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Identification of Respiratory Syncytial Virus Fusion Protein Inhibitor: *In silico* Screening and Molecular Docking Approach

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

In young children, immunocompromised individuals, and elderly people, the respiratory syncytial virus (RSV) is the primary source of acute lower respiratory tract infection. Intervention with RSV-specific small-molecule antivirals may provide significant therapeutic potential. For virus entry, the RSV fusion protein (F) is crucial as it facilitates viral and hosts membrane fusion. To date, no approved vaccine or drug molecule is available to treat RSV. With this purpose, in the present study, virtual screening of a library of natural compounds against the active site of F protein was performed, followed by an in-depth molecular docking study of top-scored compounds. Selected hits ZINC8740013, ZINC4029781 and ZINC898642were found to strongly bind with RSV F protein relative to the other compounds as well as the control. The binding energy (BE) and inhibition constant for 'ZINC8740013-RSV F', 'ZINC4029781-RSV F', and 'ZINC898642-RSVF' complexes were found as '-7.8 kcal/mol and 63.27 μM', '-7.7 kcal/mol and 19.04 μM', and '-7.5 kcal/mol and 3.31 μM', respectively. However, BE and inhibition constant of control (JNJ-53718678) with RSV F protein was found as -6.1 kcal/mol and 563.26 μM, respectively. Phe140 and Phe488 are the main interacting residues of RSV F protein with JNJ-53718678 and selected hit compounds. The finding of this study suggests that these hits can be utilized as the RSV Fprotein inhibitor to prevent the fusion of the viral envelope with the host cells. Further, bench work experiments are required to optimize these hit compounds as RSV Fprotein inhibitors.

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1. INTRODUCTION

In infants and young children, human RSV is the leading cause of respiratory infection. Bronchiolitis is a serious clinical manifestation of infection with RSV. Indeed, in children younger than 1 year of age in the United States, RSV is the most common cause of bronchiolitis and pneumonia. In older people, it is also a major cause of respiratory illness [1]. In a week or two, most people recover, but RSV can be serious, especially for infants and older adults [2]. RSV-infected individuals are typically infectious for 3 to 8 days [3]. However, even after they stop displaying symptoms, some children, and people with compromised immune systems, will continue to transmit the virus for as long as 4 weeks. RSV leads to an average of 2.1 million pediatric visits per year in the United States for children younger than 5 years of age, 57527 hospitalizations among children younger than 5 years of age, 177000 hospitalizations among adults older than 65 years of age, 14000 deaths among adults older than 65 years of age (https://www.cdc.gov/rsv/research/us-surveillance.html).

RSV is the main viral cause of child mortality, with 7 to 9 times more deaths than those associated with the influenza [4]. For infants aged less than 6 months, the incidence of respiratory admissions due to RSV is significantly higher than for any other viral cause, like influenza [5]. Apnea, which can be one of the first symptoms of RSV infection, is present in 16-20 percent of young children [6].The occurrence of apnea at hospital admission was shown to be linked to the expression of chronic apnea and, as a result, enhanced the probability of mechanical ventilation [7].

The length of the RSV viral genome is approximately 15 kb, encoding 11 distinct proteins. Proteins G and F are viral membrane proteins responsible for virus binding to the host cell and viral and cell membrane fusion, respectively [8]. Human RSV disease pathogenesis is still not well described and the pathways associated with many diseases caused by RSV infection have not been delineated [9]. Initially, the F protein is expressed as a precursor (F0) that is broken into a furin-like protease in the trans-Golgi network at two sites, resulting in two disulfide-linked subunits, F1 and F2, and releasing an undisclosed 27-amino-acid (aa) fragment, p27 [10,11].

Trimerized and completely cleaved F endorses a fusion-competent, metastable prefusion state that is located on the surface of infected cells in which it is inserted into developing infectious virons [10].

