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High throughput virtual screening strategy to develop a potential treatment for bronchial asthma by targeting interleukin 13 cytokine signaling

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Abstract
Chronic inflammation in the airway passage leads to the clinical syndrome of pediatric asthma. Allergic reactions caused by bacterial, viral, and fungal infection lead to the immune dis-balance which primes T helper cells (Th2), a specific cluster of differentiation 4 (CD4) T cell differentiation. This favors the Th2-specific response by activating the interleukin 4/interleukin 13 (IL-4/IL-13) cytokine signaling and further activates the secretion of immunoglobulin E (IgE). IL-13 develops bronchial asthma by elevating bronchial hyperresponsiveness and enables production of immunoglobulin M (IgM) and IgE. The present study aims to target IL-13 signaling using molecular docking and understanding molecular dynamic simulation (MDS) to propose a compelling candidate to treat asthma. We developed a library of available allergic drugs (n=20) and checked the binding affinity against IL-13 protein (3BPN.pdb) through molecular docking and confirmed the best pose binding energy of -3.84 and -3.71 for epinephrine and guaifenesin, respectively. Studying the interaction of hydrogen bonds and Van der Walls, it is estimated that electrostatic energy is sufficient to interact with the active site of the IL-13 and has shown to inhibit inflammatory signaling. These computational results confirm epinephrine and guaifenesin as potential ligands showing potential inhibitory activity for IL-13 signaling. This study also suggests the designing of a new ligand and screening of a large cohort of drugs, in the future, to predict the exact mechanism to control the critical feature of asthma.

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Introduction

Allergy is an a complex immunological reaction that begin when a harmless foreign substance such as dust, mold, or pollen cause strong immunological reactions through immunoglobulin E (IgE)-mediated or non-IgE mediated (T lymphocyte) to attack an allergen by the immune system.[1] Allergic rhinitis is a type of seasonal hay fever that occurs when the immune system overreacts to an allergen in the air. Currently, the diseases affected between 10% and 30% of the population.[2] Globally, asthma ranked 46th, and 28th leading cause of burden is approximately 2%-5% of children who are affected,[3] and they are heterogeneous, indicating many pathological, clinical, and physiological phenotypes.[4] Acute rhinosinusitis (ARS) is an infection of both your nasal cavity and sinuses that occurs due to viral, bacterial, or fungal infections, or allergic reactions, or environmental factors.[5] Allergic diseases are caused by the expansion of a subset of T cells such as "T-helper 2 cells" (T_H 2-cell), which switch to generate the IgE antibodies to specific allergens[6] by releasing interleukin-4 (IL-4) and IL-13 to promote IgE production[7]. This in turn causes allergic asthma triggered by other cytokines such as IL-4, IL-5, IL-25, and IL-33. Then mast cells release histamine response to allergens leading to allergic symptoms and causing asthma.[8,9] All these cytokines play a key role in initiating airway inflammation. Importantly, IL-13 is a 17-kDa glycoprotein that plays as the role of a central mediator in asthma by eliciting independent pathological and physiologic characteristics which are activated by mast cells, basophils, natural killer cells, eosinophils, CD4+ TH2 cells, and CD8+ T cells.[10]

Bronchial asthma is a disorder of the airways due to the accumulation of inflammatory cells, and the primary treatment is inhaled corticosteroids (ICSs), which act as an anti-allergic agent on inflammatory cells.[11,12] Severe asthma is lower in childhood as compared to adult asthma.[13] A cohort study of 323 12-year-old children in Sweden, had shown severe asthma defined by the World Health Organization (WHO),[14] and suggested that in a population about 0.23% of prevalence had 2.1% in children.[15] Asthma phenotypes are related to airway inflammation pathways, defined by distinct inflammatory endotypes, cell mediators, and immune pathways.[16] One of the primary aims to reverse asthma is the reversal of existing airway inflammation. Hence, strategies for therapeutics focus on reducing inflammation[17], in the field of biological treatment blockade of IgE is the milestone[18], and Omalizumab is the available humanized monoclonal anti-IgE with the pediatric indication (age >6 years).[19] Generally, there is no cure for allergies but, many medications are available including antihistamines such as Cetirizine, Chlorpheniramine, Ketotifen, etc, and decongestants such as pseudoephedrine, phenylephrine with oxymetazoline, corticosteroids, and combination drugs.[20] The biopharmaceutical targeted the IL-13 (lebrikizumab) and shows IL-13 blocking through the IL-13 receptor (R) α1/IL-4Rα.[21] The pathophysiology of asthma through treatment options in use includes corticosteroids, β2-adrenoceptor agonists, mediator antagonists, synthesis inhibitors, and phosphodiesterase inhibitors.[22]

