STRUCTURE BASED VIRTUAL SCREENING OF INDOLE DERIVATIVES AS ANTI NIPAH VIRAL AGENTS

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Abstract: **Nipah virus (NiV), a newly emergent zoonotic paramyxovirus, has reported to cause several outbreaks in humans and is mainly associated with severe encephalitic diseases. Till these days, neither vaccines nor drugs with best possible accession against the virus are available. To identify novel inhibitors of NiV-G using structure based virtual screening of indole derivatives was performed using CHEMBL database and PyRx Software. The Screened molecules were subjected to structure based molecular docking studies using the crystal structure of the NiV-G to virtually screen for novel inhibitors of NiV-G and 4 potential compounds with highest potential ability to inhibit the NiV-G were found.**

IndexTerms **- Nipah virus, indole derivatives, virtual screening, molecular docking.**

I. Introduction to Nipah virus infections

Nipah virus infection is rising zoonotic disease which is associated with high mortality rate in humans which ranges from 40% to 100%. It is associated with predominant respiratory and neurologic features. Not long ago there was an outbreak in Perambra, Calicut district of Kerala, India.

Epidemiology: The first human outbreak of Nipah virus was reported in 1998 from Malaysia among pig farmers which was associated with 40% fatality rate. The virus was named nipah following the name of the village of "Sungai Nipah", in Malaysia, were it was first recognized. The outbreak in Singapore in the year 1991 was associated with 9% mortality, whereas the outbreak in Siliguri district of West Bengal in the year 2001 was associated with 74% mortality. There were number of outbreaks in Bangladesh and the first reported outbreak was in the year 2001 and it was an epidemic in Bangladesh. Most of the cases were in the northern- central districts of Bangladesh where date palm sap collection is very common and the area is referred as "Nipah belt". 2007 outbreak of nipah in Nadia district, West Bengal, India was associated with 100% case fatality. Nipah virus Infection, which is fatal zoonotic infection, has got many features which made it a potential agent for Bioterrorism and is categorized as Biosafety level organisms. In the recent outbreak that is 2018 may in Perambra, Kerala, India patients developed both neurological as well as respiratory symptoms and there was high human to human transmission. Most appreciable fact about the Kerala outbreak was that the team of doctors were able to identify the nipah virus infection in the second patient itself, compared to other outbreaks where it took months to identify causative organism. Speedy diagnosis of Nipah outbreak aided to effectively implement preventive measures (wahed 2011).

Nipah Virus: Nipah virus belongs to genius – henipavirus, family - paramyxovirus family and closely related to Hendra virus and Cedar virus. The virus in an envelope virus having negative sense single stranded non segmented RNA genome. It is inactivated by 600c for 1 hour. It is sensitive to common soaps, disinfectants and lipid solvents like alcohol, ether and sodium.

Natural host: Fruit bats of Pteropus genus, Pteropodidae family are the natural host for Nipah virus and they are migratory. The bats that are carrying virus are asymptomatic. In recent past African fruit bats that belongs to family pteropodidae, genus Eidolon is also found positive for Nipah virus. It is hypothesized that geographic distribution of henipavirus overlaps with that of pteropus bats. Nipah outbreak in pigs and else domestic animals like horse, goats, sheep, cat and dogs were first described during the Malaysian outbreak in 1999. Pigs can be asymptomatic or symptomatic with neurological and respiratory features. Pigs gets infected during incubation period which ranges from 4 days to 14 days.

Human infection: Human gets infection upon exposure to infected domestic animals like pig, cow or by consumption of fresh date palm soup contaminated with infected bat saliva, urine or fecal matters. Person to person transmission through droplet was also common during the outbreak. Disclosure to patients' secretion

is a high risk factor for the occurrence of disease. Incubation period varies from 4 to 45 days. In person-to-person transmission the average incubation period is 6 days to 11 days (Hsu 2004). Transmission in Malaysian outbreak, it transferred from natural host (fruit bat) to amplification host (pigs) and then to human being, by direct contact with sick pigs or their contaminated tissue. Transmission through respiratory droplet, contact with body secretions or tissues of sick animals (Figure 1). But in Bangladesh outbreak transmission directly from fruit bats without transmission into the amplifying host. Consumption of fruits, fruit products and raw date palm sap contaminated with urine or saliva of infected bats ended in the outbreak in Bangladesh. People working under the trees also got infected with virus (Montgomery 2008). Virus transmit directly from person to person through close contact, preferentially with the patient's secretions. In Siliguri, India outbreak, 75% of infected persons were among the hospital staff or visitors indicating the high risk of transmission through close contact (Stachowaik 2012).

