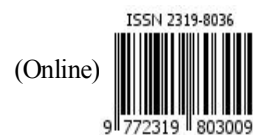




(Print)

JUC Vol. 22(4), 49-65 (2026). Periodicity 2-Monthly



(Online)



Estd. 2005

JOURNAL OF ULTRA CHEMISTRY

An International Open Free Access Peer Reviewed Research Journal of Chemical Sciences and Chemical Engineering

website:- www.journalofchemistry.org

Targeting Nipah Virus Glycoprotein: Molecular Docking, Synthesis and CPE Inhibition Evaluation of Pyrazole Scaffolds with Comparative Studies of Triazole Derivatives in Vero E6 Cell Lines

RASHMI T, VARSHA G P*, SWAROOPA H M, VINUTHA S and PALLAVI K M

Department of Pharmaceutical Chemistry, Bharathi College of Pharmacy,
Bharathinagara, Maddur Taluk, Mandya District, Karnataka - 571422 (INDIA)

Corresponding author : varsha G P*

Corresponding author Email ID : varshagowda02035@gmail.com

DOI : <http://dx.doi.org/10.22147/juc/220401>

Acceptance Date 24th September 2026

Online Publication Date 03rd October 2026

Abstract

Nipah virus (NiV) infection remains a significant public health threat due to its high mortality rate. currently there are No approved antiviral therapeutics exist, necessitating the development of selective and effective antiviral agents. The viral attachment glycoprotein (G) is a well-established molecular target, as receptor-binding interactions are critical for host cell entry and disease progression. In the present study, a library of 60 heterocyclic derivatives was designed and computationally evaluated as potential Nipah virus glycoprotein inhibitors, comprising 30 pyrazole-based (PP) and 30 triazole-based (T series) compounds. Molecular docking studies against the Nipah virus glycoprotein (PDB ID: 2VSM) revealed favorable binding energies, ranging from -7.8 to -9.5 kcal/mol for the pyrazole series and -6.9 to -8.8 kcal/mol for the triazole series. Compounds exhibited strong hydrogen bonding and hydrophobic interactions with crucial active-site residues, including GLN559, GLN530, ALA532, and GLY506. Compounds with highest binding energy were synthesized (**PP3, PP9, PP17, PP18, PP20, T2, T4, T5, T9, T18**) and characterization using IR, ¹H-NMR and Mass spectrometry, for

the structural conformation. The synthesized compounds were screened for *in-vitro* antiviral potential using the CPE inhibition assay in Vero E6 cell-line. Compound **PP3** and **T18** emerged as the most potent leads, with EC₅₀ values of 19.83 μ M and 21.18 μ M respectively when compared with Ribavirin (EC₅₀ = 12.48 μ M) as a standard drug. Overall, these findings suggest that the designed pyrazole and triazole derivatives as promising Nipah virus glycoprotein inhibitors, providing a strong foundation for further lead optimization and preclinical evaluation.

Key words : Pyrazole derivatives; Triazole derivatives; Nipah virus; Molecular docking; Glycoprotein; CPE assay; Antiviral activity.

Introduction :

Nitrogen-containing heterocyclic compounds are pivotal scaffolds in medicinal chemistry due to their exceptional biological activities, versatile synthetic accessibility, and superior electron-donating capabilities¹⁻². Nitrogen atoms form multiple hydrogen bonding interactions more effectively than sulfur and oxygen, making them highly valuable for modulating protein-ligand interactions and enhancing drug bioavailability³⁻⁴. Pyrazoles and triazole derivatives are five-membered aromatic heterocycles containing two adjacent nitrogen atoms and three adjacent atoms⁵⁻⁶. Both scaffold classes serve as privileged structures for designing potent antiviral agents with enhanced therapeutic efficacy⁷⁻⁸.

The Nipah virus (NiV) is a highly pathogenic zoonotic paramyxovirus with case fatality rates of 40-75%, causing severe respiratory and neurological disease in humans⁹. The crystal structure of the Nipah virus nucleocapsid protein (PDB:2VSM) elucidates viral RNA binding mechanisms and provides a structural foundation for developing targeted antiviral therapeutics¹⁰.

In the present study, the therapeutic potential of newly designed pyrazole and triazole compounds were studied using computational approaches. Molecular docking studies were

carried out to evaluate the binding affinity of these derivatives against the viral attachment glycoprotein with PDB ID: 2VSM¹¹. Based on the highest docking results, selected lead compounds from both series were synthesized and characterized using IR, ¹H-NMR, and mass spectrometry, for the structural conformation and were further evaluated for their *in-vitro* antiviral activity against Nipah virus using the CPE inhibition assay in Vero E6 cell line, in order to correlate the computational binding predictions with experimental antiviral efficacy¹²⁻¹³.

Materials and Methods :

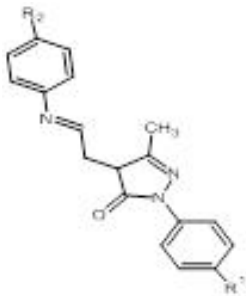
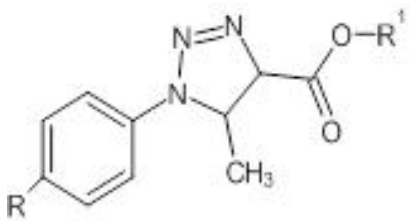
Ligand Design :

A total of 60 nitrogen-containing heterocyclic compounds were designed as potential inhibitors, including 30 pyrazole derivatives (table 1) and 30 triazole derivatives (table 2). Different substituents were introduced into the parent scaffolds to study their effect on binding affinity and to improve interaction with the target protein¹³.

Pharmacokinetic screening using swiss ADME:

To evaluate the drug-likeness properties of the designed compounds, all 60 molecules were screened using the Swiss ADME web server (<http://www.swissadme.ch>). The compounds

Table no. 1: Different substituents of Pyrazole and triazole designed (table 1)

Pyrazole designed				Triazole designed		
 R1=Substituted Phenyl hydrazine R2=Substituted Aniline				 R= Substituted Aniline R1= Substituted Beta, Keto, Ester		
SI NO.	CODE	R1	R2	CODE	R1	R2
1.	P1	H	<i>o</i> -NO ₂	T1	<i>O</i> -NO ₂	CH ₂ C ₆ H ₅
2.	P2	H	<i>m</i> - NO ₂	T2	<i>p</i> -NO ₂	CH ₂ C ₆ H ₅
3.	P3	4-Cl	<i>m</i> - NO ₂	T3	<i>m</i> -NO ₂	CH ₂ Br
4.	P4	2,4- dinitro	<i>p</i> -OH	T4	<i>m</i> -NO ₂	CH ₂ C ₆ H ₅
5.	P5	H	<i>p</i> -OH	T5	<i>m</i> -NO ₂	3 ⁰ -butyl
6.	P6	4-Cl	<i>p</i> -OH	T6	<i>m</i> -NO ₂	CH ₂ Cl
7.	P7	2,4- dinitro	<i>O</i> -OH	T7	4-OH	CH ₂ C ₆ H ₅
8.	P8	4-Cl	<i>O</i> -OH	T8	4-OH	3 ⁰ -butyl
9.	P9	4-Cl	<i>O</i> -Cl	T9	<i>O</i> -OH	CH ₂ C ₆ H ₅
10.	P10	2,4- dinitro	<i>m</i> -COOH	T10	<i>O</i> -OH	3 ⁰ -butyl
11.	P11	H	<i>m</i> -COOH	T11	<i>O</i> -OH	CH ₂ CN
12.	P12	4-Cl	<i>m</i> -COOH	T12	3-Cl	CH ₂ C ₆ H ₅
13.	P13	2,4- dinitro	<i>p</i> -COOH	T13	3-Cl	3 ⁰ -butyl
14.	P14	H	<i>p</i> -COOH	T14	<i>O</i> -Cl	CH ₂ C ₆ H ₅
15.	P15	4-Cl	<i>p</i> -COOH	T15	<i>p</i> -Cl	CH ₂ C ₆ H ₅

