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STRUCTURE-BASED VIRTUAL SCREENING, DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF INDOLEDERIVATIVES AS RESPIRATORY SYNCYTIAL VIRUSFUSION (RSV-F) INHIBITORS

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ABSTRACT

Indole constitutes extensively explored heterocyclic ring systems with a wide range of activities like anticancer, anticoagulant, anti-inflammatory, antibacterial, antifungal, antiviral, antitubercular, antidiabetic, and analgesic activities. In the present work, the indoles were subjected to structure-based virtual screening against RSV fusion glycoprotein (PDB ID: 5KWW). Top lead molecules were identified as HITS and subjected to molecular docking studies. By understanding their important pharmacophoric features and incorporating them in a single molecule, a series of novel compounds such as 1,3,4-oxadiazole substituted indoles were designed and their molecular properties and toxicity prediction studies were carried out to know the safety and efficacy of the title compounds by using Molinspiration, OSIRIS Property Explorer. Molecular docking studies were carried out for the title compounds on the PDB ID: 5KWW using AutoDock Vina 1.5.6 software. The synthesized compounds were characterized by physical and spectral analysis. The synthesized molecules are to be evaluatedfor their RSV fusion inhibitory activity.

KEYWORDS: Indoles, Structure-based virtual screening, Molinspiration, OSIRIS Property Explorer, Molecular Docking, RSV (Respiratory Syncytial Virus).

INTRODUCTION

Introduction to Heterocyclic Compound

Heterocyclic Chemistry is the branch of organic chemistry dealing with the synthesis, properties, and applications of the heterocyclic compounds.[1] Heterocyclic compounds possess a cyclic structure with two or more different kinds of atoms in the ring. Heterocyclic compounds in which the ring contains at least one carbon atom. The cyclic part means circle

of heterocyclic indicating that at least one cycle is present in a compound and hetero means other or different which refers to the noncarbon atoms or hetero atoms in the ring [2]The most common heterocyclic compounds having five or six-membered ring having which containing heteroatoms of Nitrogen (N), Oxygen (O), and Sulphur (S).The best examples of Heterocyclic compounds are furan, thiophene, pyrimidine, and pyrrole.

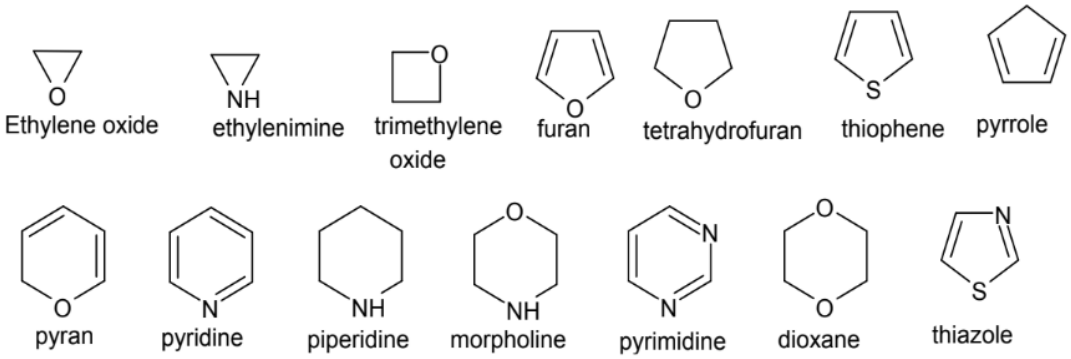


Figure 1: Examples of 3-6 membered Heterocyclic compounds.

Heterocyclic compounds are spreading widely throughout many areas of life sciences and technology like as agrochemicals, in pharmaceuticals, in veterinary products and also used as intermediates in organic synthesis. Most of the drugs are heterocyclic compounds.[4]

1.1 Introduction to Indoles

The name indole was derived from the word indigo and oleum, indole was first isolated by treatment of indigo dye with oleum. Indole also called Benzopyrrole, a heterocyclic compound that was first isolated in the year 1886, has the molecular formula C8H7N, and it was commonly synthesized from phenylhydrazine and pyruvic acid.[5] Indole has a bicyclic structure consisting of a six-membered benzene ring fused to a five-membered pyrrole ring.

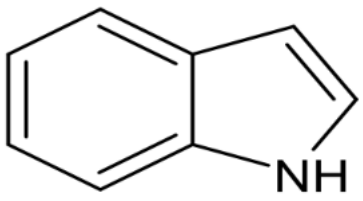


Figure 2: General structure of Indole.

Indole is reported to have various biological activities such as anti-viral, anti-microbial, anti-cancer, anti-fungal, anti-bacterial, anti-hypertensive and anti-asthmatic.

