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# Identification of Therapeutic Agents Targeting Mitogen-Activated Protein Kinase 14 in the Treatment and Management of Asthma

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**Mitogen-activated protein Kinase 14 (MAPK14) plays a pivotal role in the pathophysiology of asthma, influencing inflammation, airway remodeling, and bronchial hyperresponsiveness, highlighting its significance as a potential therapeutic target in asthma management. This study aimed to identify the inhibitory compounds from a small library of small molecule drugs curated from the DrugBank. We conducted a comprehensive exploration of the protein structure, cavity detection, molecular docking, ADMET predictions, and functional assays pertinent to lead molecules and MAPK14. Through protein structure homology modeling, the MAPK14 model exhibited exemplary quality, supported by high GMQE (0.90) and QMEANDisCo (0.82 ± 0.05) scores. Cavity detection highlighted prominent features, guiding subsequent molecular docking studies. Notably, Cobicistat emerged as a potential inhibitor, displaying strong binding affinity (-8.6 kcal/mol) across multiple binding pockets on MAPK14. ADMET predictions underscored its drug-like properties, while cytotoxicity assays on normal lung cells revealed its benign nature. Further investigations elucidated Cobicistat's inhibitory effect on MAPK14 expression and activity, validating its therapeutic potential for asthma treatment. Enzyme inhibition assay provided mechanistic insights, indicating Cobicistat's ability to modulate MAPK14 activity, thus positioning it as a promising therapeutic candidate for targeting MAPK14-associated diseases such as asthma. This comprehensive study underscores Cobicistat's multifaceted potential in therapeutic interventions, substantiating its candidacy for further validation.**

**Keywords:** Asthma; Cobicistat; Inflammation; Mitogen-Activated Protein Kinase 14 (MAPK14); Therapeutic target.

Mitogen-activated protein kinase 14 (MAPK14), also known as p38á MAPK, is a crucial signaling molecule involved in various cellular processes, including inflammation, apoptosis, cell differentiation, and cell proliferation[1]. In the context of asthma, MAPK14 plays a significant role in the pathogenesis of this chronic inflammatory disease[2]. Asthma is a heterogeneous respiratory disorder characterized by airway inflammation, bronchial hyperresponsiveness, and reversible airflow obstruction[3]. It affects people of all ages and can significantly impact their quality of life[4]. While the exact etiology of asthma remains incompletely understood, it is widely accepted that the interplay of genetic predisposition and environmental factors contributes to its development and progression[1].

Inflammation is a hallmark feature of asthma, and MAPK14 has emerged as a central

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mediator in orchestrating inflammatory responses within the airways[5]. Upon activation by various extracellular stimuli, including pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-á), interleukin-1â (IL-1â), and environmental triggers like allergens and pollutants, MAPK14 becomes phosphorylated and subsequently activates downstream effector molecules[6].

One of the primary functions of MAPK14 in asthma pathogenesis is the regulation of inflammatory cytokine production[2]. Through its ability to phosphorylate and activate transcription factors such as nuclear factor-kappa B (NF-êB) and activator protein-1 (AP-1), MAPK14 promotes the expression of pro-inflammatory mediators like interleukin-6 (IL-6), interleukin-8 (IL-8), and TNF-á[2]. These cytokines recruit and activate inflammatory cells, including neutrophils, eosinophils, and T lymphocytes, amplifying the inflammatory cascade within the airways[7]. Furthermore, MAPK14 activation promotes the synthesis of other key mediators involved in asthma pathophysiology, such as leukotrienes and prostaglandins[8,9]. These lipid mediators contribute to bronchoconstriction, airway hyperresponsiveness, and mucus hypersecretion, further exacerbating respiratory symptoms in asthma patients[9].

In addition to its role in inflammation, MAPK14 also regulates airway smooth muscle contraction, a process central to the pathogenesis of asthma[10]. Activated MAPK14 phosphorylates and activates myosin light chain kinase (MLCK), leading to myosin light chain (MLC) phosphorylation and subsequent smooth muscle contraction[11]. This process contributes to bronchoconstriction and airway narrowing, characteristic features of asthma exacerbations. Moreover, MAPK14 activation promotes airway remodeling, another hallmark of chronic asthma[12]. Persistent inflammation and repeated injury to the airway epithelium lead to structural changes, including subepithelial fibrosis, increased smooth muscle mass, and mucus gland hyperplasia[13]. MAPK14-mediated signaling pathways contribute to these remodeling processes by promoting fibroblast proliferation, collagen deposition, and extracellular matrix remodelling[14].

The role of MAPK14 in modulating the immune response extends beyond its effects on inflammatory cells and cytokine production. Studies have demonstrated that MAPK14 activation influences T helper cell differentiation, skewing the balance towards a Th2-dominated immune response characteristic of allergic asthma[15]. Th2 cells produce cytokines such as interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13), which promote eosinophil recruitment, immunoglobulin E (IgE) production, and mucus secretion, contributing to allergic inflammation and airway hyperreactivity[16].

Furthermore, MAPK14 activation in airway epithelial cells contributes to barrier dysfunction and impaired epithelial repair mechanisms, exacerbating airway inflammation and susceptibility to environmental insults[17]. Disruption of the airway epithelial barrier allows for increased penetration of allergens, pathogens, and environmental pollutants, further amplifying the inflammatory response in asthma[18].

The significance of MAPK14 in asthma pathogenesis is further underscored by preclinical and clinical studies demonstrating the efficacy of MAPK14 inhibitors in attenuating airway inflammation, bronchoconstriction, and airway hyperresponsiveness[19]. Pharmacological inhibition of MAPK14 has been shown to reduce inflammatory cytokine production, airway smooth muscle contraction, mucus hypersecretion, and airway remodeling in experimental models of asthma[20]. However, despite the promising therapeutic potential of MAPK14 inhibitors, challenges remain in translating these findings into clinical practice. The development of selective and potent MAPK14 inhibitors with favorable pharmacokinetic properties and minimal off-target effects remains a priority for drug discovery efforts in asthma and other inflammatory diseases. Overall, Mitogen-activated protein kinase 14 (MAPK14) plays a pivotal role in the pathogenesis of asthma by regulating inflammatory responses, airway smooth muscle contraction, airway remodeling, and immune dysregulation. Targeting MAPK14 signaling pathways represents a promising therapeutic approach for the treatment of asthma and other inflammatory respiratory diseases.