16.	P16	2,4- dinitro	3-Cl	T16	H	CH ₂ C ₆ H ₅
17.	P17	4-Cl	3-Cl	T17	H	CH ₂ CN
18.	P18	2,4- dinitro	C ₆ H ₅	T18	C ₆ H ₅	CH ₂ C ₆ H ₅
19.	P19	H	C ₆ H ₅	T19	C ₆ H ₅	3 ⁰ -butyl
20.	P20	4-Cl	C ₆ H ₅	T20	C ₆ H ₅	CH ₃
21.	P21	H	<i>p</i> -NO ₂	T21	4-Cl	2-OCH ₃
22.	P22	2,4- dinitro	<i>m</i> - NO ₂	T22	<i>O</i> -NO ₂	3 ⁰ -butyl
23.	P23	4-Cl	<i>o</i> -NO ₂	T23	<i>m</i> -NO ₂	CH ₃
24.	P24	H	<i>o</i> -OH	T24	<i>p</i> -Cl	3 ⁰ -butyl
25.	P25	2,4- dinitro	<i>o</i> -Cl	T25	4-OH	CH ₂ Br
26.	P26	2,4- dinitro	<i>p</i> -NO ₂	T26	4-OH	CH ₂ Cl
27.	P27	2,4- dinitro	<i>o</i> -NO ₂	T27	3-Cl	CH ₂ CH ₃
28.	P28	4-Cl	<i>p</i> -NO ₂	T28	3-Cl	CH ₂ Br
29.	P29	H	<i>O</i> -Cl	T29	3-Cl	CH ₂ Cl
30.	P30	H	3-cl	T30	4-OH	CH ₂ CH ₃

were assessed based on Lipinski's Rule of Five, gastrointestinal absorption, and synthetic accessibility. All designed molecules are satisfied the required physicochemical and pharmacokinetic parameters according to Lipinski's Rule of Five were selected for further molecular docking studies¹⁴⁻¹⁵.

Preparation of protein and ligands :

Ligand preparation :

The selected 60 ligand molecules (30 pyrazole and 30 triazole derivatives) subjected to systematic preparation to optimize their conformational states for docking studies. Gasteiger charges were assigned to all ligand atoms to represent electrostatic properties, while rotatable

bonds were identified and defined to allow conformational flexibility during docking studies¹⁶.

Protein preparation :

The three-dimensional (3D) crystal structure of Nipah virus nucleocapsid protein (PDB ID: 2VSM) was obtained from the RCSB Protein Data Bank (<https://www.rcsb.org/structure>) and prepared using AutoDock Tools (ADT) 4.2. The protein subjected to sequential processing including removal of crystallographic water molecules, ions, and co-crystallized ligands, followed by addition of polar hydrogen atoms and assignment of Kollman partial charges to accurately represent hydrogen bonding networks and electrostatic interactions essential for molecular

docking studies. Both the prepared protein structure and all ligand molecules were converted and saved in PDBQT format, the required input format for AutoDock molecular docking analysis¹⁷⁻¹⁸.

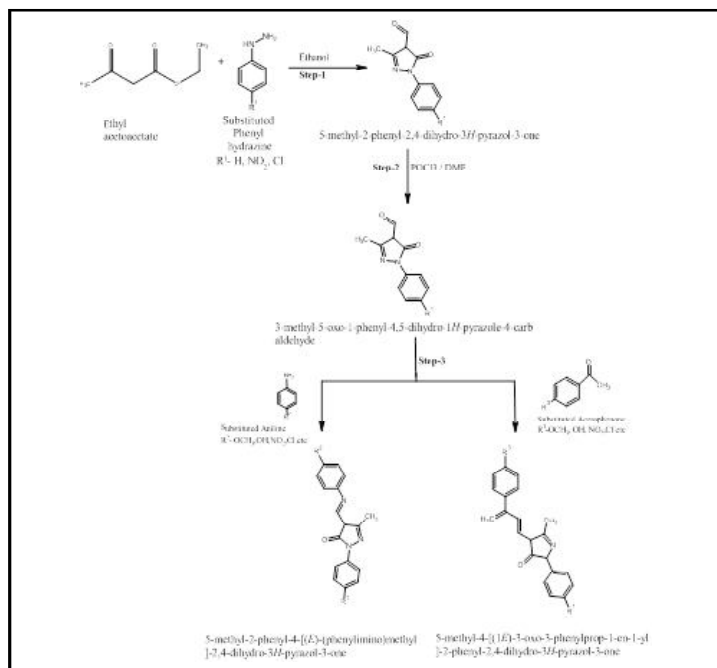
Molecular Docking Studies :

Molecular docking was performed using Auto Dock Vina 4.2. A grid box was set around the active site of the 2VSM protein based on the binding region of the native ligand to ensure accurate docking. The docked complexes were ranked according to their binding energies, and the best-scoring poses were selected for further analysis. Important non-covalent interactions, including hydrogen bonding and π -stacking interactions, were also studied to understand ligand-protein binding stability¹⁹⁻²¹.

Experimental section :

Synthesis of the target compounds was

Scheme-1. Pyrazole derivatives were synthesized using conventional synthetic methods involving three-step.



accomplished as per scheme-1 and scheme-2 . All materials used for the experimental work were of Analytical Reagent (AR) and Laboratory Reagent (LR) grade and were purchased from Aldrich and Merck. The newly synthesized compounds will be subjected to detailed physical, chemical, and analytical investigations for structural confirmation. Their melting points will be determined using the open capillary method and expressed in degrees Celsius (°C). Thin-layer chromatography (TLC) will be used to examine the purity of the compounds, while solubility tests will be carried out in suitable solvents. The synthesized pyrazole and triazole derivatives will be characterized through spectroscopic techniques such as IR, ¹H NMR using DMSO, and mass spectrometry. All synthesized compounds will be subjected to biological evaluation to determine their activity profiles.

*General Procedure :**Scheme-1: Pyrazole Synthesis :**Step-1: Synthesis of Hydrazone :*

In a 50ml round-bottom flask add 1m mol of ethyl acetoacetate was dissolved in 10ml of ethanol. To this solution, 1m mol of substituted Phenyl hydrazine was added, followed by addition of 2-3 drops of glacial acetic acid as a catalyst. The reaction mixture was then heated under reflux with continuous stirring for 2-4 hrs. After completion of the reaction, the reaction mixture was poured into ice-cold water. Resulting in the formation of a precipitate. The precipitate was then filtered and recrystallize with ethanol to obtain the product.

