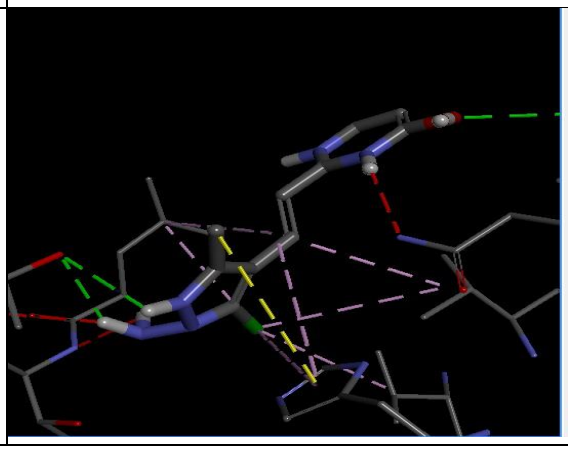
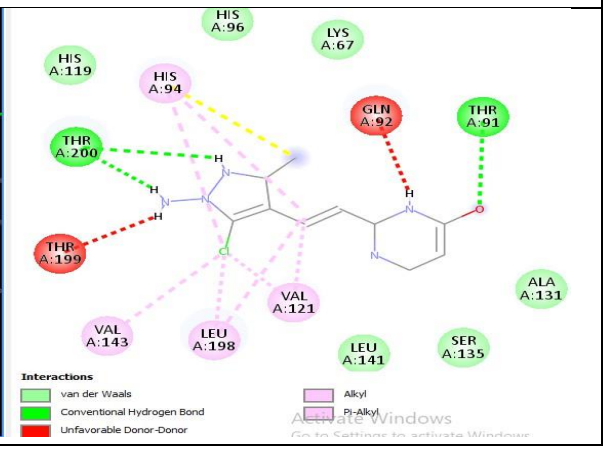
Table 2: The formula of ligands tested in molecular docking

Table3: 3D and 2D interaction of best conformer with Lyase receptor with 1JCZ Protein

COMPOUNDS	3D	2D

AV1		 TRP A:5 PRO A:202 PRO A:201 THR A:200 LEU A:198 SER A:135 ASN A:136 ASN A:204 Interactions van der Waals Conventional Hydrogen Bond Alkyl Pi-Alkyl

AV2		
AV3		
AV4		
AV5		

AV6		
AV7		
AV8		
AV9		

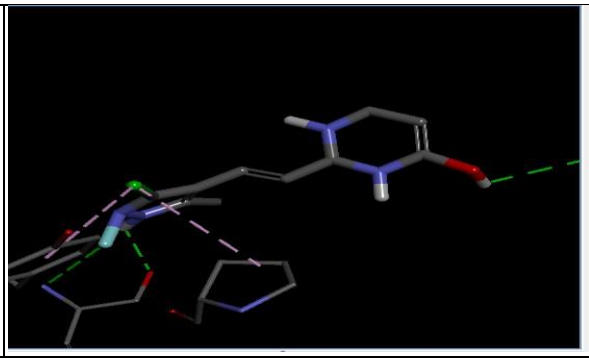
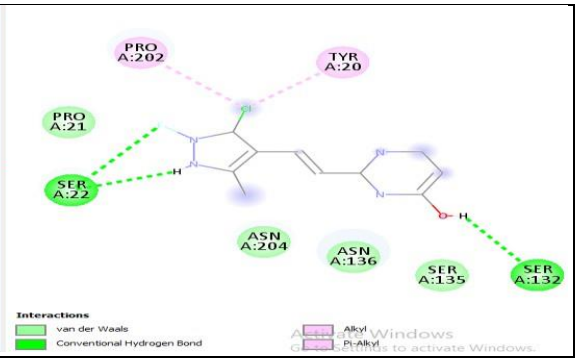
AV10		

Table4: Hydrobhobic Interaction and binding energy of compounds

Compound	Hydrophobic interaction	Binding energy (K.Cal/Mol)
AV1	TRP:5, PRO:201, ASN:136, ASN:204	-5.1
AV 2	LEU:84, SER:86, LEU:141,LEU:27	-6.7
AV 3	LEU:141, GLN:92, THR:200, HIS:64, SER:65	-6.7
AV 4	ASN:22, SER:22, ASN:136	-4.6
AV 5	LEU:47, GLU:48,PRO:46, ALA:189, GLU:187	-5.4
AV 6	LEU:47, GLU:48, PRO:46, ALA:189, GLU:187,	-5.5
AV 7	SER:86, LEU:84, LEU:44, TYR:257, TYR:194, GLU:139	-6.1
AV 8	THR:206, LYS:137, PRO:205, ASN:204, PRO:21 GLY:25, LEU:26	-5.2
AV 9	HIS:119, HIS:96, LYS:67, ALA:131, SER:135, LEU:141	-7.0
AV 10	PRO:21, SER:135, ASN:136, ASN:204	-5.0
Celecoxib (standard)		-4.6