The rationale for conducting further research in this area stems from the significant impact that small molecule inhibitors targeting specific molecules and pathways have been demonstrated in disease therapeutics. As highlighted in the provided text, these inhibitors play a crucial

role in controlling disease pathology by selectively targeting key molecules involved in disease progression, thus offering a promising avenue for effective treatment. The focus on small molecule inhibitors that inhibit the activity of MAPK14 presents a persuasive rationale for investigation in asthma. This study aims to identify penitential inhibitor small molecules of MAPK14 using in-silico and in-vitro methods for the therapeutics of asthma.

## MATERIAL AND METHODS

**Protein structure homology modeling and preparation of MAPK14 receptor**

Homology modeling, a widely used technique, predicts a protein's 3D structure based on homologous proteins with known structures. The popular web tool SWISS-MODEL (https://swissmodel.expasy.org/) facilitates this process and was utilized to predict the high-resolution MAPK14 model[21,22,23]. Starting with the FASTA sequence of MAPK14 retrieved from PubMed, template sequences from the Protein Data Bank were identified to find a suitable homologous structure (MAPK14: PDB ID - 6y4x) based on sequence similarity, quality, and relevance. Structural analysis was then performed using PyMOL and Chimera before preparing for molecular docking by refining MAPK14's structure through standard protocol practices including addressing steric clashes, missing atoms, and assigning charges[24,25]. The final step involved saving the refined structures in PDBQT format for docking compatibility.

**Selection and preparation of ligands for molecular docking with target protein**

1936 small molecule drugs from DrugBank (https://go.drugbank.com/) approved by the US were sourced. Their three-dimensional (3D) structures in SDF format were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Each structure underwent rigorous validation to correct bond lengths, angles, and torsional angles. After validation, the structures were converted to PDBQT format for compatibility with docking software, ensuring accurate representation during simulations.

**Structure-based cavity detection on target protein**

Identifying potential binding sites on MAPK14 involves cavity detection using CB-Dock2 (http://cadd.labshare.cn/cb-dock2/), which automates the process. By utilizing query ligands, CB-Dock2 calculates and customizes docking box size through AutoDock Vina. The initiation of process begins with uploading the MAPK14 PDB file while specifying parameters like probe radius and cavity size to optimize detection accuracy. This setup ensures precise identification of potential binding pockets on MAPK14[26].

**Screening and molecular docking of the lead molecule with the target protein**

A thorough screening of 1936 small molecule drugs was conducted to evaluate their binding affinity against MAPK14, a key target in asthma therapeutics. The top hit, chosen based on fitness score and binding affinity, underwent molecular docking using CB-Dock2, well-known for its blind docking capability[26]. CB-Dock2 combines cavity detection, docking processes, and template fitting to accurately predict the binding site. This approach sheds light on potential interactions at the molecular level. To verify this, the ligand-protein complex underwent redocking using SeamDock, which enhances the reliability of assessing binding affinity[27]. SeamDock adds an extra layer of validation to the docking results obtained from CB-Dock2.

**Assessment of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of lead molecule**

Assessing the ADMET properties is crucial for understanding pharmacokinetics and toxicity. We used the pkCSM online tool (https://biosig.unimelb.edu.au/pkcsm/.) to evaluate these properties for the lead molecule. pkCSM predicts pharmacokinetics and toxicity comprehensively by offering an analysis of ADMET parameters based on chemical structure in SMILES format. This assessment sheds light on how the molecule may be absorbed, distributed, metabolized, and excreted and its potential toxicity profile; thus aiding in drug development decisions.

**Prediction of Activity Spectra for lead ligand molecule using SwissTargetPrediction tool**

Chemoinformatics and drug discovery rely on predicting ligand activity spectra to identify targets and understand pharmacological effects. The study utilized SwissTargetPrediction (http://www.swisstargetprediction.ch/) to explore the

lead molecule's biological activity spectrum. This tool predicts potential targets for small molecules, helping with protein interaction identification and understanding pharmacological profiles. By analyzing compound chemical structures, it identifies targets based on structural similarities and known interaction patterns. Inputting ligand SMILES notation into SwissTargetPrediction reveals potential biological targets and activity spectrum, deepening our understanding of molecular mechanisms essential for drug discovery and informing experimental studies for therapeutic exploration.

**Test compound and cell lines**

Cobicistat was dissolved in phosphate-buffered saline to create a stock solution with a concentration of 1000 nM/mL. The cell lines used in this study, HEK-293 (human embryonic kidney) and BEAS-2B (normal human bronchial epithelial cell line), were obtained from the ATCC. HEK-293 cells were cultured in Dulbecco's Modified Eagle Medium, while BEAS-2B cells were cultured in Airway Epithelial Cell Basal Medium supplemented with Bronchial Epithelial Cell Growth regulators. Both culture media were supplemented with 10% fetal bovine serum and 1% antibiotics to support cell growth and maintain sterility.

**Cell culture and cytotoxicity assay**

HEK-293 cells were seeded at a density of 5,000 cells per well in a 96-well microplate and incubated for 24 hours in cell culture incubator at 37 °C and 5% $CO_2$. They were then exposed to varying concentrations of Cobicistat (0-1000 nM) for 72 hours to evaluate the dose-dependent effects on cell viability. Subsequently, the medium was replaced with an MTT reagent (0.5 mg/mL) and incubated for 4 hours to form formazan crystals. The formed formazan was solubilized using DMSO, and the absorbance at 570 nm was measured. Net absorbance values were determined by subtracting blank readings. Cell viability was assessed by comparing the net absorbance of treated wells to that of untreated control wells and multiplying by 100. For examining time-dependent effects, HEK-293 cells were exposed to the mean $IC_{50}$ concentration of Cobicistat (293.11 nm) for durations of 24, 48, and 72 hours.

**mRNA expression of MAPK14 in Cobicistat treated BEAS-2B cells**

To investigate the effect of Cobicistat on MAPK14 gene expression, BEAS-2B cells were treated with a concentration of 293.11 nM, which is the $IC_{50}$, for 48 hours. Subsequently, RNA extraction was carried out using TRIzol™ Reagent following standard protocol[28]. The extracted RNA was then used for cDNA synthesis and qRT-PCR analysis using SYBR™ Green Master Mix. The expression level of MAPK14 was assessed and normalized to that of the housekeeping gene GAPDH, followed by a calculation of the relative fold change in MAPK14 expression. The primer sequences utilized were as follows: MAPK14: forward 5'-GAGCGTTACCAGAACCTGTCTC-3' and reverse 5'-AGTAACCGCAGTTCTCTGTAGGT-3', GAPDH: forward 5'-GTCTCCTCTGACTTCAACAGCG-3' and reverse 5'-ACCACCCTGTTGCTGTAGCCAA 3'.