In protein dynamics, integrally associated mechanics, forces, and motions with its structure will determine the function and its ability to adapt to various driven sets of conditions. Studying ligand-driven molecular protein dynamics, also known as the induced fit (IF) hypothesis, helps to understand biology at the molecular level.[23-25] In this regard, studying protein dynamics influences ligand on protein dynamics, and it is a core issue in biology. With this background, it is necessary to address the potential candidate to control and manage the inflammation associated with asthma at this scenario. To address this, the study aimed to screen the drug candidates by applying high throughput virtual screening and molecular dynamics for allergies that cause asthma. To understand the mechanism of these with target inflammatory determinants rule out the possible way to treat asthma in the future. Ligand docking and ligand–protein molecular dynamics highlight the marker IL-13 cytokine which plays an important role in the inflammatory pathway and this helps in analyzing asthma.

Materials and methods

Selection of drug candidates

To understand the suitable candidate for target inhibition and disease prevention, *in silico* molecular assessment, we targeted the drugs which manage allergic asthma. There are several categories of allergic medicines available on Drugs.com and we selected 20 drugs (Table 1) based on the literature survery pertaining to drugs having target action on IL-13 and few side effects compared to others. Further, screen the best suitable candidate to understand the designing strategy for treating asthma.

Molecular docking study to investigate potential lead for inhibiting IL-13 cytokine signaling

Target selection

The putative three-dimensional (3D) target structure PDB ID: 3BPN for IL-13 receptor, involved in the inflammatory signaling pathway, was selected based on literature reports suggesting this is the key to the development of T cell-mediated immune responses which cause allergy and asthma.[26] Also, lebrikizumab, a monoclonal antibody (5L6Y.pdb) was used as a control to compare the drug candidates. This protein crystal structure was retrieved from Research Collaborative for Structural Bioinformatics (RCSB) protein databank and helps to screen the targeted drugs to understand those drug–target interactions.

Ligand preparation

The selected drug analog (Table 1) structures were further drawn from ChemSketch and saved the molecules in mol2 format to use for further study.

Table 1 The available drug candidate binding energy to protein IL-13 receptor used to treat allergy which they are used during asthma conditions.

Sl.No.	Drug candidates	Generic name	Est. Free Energy of Binding in kcal/mol
1	Albuterol	Albuterol inhalation	+1.13
2	Beclomethasone	Beclomethasone inhalation	+1.27
3	Benadryl	Diphenhydramine	+4.55
4	Budesonide	Budesonide	+1.24
5	Ciclesonide	Ciclesonide inhalation	+1.56
6	Cyproheptadine	Cyproheptadine	+51.58
7	Dexamethasone	Dexamethasone	+285.89
8	Epinephrine	Epinephrine injection	-3.84
9	Flunisolide	Flunisolide inhalation	+608.71
10	Ipratropium	Ipratropium inhalation	+32.96
11	Levalbuterol	Levalbuterol	+15.50
12	Loratadine	Loratadine	+60.32
13	Methylprednisolone	Methylprednisolone	+280.09
14	Guaifenesin	Mucinex	-3.71
15	Prednisone	Prednisone	+382.14
16	Promethazine	Promethazine	+20.86
17	Racepinephrine	Racepinephrine	-3.08
18	Salmeterol	Salmeterol	+651.73
19	Singulair	Montelukast	+1.65
20	Terbutaline	Terbutaline	-2.81
21	Theophylline	Theophyline	-2.43

Ligplot analysis using PDBsum

The LIGPLOT[27,28] program was used to predict the binding sites of drugs (Table 1) for protein 3BPN, and the program generates the 2D representation of protein-ligand complexes from the PDB file as input. Then, it displays all the possible interactions (hydrogen bond and non-bonded contacts) held between ligand and residue of protein structure to understand the drug potentiality in target inhibition.

Lead optimization

All drug analogs were generated to the guide molecule structure to that of the binding pocket of the 3BPN protein molecule, which was used to identify the active sites,[27] that is, the pocket where the ligand is likely to bind. To compute this, the mol2 3BPN file was read using the main menu to display the number of cavities.