Structural screening of targeted chemical compound libraries identified a variety of structurally diverse compounds that inhibited RSV fusion with target cells [12,13]. In clinical trial Phase II double blind, placebo-controlled, human-challenge trials, the fusion inhibitors GS-5806 and JNJ-53718678, respectively, accom plished well. Oral dosing either of the compound led to a decrease in viral load and disease incidence among healthy adults with a clinical isolate of RSV who were experimentally infected intranasally [14,15]. We conducted state-of-the-art *in silico* research here in this article, including virtual screening (VS) accompanied by in-depth molecular docking analysis to discover potential inhibitors of RSV.

2. METHODOLOGY

2.1 Protein Structure Preparation

From the protein data bank, the RSV F protein 3D structure [PDB ID: 5kww] was retrieved. Co-crystallized ligands and water molecules were separated and protein was prepared in monomer form using the protein preparation wizard by Discover Studio (DS) 2020 and processed in .pdb format.

2.2 Natural Compound Library Pre paration

Natural compounds were retrieved from the ZINC database (https://zinc.docking.org) by selecting 'substances' accompanied by selecting 'natural products' as a subset, resulting in a total of 224205 compounds and downloaded in sdf format. Using Chemdraw Ultra, the retrieved compounds were prepared and the molecules in sdf format were minimized using online minimization methods for further screening studies.

2.3 Structure-Based Virtual Screening

Using the AutoDock Vina program under PyRx (version 0.8) software, the prepared natural compounds library was screened against the

active site of RSV F protein. These compounds were imported into the workspace of PyRx and converted into .pdbqt format by Open Babel in PyRx. Finally, the top-ranked screened compounds were further processed for in-depth docking experiments.

2.4 Molecular Docking Analysis

Two top-screened natural compounds were processed for molecular docking analysis to the active site of RSV F protein using the 'Autodock' program. The energy minimization of ligands was done by the 'MMFF94' force field. The size of grid box parameters was set to 20.28×18.97×19.24 Å. All other parameters have been set to customary.

2.5 Ligplot+ Analysis

After the docking, LIGPLOT+ Version v.2.1 analyzed the docked complexes to classify hydrogen and hydrophobic interactions between the essential important amino acid residues of RSV F protein and natural compounds. The 3-D structures of the docked complex interaction generated were converted into 2-D using the LIGPLOT algorithm.

3. RESULTS AND DISCUSSION

Human Respiratory Syncytial Virus (RSV) is the main cause of severe respiratory tract infections in pediatric and patients of different age groups and continues a significant problem to worldwide health issue [16,17]. F protein is vital for the replication of RSV and is the main target for drug development [18]. Ribavirin is the only approved drug for the treatment of RSV. However, the use of this drug is limited due to its genotoxicity [19]. Therefore, the development of highly selective and effective anti-RSV drugs is a need of time. With this purpose, in this study, 224205 natural compounds from the ZINC database have been screened against the RSV F protein using the *in silico* art of techniques. Among the screened compounds, ZINC8740013, ZINC4029781, and ZINC898642 were the selected hits and were found to strongly bind with RSV F protein. Phe137, Gly139, Phe140, Gln354, Met 396, Ser398, Asp486, Phe488 are the main interacting residues of RSV F protein with the selected hits compounds (Figs. 1-3). Consistent with this, in a recent study, these residues of RSV F protein have also been documented to interact with Ziresovir, which has been reported as a promising RSV F protein inhibitor and currently in phase II clinical trials [20].

The binding energy (BE) and inhibition constant for 'ZINC8740013-RSV F', 'ZINC4029781-RSV F', and 'ZINC898642-RSVF' complexes were found as '-7.8 kcal/mol and 63.27μM', '-7.7 kcal/mol and 19.04μM', and '-7.5 kcal/mol and 3.31μM', respectively (Table 1).

JNJ-53718678 is a selective and effective inhibitor of RSV F protein and in phase II clinical trial [21]; in this study, this compound has been used as a control. BE and inhibition constant of JNJ-53718678 with RSV F protein were found as -6.1 kcal/mol and 563.26 μM, respectively (Table 1). Recently, the binding site RSV F proteinwithJNJ-53718678has been documented [21], in which Phe140 and Phe488 residues are vital for the interaction of RSV F protein with JNJ-53718678. Interestingly, in our study, Phe140 and Phe488 are the common interacting residues of RSV F protein with JNJ-53718678 and selected hit compounds (Figs. 1-4).