Further, cellular protein MAPK14 activity in BEAS-2B cells was quantified using the Human MAPK14 ELISA Kit from Abcam following the protocol as described by the manufacturer.

**Enzyme inhibition assay**

A series of experiments investigated the impact of Cobicistat on MAPK14 kinase activity. Fixed concentrations of Cobicistat (0 to 1000 nM) were added to 2 µM of MAPK14 in a 96-well plate and incubated for an hour at 25°C. Following this, a reaction mixture containing 100 µM ATP and 10 mM MgCl2 was introduced and further incubated for another 30 minutes. The reaction was then terminated with BIOMOL® reagent, which formed a green-colored complex monitored at 620 nm. Malachite green reagent facilitated the detection of inorganic phosphate released during ATP hydrolysis, enabling the reflection of MAPK14 kinase activity based on color intensity - directly indicating enzyme activity levels. Additionally, native MAPK14's activity without Cobicistat was set as the baseline at 100%.

## RESULTS

**Protein structure homology modeling and preparation of MAPK14**

Fig. 1a illustrates the high-resolution structure of the MAPK14 model from Swiss-

**Table 1.** Detailed information on the size, volume, or other relevant characteristics of the structure-based cavities (C1-C5) detected on MAPK14

| CurPocket ID | Cavity volume (Å$^3$) | Center (x, y, z) | Cavity size (x, y, z) |
|---|---|---|---|
| Cavity 1 | 3650 | -2, -8, -2 | 30, 19, 29 |
| Cavity 2 | 3151 | 1, 15, -11 | 30, 23, 30 |
| Cavity 3 | 385 | -8, 9, 19 | 14, 8, 7 |
| Cavity 4 | 353 | 5, 5, -3 | 9, 9, 13 |
| Cavity 5 | 323 | -1, 15, 10 | 15, 8, 9 |

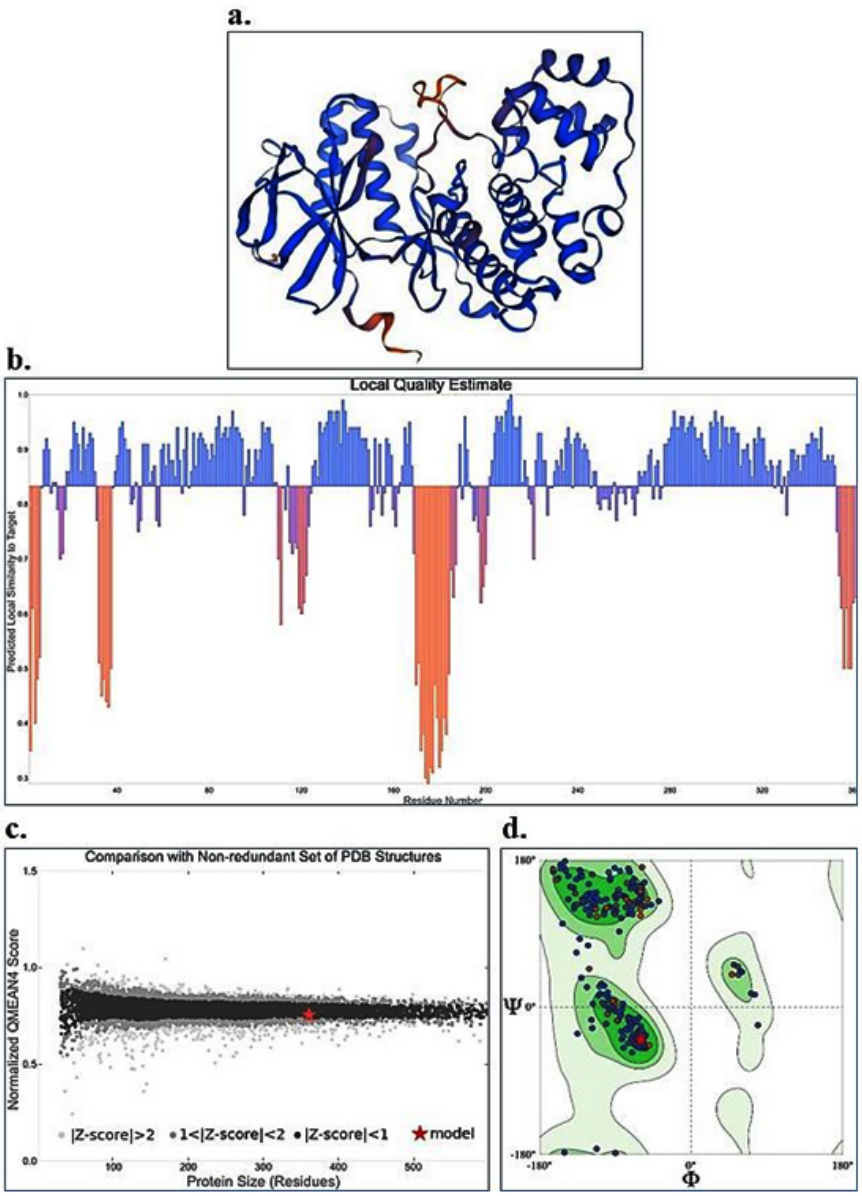


**Fig. 1.** (a) 3D structure of the MAPK14; (b) Graphical representation of the QMEANDisCo local quality estimate; (c) Non-redundant set of PDB structures and; (d) Ramachandran Plot.