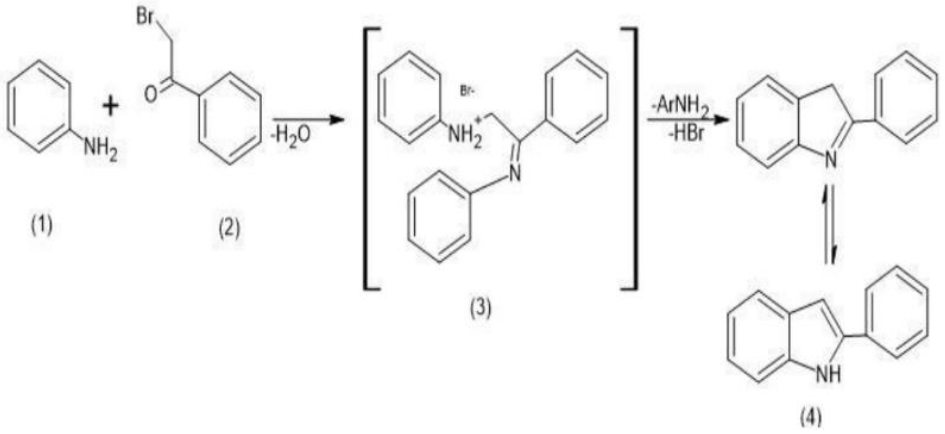
Physical properties of Indole

Indoles are white to brown crystalline solids. It is weakly basic. The compound has melting point range of 52-54oC (126-129oF) and a boiling point of 254oC. It is soluble in DMSO and Dimethyl formamide. At very low concentrations, it has a flowery smell[7] and is a constituent of many flower scents (such as orange blossoms) and perfumes. They can be easily dissolved in any polar solvents.

1.1.1 General methods for synthesis of indole

Bischler Indole Synthesis

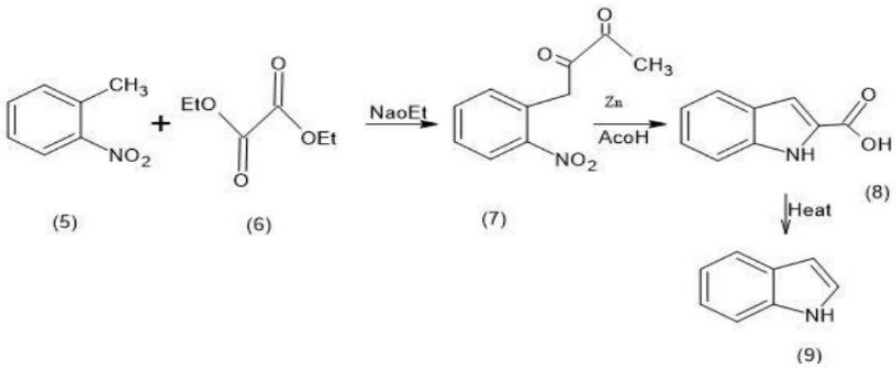
It is also known as Bischler Mohlau indole synthesis was first reported in 1892. It generally involves an alkylation reaction of α-halo ketones with anilines followed by ring-closer cyclization in presence of an acid. Fischer et al., (1888) have reported this synthesis by the reaction of an excess of aniline (1) with α-bromoacetophenone (2) to form 2-arylindole. This reaction proceeds via the formation of α-anilinehaloketones [phenyl-(2-phenylimino-propyl)-ammonium bromide] intermediate (3) which upon tautomerization results in the formation of indoles (4).[8]



Reissert Indole Synthesis

It is a multistep synthetic process involving condensation of o-nitro toluene (5) with oxalic esters (6) to afford o-nitrophenyl pyruvic esters (7), which on reduction of the nitro group in presence of Zn dust as a reducing agent in acetic acid resulted in cyclization to indol-2-carboxylic acid (8).The later decarboxylation under thermal conditions afforded indole (9). Other reductive conditions used for this reaction may include iron filling in HCl, sodium

dithionite in ethanol, and iron powder in acetic acid or ethanol.[9]



1.2 Introduction to Target

Respiratory Syncytial Virus also called human respiratory syncytial virus and human orthopneumovirus is a very common, contagious virus that causes infections of the respiratory tract. The genus orthopneumovirus consists of pathogens that target the upper respiratory tract within their specific host and it belongs to the family- pneumovirus, and kingdom-orthonavirae. The common symptoms are runny nose, decrease in appetite, coughing, sneezing, fever, and wheezing. The incubation period for RSV infection ranges from 2 to 8 days. The characteristics of RSV infection are increased mucous secretion and bronchial obstruction that leads to prolonged cough, wheezing, and radically altered lung function. The course of the illness is variable and can last from one to several weeks. Treatment options for RSV are limited. Palivizumab, a monoclonal antibody (mAb), is approved for prophylactic use but is only 60% effective at reducing hospitalization rates.

Virus Structure and Life Cycle

RSV is s Paramyxovirus belonging to the genus Pneumoviridae. It contains an enveloped, nonsegmented, negative (−), single-stranded linear RNA genome with 10 genes encoding 11 proteins with two open reading frames of gene M2.[10,11] It also includes Specific genes such as nonstructural (NS) proteins NS1 and NS2, large (L) protein (the RNA polymerase), nucleoprotein (N), phosphoprotein (P) which serves as the cofactor for the L protein, matrix(M) protein, M2.1 and M2.2 which is required for transcription, small hydrophobic (SH) protein, glycoprotein (G protein), and fusion (F) protein. RSV can be divided into two subgroups, RSVA and RSV-B, which contain different genotypes based on the nucleotide sequence of the carboxy-terminal ectodomain of the G protein.[12] The surface of RSV is coated with two glycoproteins: the attachment glycoprotein (G) and the fusion glycoprotein

(F). RSV F is essential for entry, as it facilitates pH-independent fusion of the viral membrane with the host cell plasma membrane, leading to infection of the host cell. The F protein is indispensable for viral attachment to the host and entry into the host cell. Although the G protein is responsible for the preliminary attachment, the F protein is necessary for the fusion, budding, and spread of the virus.[58] RSV fusion inhibitors are thought to be able to disrupt the formation of a six-helix bundle that is essential to start the fusion between the viral and the cellular membrane.