Clinical Features: Nipah virus infected individual mostly complains about high fever, headache, myalgia and sore throat. The respiratory and neurological symptoms start 4 days after the onset of fever. The neurological symptoms include dizziness, vomiting, impairment in spatial perception, myoclonus, altered consciousness, drowsiness, seizure and abnormal plantar response which can be further rapidly progressed to comma within 24-48 hours suggesting acute encephalitis. Symptoms related to brain stem dysfunction like hypertension and tachycardia were also seen. Patients also acquired respiratory symptoms like cough, breathlessness and features of atypical pneumonia and acute respiratory distress. Seriously infected patients develop septicemia, renal impairment and gastro-intestinal bleeding. In Malaysian outbreak it was predominantly neurological signs (encephalitis presentation) where as in Bangladesh, both respiratory and neurological symptoms were common. This may explain the high risk of human- to-human transmission noticed in Bangladesh outbreak. The variation in the clinical presentation may be because of genetic difference in the nipah virus strain. The outbreak in Malaysia was associated with single strain, where as in Bangladesh it is because of diverse strain (Bellini 2005). Case fatality rate ranges from 40-100% in different outbreaks. The patients who were infected with Nipah virus have high fever, respiratory symptoms and absence of plantar reflex and it is associated with high risk of mortality. About 20% of infected patients who got cured were having residual neurological consequence like persistent convulsions and personality changes. Recovered patient may experience relapse in the coming years and in sub clinically infected individual there may be development of neurological features years later.

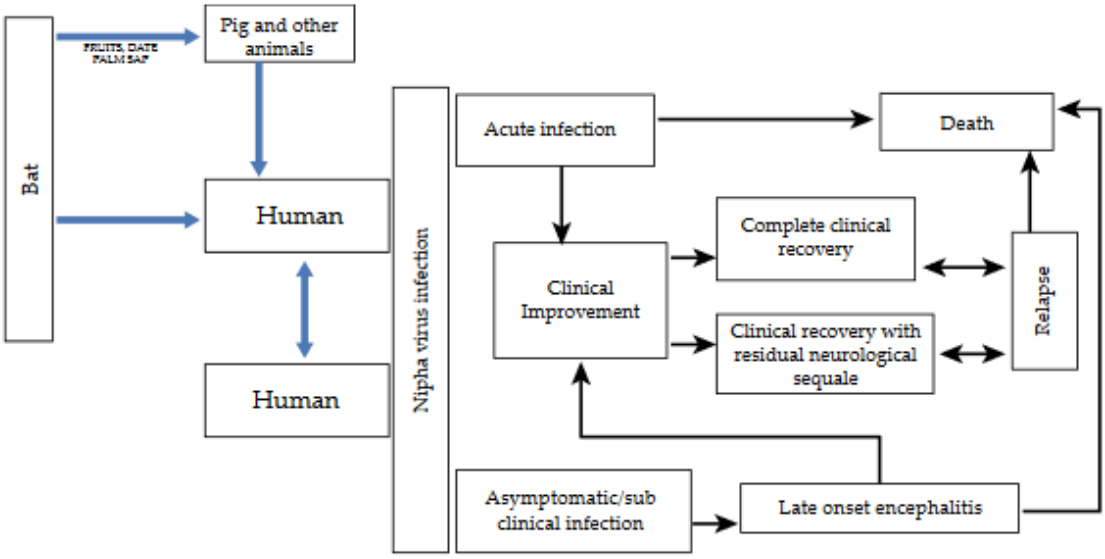


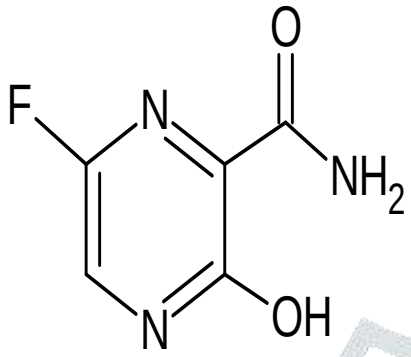
Fig 1: Overview on Nipah Virus infections

Treatment: There are no specific antiviral agents effective against Nipah presently. The utmost treatment is intensive support care for ARDS and encephalitis and treatment of symptoms. Ribavirin, a broad spectrum antiviral agent active against both RNA and DNA virus was given to Nipah virus infected patients tried in patients. It can penetrate blood brain barrier following oral administration of drug making it useful in the treatment of viral encephalitis.