Molecular docking study

The docking of the ligands for 3BPN was performed using an online molecular docking server.[27,29] Also, lebrikizumab, a monoclonal antibody (5L6Y.pdb) with 3BPN, is used as a control to compare the drug candidates. This predicts the ligand interactions with the target by separating compounds with micro and nanomolar binding constants from those with millimolar binding constants and may typically rank molecules with finer variations in affinity. The first step is to retrieve ligands and target 3BPN.pdb files from the database. The second step is to prepare PDBQT format files for target, ligand, and grid parameter files to finally dock the complex interactions.

Molecular dynamics simulations

The ligand-protein complex was used to study the molecular dynamics using LARMD[30] aiming to solve the problem of ligand-driven protein dynamics. This bioinformatics tool may help to guide and direct dynamic processes and give a reasonable hypothesis. Also, this toolkit simplifies the challenges to correlate ligand recognition with protein structure.

Results

Identification of potential inhibitory compound by molecular docking study

Currently, several drugs are used to treat inflammation and asthma in which IL-13 is the main cause of asthma (Figure 1). The drugs which are listed in Table 1 are initially tested to know their interactions with protein 3BPN and their

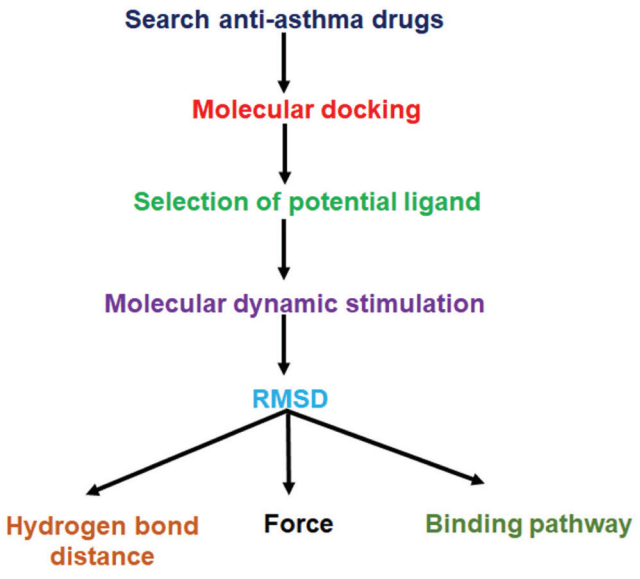

Figure 1 Designing of ligand-protein interaction study for asthma control. The current investigation is designed to explore the suitable drug candidate for the treatment of asthma. The figure shows the investigation studies by suitable drug screening.

binding property was reported in the present study. The 3BPN is a type of IL-4 and IL-13 cytokines, which are important keys for the development of T cell-mediated humoral immune responses, associated with allergy and asthma. They exert actions through three different combinations (type I to type III) of shared receptors. Among screened candidates, the estimated epinephrine and guaifenesin have high binding energy towards protein 3BPN (-3.84 and -3.08 kcal/mol) compared to tralokinumab (PDB-5L6Y) (Supplementary Figure S1) having -882.8 kcal/mol docking score which is strong enough to block the Th2 associated IL-13; and many other drugs that cause asthma are also tabulated (Supplementary Table S1).

The interaction of the ligand with the IL-13 receptor is tabulated in Table 2 to overview the strong enough Van der walls and electrostatic interaction of epinephrine and guaifenesin drugs which reduce the downstream signaling of IL-13 and increase the inflammatory activities that induce the allergy.

Both epinephrine and guaifenesin are potential candidates to bind efficiently with IL-13 with their side chains and atomic interaction shows hydrogen bonds LEU17 (-4.9046) and THR25 (-0.0501) with epinephrine, and THR25 (3.8366) with guaifenesin (Table 2) which is important in blocking the IL-13 signaling as expected. Also, the geometry of epinephrine and guaifenesin drugs with IL-13 analyzing ligand-protein complex by LigPlot+ program and molecular interactions of bond distance with the protein and lead molecule was plotted by using PyMOL software (Figure 2). This confirms that epinephrine and guaifenesin are potentially able to interact with IL-13 to efficiently suppress its allergic pathways of signaling to release the mediators of inflammation by the mast cells.

Molecular dynamic simulation

The molecular dynamic simulation (MDS) is a computer program to analyze the physical movements of atoms and

Table 2 Atomic interactions of epinephrine and guaifenesin with IL-13.