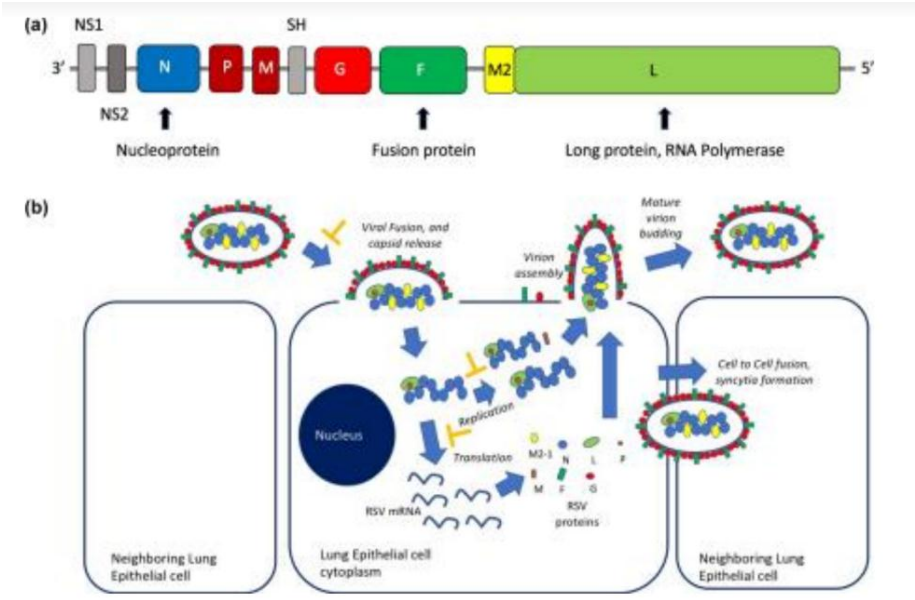


Figure 3: Life cycle of Respiratory Syncytial Virus.

a. RSV genome with drug involvement targets of viral fusion and replication.

b. The RSV infection and replication process involves the virus entry into ciliated epithelial cells is mediated through the glycoprotein (G protein), which binds to the receptor on the cell surface,[59] and the fusion (F protein). The ribonucleoprotein complex functions as the template for the RSV polymerase in complex with large, phosphoprotein and Messanger protein to synthesize progeny mRNAs.[60,61] Nucleocapsids are packaged into virus particles and subsequently released by budding from the cell plasma membrane. Nonstructural proteins, NS1 and NS2 hinder the innate immune response to infection, while M2-2 regulates replication.[62] The key steps are targeted by fusion and replication inhibitors.

RSVF protein plays a critical role in establishing and propagating RSV infection by catalysing membrane fusion and virus entry as well as cell-cell fusion resulting in the

formation of syncytia.[63,64] Different chemotypes have been shown to inhibit the RSV F protein by binding into a threefold symmetrical central cavity of the pre-fusion metastable state of F, thereby stabilizing it and preventing it from refolding to the post-fusion state which is required for membrane fusion.[65]

Replication Inhibitors:- Inhibition of viral replication is the most common approach to antiviral chemotherapy in numerous viruses from Herpes simplex virus with acyclovir, through HIV to most recently RNA viruses with sofosbuvir in hepatitis C.

One of the examples for RSV fusion inhibitors is JNJ-53718678 a sub-nanomolar indole-based fusion inhibitor, showing the clinical activity in a Phase 2a challenge study conducted in healthy adult subjects experimentally infected with RSV, and currently being evaluated in both infants and adults and another example is PRESATOVIR is an antiviral drug which was developed as a treatment for the respiratory syncytial virus. It acts as a fusion inhibitor and has shown promising results in phase II clinical trials.

About Protein

5KWW is the PDB ID of the crystal structure of inhibitor JNJ-53718678 In complex with prefusion RSV Glycoprotein. The experimental data for the protein is given as follows

- Method :- X-RAY DIFFRACTION
- Resolution:- 2.50 A
- R-Value free:- 0.239
- R-value work:- 0.210

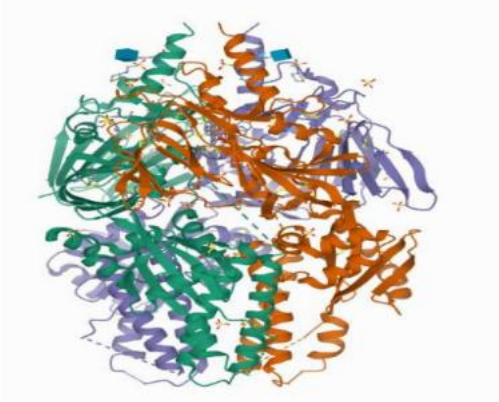


Figure 4: Structure of RSV glycoprotein.