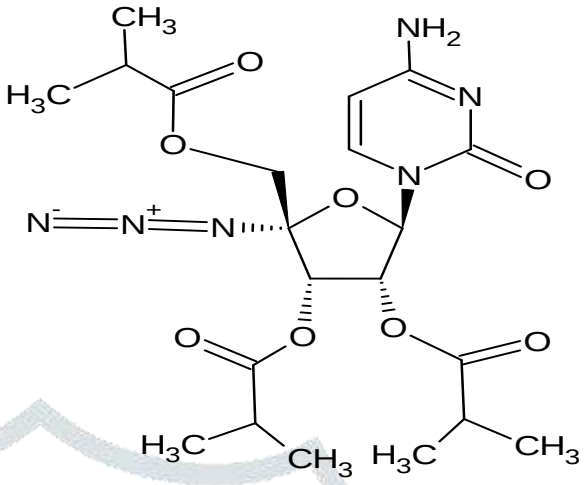
Initial trial with ribavirin in Malaysia showed 36% reduction in mortality and increased survival rate without neurological deficits. Ribavirin also subdued the duration of ventilator support and full hospital stay in patients with nipah virus infection (Chong 2001). Neutralizing human mechanical antibody the m102.4, that identified the receptor binding domain of the nipah virus G glycoprotein is successfully tested in animal model (Bossart 2009). Favipiravir (T-705; 6-flouro-3-hydroxy-2- pyrazinecarboxamine) a purine analogue that inhibit viral RNA-dependent RNA polymerase (RdRp), got potent anti-influenza activity which also act

against different RNA viruses including bunyaviruses, arenaviruses, filoviruses, norovirus, flaviviruses, alphaviruses, enteroviruses, paramyxoviruses, Ebola virus and rhabdoviruses.

Various trails with Favipiravir elicit promising results in nipah virus infection (Dawes 2018). Adenosine nucleoside analogue GS-441524, its monophosphate prodrug GS-5734 and other nucleoside analogue, R1479 (balapiravir), was also found to be effective against NiV and HeV in various studies (Lo 2017, Hotard 2017).



Favipiravir



Balapiravir

Computer-aided drug design (CADD) has become utmost part of the rational drug design process now a day. This CADD involves extensive computer modeling methods to reduce the costs and speed up the drug developing process (Douglass 2011). Designing a drug is not a talk of overnight it is a very sensitive, time-consuming, costly, sophisticated, and inefficient process. Collier et al. estimated that, the cost for the new drug development is about US$ 1.3-1.7 billion and it takes about eight - ten years (Robinson 1983). CADD helps to identify potential drug by its speed and efficiency. High-throughput robotic screening methods accelerate this process, but still, this is a time-taking process as a large number of compounds must be trialed (Lehninger 1993). By making use structure-based drug designing process, it is hoped that at least a smaller number of compounds will be identified which are active against the target and that very small number of compounds are then can be taken for trial. The availability of the 3D structure of the targeted macromolecule, usually by X-ray crystallography or nuclear magnetic resonance (NMR) or in few instances, homology models, enhances the procedure (Cornforth 1938). In general, the more specific the 3D structure and their information are the more accurate predictive results shall be identified in the context of drug discovery, significane of high-throughput docking has getting elevation day by day (Stanley 1967, Yamamoto 1982). Though there are many technical challenges in predicting the mode of binding of a molecule with the target and making comparison among the binding affinities to other compounds, molecular docking campaigns have produced important upliftment of hit rate when compared to random screening in a commencing number of cases (Bergman 1989, Dalian 1991, Watson 2000, Murakami 1995). In this particular study, we have incorporated structure based virtual screening method to search out the potential drug like compounds for the treatment against NiV-encephalitis targeting the NiV attachment glycoproteins (Niv-G).

Preparation of G-glycoprotein structure: The 3D crystal structure of G-glycoprotein of NiV (PDB ID: 6jb3) in complex with N-glycosylation site was retrieved from the Protein Data Bank (Yu 2013). All the water molecules were evacuated to make the structure of G-glycoprotein of NiV ready for structure based virtual screening and molecular docking processes.