Drug	vdW + Hbond + desolv Energy in kcal/mol	Electrostatic Energy in kcal/mol	Total Intermolec. Energy in kcal/mol	Interact. Surface	Other
Epinephrine	-4.96	+0.10	-4.86	469.961	Not applicable
Guaifenesin	-4.48	+0.15	-4.33	500.514	Not applicable

	Decomposed interaction energies in kcal/mo				
Drug	**Hydrogen bonds**	**Polar**	**Cation-pi**	**Hydrophobic**	**Other**
Epinephrine	LEU17 (-4.9046) THR25 (-0.0501)	GLN20 (-1.1191) GLN78 (-0.114)	PHE82 (-0.1084)	LEU79 (-0.4984) LEU113 (-0.0124)	GLU110 (-0.1891) THR22 (0.2064)
Guaifenesin	THR25 (3.8366)			LEU79 (-1.8536) LEU113 (-0.9205) LEU109 (-0.6618)	LEU17 (-0.9158) THR18 (-0.5055) GLN78 (-0.4224) GLU110 (0.149) THR22 (1.6516)

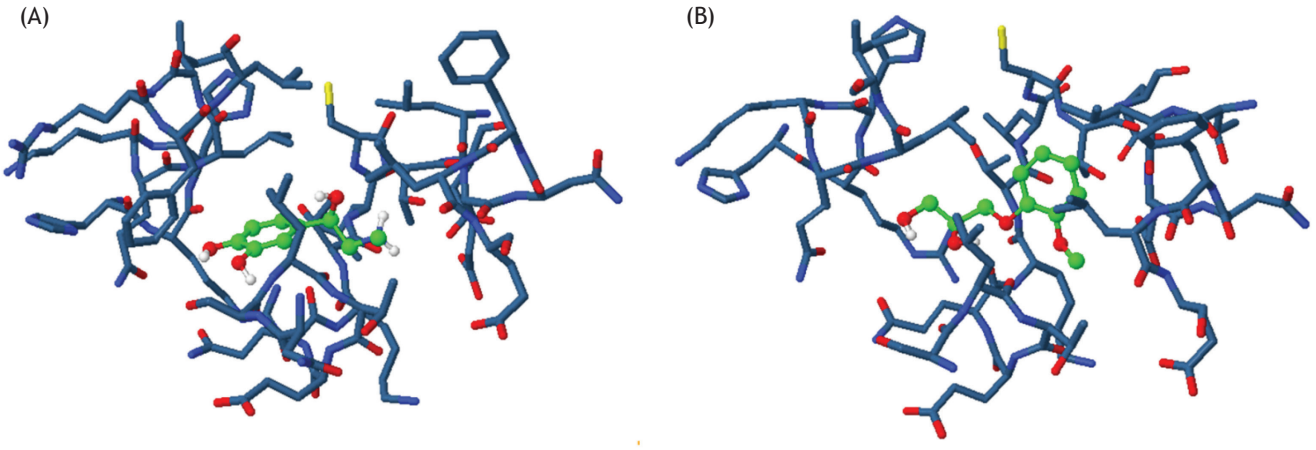

Figure 2 Interaction of epinephrine and guaifenesin with IL-13. The ligand-protein complex interaction with lead molecule is plotted using PyMOL software: (A) ligand-protein interaction of epinephrine; (B) guaifenesin geometry.

molecules. In the current investigation, screened candidates for the drug interaction suggest that they would have strong interaction with the target IL-13. Therefore, the ligand-protein of epinephrine-IL-13 (e-IL-13) and guaifenesin-IL-13 (g-IL-13) were processed for the MDS for 5 ns timescale in order to analyze the dynamic properties of these interactions through Int_mod to explore the binding mode. The results show protein structure, residue correlation, deformation energy, and energy decomposition of e-IL-13 (Figure 3) and g-IL-13 (Figure 4).

The e-IL-13 interacting pose is depicted in Figure 3A which has RMSD of 1.5–2.0 and 0.5–1.0 for IL-13 and epinephrine, respectively, confirms the average distance between the atom and superimposed structure at the equilibrium of the system (Figure 3B). The radius of Gyration (Rg) of 30.0 to 30.5 indicates the body axis rotation defined as the radial distance assuming the whole mass is concentrated. The molecular mechanics energies combined with the Poisson–Boltzmann or generalized Born and surface area continuum solvation (MM/PBSA and MM/GBSA) consists of electrostatic energy (ELE, –89.55), Van der Waals contribution (VDW, –23.21), total gas-phase energy (GAS, –112.76), non-polar and polar contribution to solvation (PBSOL/GBSOL, 95.69/96.91), entropy (TS, 15.82 unit is kcal/mol) and, etc. The binding energy (deltaPB/deltaGB, –1.25/–0.03) was calculated as PBTOT/GBTOT, –17.07/–15.82.