The amino acids present in the Active site of the protein were found to be VAL300, ILE288,

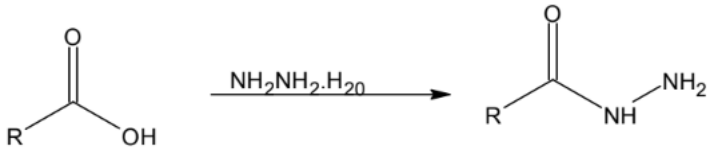
MET251, GLN302, TYR299, MET289, SER287, TYR286, THR244, SER285, GLY242, ILE57, SER55, LEU260.

Experimental Methods

Table 1: List of Chemicals and Reagents used.

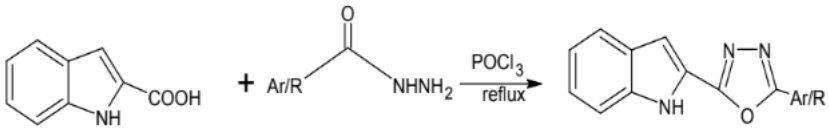
Benzoic acid	Hydrazine hydrate
4-chlorobenzoic acid	Phosphorus oxychloride
4-bromobenzoic acid	Sodium bicarbonate
4-Nitrobenzoic acid	Distilled water
4-Methoxybenzoic acid	Methanol
4-Methylbenzoic acid	Ethylacetate
Indole-2-carboxylic acid	Hexane
Indole-3-acetic acid	Silica gel

Step 1:-General procedure for the synthesis of carboxylic acid hydrazides:-



Carboxylic acid (0.01 moles/1gm) and hydrazine hydrate (0.012 mole/0.01 ml) were taken in a 150 ml conical flask. The reaction mixture was irradiated under microwave for 60-200 seconds at 900 watts at 2.45 GHz. Then the reaction mixture was cooled to -200 C and then it was lyophilized at -500 C. The products obtained were recrystallized from ethanol. The hydrazides were characterized based on physical and spectral data.

Step 2:- General procedure for the synthesis of 1,3,4 –oxadiazolyl indoles :-



Aryl hydrazide (1M/1gm) was dissolved in phosphorus oxychloride (5ml) and its compounds indole-2-carboxylic acid or indole-3-acetic acid (1M/1gm)weres added to the reaction mixture, after refluxing for 6-7 hours, was cooled to room temperature and poured onto crushed ice. On neutralization of the contents with sodium bicarbonate solution (20%), a

solidmass separatede. This was filtered and washed with water. It was crystalized by using ethanol to give a product. They were characterized by using physical and spectral data.

Virtual screening studies on Indole derivatives

- Virtual screening of 100 molecules was performed by retrieving the indole molecules from the CHEMBL database for various activities like anticancer, antidepressant, antihypertensive, antiviral, and antiemetic activity.
- Among all the activities performed, indoles were found to have good antiviral activity as RSV fusion inhibitors against the Respiratory syncytial virus. Here we have screened indole structures against the crystal structure of inhibitor JNJ-53718678 In complex with prefusion RSV Glycoprotein (PDB ID:- 5KWW).
- Therefore, the above results are obtained by using virtual screening of indole structures with PDB ID: 5KWW which are having good antiviral activity against RSV.
- From the above results, we have selected the highest score of 6 molecules for further studies which are selected as HITS.

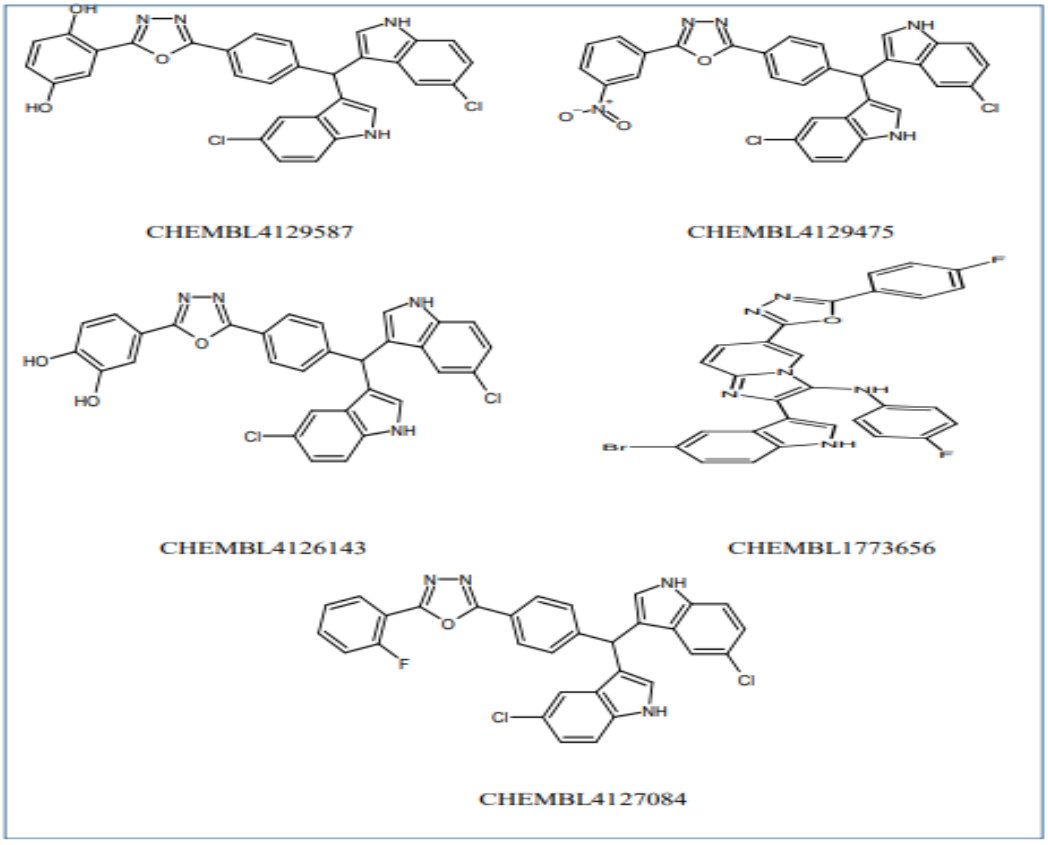
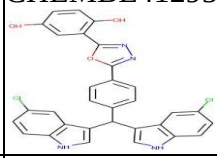
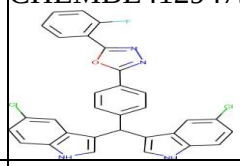
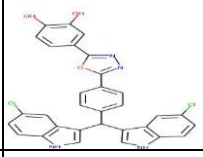
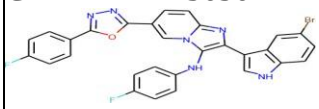
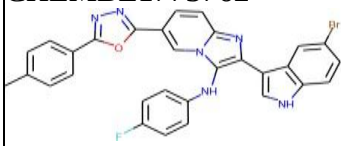
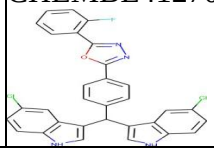