Ligand preparation for virtual screening: Compound input libraries of 2000 drug like small compounds from about 3500 drug-like compounds, based on Lipinski rule of five from CHEMBL database and 50 Indole derivatives compounds with a rich structural as well as pharmacophore diversity, were chosen as the molecules in demand for virtual screening. The pharmacophores for the current structure were weighted to all other pharmacophores identified in structures already.

Virtual screening Using PYRx: For this present study, we have used **PYRx** as the primary program for performing virtual screening (Amira). We prepared the 6jb3 pdbqt file. SBVS was performed on the common gene in all nine pathogens, MetK, and the metabolite that it produces, SAM which is next utilized in methylation reactions. Before performing molecular docking, the proteins as well as ligands were prepared for efficient and accurate docking results. Protein preparation was done by removing water molecules, adding

hydrogen atoms, merging non-polar bonds, and computing Gasteiger charges in Auto Dock-Tools (http://mgltools.scripps.edu/). Similarly, ligand preparation was done in Openbabel GUI (Prathipati 2007) available in PyRx interface by adding hydrogens, energy minimization and converted to pdbqt file format, a use-able file format for docking afterwards.

Molecular Docking using Autodock Vina: The conformation which is having the lowest docked energy was selected after the docking interactions since, the greater the negative binding energy value, the stronger is the binding of the ligand with the target [determined grid box sizes using Auto dock Tools version 4.2 (The Scripps Research Institute, La Jolla, USA). Auto dock Vina is reported for its accurate results and speedy, which is far faster than its ancestor program, Auto dock4. we prepared the input pdbqt file of 3D11 and have set the size and also the center of the grid box by employing Auto dock Tools. The 3D11 structure was incorporated with the Kollman charges and polar hydrogen atoms. Over and above, we have set the grid box sizes at 60, 60, and 60 Å and have given space between grid points at 0.375 angstroms. The predicted binding energy (kcal/mol), which questioned at how strongly a ligand binds to the receptor, is computed depending on the scoring function used in Auto Dock Vina. The more the negative value of binding affinity is, the stronger the affinity is. Pursuant that, the scoring function of the software Auto Dock Vina is divided into 2 portions: i) Conformation-dependent portion, which can be regarded as a sum of intramolecular and intermolecular contributions, and ii) Conformation-independent portion, which relies on the number of rotatory bonds between heavy atoms in the ligand. Contributions of each of the portions are given an unidentical weighted value in the scoring function of the Auto Dock Vina (Xiaoqian 2014). The contributions include the steric, hydrophobic, hydrogen bonding and number of rotatory bonds

ADMET properties prediction: The toxic profiles and drug-likeness based on the binding energies were predicted using the OSIRIS program (Gul 2005). OSIRIS calculates different drug relevant properties like molecular weight, cLogP, cLogS, Druglikeness and toxicities like mutagenicity, tumorigenicity, reproductive effects and irritant effects in the lead molecules based on functional groups present in their structures

Table :1 Physico-chemical Parameters used for screening compounds using CHEMBL database

HBD	0-5
HBA	0-10
Log P	0-5
Molecular weight (M. wt)	160-500
TSPA	20-140A2
Number of rotatable bond	0-10

IV. RESULTS AND DISCUSSION

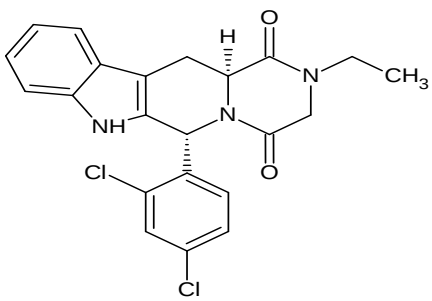
4.1 Virtual screening results of for Antiviral activity with Indoles.