The heatmap of decomposition range from –1.5 to 1.0 of amino acid of protein residues red to blue, which pose with a ligand for binding as given in Figure 3C for the contribution of binding energies as compared to structure superimpose (Figure 3A). Also, the molecular trajectories of conformational differences in residues in PC1, PC2, and PC3, respectively; frame colored blue and black to red in order of time clustered in distance PC space (Figure 3D).

The interaction of g-IL-13 was further understood for the complex poses to best fit and shows guaifenesin with IL-13 (Figure 4A) and the RMSD of 1.5–2.0 for IL-13 and 0.5–1.5 for guaifenesin with 1.4 having best poses for protein superimpose with it as indicated (Figure 4B) and MM/PBSA and MM/GBSA having ELE, –5.72; VDW, –22.02; GAS, –27.74; PBSOL/GBSOL, 11.45/10.09; TS, 19.50 unit is kcal/mol and deltaPB/deltaGB, 3.21/1.85 was calculated as PBTOT/GBTOT of –16.29/17.65. The decomposition of amino acid residues shown in the heatmap (Figure 4C) from -1.5 to 1 red to blue of ligand residues binding poses as compared to complex interaction (Figure 4A) of structure superimpose. The principal component analysis (PCA) trajectory of g-IL-13 as depicted from blue and black to red changes upon time scale by crusting (Figure 4D). With these notes, the *in silico* identified potential candidates epinephrine and guaifenesin how they behave *in vivo* is unknown and the limitation of the study is that until the drugs are characterized experimentally for asthma, it is difficult to conclude the suitability of the drugs for the therapy.

Discussion

In the present study, to understand the role of available drugs that are used to treat asthma were analyzed to predict the potentiality of drug efficacy in terms of interaction with target IL-13 to alter its molecular pathway involved