Drug development against RSV is mainly focused on targeting the viral entry machinery and the viral RNA dependent RNA polymerase complex. Inhibiting the RSV F protein results to block the viral entry [14]. The RSV envelope is coated with the G protein (attachment protein) and the F protein (fusion protein) [18]. RSV G protein is not vital for the entry of virus and but contribute to its pathogenesis; however, RSV F is essential for entry and aids fusion of the viral envelope with host cells [22].

Amino acid residue Gln354 of RSV Fprotein was found to make H-bond with ZINC8740013 (Fig. 5a). Besides, Phe140 and Phe488 are the common hydrophobic interacting residues of RSV Fprotein with JNJ-53718678 and selected hit compounds (Fig. 5a-5d). The H-bond and hydrophobic interaction assist to explain the strength of inhibitors to the target protein and have an important role in the stability of the 'inhibitor-protein' complex [23].

In computational docking, BE determined the binding strength between the ligand-protein complex, and a high (negative) BE signifies the efficient binding of the ligand with the target protein [24]. Based on the BE, the selected hit compounds (ZINC8740013, ZINC4029781, and ZINC898642) show efficient binding with RSV Fprotein relative to the other compounds as well as the control (Table 1), suggesting that these hits can be utilized as the RSV Fprotein inhibitor to prevent the fusion of viral envelope with host cells.

Table 1. Binding energy of hit compounds with RSV

Compound	Binding energy (kcal/mol)	Inhibition constant (μM)
ZINC8740013	-7.8	63.27
ZINC4029781	-7.7	19.04
ZINC898642	-7.5	3.31
JNJ-53718678*	-6.1	563.26