Figure 5: Structures of five potent HITS.

Molecular Docking Results of Potent HITS

We carried out docking studies of five HITS obtained from virtual screening to know the correct binding pose of novel molecules with PDB ID:5KWW.

Table 3: Molecular Docking interactions of 5 potent HIT molecules with PDB ID: 5KWW obtained from virtual screening results.

S.N0	STRUCTURE OF THE LEAD MOLECULE	HYDROGEN BONDS	OTHER INTERACTIONS	DOC KING SCORE ES
01	CHEMBL4129587 	None	**Hydrophilic interactions** with LYS228, GLU125, ARG315.	-9.0
02	CHEMBL4129475 	None	**Hydrophilic interactions** with ASN460, ARG339. **Hydrophobic interactions** with VAL406, ILE407.	-9.2
03	CHEMBL4126143 	Hydrogen bonding with ARG429	**Hydrophilic interactions** with ARG420, ASN426. **Hydrophobic interactions** with ILE432, VAL442.	-8.9
04	CHEMBL1773656 	None	**Hydrophilic interactions** with LYS15, ARG330. Hydrophobic interactions with ASN460.	-7.8
05	CHEMBL1773762 	Hydrogen bonding with LYS315	**Hydrophilic interactions** with ASN460, LYS315. **Hydrophobic interactions** with LEU465, VAL408, ILE407, VAL80.	-9.4
06	CHEMBL4127084 	Hydrogen bonding with LYS315, ASN460	**Hydrophilic interactions** with ARG330, LYS315. **Hydrophobic interactions** with ILE407, VAL406.	-7.3