S.NO	CHEMBL ID	ZINC ID	ENERGY	PYRX VALUE (B.E)
1	1094073	ZINC000049070965	714.95	-8.5
2	3040484	ZINC000096285114	438.81	-7.1
3	1533368	ZINC000003503757	267.8	-6.9
4	2008069	ZINC000001653330	574.46	-7.0
5	1254204	ZINC00010617027	403.64	-7.9

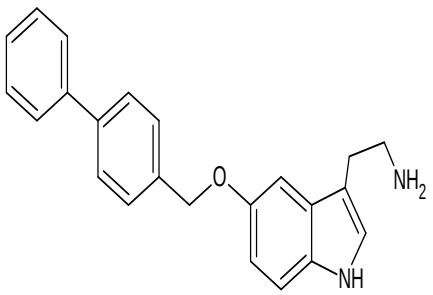
6	1622046	ZINC000003460446	245.65	-6.6
7	1205589	ZINC0000027104491	451.01	-7.7
8	2159625	ZINC 0000095574934	611.83	-7.8
9	163916	ZINC000013492827	393.51	-7.4
10	234715	ZINC000028817993	452.17	-6.8
11	3086118	ZINC000103285452	359.51	-7.4
12	417713	ZINC00013779438	289.3	-7.3
13	1933103	ZINC0003938300	636.61	-8.7
14	180067	ZINC0001890596	732.04	-6.7
15	1288259	ZINC0003816716	398.64	-6.3
16	1766320	ZINC00071315826	808.33	-8.0
17	2442586	ZINC00038189739	598.08	-6.1
18	499067	ZINC00040973170	631.71	-9.5
19	221765	ZINC000013585526	445.57	-6.9
20	1907348	ZINC000004114773	382.01	-7.6
21	603081	ZINC0000045375185	716.09	-7.7
22	295135	ZINC000001484671	542.96	-7.6
23	1619347	ZINC000005137889	505.19	-8.6
24	94773	ZINC000003831916	493.74	-9.4
25	1650867	ZINC0000066104757	536.25	-8.0
26	16424	ZINC0000043176090	973.16	-7.5
27	1099245	ZINC0000049792576	619.95	-7.9
28	381035	ZINC0000028524705	531.65	-8.1
29	163034	ZINC000001489440	626.36	-7.8
30	3133833	ZINC00000103297578	424.48	-7.6
31	2313790	ZINC000095595626	621.74	-7.1
32	3809343	ZINC000653819851	513.97	-7.7

33	1213854	ZINC000100752548	410.03	-8.0
34	562978	ZINC000042920242	398.52	-6.5
35	347133	ZINC000027716098	550.11	-7.6
36	1181735	ZINC000028468542	968.17	-8.2
37	3304393	ZINC0000139093762	684.8	-9.1
38	388176	ZINC000028651317	408.44	-6.7
39	329441	ZINC000026647126	642.27	-7.9
40	1195589	ZINC000043013568	250.61	-9.1
41	40149	ZINC000029326534	503.26	-6.8
42	2365277	ZINC000095597064	469.56	-8.8
43	2079144	ZINC000084687177	799.71	-8.7
44	2079184	ZINC000084652418	1212.73	-9.6
45	1188953	ZINC000027873967	400.6	-8.1
46	1740663	ZINC00003244555	572.5	-9.1
47	529859	ZINC000045303149	743.83	-8.7
48	1179679	ZINC000013555507	411.23	-7.7
49	1187710	ZINC000038457296	450.22	-7.0
50	2021691	ZINC000084587149	673.6	-9.6

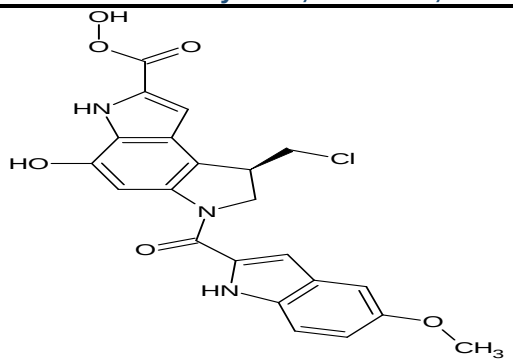
That potent molecules having highest anti-Nipah viral activity are shown below:



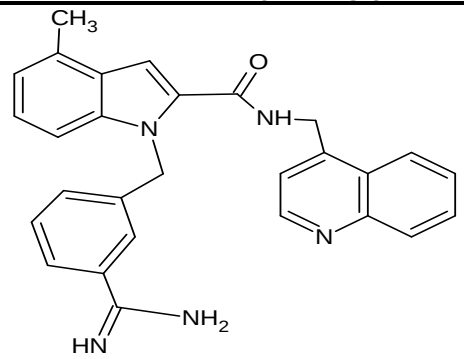
CHEMBL ID – 1650867 E=536.25



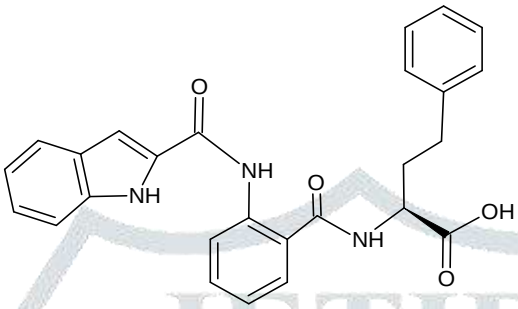
CHEMBL-ID – 2365277 E= 469.56



CHEMBL ID – 1094073 E= 714.95



CHEMBL ID – 94773 E=493.74

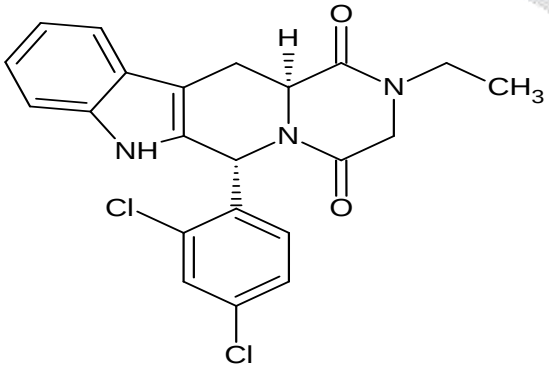


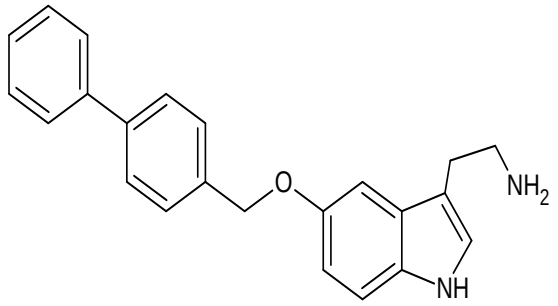
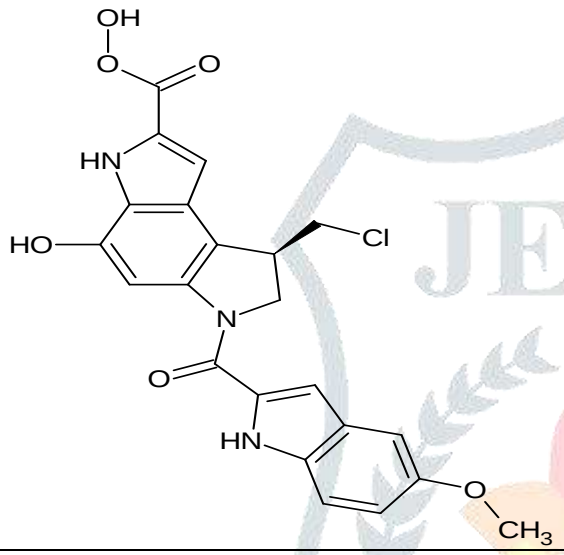
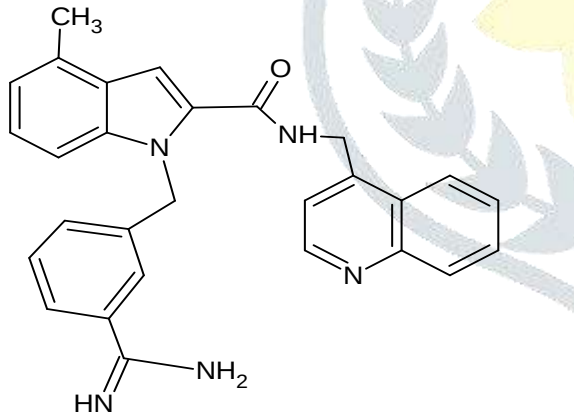
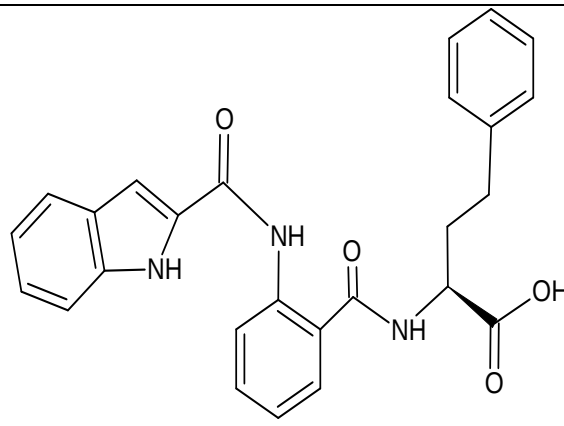
CHEMBL ID – 1933103 E=636.61