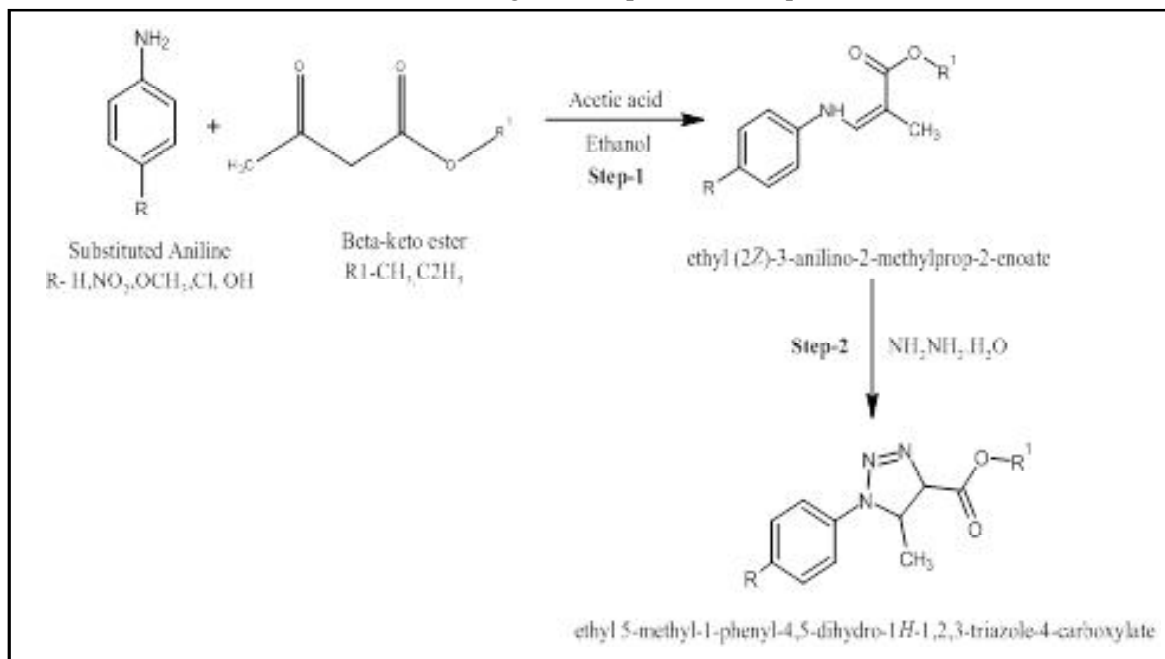
Step-2: Synthesis of pyrazole carbaldehyde :

Vilsmeyer-Hack reagent was prepared by the addition of phosphorus oxychloride (3.8ml)

dropwise to dimethyl formamide DMF (20 ml) in an ice bath with continuous stirring. The previously prepared step-1 product (10 m mol) Was added to this reagent. The reaction mixture was refluxed for 3-4hrs. After completion of the reaction. The mixture was poured into ice cold water and neutralized using sodium bicarbonate. The resulting solid precipitate was filtered and recrystallized to obtain pyrazole carbaldehyde.

Step-3: In an ice bath, a 5% NaOH (5ml) solution was added dropwise to a mixture of respective carbaldehyde (10m mol) and the appropriate acetophenone (10m mol) or aniline (10m mol) derivatives dissolved in 30ml of ethanol. The reaction mixture was stirred at 0°C for 1hr. Then 8h at room temperature. The mixture was poured into ice-cold water, resulting in the formation of solid precipitate. The precipitate was filtered and recrystallized to obtain desired pyrazole derivatives.

Scheme-2: Triazole derivatives were synthesized using conventional synthetic methods involving two -step reaction sequences.



Scheme 2: Triazole Derivatives :

Step-1: In a round bottom flask, 25ml of ethanol and 1ml of aniline were taken and stirred. To this mixture, 1.3ml of ethyl acetoacetate was added slowly in a dropwise manner with continuous stirring. Then 2-3 drops of glacial acetic acid were added as a catalyst. The reaction mixture was refluxed for 4-hrs, resulting in the formation of -enaminone intermediate.

Step-2: In the above reaction, 0.60ml of hydrazine hydrate was added to the reaction mixture and the mixture was kept under reflux for 2.5 hours. After completion of the reaction, the reaction mixture was poured into ice-cold water. A precipitate was formed, which was then filtered and recrystallized using ethanol to obtain the triazole derivatives.

In-vitro antiviral activity :

The antiviral activity of the synthesized pyrazole derivatives (PP3, PP9, PP17, PP18, PP20) and triazole derivatives (T2, T4, T5, T9, T18) was determined by using a CPE (cytopathic effect) inhibition assay against Nipah virus in Vero E6 cell cultures. All the compounds were dissolved in DMSO and diluted in cell culture medium to a maximum final DMSO concentration of 1%. The results were expressed as the 50% effective concentration (EC_{50}). The virus cells were grown to confluency in 96-well plates and

serial dilutions of the test compounds were added to the media. The reference compound Ribavirin, a broad-spectrum antiviral agent, was also used for comparison. The cultures were incubated at 37°C for 3-6 days and were examined under the microscope in order to score the inhibitory effects of the synthesized compounds according to their virus-induced CPE, expressed as the 50% effective concentration (EC_{50}).

$$SI = \frac{CC_{50}}{EC_{50}}$$

where CC_{50} represents the concentration of the compound producing 50% cytotoxicity in uninfected cells and EC_{50} represents the concentration producing 50% antiviral protection. Compounds exhibiting concentration-dependent inhibition of CPE, significant protection of infected cells, and a favorable selectivity index were considered promising antiviral candidates.

Result and Discussion :

A total of 30 pyrazole (P1-P30) and 30 triazole (T1-T30) derivatives were selected as based on their highest binding energies obtained from Auto Dock Vina 4. 2 docking studies against the target protein (PDB ID: 2VSM). The docking results of the pyrazole and triazole derivatives are summarized in Table 2 and 3 respectively, and compared against the standard antiviral drug Acyclovir.

Table no. 2 : The Binding energy analysis of Pyrazole and Triazole analogues against viral protein with PDB ID:2VSM

Sl No.	PYRAZOLE DERIVATIVES				TRIAZOLE DERIVATIVES			
	Code (pyrazole)	Binding energy (kcal/mol)	No. Of hydrogens	Hydrogen bond residues	Code (triazole)	Binding energy (kcal/mol)	No. Of hydrogens	Hydrogen bond residues
1.	P1	8.1	1H	ALA532	T1	8.3	1H	GLN559
2.	P2	8.8	1H	GLN559	T2	7.9	1H	GLN559
3.	P3	8.8	1H	GLN559	T3	7.2	1H	GLN559