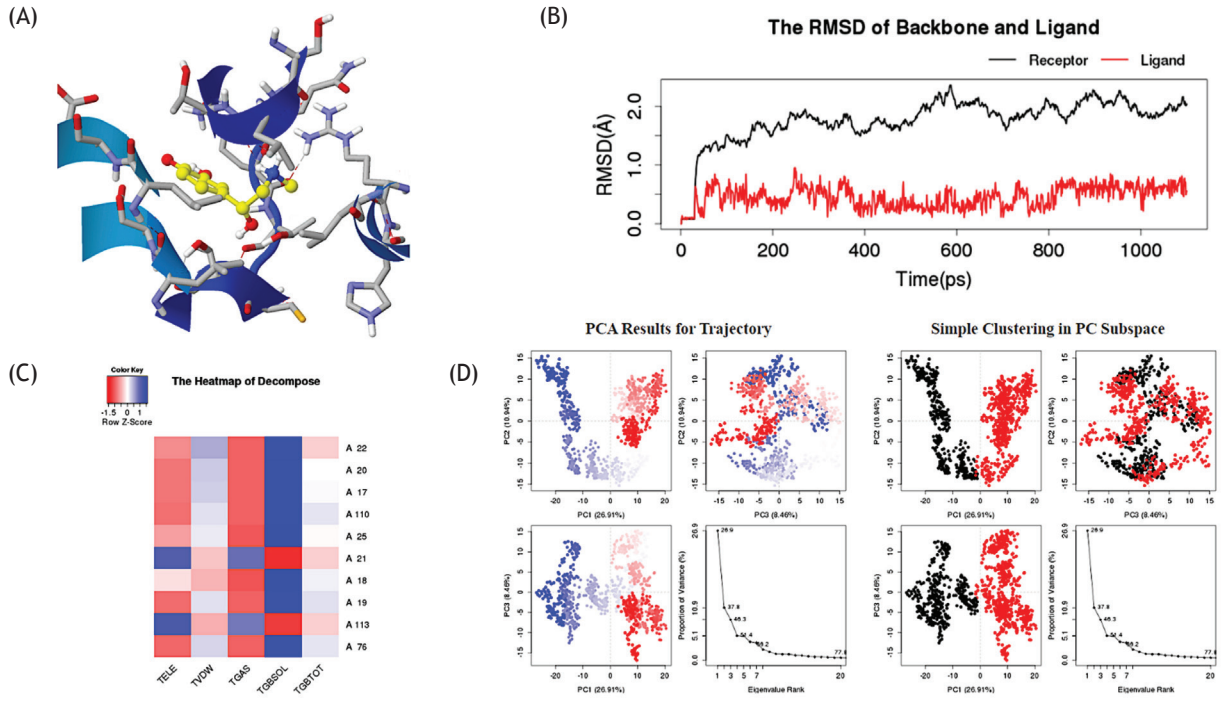

Figure 3 Molecular dynamics of e-IL-13. The residue correlation, deformation energy, and energy decomposition of e-IL-13 are depicted. Molecular interaction poses of ligand-protein complex (e-IL-13) (A), the distance of molecular interaction distance of e-IL-13 RMSD (B), heatmap of decomposition of amino acid of protein residues red to blue which pose with a ligand for binding that is depicted by decomposing of the residues (C), and PCA of the complex with time (D).

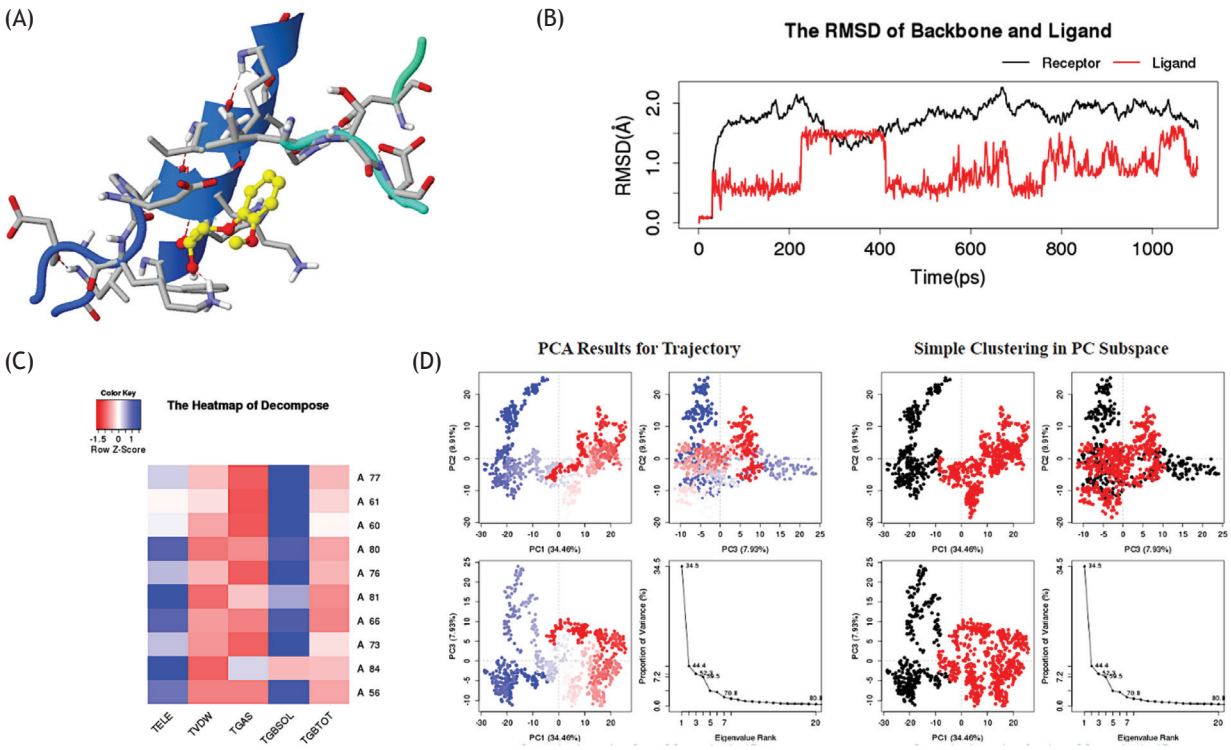

Figure 4 Molecular dynamics of g-IL-13. The residue correlation, deformation energy, and energy decomposition of e-IL-13 are depicted. Molecular interaction poses of ligand-protein complex (g-IL-13) (A); the distance of molecular interaction distance of g-IL-13 RMSD (B); heatmap of decomposition of amino acid of protein residues red to blue which pose with a ligand for binding that is depicted by decomposing of the residues (C), and PCA of the complex with time (D).