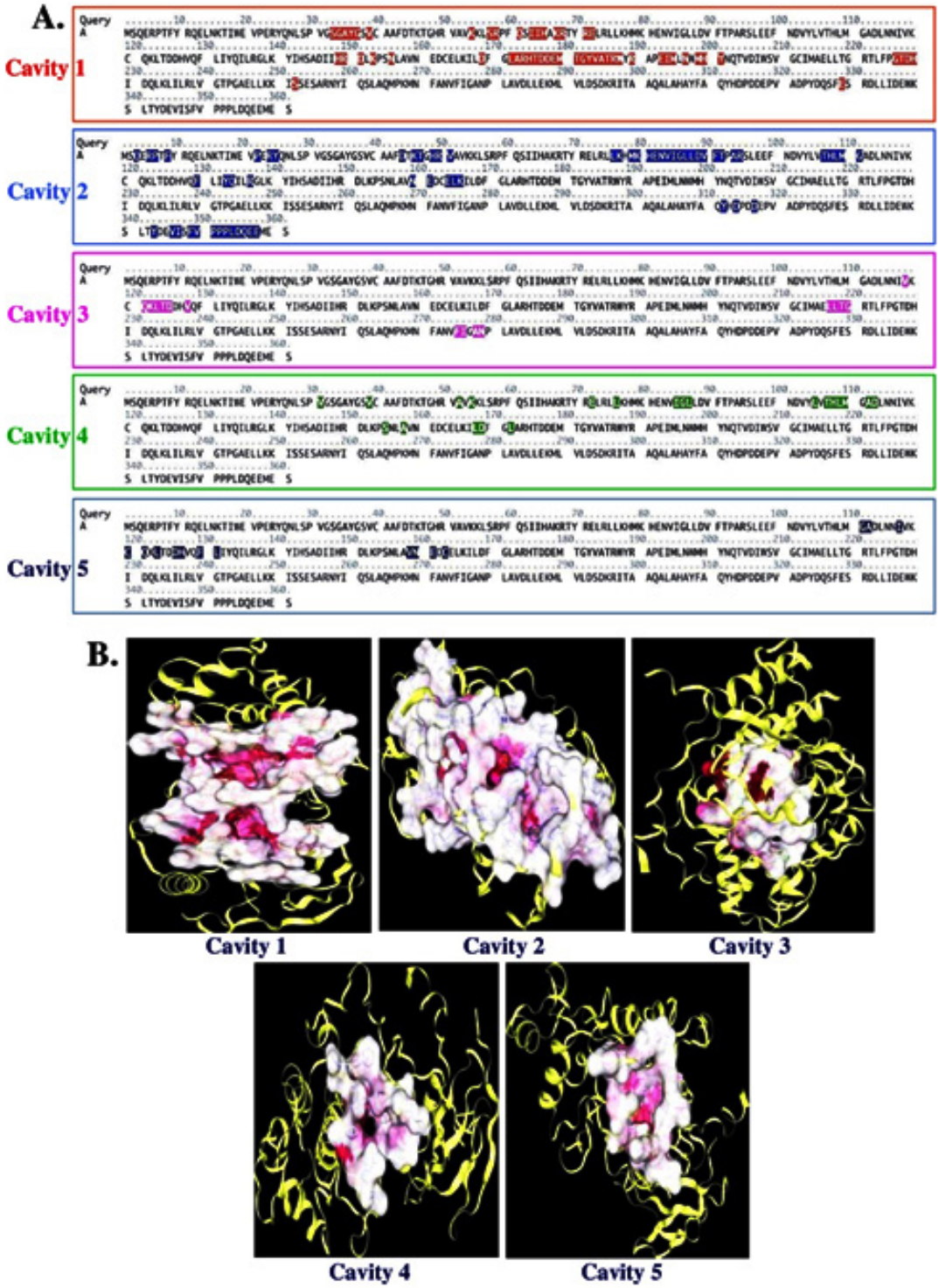


**Fig. 2.** (A) Sequence of cavities C1, C2, C3, C4, and C5 detected on MAPK14. (B) Structural visualization of all identified cavities (C1, C2, C3, C4, and C5) within MAPK14.

MODEL, boasting a remarkable sequence identity and 100% coverage, signifying precise alignment with the target sequence. Quality assessment revealed excellence, with a GMQE score of 0.90 and a QMEANDisCo Global score of 0.82 ± 0.05. These scores, ranging from 0 to 1, denote overall model quality, with higher values indicating superior expected quality. GMQE considers alignment coverage, while QMEANDisCo evaluates quality independently. Fig. 1b presents

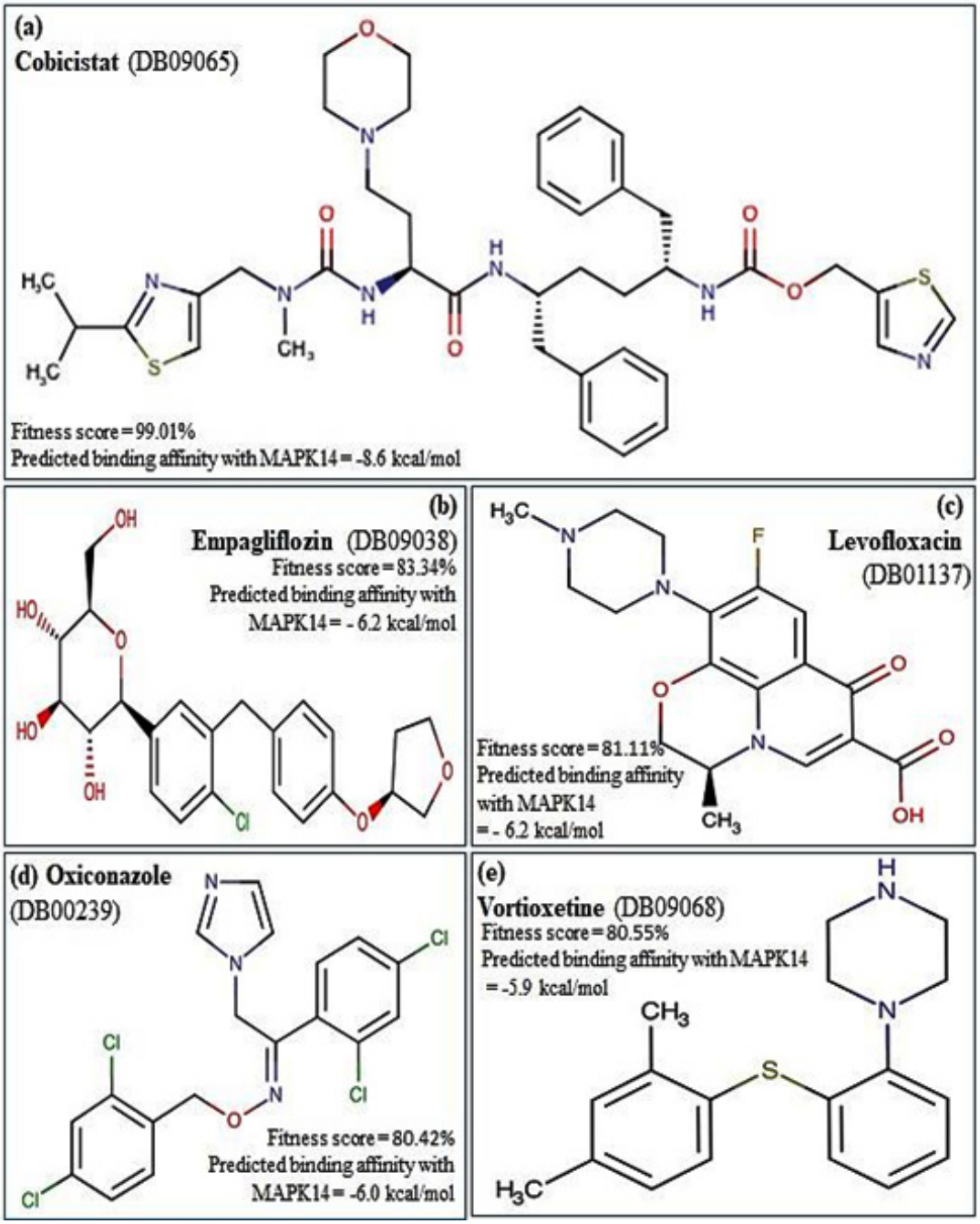


**Fig. 3.** Detailed information on binding affinity of top 5 small molecule drugs. Chemical structures of these compounds were obtained from Drug Bank (https://go.drugbank.com/).https://go.drugbank.com/