4.	P4	8.3	1H	ALA532	T4	8.0	2H	ALA532, GLN559
5.	P5	8.7	2H	GLN559, PRO488	T5	7.7	1H	GLY506
6.	P6	8.5	1H	GLN559	T6	7.2	1H	GLN559
7.	P7	8.2	-	-	T7	8.7	1H	GLN490
8.	P8	8.5	1H	GLN559	T8	7.6	1H	GLN559
9.	P9	8.7	1H	GLN559	T9	8.7	1H	GLN490
10.	P10	8.2	-	-	T10	7.4	1H	GLN559
11.	P11	8.9	2H	GLN559, GLY506	T11	7.4	1H	GLN559
12.	P12	9.1	2H	GLN559, GLY506	T12	8.0	1H	GLN559
13.	P13	8	2H	ARG236, CYS240	T13	7.4	1H	GLN559
14.	P14	8.5	2H	ALA532, GLN530	T14	8.5	1H	GLN559
15.	P15	8.1	2H	GLN530, ALA532	T15	8.1	1H	GLN559
16.	P16	8	1H	TYR581	T16	8.6	1H	ALA532
17.	P17	8.8	1H	GLN559	T17	7.1	1H	GLN559
18.	P18	8.1	1H	ALA532	T18	8.8	1H	GLN490
19.	P19	9.5	2H	ALA532, GLN530	T19	7.6	-	-
20.	P20	8.7	-	-	T20	7.6	1H	ALA532
21.	P21	-8	2H	ARG236, CYS240	T21	-7.1	-	-
22.	P22	-8	1H	ILE580	T22	-7.1	-	-
23.	P23	-7.9	1H	ARG236	T23	-7	-	-
24.	P24	-7.9	1H	GLY506	T24	-6.9	1H	GLN559
25.	P25	-7.8	1H	TYR581	T25	-7	1H	GLN559
26.	P26	-7.6	2H	GLY506, ARG242	T26	-7	3H	GLY489, GLN530 GLN559
27.	P27	-7.5	1H	ALA558	T27	-7	1H	ALA532
28.	P28	-7.5	1H	ARG236	T28	-7	1H	ALA532
29.	P29	-8.2	1H	GLN559	T29	-7	1H	GLN559
30.	P30	-8.6	1H	GLN559	T30	-7	2H	GLN559, GLY489
31.	Acyclovir	5.9	1H	SER241	Acyclovir	-6	1H	SER241

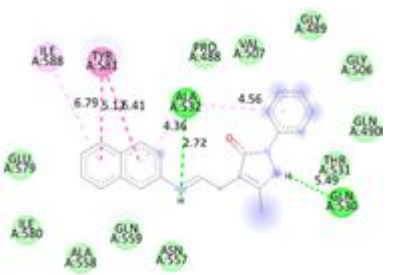
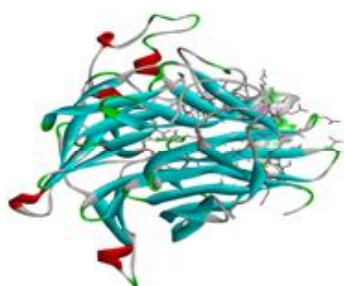
The binding energies thirty pyrazole derivatives (P1-P30) ranged from -7.8 to -9.5 kcal/mol, all surpassing the standard antiviral drug Acyclovir (-5.9 kcal/mol), which formed only one hydrogen bond with SER241. Among all derivatives, P19 exhibited the strongest binding affinity at -9.5 kcal/mol, forming two hydrogen bonds with ALA532 and GLN530, marking it as the most promising lead compound. P12 (-9.1 kcal/mol) and P11 (-8.9 kcal/mol) both interacted with GLN559 and GLY506, while P13 and P14 showed binding affinities of -8.0 and -8.5 kcal/mol, interacting with ARG236/CYS240 and ALA532/GLN530, respectively. These findings confirm that multiple hydrogen bond interactions significantly enhance ligand binding. Among the active site residues, GLN559 was the most commonly involved amino acid in ligand interactions and was observed in compounds P2, P3, P5, P6, P8, P9, P11, P12, P17, and P19. This indicates that GLN559 plays an important role in ligand recognition and stabilization within the active site of the 2VSM protein (table 1).

The binding energies of thirty triazole derivatives (T1-T30) ranged from -6.9 to -8.8 kcal/mol, which were better than the standard drug Acyclovir (-6.0 kcal/mol). Among them, T18 exhibited the highest binding affinity (-8.8 kcal/mol) with one hydrogen bond interaction at

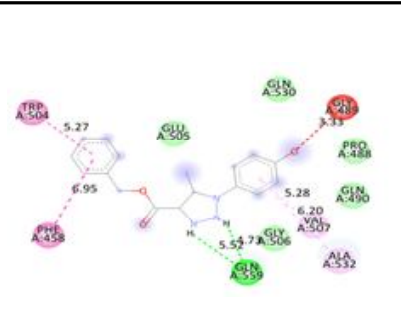
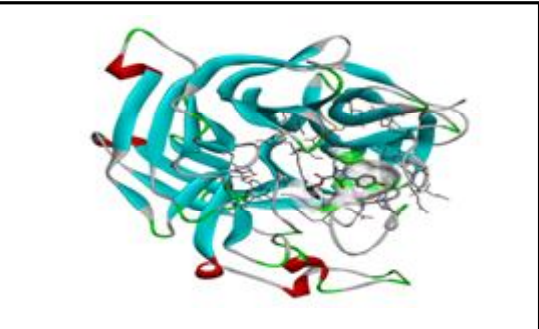
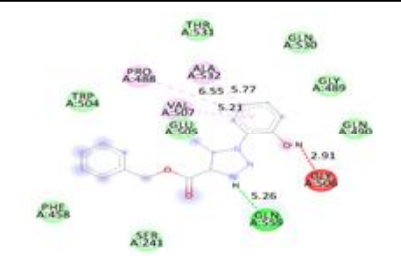
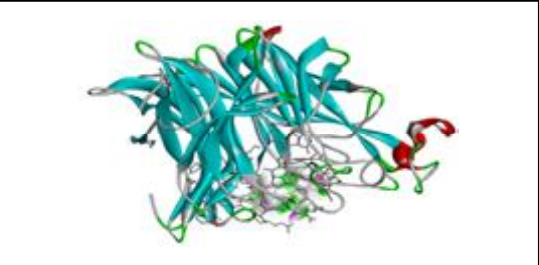
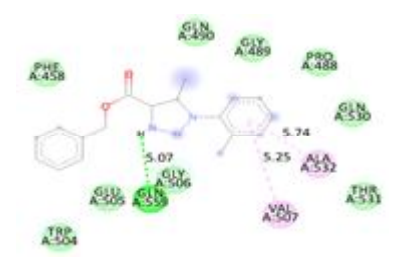
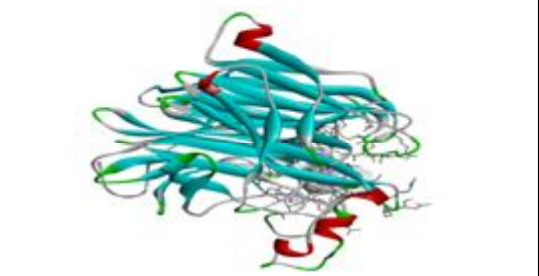
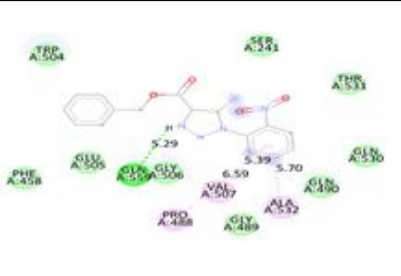
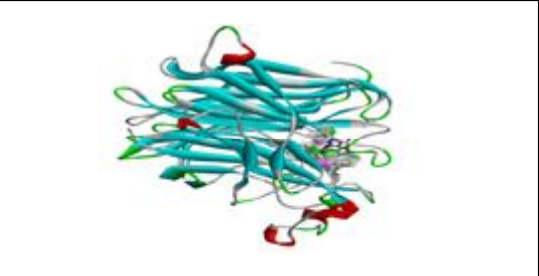
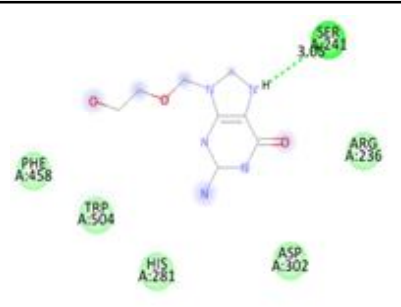
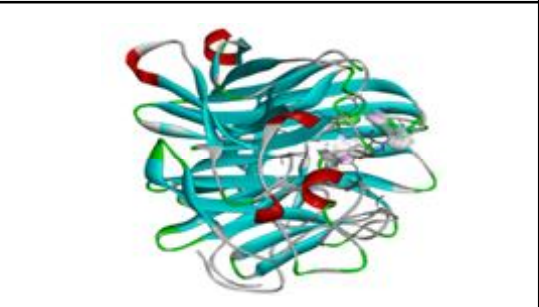
GLN490. Compounds T7 and T9 also showed strong binding energies of -8.7 kcal/mol with interactions involving GLN490, while T16 displayed a binding energy of -8.6 kcal/mol with ALA532. Only compound T4 formed two hydrogen bonds with ALA532 and GLN559, suggesting that multiple hydrogen bond interactions were less common in the triazole series compared to the pyrazole derivatives. GLN559 was the most frequently involved residue in triazole binding interactions, whereas GLN490 appeared important for compounds with higher binding affinity. Compound T19 showed moderate binding without hydrogen bond interactions (table 2).