Positive control

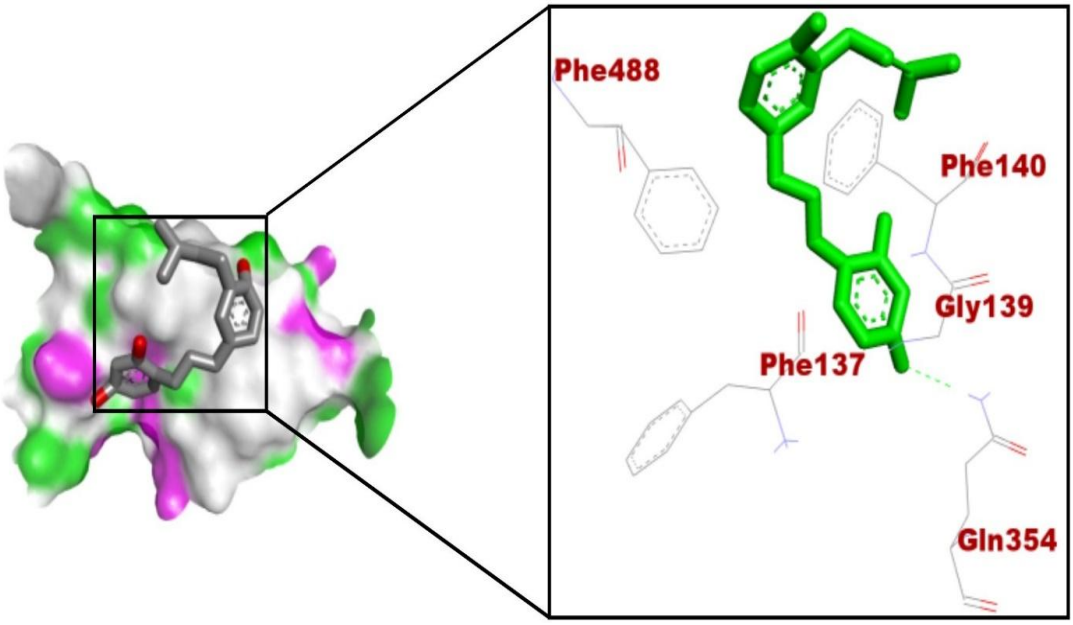

Fig. 1. Interaction of ZINC8740013 (stick representation) with RSV F protein

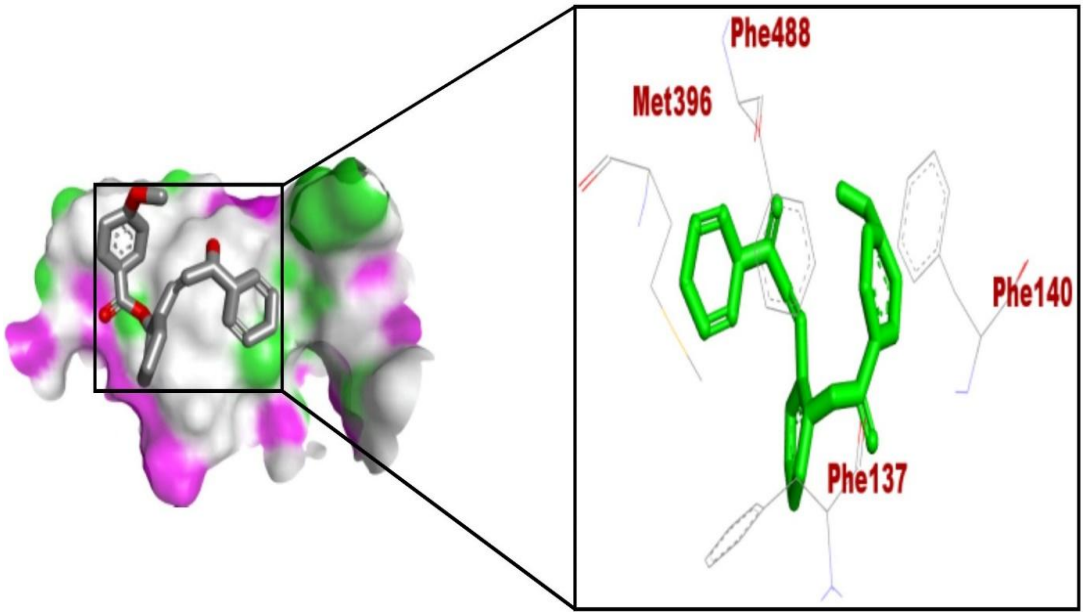

Fig. 2. Interaction of ZINC4029781 (stick representation) with RSV F protein