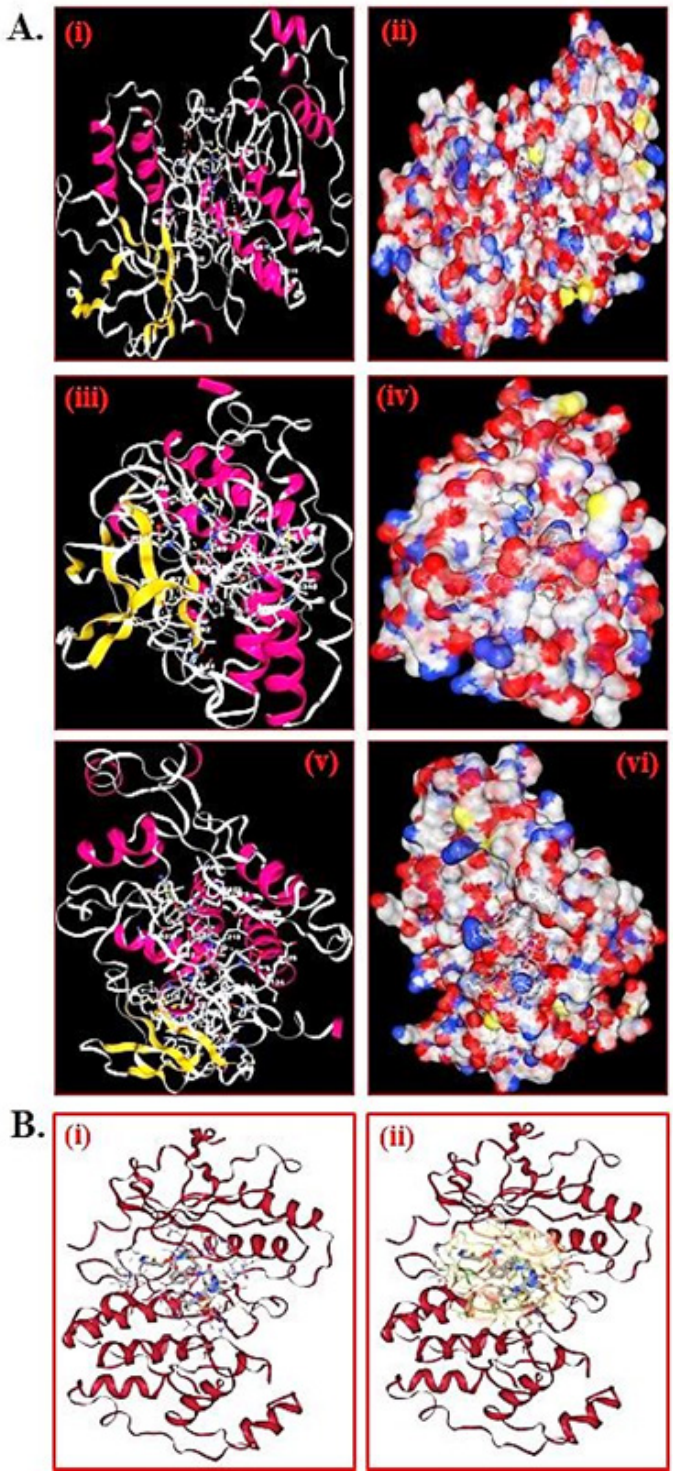


**Fig. 4.** (A) Visual representation of the Cobicistat: MAPK14 molecular docking complexes formed at C1, C2, and C3 cavities. (i, iii, and v) cartoon presentation of the complex; (ii, iv, and vi). Surface view of the complex. (B) Redocking validation of Cobicistat-MAPk14 complex. (i) Without representation of pocket and; (ii) with representation of pocket.

a graphical representation of the QMEANDisCo local quality estimate, providing insights into the local quality of the model. Additionally, Fig. 1c compares with a non-redundant set of PDB structures, and Fig. 1d depicts the Ramachandran Plot, showing energetically favored regions for backbone dihedral angles of amino acid residues, crucial for assessing structural integrity and reliability of the MAPK14 model.

**Cavity detection on MAPK14**

Table 1 outlines findings on cavities within MAPK14, detailing the number and characteristics via CB-Dock2 analysis. Notably, five prominent cavities (C1 to C5) were identified, with Table 2 providing comprehensive data on size, volume, and attributes. Cavity 2 (C2) stood out for its maximum size, warranting further investigation through molecular docking experiments. Sequenced-based depiction of cavities (C1-C5) is in Fig. 2A, while Fig. 2B visually represents all structure-based cavities within MAPK14. These analyses yield insights into MAPK14's structural traits, crucial for subsequent molecular docking studies, especially regarding interactions with ligands like Cobicistat.

**Molecular docking revealed Cobicistat as a potential inhibitor of MAPK14**

After screening 1936 US-approved small molecule drugs against MAPK14, Cobicistat emerged as the most promising candidate with a remarkable fitness score of 99.01% and the highest predicted binding affinity. Figure 3a-e presents detailed information on the fitness scores and binding affinities of the top 5 small molecule drugs. Following the identification of Cobicistat as the top hit, it was prioritized for molecular docking against the target protein MAPK14. The results of molecular docking show Cobicistat specifically at the C1, C2, and C3 binding pockets on MAPK14, visually represented in Fig. 4. Fig. 4A (i) and 4A (ii) show the molecular docking at C1, where Cobicistat interacts with multiple amino acid residues of MAPK14, including GLY32,