Overall, the pyrazole derivatives demonstrated stronger binding affinity than the triazole derivatives. Compound P19 showed the best docking score (-9.5 kcal/mol), which was higher than the best triazole compound T18 (-8.8 kcal/mol). Both series performed better than Acyclovir. The improved activity of pyrazole derivatives may be due to their ability to form more stable dual hydrogen bond interactions with important residues such as ALA532, GLN530, and GLY506. The repeated involvement of GLN559 in both series indicates its important role in ligand binding and stabilization within the active site.

TABLE 3: 2D AND 3D STRUCTURES OF THE INTERACTION OF LIGAND WITH SELECTED PROTEINS (PDB ID: 2vsm)

SI NO.	Compound	2D structure of compound with PDB ID: 2VSM	3D Structure of compounds with PDB ID: 2VSM
1.	P19		

2.	P12		
3.	P11		
4.	P3		
5.	P2		
6.	T18		

7.	T7	 Chemical structure of T7 ligand showing hydrogen bonds (dashed lines) with protein residues: TRP A:504 (5.27), PHE A:458 (6.95), GLU A:505, GLN A:530 (3.11), PRO A:488, GLN A:490 (6.20), VAL A:507, ALA A:532, and GLY A:506 (5.51, 5.74).	 3D ribbon diagram of the protein structure with the T7 ligand (green sticks) bound in the active site.
8.	T9	 Chemical structure of T9 ligand showing hydrogen bonds (dashed lines) with protein residues: THR A:533, GLN A:530, GLY A:489, VAL A:507 (5.21), ALA A:532 (6.55, 5.77), PHE A:458, SER A:241, THR A:531, and VAL A:507 (2.91, 5.26).	 3D ribbon diagram of the protein structure with the T9 ligand (green sticks) bound in the active site.
9.	T16	 Chemical structure of T16 ligand showing hydrogen bonds (dashed lines) with protein residues: GLN A:490, GLY A:489, PRO A:488, GLN A:530, ALA A:532, THR A:531, VAL A:507 (5.25), GLY A:506 (5.07), GLU A:505, TRP A:504, and PHE A:458.	 3D ribbon diagram of the protein structure with the T16 ligand (green sticks) bound in the active site.
10.	T14	 Chemical structure of T14 ligand showing hydrogen bonds (dashed lines) with protein residues: TRP A:504, PHE A:458, GLU A:505, GLY A:506 (5.29), GLN A:530 (5.39), VAL A:507 (6.59), PRO A:488, GLY A:489, ALA A:532 (5.70), GLN A:490, SER A:241, and THR A:533.	 3D ribbon diagram of the protein structure with the T14 ligand (green sticks) bound in the active site.
11.	Acyclovir	 Chemical structure of Acyclovir showing hydrogen bonds (dashed lines) with protein residues: PHE A:458, TRP A:504, HIS A:281, SER A:241 (3.06), ARG A:236, and ASP A:302.	 3D ribbon diagram of the protein structure with the Acyclovir ligand (green sticks) bound in the active site.

Synthesis : Spectral characterization of synthesized compounds

The spectral data of synthesized compounds is as follows:

Pyrazole derivatives :

Compound (PP3): (E)-2-(4-chlorophenyl)-5-methyl-4-(2-((3-nitrophenyl)imino)ethyl)-2,4-dihydro-3H-pyrazol-3-one :

FT-IR (cm⁻¹): 3425, 3327 cm⁻¹ (N-H stretching), 3083 cm⁻¹ (aromatic C-H stretch), 2918, 2849 cm⁻¹ (aliphatic C-H stretch), 1615 cm⁻¹ (C=N aromatic ring stretch), 1501 cm⁻¹ (aromatic C=C), 1330 cm⁻¹ (C-N stretch), 1073, 990, 918 cm⁻¹ (in-plane bending finger print region), 866, 801, 724 cm⁻¹ (C-O stretch and aromatic C-H out-of-plane bending).

¹H NMR (400 MHz, DMSO-d₆) δ ppm: 2.20 (s, 3H, CH₃ -pyrazole), 6.05 (s, 2H, O-CH₂ O, methylenedioxy proton), 6.85-7.05 (m, 3H, benzodioxole aromatic H), 7.25 (d, J = 8.4 Hz, 2H, Ar-H, p-chlorophenyl), 7.45 (d, J = 15.6 Hz, 1H, CH=CH-CO, β-olefinic H), 7.55 (d, J = 8.4 Hz, 2H, Ar-H, p-chlorophenyl), 7.65 (d, J = 15.6 Hz, 1H, CH=CH-CO, α-olefinic H), 7.75-7.90 (m, 2H, pyrazole/benzodioxole aromatic protons).

mass: m/z 370.79(M), 370.85(M+1,100%).

Compound (P-P9): (E)-2-(4-chlorophenyl)-4-(2-((2-chlorophenyl)imino)ethyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one):

FT-IR (cm⁻¹): 2981 cm⁻¹ (aliphatic C-H stretch), 1672 cm⁻¹ (C=O stretch), 1629 cm⁻¹ (C=N stretching vibration), 1587, 1561, 1488, 1438 cm⁻¹ (C=C ring stretching), 1388 cm⁻¹ (aromatic C=C / C-H bending), 1330, 1304, 1264 cm⁻¹ (C-N stretching), 1143, 1092, 1043, 1010 cm⁻¹ (C-N stretch), 869, 827, 738 cm⁻¹ aromatic (C-H out-of-plane bending).

¹H NMR (400 MHz, DMSO-d₆) δ ppm: 8.25-8.35 (s, 1H, -CH=N-), 7.65-7.72 (dd, J = 7.8, 1.6 Hz, 1H, Ar-H), 7.48-7.55 (d, J = 8.4 Hz, 2H, 4-chlorophenyl-Ar-H), 7.30-7.42 (m, 3H, Ar-H), 7.15-7.25 (t, J = 7.6 Hz, 1H, Ar-H), 7.05-7.12 (d, J = 7.8 Hz, 1H, Ar-H), 6.95-7.05 (t, J = 7.6 Hz, 1H, Ar-H), 3.35-3.50 (s, 2H, -CH₂ -CH=N-), 2.15 (s, 3H, =C-CH₃).

mass: m/z 360.24 (M), 360.64 (M+1,100%).