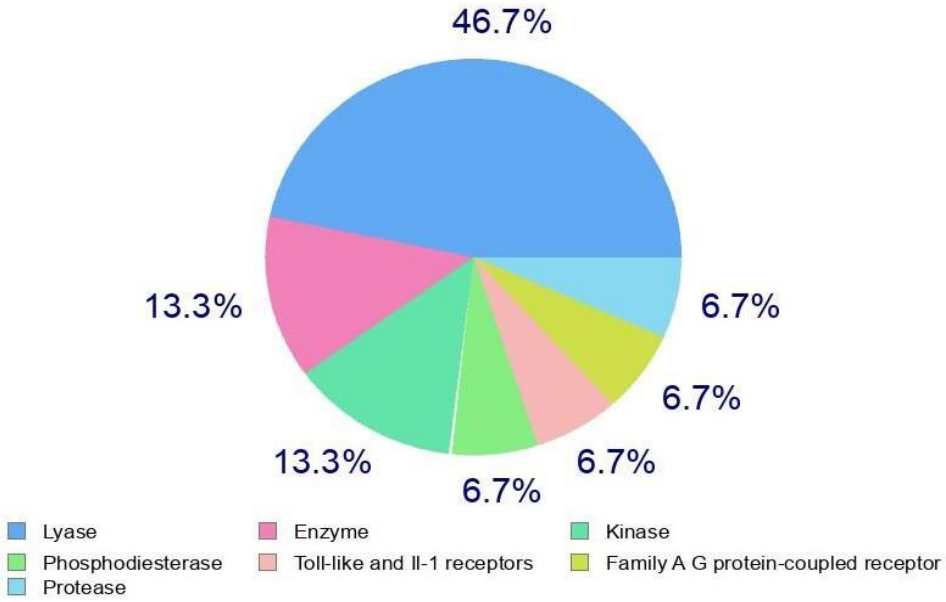


Figure4: Result of Interaction in percentage of Ligands with different Enzymes and Receptors

Docking Result

The molecular docking technique is used to predict their possible targets.The protein enzyme Lyase(PDB ID:1JCZ) was used and the docking score values of the synthesized compounds are given in the Table-3.The compound**AV9 and AV2,AV3**shows Excellent binding score-7.0 and -6.7,-6.7 for an enzyme Lyase(PDB ID:1JCZ) with grid box dimensions **X=35.112, Y=0.502,Z=25.518.**In the Table 4the compound AV1 has the binding energy(-5.1) andhydrophobic interactions**TRP:5, PRO:201, ASN:136, ASN:204.**The compound AV2 has the binding energy(-6.7)and hydrophobic interactions**LEU:84, SER:86, LEU:141,LEU:27.**The compound AV3 has the binding energy(-6.7)and hydrophobic interactions**LEU:141, GLN:92, THR:200, HIS:64, SER:65.**The compound AV4 has the binding energy(-4.6)and hydrophobic interactions**ASN:22, SER:22, ASN:136.**The compound AV5 has the binding energy(-5.4)and hydrophobic interactions**LEU:47, GLU:48,PRO:46, ALA:189, GLU:187.**The compound AV6 has the binding energy(-5.5)and hydrophobic interactions**LEU:47, GLU:48, PRO:46, ALA:189, GLU:187.**The compound AV7 has the binding energy(-6.1)and hydrophobic interactions **SER:86, LEU:84, LEU:44, TYR:257, TYR:194, GLU:139.**The compound AV8 has the binding energy(-5.2)and hydrophobic interactions**THR:206, LYS:137, PRO:205, ASN:204, PRO:21 GLY:25, LEU:26.**The compound AV9 has the binding energy(-7.0)and hydrophobic interactions**HIS:119, HIS:96, LYS:67, ALA:131, SER:135, LEU:141.**The compound AV10 has the binding energy(-5.0)and hydrophobic interactions**PRO:21, SER:135, ASN:136, ASN:204.**The compounds shows inhibitory action against various enzymes and receptors like Family AG protein –coupled receptor(6.7%), Toll like and II-1 receptors(6.7%),Kinase(6.7%), Protease(6.7%),Phosphodiestrase Enzyme(13.3%),and with Lyase (46.7%)which is the best inhibiton action among all the derivatives**.**

Conclusion

In the current study, ten derivatives of pyrazole-bearing Pyrimidine derivatives were analysed for docking against celecoxib to understand the drug target interactions. The structure of derivatives were modeled and docked by using Auto-DockVina Sotware. Our results showed that all derivatives has the highest binding affinity on binding to**1JCZ**due to electron withdrawing groups like NO_2,Cl,Br,F,OCH_3,NH_2 but **AV9 and AV2,AV3** shows best activity than celecoxib as a standard. Thus all derivatives appears to be more effective comparative Celecoxib.In conclusion, our findings suggest the possible use of derivatives of Pyrazole bearing Pyrimidine which may serves as a promising anti-inflamatory strategy for overcoming various the cancers with the best prognosis were those of the colon, breast, prostate, and head and neck etc.the most recent studies to provide light on Pyrazole bearing Pyrimidine derivatives application in the treatment and avertance of cancer in the future.

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