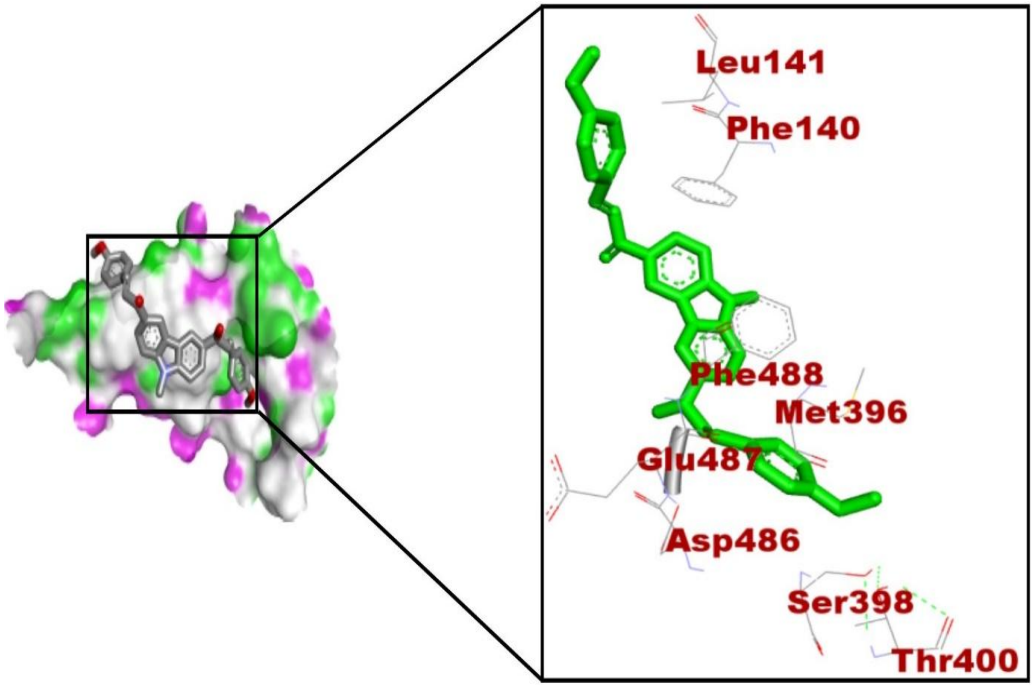

Fig. 3. Interaction of ZINC898642 (stick representation) with RSV F protein

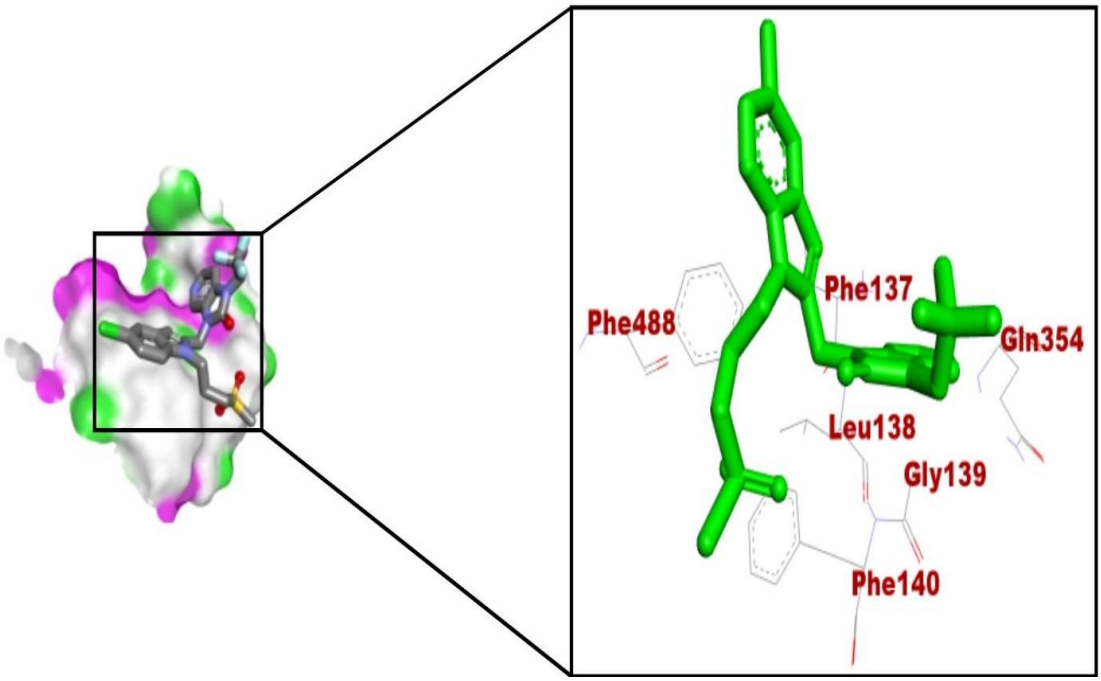

Fig. 4. Interaction of control compound JNJ-53718678 (stick representation) with RSV F protein