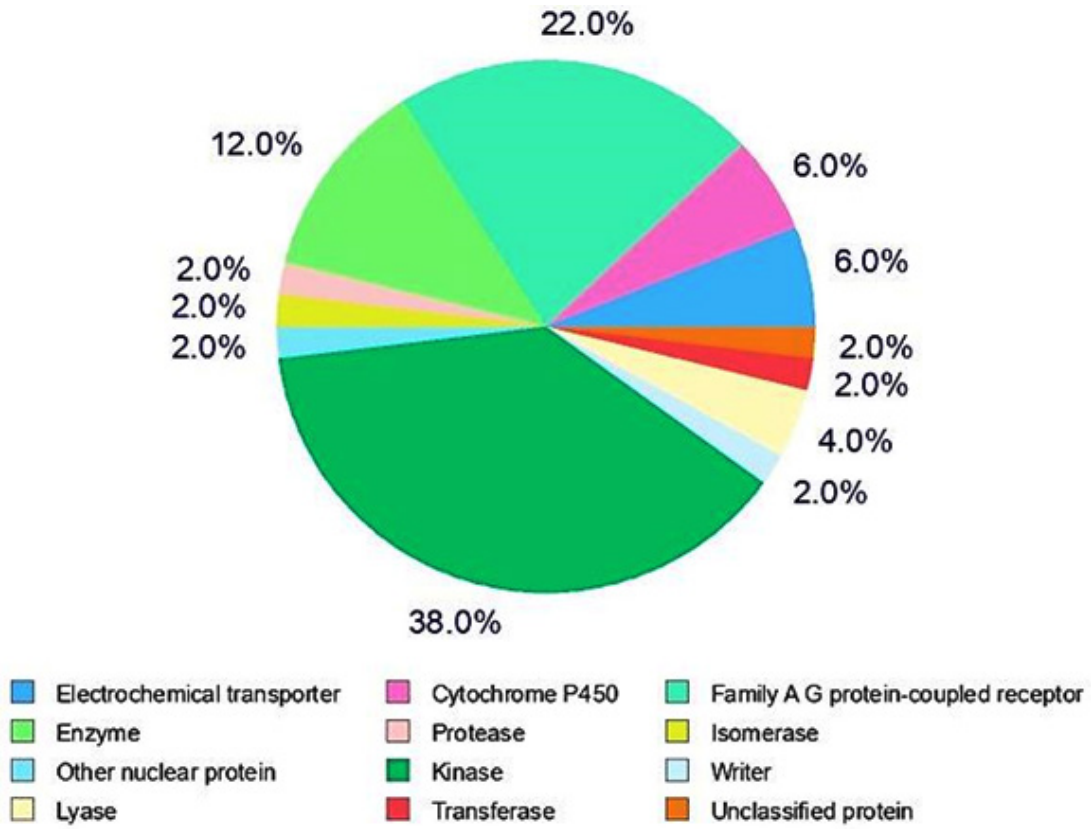


**Fig. 5.** Swiss Target Prediction analysis showing diverse biological properties linked to Cobicistat

**Table 2.** In silico prediction of ADME & Tox properties for Cobicistat (CBST)

| | Absorption | | Distribution | | Metabolism | Properties | | | | | Excretion | Toxicity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Models | Intestinal absorption (human) | VDss (human) | BBB permeability | CNS permeability | CYP Substrate 2D6 | Inhibitor 3A4 | 1A2 | 2C19 | 2C9 | 2D6 | 3A4 | Total clearance | AMES toxicity |
| Unity Predicted values | Numeric (% absorbed) | Numeric (log L/kg) | Numeric (Log BB) | Numeric (Log PS) | Categorical (yes/no) | | | | | | | Numeric (log ml/min/kg) | Categorical (yes/no) |
| CBST | 76.503 | 0.668 | -1.761 | -3.454 | NO | Yes | No | No | No | NO | Yes | 0.845 | NO |

SER33, GLY34, ALA35, TYR36, GLY37, VAL39, LYS54, ARG68, ASP113, ASN115, LYS119, ASP151, LYS153, SER155, ASN156, LEU168, ASP169, LEU172, ALA173, ASP177, GLU179, MET180, VAL184, and ALA185. Fig. 4A (iii) and 4A (iv) show the molecular docking at C2, where Cobicistat formed interaction with MET2, GLN4, GLU5, ARG6, PRO7, PHE9, PRO22, ARG24, LYS46, ASP89, VAL90, PHE91, THR92, ALA94, VAL346, ILE347, SER348, PHE349, VAL350, and PRO351 amino acid residues of MAPK14. Fig. 4A (v) and 4A (vi) show the molecular docking at C3, where Cobicistat interacts with multiple amino acid residues of MAPK14, including VAL118, LYS119, CYS120, GLN121, LYS122, LEU123, THR124, ASP125, LEU217, LEU218, THR219, GLY220, ARG221, LEU223, VAL274, PHE275, ILE276, GLY277, and ALA278. In this docking analysis, we generated affinity scores and docked poses which revealed a notable binding affinity score of -8.6 kcal/mol between Cobicistat and MAPK14. Fig. 4 (i), 4A (iii), and 4 (v) provide a cartoon presentation of the MAPK14: Cobicistat complex for C1, C2, and C3 respectively to offer insights into the spatial arrangement of the molecules. Meanwhile, Fig. 4A (ii), 4 (iv), and 4 (vi) represent a surface view that offers additional perspectives on their interaction for C1, C2, and C3 respectively. All these interactions highlight the comprehensive nature of the binding between Cobicistat and MAPK14, strongly suggesting a strong and multi-faceted affinity between the two molecules. Furthermore, to confirm the binding affinity of the MAPK14-Cobicistat complex, redocking was performed using SeamDock. The results of the redocking poses are shown in Fig. 4B (i) and 4B (ii). In these poses, Cobicistat consistently interacted with the binding site of MAPK14, demonstrating a binding affinity of -8.3 kcal/mol. This consistent affinity supports Cobicistat's potential as a promising therapeutic candidate for targeting MAPK14 in asthma.