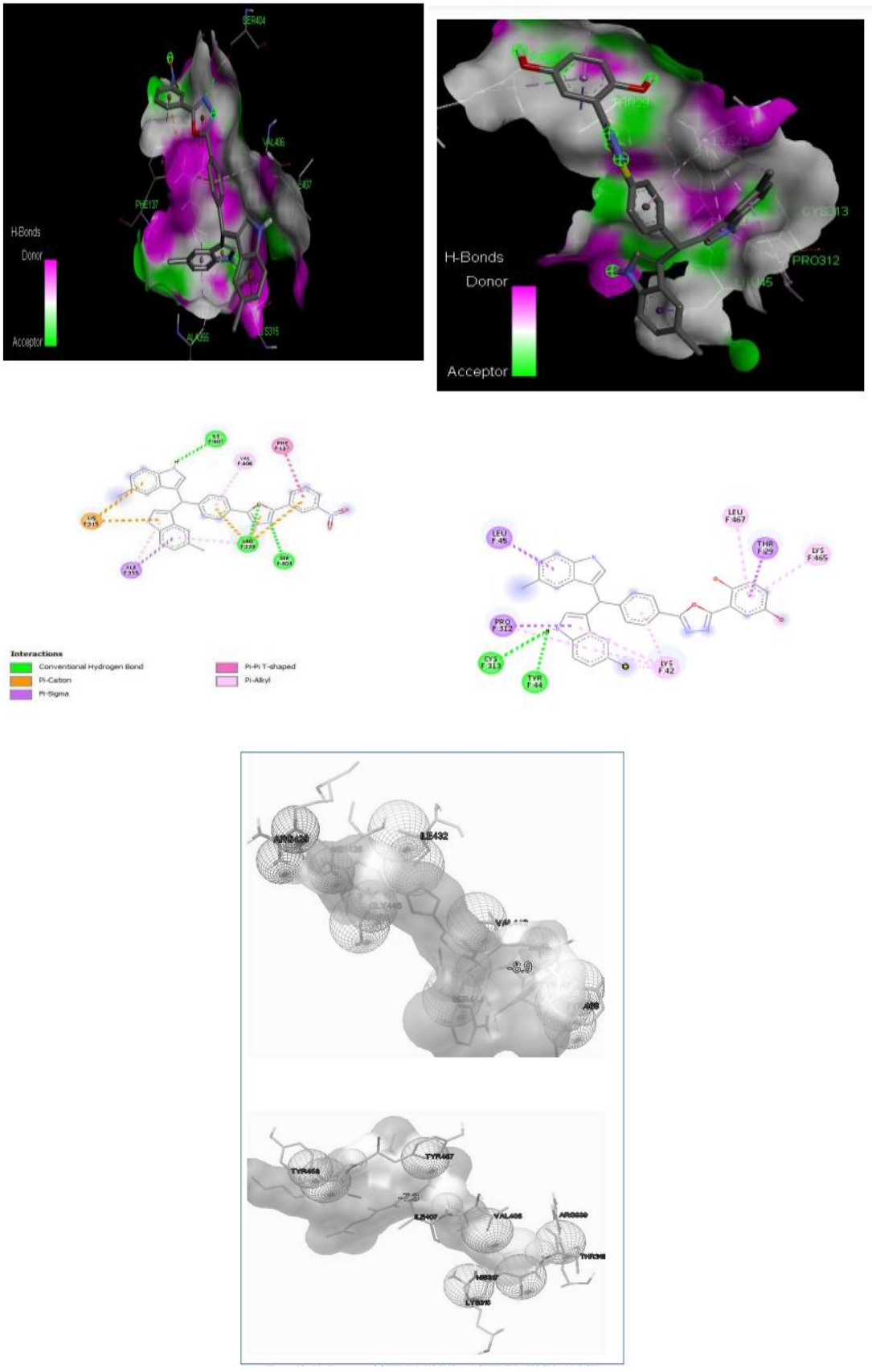


Figure 6: Binding pose and binding interaction of HITS with RSVGlycoprotein PD.

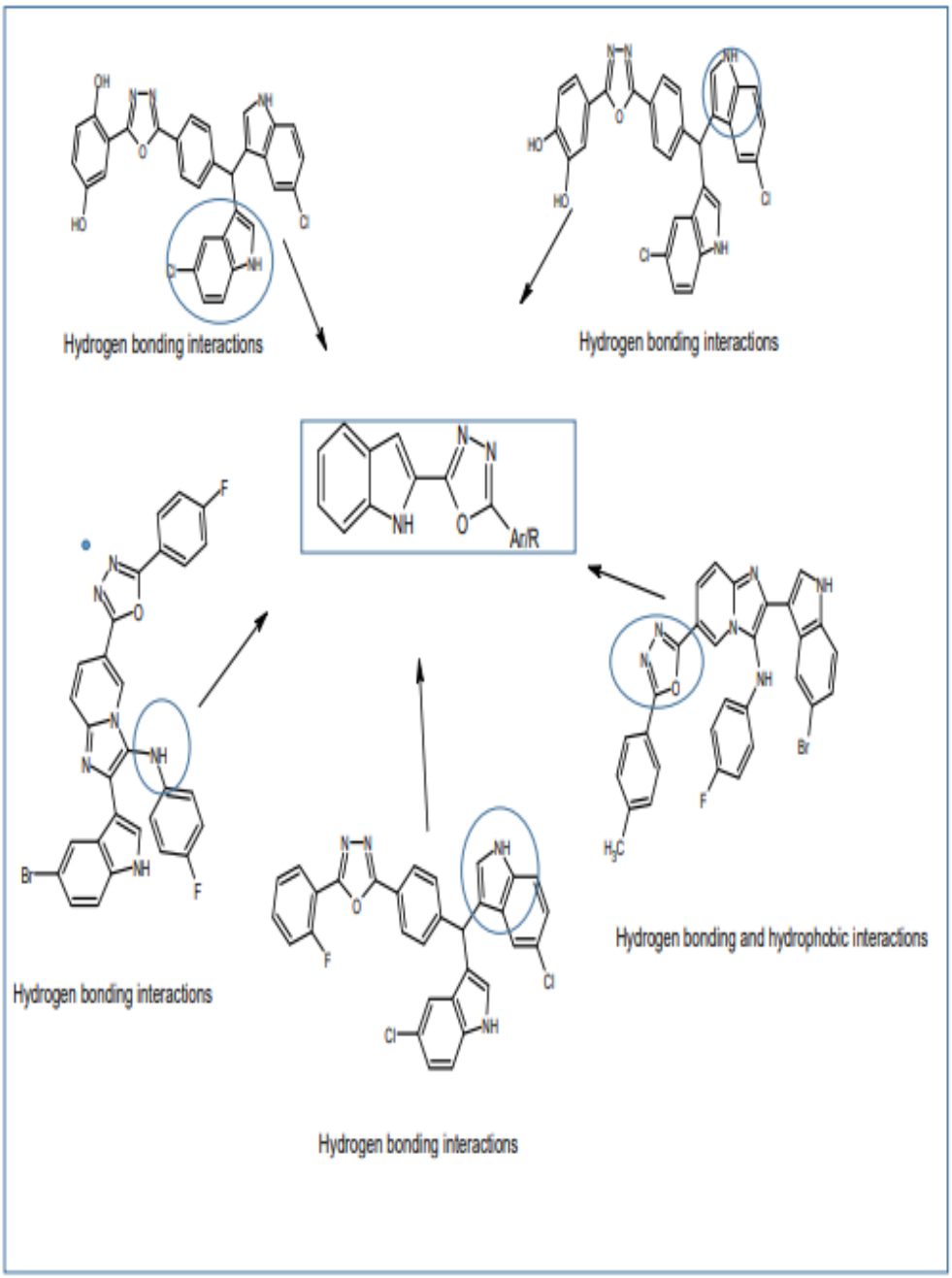
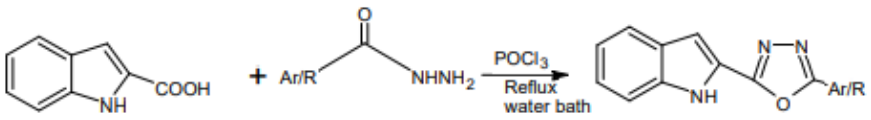