Compound (P-P17): (E)-2-(4-chlorophenyl)-4-(2-((3-chlorophenyl)imino)ethyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one:

FT-IR (cm⁻¹): 3357, 3214 cm⁻¹ (N-H stretching vibrations), 2982 cm⁻¹ (aliphatic C-H stretch), 1592 cm⁻¹ (C=N stretch (pyrazole ring), 1481 cm⁻¹ (aromatic C=C stretch), 1298, 1264 cm⁻¹ (C-O stretching), 1069, 1041, 1014 cm⁻¹ (C-N stretch), 883, 830, 765, 676 cm⁻¹ (aromatic C-H out-of-plane bending). **¹H NMR (400 MHz, DMSO-d₆) δ ppm:** 8.25-8.35 (s, 1H, -CH=N-), 7.65-7.72 (d, J = 8.4 Hz, 2H, 4-chlorophenyl-H), 7.35-7.45 (m, 2H, 3-chlorophenyl-H), 7.20-7.30 (m, 3H, 3-chlorophenyl-H), 7.05-7.12 (d, J = 8.0 Hz, 1H, 3-chlorophenyl-H), 4.55-4.70 (dd, J = 8.4, 5.2 Hz, 1H, pyrazolone C4-H), 3.35-3.50 (m, 2H, -CH₂-CH=N-), 2.15 (s, 3H, pyrazolone -CH₃). **mass:** m/z 360.24 (M), 360.58 (M+1,100%).

Compound (P-P18): (E)-2-(2,4-dinitrophenyl)-5-methyl-4-(2-(naphthalen-2-ylimino)ethyl)-2,4-dihydro-3H-pyrazol-3-one.

FT-IR (cm⁻¹): 3412, 3340, 3225 cm⁻¹ (N-H stretching), 3041 cm⁻¹ (aromatic C-H stretch), 1622 cm⁻¹ (C=N stretch (pyrazole ring), 1573, 1510, 1455 cm⁻¹ (aromatic C=C ring stretching), 1400, 1372, 1286 cm⁻¹ (C-N stretching),

1084 cm^{-1} (C-O stretch), 1010, 853, 765 cm^{-1} (aromatic C-H out-of-plane bending).

^1H NMR (400 MHz, DMSO- d_6) δ ppm: 9.05-9.15 (s, 1H, -CH=N-), 8.75-8.85 (d, J = 2.4 Hz, 1H, Ar-H), 8.45-8.55 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 8.15-8.25 (d, J = 8.8 Hz, 1H, Ar-H), 7.80-7.90 (m, 4H, naphthalenyl-H), 7.65-7.75 (m, 2H, naphthalenyl-H), 7.45-7.60 (m, 4H, naphthalenyl-H), 4.45-4.60 (dd, J = 8.4, 5.2 Hz, 1H, pyrazolone C4-H), 3.45-3.60 (m, 2H, -CH₂-CH=N-), 2.20-2.30 (s, 3H, pyrazolone -CH₃).

mass: m/z 431.40 (M), 431.29 (M+1, 100%).

Compound (P-P20): (E)-2-(4-chlorophenyl)-5-methyl-4-(2-(-naphthalen-2-ylimino)ethyl)-2,4-dihydro-3H-pyrazol-3-one.

FT-IR (cm^{-1}): 3412, 3339, 3225 cm^{-1} (N-H stretching), 3041 cm^{-1} (aromatic C-H stretch), 1622 cm^{-1} (C=N stretch), 1571, 1508, 1455 cm^{-1} (aromatic C=C), 1400, 1372, 1286 cm^{-1} (C-N stretching), 1171, 1086 cm^{-1} (C-O stretch), 1010, 853, 764 cm^{-1} (aromatic C-H out-of-plane bending).

^1H NMR (400 MHz, DMSO- d_6) δ ppm: 8.55-8.65 (s, 1H, -CH=N-), 7.80-7.90 (m, 4H, naphthalenyl-H), 7.65-7.75 (m, 2H, naphthalenyl-H), 7.45-7.55 (m, 1H, naphthalenyl-H), 7.35-7.45 (d, J = 8.4 Hz, 2H, 4-chlorophenyl-H), 7.15-7.25 (d, J = 8.4 Hz, 2H, 4-chlorophenyl-H), 4.45-4.60 (t, J = 6.8 Hz, 1H, pyrazolone C4-H), 3.45-3.60 (m, 2H, -CH₂-CH=N-), 2.35-2.45 (s, 3H, C5-CH₃).

mass: m/z 375.85 (M), 375.49 (M + 1, 100%).

Triazole derivatives:

Compound (T2): Benzyl-5-methyl-1-(4-nitrophenyl)-4,5-dihydro-1H-1,2,3-triazole-4-carboxylate

FT-IR (cm^{-1}): 3477, 3350 cm^{-1} (N-H stretching), 1626 cm^{-1} C=N stretch (triazole), 1583 cm^{-1} (aromatic C=C), 1468, 1439 cm^{-1} (C-H bending), 1293 cm^{-1} (strong C-N stretch), 1106 cm^{-1} (C-H stretch), 833, 747, 692 cm^{-1} (aromatic C-H out-of-plane bending).

^1H NMR (400 MHz, CDCl₃) δ ppm: 8.20-8.28 (d, J = 8.8 Hz, 2H, 4-nitrophenyl-H), 7.55-7.65 (d, J = 8.8 Hz, 2H, 4-nitrophenyl-H), 7.30-7.38 (m, 5H, benzyl-Ar-H), 5.20-5.30 (s, 2H, OCH₂ Ph), 4.85-4.95 (d, J = 6.8 Hz, 1H, triazoline-H5), 4.35-4.45 (d, J = 6.8 Hz, 1H, triazoline-H4), 1.65-1.75 (d, J = 6.8 Hz, 3H, CH₃).

mass: m/z 340.12 (M), 340.29 (M+1, 100 %).

Compound (T4): Benzyl-5-methyl-1-(3-nitrophenyl)-4,5-dihydro-1H-1,2,3-triazole-4-carboxylate:

FT-IR (cm^{-1}): 3422, 3324 cm^{-1} (N-H stretching), 3077 cm^{-1} (aromatic C-H stretch), 1612 cm^{-1} (C=N stretch), 1497 cm^{-1} (aromatic C=C), 1327 cm^{-1} (C-N stretch), 1069, 977, 862, 798, 727 cm^{-1} (aromatic C-H out-of-plane bending).

^1H NMR (400 MHz, CDCl₃) δ ppm: 8.15-8.25 (s, 1H, Ar-H), 8.05-8.12 (d, J = 8.0 Hz, 1H, Ar-H), 7.65-7.72 (d, J = 7.6 Hz, 1H, Ar-H), 7.45-7.52 (t, J = 7.8 Hz, 1H, Ar-H), 7.30-7.38 (m, 5H, Ar-H), 5.20-5.30 (s, 2H, OCH₂ Ph), 4.85-4.95 (d, J = 6.8 Hz, 1H, triazoline-H5), 4.35-4.45 (dd, J = 6.8, 4.8 Hz, 1H, triazoline-H4), 1.65-1.75 (d, J = 6.8 Hz, 3H, CH₃).

mass: m/z 340.34 (M), 341.10 (M+1, 100 %).

Compound (T5): Tert-butyl-5-methyl-1-(3-nitrophenyl)-4,5-dihydro-1H-1,2,3-triazole-4-carboxylate

FT-IR (cm^{-1}): 3425, 3327 cm^{-1} (N-H

stretching), 3083 cm^{-1} (aromatic C-H stretch), 1615 cm^{-1} (C=N stretch), 1502 cm^{-1} (aromatic C=C stretch), 1333, 1255 cm^{-1} (C-N stretch), 1071, 982, 916 cm^{-1} (C-H in plane finger print region), 865, 801, 727 cm^{-1} (aromatic C-H out-of-plane bending).