**ADMET predictions and SwissTarget Prediction**

Cobicistat's drug-like properties were validated through ADMET predictions and SwissTargetPrediction. The ADMET predictions, summarized in Table 2, indicate compliance with key pharmacokinetic criteria. Furthermore, the SwissTargetPrediction analysis confirms and expands on the biological properties of Cobicistat

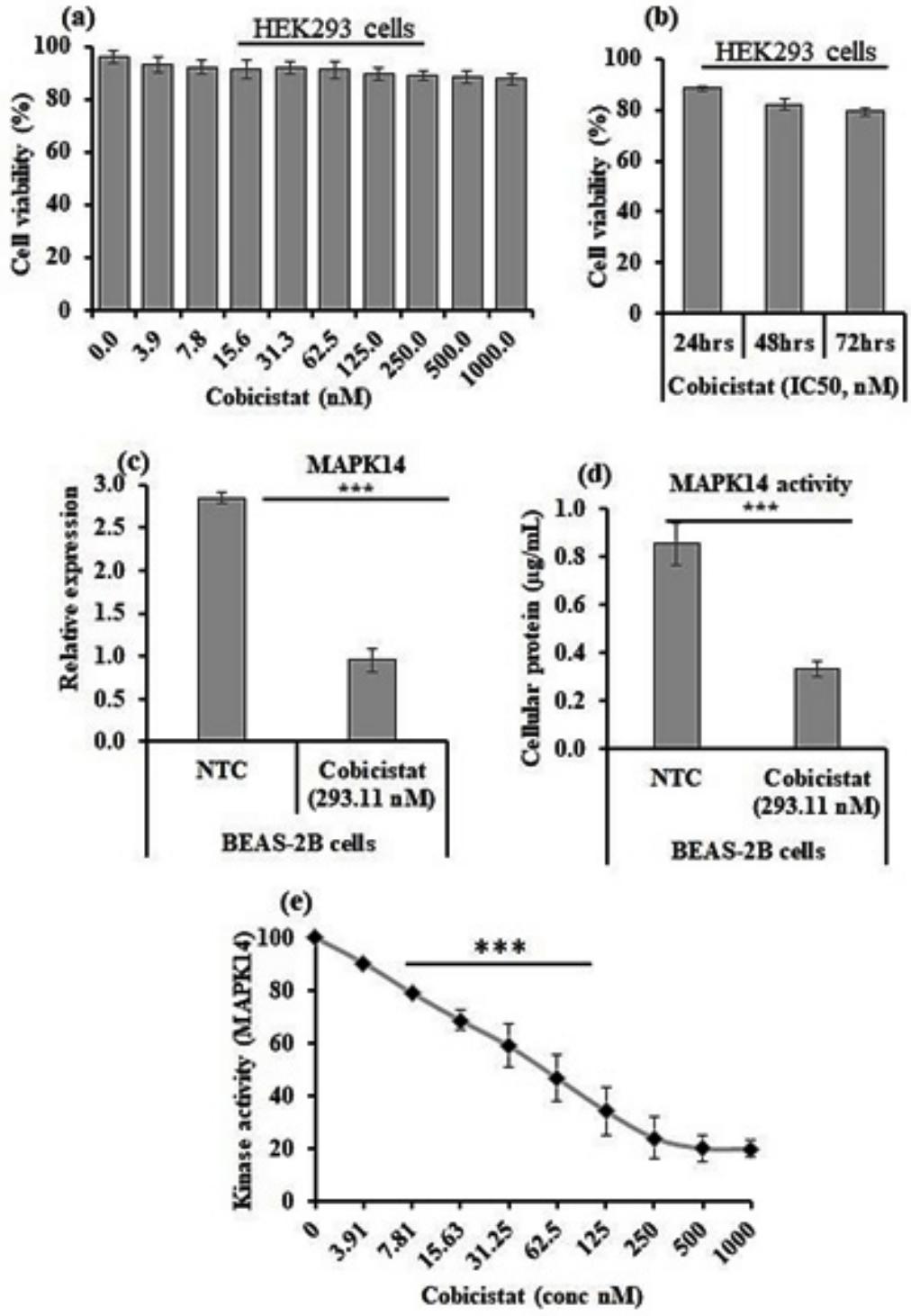


**Fig. 6.** (a) concentration-dependent cell toxicity; (b) time-dependent cell toxicity. Effect of $IC_{50}$ dose of Cobicistat on the (c) mRNA expression and; (d) activity of MAPK14 in BEAS-2B cells. *** $p<0.001$. (e) Enzyme inhibition assay showing inhibition of MAPK14 by Cobicistat. *** $p<0.001$

by suggesting promise in targeting MAPK14 inhibition for asthma treatment as detailed in Fig. 5.

**Cytotoxic effect of Cobicistat on HEK-293 normal lung cells**

Fig. 6a depicts the concentration-dependent effect of Cobicistat on HEK-293 cell viability. Analysis revealed no significant concentration-dependent impact on cell viability, suggesting Cobicistat lacks notable effects within the tested concentration range on these cells. HEK-293 cells were treated in concentration concentration-dependent manner for 72 hrs. Cells were harvested for MTT assay and absorbance was recorded to determine the respective $IC_{50}$ concentrations. The $IC_{50}$ value for Cobicistat was found to be 293.11 nM for HEK-293 cells.

Fig. 6b depicts the investigation into Cobicistat's effects on HEK-293 cell viability over varying periods. HEK-293 cells were exposed to increasing durations of Cobicistat (293.11 nM) - 24, 48, and 72 hours. Analysis revealed consistent viability across all durations, indicating Cobicistat had no adverse impact on non-cancerous HEK-293 cells. This suggests its benign nature over the specified periods, as it did not compromise cell viability.

**Expression and activity of MAPK14 in Cobicistat-treated BEAS-2B cells**

Fig. 6c and 6d provide a visual representation of the impact of Cobicistat on the mRNA expression and activity of MAPK14 in BEAS-2B cells, respectively. The experimental setup involved treating cells with Cobicistat at its specific $IC_{50}$ concentration. Upon analysis, it was observed that treatment with Cobicistat led to a significant decrease ($p < 0.001$) in the mRNA expression and activity of MAPK14 in treated cells compared to NTC. This significant reduction in MAPK14 mRNA expression and activity underscores the inhibitory effect of Cobicistat on MAPK14, highlighting its potential as a therapeutic agent for targeting MAPK14 in asthma treatment.

**Enzyme inhibition assay showed inhibition of MAPK14 by Cobicistat**

The kinase assay showed a notable ($p<0.001$) decline in MAPK14 activity with rising Cobicistat levels (Fig. 6e). This hints at Cobicistat's potential to inhibit MAPK14 kinase activity. The dose-dependent response underscores its probable modulation of MAPK14, via direct interaction or downstream regulatory pathways.