Figure 7: Design of the lead molecule.

SCHEME: Synthesis of the designed molecules is achieved by the following scheme:



1H-indole-2-carboxylic Acid | 2a-f | 5a-f

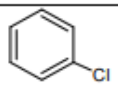
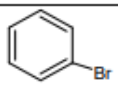
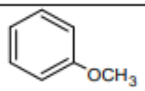
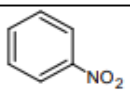
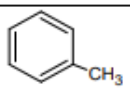
S. No	-Ar
5a	
5b	
5c	
5d	
5e	
5f	

Table 4: Physical properties of final compound.

Compound	Name of the compound and Molecular Formula	Mol. wt	Physicalstate	Melting point	Yield %	Rf value Ethylacetate : Hexane8:2
5a	2-(5-phenyl-1,3,4-oxadiazol-2-yl)- 1*H*-indole C16H11N3O	261.28	Brown colour powder	162-164^{0}C	72%	0.4
5b	2-[5-(4-bromophenyl)- 1,3,4-oxadiazol-2-yl]-1*H*-indole	340.18	Yellowcolour powder	152-154^{0}C	68%	0.32
5c	2-[5-(4-chlorophenyl)- 1,3,4-oxadiazol-2-yl]-1*H*-indole	295.73	Yellowcolour powder	160-162^{0}C	65%	0.43
5d	2-[5-(4-methoxyphenyl)- 1,3,4-oxadiazol-2-yl]-1*H*-indole	291.31	Light green colour powder	166-168^{0}C	72%	0,5
5e	2-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]- 1*H*-indole	306.28	Creamywhite colour powder	158-160^{0}C	70%	0.35

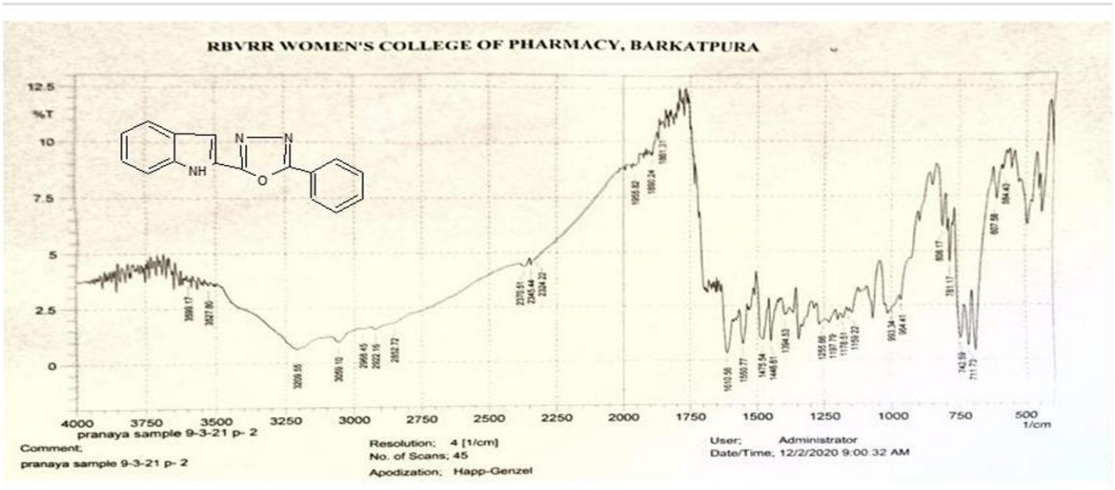


Figure 8: IR spectroscopy of the final compound.