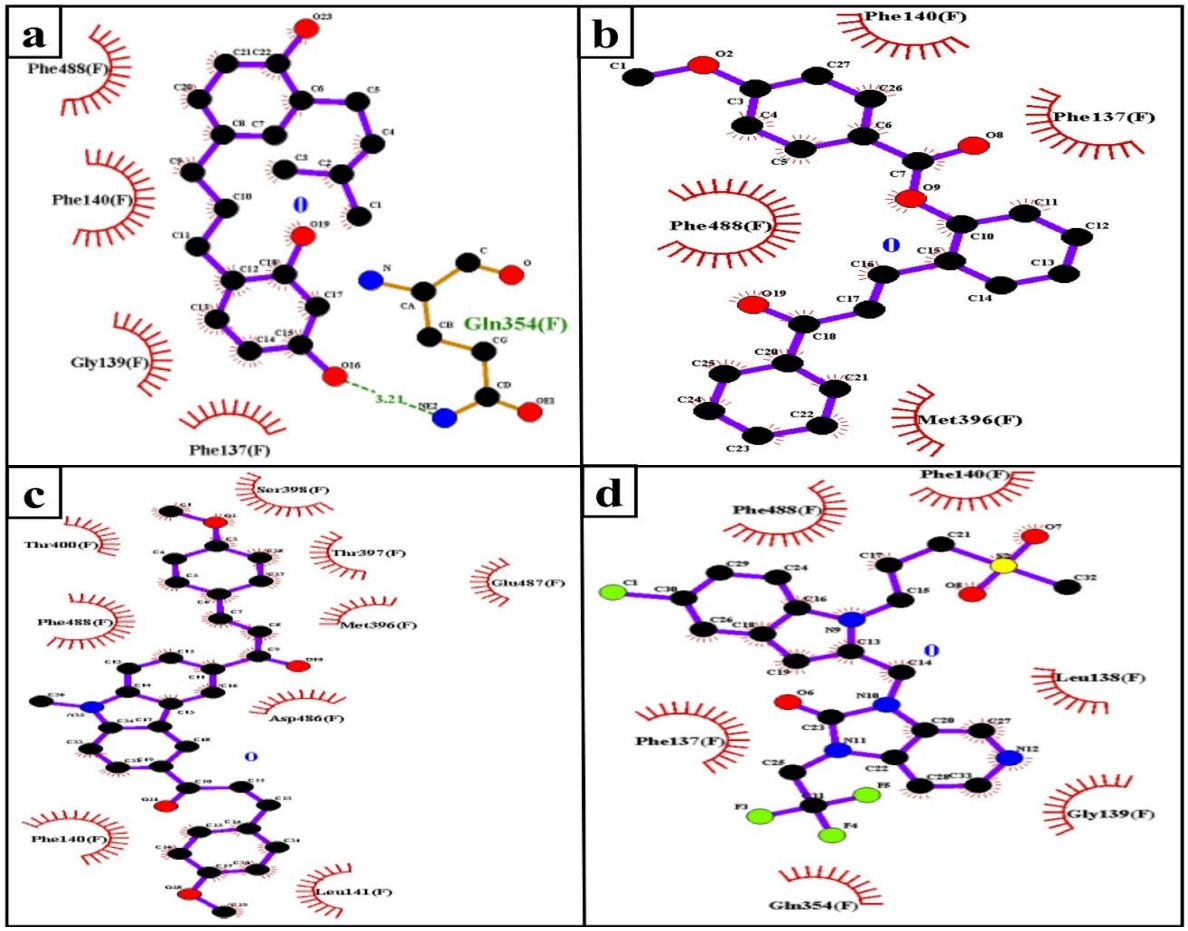

Fig. 5. H-bond (green dashed line) and hydrophobic interacting residues (red arc) of RSV F protein with a)ZINC8740013, b) ZINC4029781, c) ZINC898642, and d) JNJ-53718678

Treatment for RSV usually includes measures of self-care. Nasal suction may provide symptomatic relief in infants, but nasal edema and congestion may be exacerbated by prolonged suction. As there is no RSV vaccination to date, only symptomatic treatment is given in practice.

4. CONCLUSION

In this study, 224205 natural compounds from the ZINC database have been screened against the RSV F protein using the *in silico* art of techniques. Among the screened compounds, ZINC8740013, ZINC4029781, and ZINC898642 were found to strongly bind with RSV F protein relative to the other compounds as well as the control, suggesting that these hits can be utilized as the RSV Fprotein inhibitor to interrupt the fusion of viral envelope with host cells. Further, bench work experiments are required to optimize these hit compounds as RSV Fprotein inhibitors.

DISCLAIMER

The products used for this research are com monly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. In addition, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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