in inflammatory-induced asthma. In the current investigation after literature screening,[21] the potentially used candidates were selected for the drug suitable screening and selected the potential targeting binding epinephrine and guaifenesin with protein 3BPN compared to tralokinumab. This indicates that those two are potentially important for the development of next-generation drug candidates for asthma. The MDS and heatmap study interactions show strong supporting evidence of having a strong correlation to binding and inhibition. IL-13 is sufficient to cause mucus hypersecretion. A recent study shows β2AR-agonists contribute to asthma through the activation of β2ARs on epithelial cells.[31,32] In which, a strong association of Th2-associated IL-13 may cause asthma by inducing a chronic inflammatory state[33] and aberrant production of two cytokines, such as IL-4 and IL-13, that are involved in the association of pathogenesis and its allergic pathological disorders.[34] The treatments refer based on the act on the secondary consequences of asthma that is inflammation and bronchospasm and the etiological cause of diseases.[35] The release of mediators from mast cells, and also mast cell activation elevate the chronic diseases of asthma by inhibiting the agents involved in mast cell activation and mediator release.[36] Dupilumab Anti-IL4 receptor α mAb (blocks IL-4 and IL-13) is recently approved by the Food Drug Administration (FDA) to treat patients with severe asthma at the age of 12 years. The recommended dose is 600 mg (300 mg dose of two doses for every 2 weeks) as initial, followed by 300 mg for every 4 weeks[37] (https://www.regeneron.com/sites/default/files/Dupixent_FPI.pdf) to control the following inflammation pathways to reduce the consequential asthma issues that could be highlighted.

The molecular docking prediction of epinephrine and guaifenesin shows that these are the most suitable drug likely candidates for the target IL-13 as compared to tralokinumab. Nowadays, a variety of computational tools are available to study the conformational dynamics of ligand-driven protein structure to predict the superimposed ligand and proteins over time. The system stability was elevated by the root mean square deviation (RMSD), the radius of gyration (Rg), and a fraction of native contacts (Q). Among them, RMSD is used for the calculation of the atomic position to measure the distance between superimposed ligand and protein.[38] Through the interaction of molecules with the IL-13, more cross-interaction with other host molecules need to be investigated for these two candidates.[39] The MDS is a well-known theoretical technique used mainly to evaluate any predicted ligand-protein model and to visualize the interaction of protein interaction by Jsmol[40] and structure profile was retrieved from Str mod,[41] and the corresponding conformational system was discussed. In the present study, PCA is estimated to show the relationship of structures based on their residues.[42] The interaction and RMSD of IL-13 show an efficient target to interact during the chronic condition, which may have sufficient impact on asthma treatment in future was exploited.

The current study has limitations in selecting the drug candidates for the target. Not all the available *in silico* design can be a true candidate for the success of *in vivo* studies with targeted disease treatment. Currently, IL-13

has broad activity in asthma such as synthesis of IgM to IgE production from plasma cell,[43] synthesis of eotaxin in the lung by eosinophils,[44] high expression of adhesion molecules in eosinophils,[45] increased mucus production,[46] sensitizing airway smooth muscle,[47] and hyperresponsiveness.[48] The present investigation shows the potent nature of epinephrine and guaifenesin in molecular docking interactions and MDS studies. Additionally, a lot of clinical studies are necessary for the approval of a drug to treat any disease. In this case, *in-silico* prediction cannot work *in vivo* due to drug characteristics, interaction with immune system components, and many other unknown parameters that are the limitations of this study.

Currently, the world is affected by the Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) caused by Coronavirus disease 2019 (COVID-19). This spread has impacted the health especially respiratory virus COVID-19.[49] Asthma treatment is a topic of interest and risk factors associated with severe diseases or negative motivation are a great matter of debate. A study from the Albert Einstein College of Medicine/Montefiore Medical Center 4558 COVID-19 patients, 951 patients had asthma, 8 of whom were on biologics. The study does not find a clinical association between treatment biologicals and odds admitted to the emergency department.[50] This study states the use of omalizumab confers some protection against severe forms of COVID-19 or even helps to manage them, but[51] some other studies reveal that no evidence was concluded for the safe use for the control of COVID-19.[52] The control of the spread of COVID-19 is to improve the defence mechanism of the host system through anti-Th2 inflammation therapies that may be able to provide some beneficial effects in treated patients developing COVID-19.[53] The virus-infected cells are killed by CD4+ T cells. The virus-activated B lymphocytes can interact with CD4+ T cells. The first week of virus infection shows an increased amount of IgM followed by IgGs antibodies. If the adaptive immune system is insufficient, an innate immune response can be reinforced through cytokine storm which is responsible for multi-organ damage.[54] Current evidence suggest that severe asthma patients should maintain their medications during the COVID-19 pandemic, regardless of therapy.