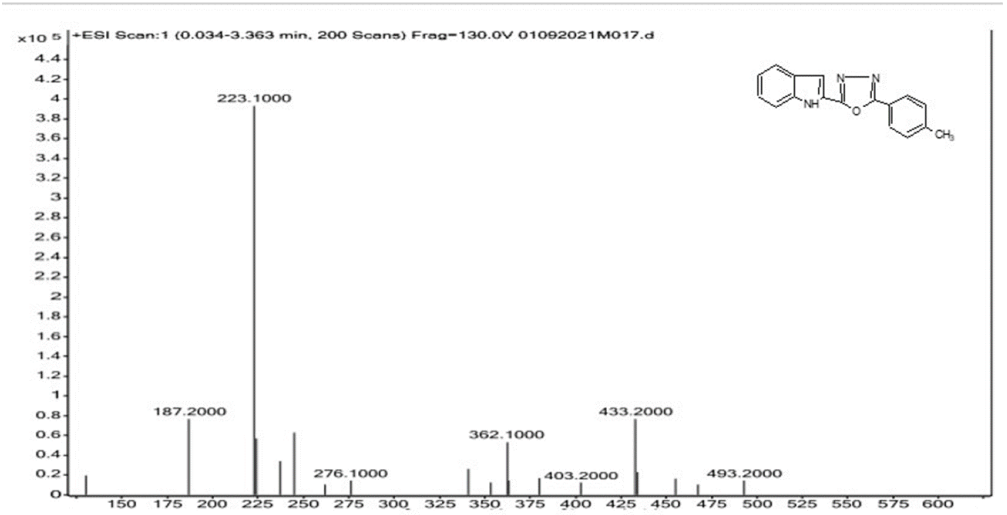


Figure 9: Mass Spectroscopy of The Final Compound.

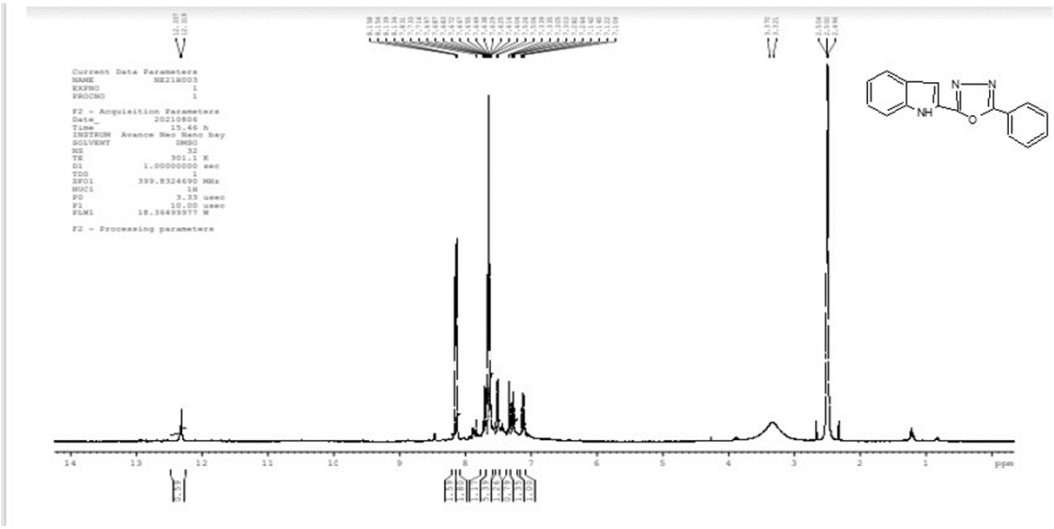


Figure 10: NMR Spectroscopy of Final Compound.

DISCUSSION

- Virtual screening of 100 molecules was performed by retrieving the Indole molecules from the CHEMBL database for various activities like anti-cancer, anti-bacterial anti-hypertensive, and anti-viral.
- Among all the activities performed, indole derivatives were found to have interesting anti-viral activity against the Respiratory Syncytial Virus.
- Therefore, from the above results obtained by virtual screening of indole derivatives with RSV glycoprotein (PDB ID: 5KWW), it is understood that the following molecules could have possible activity against RSV. Then 5 molecules with the highest binding scores were considered HITS.

- 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 of five HITS obtained from virtual screening to investigate the correct bindingpose of the novel molecules with RSV glycoprotein (PDB ID: 5KWW).
- By understanding the basic pharmacophoric features of the selected HITS, novel Indole substituted derivatives were designed.
- Based on drug-likeness, compounds were predicted to be promising druggable candidates. Further, the result of molecular docking studies supports the accuracy of prediction.
- The toxicity of the compounds was also predicted using OSIRIS, most of the compounds amongst the synthesized ones showed non-tumorigenic and non-reproductive effects, which further supports the drug features in the molecules. This toxicity prediction would be useful forthe selection of compounds to test in animal models.
- Molecular docking studies of the title compounds were carried out to understand the correct binding interactions of the title compounds with RSV GLYCOPROTEIN (PDB ID: 5KWW).
- New series of 5a-f were synthesized by a known convenient method. Indole-substituted derivatives were synthesized from Aryl hydrazides (2a-f). The compounds were characterizedby IR, NMR and Mass Spectrometry.
- The synthesized compounds were characterized with physical and spectral data.

CONCLUSION

In the present investigation, 100 molecules from the CHEMBL database were retrieved and subjected to virtual screening against (PDB ID: 5KWW). Five HITS with the highest scores were selected and by understanding the basic pharmacophoric features, a new lead molecule was designed and from that, a new series of Indole derivatives (5a-f) were subjected to molecular docking and toxicity prediction studies. The molecular docking results showed that all the six compounds that were screened exhibited a good binding affinity which was similar to that of the standard Presatovir. Compound 5f (2-[5-(4-methyl phenyl)-1,3,4-oxadiazol-2-yl]-1H-indole) shows the highest binding affinity -7.6 Kcal/mol. All the designed molecules were synthesized and characterized by physical and spectral data. Further invivo biological activity of the compounds has to be carried out.

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