MOLECULAR DOCKING

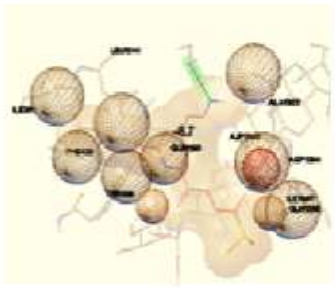
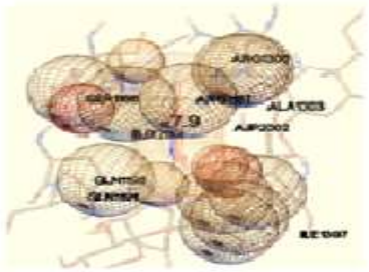
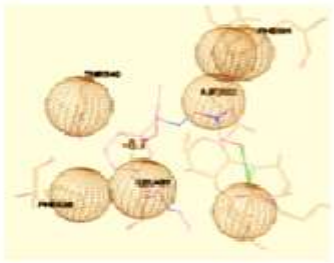
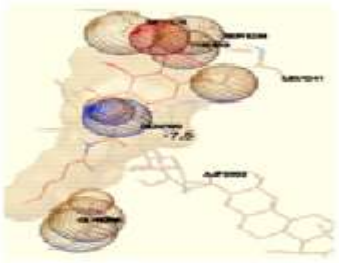
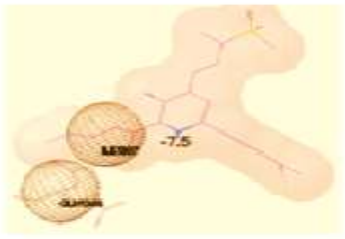
In the field of molecular modelling, docking is a method which predicts the preferred orientation of ligand in the binding pocket of the target protein. As the binding affinity studies between ligands and their receptors form the basis of physiological activity and pharmacological effects of chemical compounds. We carried out docking studies to investigate the correct binding pose of potent & derived (novel) compounds in the active site pocket of the G-Glycoprotein to evaluate the affinity of the title compounds towards the protein in order to assess their potency in anti-Nipah viral activity.

Table :2 Molecular Docking Studies of Potent Molecules (Screened by using Virtual Screening)

S.NO	STRUCTURE OF LEAD MOLECULES	Interactions With G-Glycoprotein		Scores
		Hydrogen bonding	**Other interactions**	
1		ASP503	**Hydrophobic interactions** VAL431, LEU405. **Hydrophilic interactions** LYS432, LYS466, GLU345.	-8.7

2		ASP503	**Hydrophobic interactions** ILE484, LEU481, GLN482, LEU493, LEU504. **Hydrophilic interactions** TYR501, ASP503.	-7.9
3		LYS-483	**Hydrophobic interactions** ILE484, VAL475. **Hydrophilic interactions** LYS485, GLN482, LYS502.	-8.7
4		ASP503	**Hydrophobic interactions** LEU481, ILE484, LEU493, VAL478. **Hydrophilic interactions** GLN479, ASP503, GLN482, TYR501.	-7.5
5		LYS-483	**Hydrophobic interactions** PRO394. **Hydrophilic interactions** GLN479, GLU476, ASP390.	-7.5

Molecular Docking Results with Potent Molecules:

MOLECULAR PROPERTY CALCULATION AND TOXICITY PREDICTION

Bioactivity prediction of indole derivatives using Molinspiration software.

The molecular property of the newly synthesized compounds were calculated values of some basic molecular description such as logp, logs, molecular weight, Polar Surface Area, number of hydrogen bonds donor and number of hydrogen bonds acceptor in molecule membrane hydrophobicity and bioavailability were predicted.