^1H NMR (400 MHz, CDCl_3) δ ppm:

8.20-8.28 (s, 1H, Ar-H), 8.05-8.12 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.55-7.65 (d, $J = 7.6$ Hz, 1H, Ar-H), 7.40-7.48 (t, $J = 7.8$ Hz, 1H, Ar-H), 5.05-5.15 (d, $J = 6.8$ Hz, 1H, triazoline-H4), 4.20-4.30 (dq, $J = 6.8, 6.4$ Hz, 1H, triazoline-H5), 1.45 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.25-1.32 (d, $J = 6.4$ Hz, 3H, 5- CH_3).

mass: m/z 306.32(M), 306.28 (M+1,100%).

Compound (T9): Benzyl-1-(2-hydroxyphenyl)-5-methyl-4,5-dihydro-1H-1,2,3-triazole-4-carboxylate

FT-IR (cm^{-1}): 3373, 3301 cm^{-1} (N-H stretching), 2980 cm^{-1} (aliphatic C-H stretching), 1597 cm^{-1} (C=N stretch), 1508, 1459, cm^{-1} (aromatic ring C=C stretch), 1396,1264 cm^{-1} (C-N stretching), 1215 cm^{-1} (C-O bending), 1074, 1028 cm^{-1} (C-N stretch), 890, 844, 798, 738 cm^{-1} (aromatic C-H out-of-plane bending).

^1H NMR (400 MHz, CDCl_3) δ ppm:

2.20-2.50 (s, 3H, CH_3 -pyrazole), 5.32-5.52 (s, 2H, CH_2 , Aliphatic H), 6.823 (d, $J = 8.0$ Hz, 1H, Ar-H, o-hydroxyphenyl), 6.928 (t, $J = 7.6$ Hz, 1H, CH, Pyrazoline ring), 7.106-7.354(m, 5H, benzyl phenyl protons), 7.37-7.39 (m, 2H, o-hydroxyphenyl aromatic H), 7.511 (s, 1H, pyrazole-H at C5), 7.65 (d, $J = 8.0$ Hz, 1H, o-hydroxyphenyl aromatic H), 8.103 (s, 1H, Ar-OH).

mass: m/z 311.13, 311.48 (M+1,100%).

Compound (T18): Benzyl-5-methyl-1-(naphthalen-2-yl)-4,5-dihydro-1H-1,2,3-triazole-4-carboxylate

FT-IR (cm^{-1}): 3414, 3336, 3224 cm^{-1} (N-H stretching vibrations), 3043 cm^{-1} (aromatic C-H stretching), 1626 cm^{-1} (C=N stretch (triazole ring), 1574, 1508, 1454 cm^{-1} (aromatic C=C ring stretching), 1399, 1284 cm^{-1} (C-O stretching), 1169, 1083, 1008 cm^{-1} (C=N stretching), 856, 761 cm^{-1} (aromatic C-H out-of-plane bending).

^1H NMR (400 MHz, CDCl_3) δ ppm:

7.90-8.05 (m, 3H, naphthalen-2-yl-H), 7.65-7.75 (d, $J = 8.4$ Hz, 1H, naphthalen-2-yl-H), 7.45-7.58 (m, 3H, naphthalen-2-yl-H), 7.30-7.40 (m, 5H, benzyl-Ar-H), 5.20-5.35 (s, 2H, OCH_2 Ph), 4.85-4.98 (dd, $J = 8.0, 5.2$ Hz, 1H, triazoline-H5), 4.35-4.48 (dd, $J = 8.0, 6.0$ Hz, 1H, triazoline-H4), 1.55-1.70 (d, $J = 6.0$ Hz, 3H, CH_3).

mass: m/z 345.40 (M).

Biological activities: Anti-viral activity :

Table no. 4: Antiviral activity of synthesized compounds pyrazole (P-P9,P-P18,P-P27, P-P28.P-P30) and triazole (T10,T17, T18,T31,T66) against nipah virus in Vero E6 lines

Sl. no	Code	EC_{50} (mm)Vero E6
1	P-P3	19.83
2	P-P9	35.81
3	P-P17	26.46
4	P-P18	62.57
5	P-P20	41.39
6	T2	52.69
7	T4	43.75
8	T5	72.51
9	T9	31.27
10	T18	21.18
11	Ribavirin	12.48

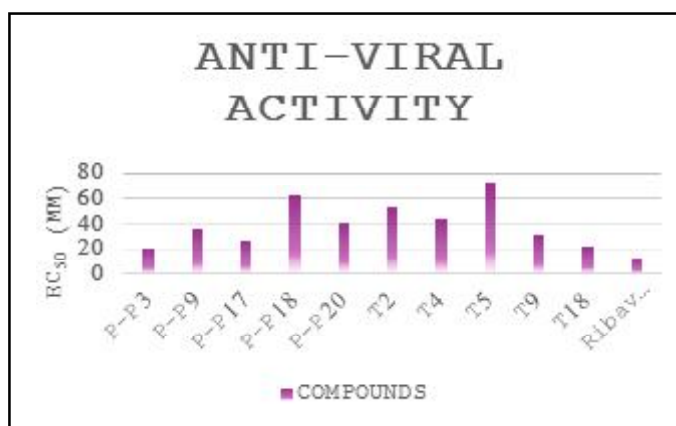


Fig no.1 : Graphical representation of EC₅₀ values(mm) of pyrazole and triazole derivatives against nipah virus compared with Ribavirin

Among the tested pyrazole derivatives, compound **PP3** exhibited the most potent antiviral activity with the lowest EC₅₀ value of **19.83 μ g**, followed by PP17 (26.46mm), PP30 (41.39 μ g) and P-P20 (41.39mm), while PP18 showed the weakest activity (62.57mm). Among the triazole derivatives, **T18** demonstrated the highest potency with an EC₅₀ of **21.18 mm**, closely followed by T9 (31.27 mm), whereas T5 showed comparatively lower activity (72.51 mm), T4 (43.75mm) and T2 (52.69mm). Although all of the synthesized compounds surpassed the reference drug Ribavirin (EC₅₀ = 12.48 mm), Compounds PP3 and T18 showed EC₅₀ values reasonably close to the standard, indicating promising antiviral potential against Nipah virus which supports the need for further structure-activity relationship investigation.

Conclusion

In the present study, a series of 60 nitrogen-containing heterocyclic derivatives (30 pyrazole and 30 triazole) were designed and evaluated as potential inhibitors of the Nipah virus glycoprotein (PDB ID: 2VSM). Molecular docking studies revealed that both series exhibited superior binding affinities compared to the standard

drug Acyclovir, with the pyrazole derivatives (P1-P30, -7.8 to -9.5 kcal/mol) showing stronger overall interactions than the triazole derivatives (T1-T30, -6.9 to -8.8 kcal/mol), largely attributed to consistent hydrogen bonding with key active-site residues such as GLN559, GLN530, ALA532, and GLY506. Based on favorable docking scores, five pyrazole (PP3, PP9, PP17, PP18, PP20) and five triazole (T2, T4, T5, T9, T18) derivatives were synthesized and structurally confirmed through IR, ¹H-NMR, and mass spectrometry. Subsequent *in-vitro* evaluation via the CPE inhibition assay in Vero E6 cells identified P-P3 (EC₅₀ = 19.83 mm) and T18 (EC₅₀ = 21.18 mm) as the most potent antiviral leads, with activity approaching that of the reference drug Ribavirin (EC₅₀ = 12.48 mm). These findings collectively establish the designed pyrazole and triazole scaffolds as promising candidates. In this comparative study pyrazole as promising candidates against nipah virus glycoprotein inhibitors and provide a strong structural basis for further lead optimization, cytotoxicity profiling, and preclinical development.