## DISCUSSION

The comprehensive investigation presented in this study sheds light on the structural attributes of MAPK14 and its potential inhibition by Cobicistat, offering promising insights into its therapeutic application in asthma treatment and management. Modeling the 3D structure of a target protein is crucial for identifying therapeutic agents in disease management[23]. Understanding protein structures aids in designing molecules that interact specifically with the target, enhancing drug efficacy and minimizing side effects[29]. Through computational modeling, scientists predict how potential drugs bind to the protein, guiding drug development. This approach accelerates drug discovery by screening virtual compound libraries, saving time and resources compared to traditional methods[24]. Additionally, structure-based drug design allows for the optimization of lead compounds, fine-tuning their properties for improved therapeutic outcomes[25]. Ultimately, this methodology revolutionizes the treatment and management of diseases, offering tailored solutions with higher precision. In the current study, we aimed to target to inhibit MAPK14 for the therapeutics and management of asthma. In this study, the high-resolution model of MAPK14 generated through protein structure homology modeling exhibited remarkable sequence identity and coverage, indicative of precise alignment with the target sequence. The quality assessment metrics, including GMQE and QMEANDisCo scores, underscored the excellence of the model, highlighting its reliability and suitability for subsequent analyses.

The modeled structure was further subjected to predict the binding cavities on MAPK14. Identifying binding cavities on target proteins is pivotal for structure-based identification of therapeutic agents in disease management[30]. These cavities are regions where small molecules can bind, influencing protein function and disease progression. Through computational methods investigators pinpoint potential binding sites, facilitating drug discovery[24]. By understanding the spatial arrangement and chemical properties of

these cavities, researchers design molecules tailored to fit and interact with high affinity, increasing drug specificity and efficacy[25]. This approach streamlines the search for lead compounds, accelerating the development of novel therapeutics with enhanced therapeutic potential for treating and managing a wide array of diseases. The identification of five prominent cavities within MAPK14, along with detailed characterization of their size, volume, and attributes, provided crucial insights into the structural traits of the protein. Particularly, the prominence of Cavity 2 (C2) warranted further investigation through molecular docking experiments, paving the way for targeted ligand interaction studies.

Molecular docking plays a pivotal role in the structure-based identification of therapeutic agents for disease treatment and management[31]. It involves the computational simulation of how small molecules, or ligands, interact with the binding site of a target protein[25]. By predicting the most favorable orientation and conformation of the ligand within the binding pocket, docking enables the screening of vast libraries of compounds to identify potential drug candidates[32]. This process guides medicinal chemists in optimizing lead compounds, enhancing their binding affinity and specificity to the target protein[25]. Ultimately, molecular docking expedites drug discovery, leading to the development of effective treatments tailored to combat various diseases[31]. The molecular docking studies revealed Cobicistat as a potential inhibitor of MAPK14, with a remarkable fitness score and high predicted binding affinity. Detailed analyses elucidated the specific binding interactions between Cobicistat and the C1, C2, and C3 binding pockets on MAPK14, highlighting the multi-faceted nature of their affinity.

ADMET predictions and SwissTargetPrediction play crucial roles in the structure-based identification of therapeutic agents for disease management[33]. ADMET predictions assess a compound's Absorption, Distribution, Metabolism, Excretion, and Toxicity, crucial factors influencing drug efficacy and safety[34]. By forecasting these properties, researchers prioritize lead hits with favorable ADMET profiles, minimizing risks and optimizing therapeutic outcomes. SwissTargetPrediction complements this by predicting a compound's potential targets, aiding in understanding its pharmacological effects and off-target interactions[35]. Integrating these tools enables the selection of lead compounds with optimal drug-like properties and target specificity, accelerating the development of safe and effective treatments for various diseases. The validation of Cobicistat's drug-like properties through ADMET predictions, coupled with SwissTargetPrediction analysis suggesting its promise in targeting MAPK14 inhibition for asthma treatment, further reinforced its therapeutic potential.

Evaluating the cytotoxicity effects of lead compounds is pivotal in identifying and developing therapeutic molecules for diseases[36]. This assessment examines the compound's impact on cell viability and function, ensuring it selectively targets diseased cells while sparing healthy ones[37]. By conducting cytotoxicity assays, researchers gauge the compound's safety profile, determining its maximum tolerated dose and potential adverse effects. This information guides medicinal chemists in optimizing lead compounds, balancing efficacy with tolerability. Moreover, understanding cytotoxicity aids in predicting drug responses in vivo, facilitating the translation of promising candidates from preclinical studies to clinical trials. Ultimately, this process ensures the development of safe and effective therapeutic interventions for various diseases. The concentration-dependent cytotoxicity analysis on HEK-293 normal lung cells demonstrated no significant impact on cell viability within the tested concentration range, affirming the benign nature of Cobicistat. Additionally, the determination of $IC_{50}$ concentration provided valuable insights into its potency against normal lung cells.

Evaluating the effects of lead compounds on target genes is crucial for therapeutic purposes [38,39]. This assessment elucidates the compound's mechanism of action, revealing how it modulates gene expression to exert its therapeutic effects[38]. By studying the compound's impact on target genes, researchers gain insights into the underlying molecular pathways involved in disease pathology and drug response[40]. This information guides the selection and optimization of lead compounds, ensuring they effectively modulate the desired biological targets. Moreover, evaluating target gene effects aids in predicting the compound's efficacy and potential side effects, facilitating the

development of safer and more precise therapeutic interventions for various diseases[41,42,43,44,45,46]. The investigation into Cobicistat's effects on MAPK14 expression and activity in BEAS-2B cells revealed a significant decrease, highlighting its inhibitory effect on the target protein. These findings underscored Cobicistat's potential as a therapeutic agent for asthma treatment by modulating MAPK14 activity. The enzyme inhibition assay demonstrated a dose-dependent decline in MAPK14 activity with increasing Cobicistat levels, further corroborating its potential to inhibit MAPK14 kinase activity and suggesting its role in regulating downstream signaling pathways.

## CONCLUSION

In conclusion, this comprehensive investigation underscores the potential of Cobicistat as a therapeutic agent for asthma treatment through its inhibition of MAPK14. Molecular docking studies revealed Cobicistat's high affinity for MAPK14, supporting its candidacy as a promising inhibitor. ADMET predictions and SwissTargetPrediction further validated its drug-like properties and potential therapeutic targets. Additionally, cytotoxicity assays demonstrated Cobicistat's safety profile, while evaluation of its effects on target genes highlighted its mechanism of action in modulating MAPK14 expression and activity. These findings collectively underscore Cobicistat's therapeutic potential in asthma management and pave the way for further preclinical and clinical investigations. Ultimately, this study contributes to the advancement of precision medicine by offering a targeted therapeutic approach for asthma treatment, with implications for the broader field of disease management.

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**Conflict of Interest**

The authors declare no competing or conflicts of interest.

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**Author's Contribution**

AA: Conceptualisation, Data collection/ curation, writing the MS. draft and extensive editing of the MS.

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Not Applicable.

**Ethics Approval Statement**

The work is not related to animal/humans.

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