Table-4 the Lipinski rule of five (Lipinski rule *et al* 1997) was adopted to sort out the drug likeness of synthesized compounds. The results are presented in the following table:

Table 4: Molecular Property Calculation of the title compounds:

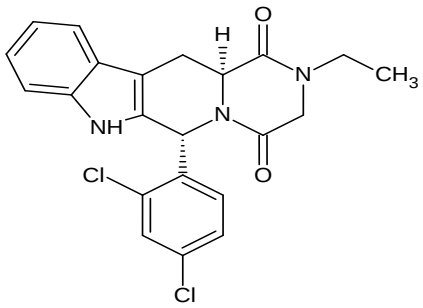
s.no	CHEMBL ID	ZINC ID	M I logp	TPSA	N atoms	Mol. Wt	no N	No. HNH	N violation	N rotb	Volume
1	1650867	66104757	4.13	56.41	29	428.32	5	1	0	2	353.97
2	94773	3831316	3.65	96.8	34	447.54	6	4	0	6	411.19
3	1094073	49070965	3.91	107.66	32	453.88	3		0	5	376.39
4	19933103	3938300	2.53	111.29	33	411.49	7	4	0	8	396.04
5	2365277	95597064	4.49	51.05	26	342.44	3	3	0	6	3260.4

Discussion

From the observations of virtual screening, Molecular studies following observations could be made:

- 2,3-Disubstituted derivatives are found reported to be more potent than other substituted derivatives. The extent of binding interactions also confirmed the significance of these substitutions.

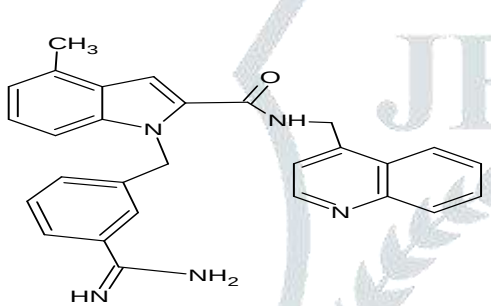
Eg: The Indole derivative with CHEMBL ID 1650867 which is a 2,3-Disubstituted derivative of indole was found be more potent in terms of binding affinity rather than other derivatives.



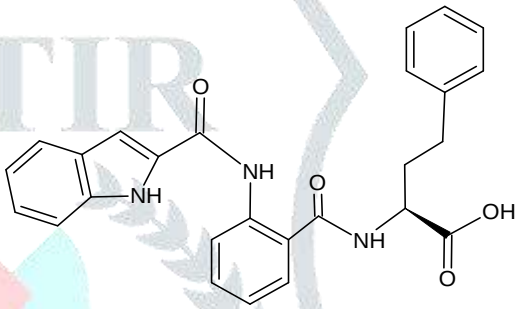
- Indole –NH at position plays a significant role in mediating the interactions of all the molecules with various amino acid residues like Asp-503, Lys-483 of G-Glycoprotein of Nipah Virus enzyme.

Therefore free –N-H is essential for mediating there interactions with the protein and substitution of position-1 decreases the antiviral activity.

- Incorporation of –CO group/-CONH at 2- position of Indole ring also displayed a significant role in mediating its interactions with G-Glycoprotein of Nipah virus.

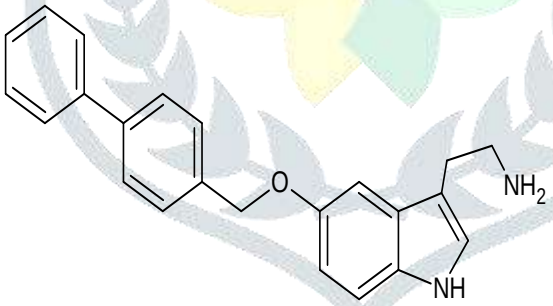


CHEMBL ID – 94773



CHEMBL ID - 1933103

- Introduction of Aryl substitution at position-5 also contributed to the antiviral activity of these derivatives.



CHEMBL ID - 2365277

Hydrophobic interactions of the molecule with ILE 484, LEU 481, GLN 482, LEU 493, LEU 504 is attributed because of the aryl substitution at position-5 of indole ring.

Conclusion

- As there are no specific antiviral drugs effective against Nipah currently and the available drugs are obtained through drug repurposing approaches, there is a strong necessity for the development of a potent anti-Nipah viral agent.
- With the observations of the SAR studies on the substituted indole derivatives screened by structure based virtual screening, a lead molecule could be designed and could be developed as a potent anti-Nipah viral agents

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