Scope for Future Work :

The outcomes of this investigation establish a framework for the rational design,

optimization, and preclinical evaluation of the active pyrazole and triazole derivatives. Initial studies will focus on determining the safety profiles of lead candidates PP3 and T18, alongside all synthesized analogues, through *in-vitro* cytotoxicity assays against non-cancerous Vero E6 and HEK-293 cell lines to establish their selectivity indices ($SI=CC_{50}$ and EC_{50}). Further structure-activity relationship studies, guided by the docking interactions with Gln559, Gln530, Ala532, and Gly506, could help design next-generation analogues with improved binding affinity and antiviral potency approaching or exceeding that of Ribavirin. Confirmation of glycoprotein binding through biophysical techniques including Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC) and evaluation against live Nipah virus using cytopathic effect (CPE) inhibition assays under BSL-4 containment would strengthen the mechanistic basis of these findings.

Comprehensive Pharmacokinetic and toxicological (ADMET) profiling of the lead molecules is also warranted to support their progression toward preclinical development. These studies will utilize advanced formulation strategies to overcome solubility limitations and advance candidate compounds toward clinical translation. Additionally, screening the pyrazole and triazole scaffolds against related paramyxoviruses could reveal a broader spectrum of antiviral applicability.

Funding Statement :

This research received no specific grant or financial support from any funding agency in the public, commercial, or non-profit sectors.

References

1. Kumar A, Singh AK, Singh H, Vijayan V, Kumar D, Naik J, Thareja S, Yadav JP, Pathak P, Grishina M and Verma A. Nitrogen containing heterocycles as anticancer agents: a medicinal chemistry perspective. *Pharmaceu*. 2023; 16(2): 299-46.
2. Mallappa, Chahar M, Choudhary N, Yadav KK, Qasim MT, Zairov R, Patel A, Yadav VK and Jangir M. Recent advances in the synthesis of nitrogen-containing heterocyclic compounds via multicomponent reaction and their emerging biological applications. *J Iran Chem Soc*. 2022; 22(1): 1-33.
3. Arora P, Arora V, Lamba HS and Wadhwa D. Importance of heterocyclic chemistry. *Int J Pharm Sci Res*. 2012; 3(9): 2947-864.
4. Chernyshov VV, Popadyuk II, Yarovaya OI and Salakhutdinov NF. Nitrogen-containing heterocyclic compounds obtained from monoterpenes or their derivatives: Synthesis and properties. *Top Curr Chem*. 2022; 380(5): 42-159.
5. Li G, Cheng Y, Han C, Song C, Huang N and Du Y. Pyrazole-containing pharmaceuticals: target, pharmacological activity, and their SAR studies. *RSC Med Chem*. 2022; 13(11): 1300-21.
6. Bakulev VA, Delisi R and Dehaen W. Chemistry of 1, 2, 3-triazoles. *Cham Curr Green hem*. 2015; 19(5): 4758-2546.
7. Oliveira FC, Zamoner LO, Baruffi MD, Augusto BS, Vaidergorn MD, Nonato MC, Bologna TS, Silva MV and Carvalho I. Synthesis and biological evaluation of 4-(4-Nitrophenyl)-1 H-1, 2, 3-triazole derivatives as anti-trypanosomal Agents. *ACS omega*. 2025; 10(20): 20299-314.
8. Faisal M, Saeed A, Hussain S, Dar P and Larik FA. Recent developments in synthetic chemistry and biological activities of pyrazole derivatives. *Int J Chem Sci*. 2019; 131(8): 70-24.
9. Angeletti S, Presti AL, Cella E and Ciccozzi M. Molecular epidemiology and phylogeny of nipah virus infection. *Asian Pac J Trop*

- Med. 2016; 9(7): 630-4.
10. Sun B, Jia L, Liang B, Chen Q and Liu D. Phylogeography, transmission and viral proteins of nipah virus. *Virol Sin.* 2018; 33(5): 385-93.
 11. Castillo JC and Portilla J. Recent advances in the synthesis of new pyrazole derivatives. *Targets Heterocycl Syst.* 2018; 22(194): 223-12.
 12. De Clercq E, Li G. Approved antiviral drugs over the past 50 years. *Clin Microbiol Rev.* 2016; 29(3): 695-747.
 13. Arathi KN, Sindhu TJ, Vishnu MV, Basith MA, Anitha SV, Roy A, Arundhathi T, George A, Asish S. Synthesis, Molecular Docking and Characterization of Pyrazole N-Mannich Base Derivatives as Antimicrobial Agents. *Res J Pharm Technol.* 2023; 16(3): 1047-52.
 14. Naseer A, Ratra S, Kumar U, Singh TG. Molecular docking, synthesis, computational studies and In-vitro evaluation of 7-substituted-2-pyrimidinyl chromen-4-one derivatives. *Res J Pharm Technol.* 2023; 16(3): 1085-94.
 15. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. *Comput Aided Drug Des.* 2011; 7(2): 146-57.
 16. Naqvi AA, Mohammad T, Hasan GM, Hassan MI. Advancements in docking and molecular dynamics simulations towards ligand-receptor interactions and structure-function relationships. *Curr. Top. Med. Chem.* 2018; 18(20): 1755-68.
 17. Aktas E, Saygılı I, Kahveci E, Tekbiyik Z, Ozgentürk NO. Bioinformatic investigation of Nipah virus surface protein mutations: Molecular docking with Ephrin B2 receptor, molecular dynamics simulation, and structural impact analysis. *Microbiol. Immunol.* 2023; 67(12): 501-13.
 18. Ibba R, Corona P, Nonne F, Caria P, Serreli G, Palmas V, Riu F, Sestito S, Nieddu M, Loddo R and Sanna G. Design, synthesis, and antiviral activities of new benzotriazole-based derivative *Pharmaceut.* 2023; 16(3): 429-36.
 19. Abdelwahab M, Mohamed AM, Mahmoud SF, Elnaggar DH, Abde Hafez NA, GabAllah M, Abou-Amra ES and Hussein AM. Synthesis, molecular docking, DFT and pharmacophore studies of new naphthalene-heterocycle hybrids of prospective antiviral activity. *Egypt J Chem.* 2025; 68(6): 43-66.
 20. Rafique I, Maqbool T, Rutjes FP, Irfan A, Jardan YA. Anti-Diabetic Activities and Molecular Docking Studies of Aryl-Substituted Pyrazolo [3, 4-b] pyridine Derivatives Synthesized via Suzuki Cross-Coupling Reaction. *Pharmaceu.* 2024 ; 17(10): 1326-68.
 21. Ostricki R, Rakas A, Rastija V, Persoons L, Daelemans D, Kraljević TG. Synthesis, Antiproliferative Activity, ADME Profiling, and Docking Studies of Novel 1, 2, 3-Triazole Derivatives of 2-Amino and 2-Mercaptobenzoxazole. *Curr. Med. Chem.* 2025; 33(10): 1993-201.