Conclusions

The present study concludes that epinephrine and guaifenesin are the two potential candidates confirmed by molecular docking which bind the inflammatory biomarker IL-13 and molecular simulation dynamics. The epinephrine and guaifenesin are promising drugs to treat asthma after exploring all the drug characteristics to use this for asthma in the coming days and available to all, including economically poor people.

The unique aspect of immunotherapy for allergy by inducing long-term immunological tolerance through suitable drug candidates is required; therefore, future directions for immunotherapy should be concerned.

Notably, further clinical and basic studies are expected to explore the relationship between COVID-19 and asthma and/or other allergic diseases.

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Supplementary

Table S1 Drug interactions with 3BPN protein (IL13).

Drug	Est. Inhibition Constant, Ki in mM	vdW + Hbond + desolv Energy in kcal/mol	Electrostatic Energy in kcal/mol	Total Intermolec. Energy in kcal/mol	Interact. Surface
Albuterol		-3.32	-0.33	-3.65	473.576
Beclomethasone		+1.27e+03	-0.08	+1.27e+03	656.9
Benadryl		+3.01	+0.31	+3.33	611.388
Budesonide		+1.24e+03	+0.01	+1.24e+03	743.464
Ciclesonide		+1.55e+03	-0.00	+1.55e+03	830.475
Cyproheptadine		+51.36	+0.22	+51.58	622.639
Dexamethasone		+281.09	-0.00	+281.09	641.755
Epinephrine	1.52	-4.96	+0.10	-4.86	469.961
Flunisolide		+607.85	-0.12	+607.73	698.918
Guaifenesin	1.90	-4.48	+0.15	-4.33	500.514
Ipratropium		+24.23	+0.02	+24.26	636.384
Levalbuterol		+12.81	+0.28	+13.10	583.642
Loratadine		+59.16	-0.10	+59.06	715.533
Methylprednisolone		+277.47	+0.10	+277.57	633.216
Prednisone		+380.09	-0.06	+380.03	626.114
Promethazine		+16.34	+0.46	+16.81	603.214
Racepinephrine	5.55	-4.57	+0.07	-4.50	471.863
Salmeterol		+637.28	-0.11	+637.17	777.573
Singulair		+1.55e+03	-0.21	+1.55e+03	957.994
Terbutaline	8.76	-4.61	-0.06	-4.67	480.44
Theophylline	16.59	-2.35	-0.08	-2.43	353.16

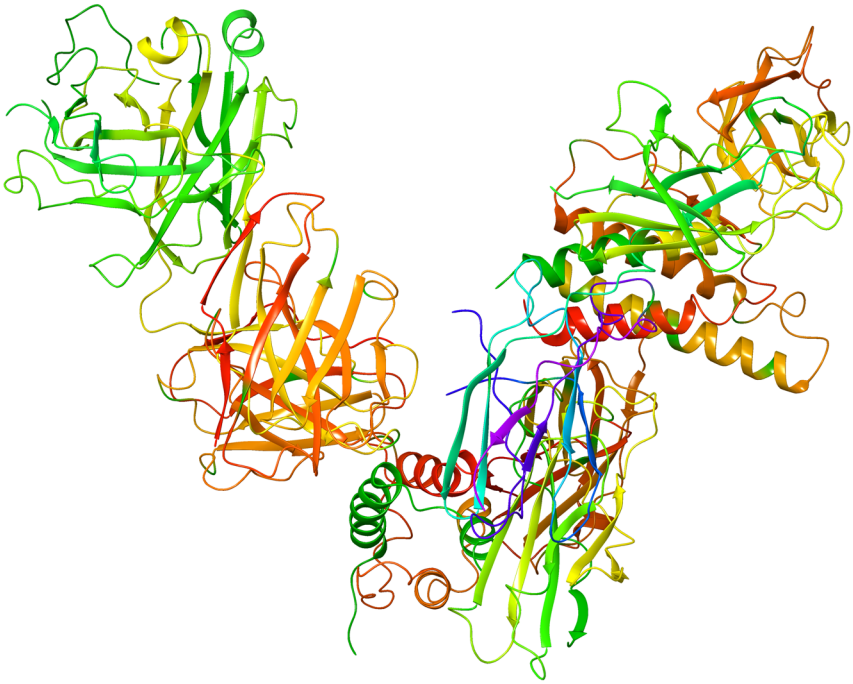

Figure S1 Tralokinumab (IL13, PDB- 5L6Y)