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In silico study reveals binding potential of rotenone at multiple sites of pulmonary surfactant proteins: A matter of concern

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ABSTRACT

Rotenone is a broad-spectrum pesticide employed in various agricultural practices all over the world. Human beings are exposed to this chemical through oral, nasal, and dermal routes. Inhalation of rotenone exposes biomolecular components of lungs to this chemical. Biophysical activity of lungs is precisely regulated by pulmonary surfactant to facilitate gaseous exchange. Surfactant proteins (SPs) are the fundamental components of pulmonary surfactant. SPs like SP-A and SP-D have antimicrobial activities providing a crucial first line of defense against infections in lungs whereas SP-B and SP-C are mainly involved in respiratory cycle and reduction of surface tension at air–water interface. In this study, molecular docking analysis using AutoDock Vina has been conducted to investigate binding potential of rotenone with the four SPs. Results indicate that, rotenone can bind with carbohydrate recognition domain (CRD) of SP-A, N-, and C- terminal peptide of SP-B, SP-C, and CRD of SP-D at multiples sites via several interaction mediators such as H bonds, C–H bonds, alkyl bonds, pi-pi stacked, Van der Waals interaction, and other. Such interactions of rotenone with SPs can disrupt biophysical and anti-microbial functions of SPs in lungs that may invite respiratory ailments and pathogenic infections.

1. Introduction

Pesticide pollution is a major global health concern. Indiscriminate application of pesticides has contaminated almost every component of the biosphere. Till date, numerous pesticides have been formulated. According to the target organism, pesticides are classified as herbicides, fungicides, insecticides, rodenticides, nematicides, and molluscicides. These chemicals target specific metabolic pathways in pests to control their population. However, they may interrupt various biomolecules in organisms other than pests to elicit toxic responses. Numerous studies have reported pesticide-induced oxidative stress, cytotoxicity, and organotoxicity on human and model organisms (Mandi et al., 2020; Khatun et al., 2018; Rajak et al., 2018; Sarkar et al., 2018; Nicolopoulou-Stamati et al., 2016; Podder and Roy, 2015; Rajak et al., 2015). Additionally, pesticide exposure can disrupt protein homeostasis and augment pathogenicity of infectious as well as fatal diseases (Rajak et al., 2021; Rajak and Roy, 2018).

Rotenone is a colorless, odorless and crystalline heteropentacyclic broad-spectrum insecticide derived from the roots and stems of *Lonchocarpus* and *Derris* species. It is lipophilic in nature and therefore can easily cross lipid bilayer of cells in several tissues. Rotenone is an established inhibitor of complex I of the mitochondrial electron

Abbreviations: ALA, Alanine; ARG, Arginine; ASN, Asparagine; ASP, Aspartic acid; CYS, Cysteine; GLN, Glutamine; GLU, Glutamic acid; GLY, Glycine; HIS, Histidine; ILE, Isoleucine; LEU, Leucine; LYS, Lysine; MET, Methionine; PHE, Phenylalanine; PRO, Proline; SER, Serine; THR, Threonine; TRP, Tryptophan; TYR, Tyrosine; VAL, Valine.

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transport system. It blocks electron transfer from the iron-sulfur centers in complex I to ubiquinone, leading to a blockade of oxidative phosphorylation with limited synthesis of ATP (Palmer et al., 1968). Furthermore, incomplete electron transfer to oxygen can generate reactive oxygen species which in turn can damage mitochondrial membranes and DNA, leading to activation of apoptotic cascades (Li et al., 2003). Moreover, rotenone inhibits microtubule assembly and cell proliferation on binding with tubulin (Brinkley et al., 1974).

Rotenone is usually non-toxic to plants; however it poses detrimental effects to human and animal health. Multiple studies have depicted the impact of rotenone in the etiology of Parkinson disease. Rotenone induced selective nigrostriatal degeneration in rat brain following the inhibition of complex I of the mitochondrial respiratory chain, has been reported (Sherer et al., 2003; Betarbet et al., 2000). Akinmoladun et al., 2018 have documented hepatotoxic potential of rotenone describing altered activities of oxidative stress markers and enzymes associated with inflammation. In addition, rotenone promotes oxidative damage and renal tissue injury in rats (Jiang et al., 2017). Siddiqui et al., 2013 investigated the impact of rotenone on human liver HepG2 cells and confirmed that rotenone fuels apoptotic changes in HepG2 cells by up-regulation of proapoptotic proteins such as p53, Bax, and caspase-3. Rotenone is cytotoxic to human dopaminergic SH-SY5Y cells as it induces neuronal cell death via phosphorylation of p38 and JNK MAP Kinases (Newhouse et al., 2004). Vomiting, respiratory depression, cardiovascular collapse, and severe metabolic acidosis have also been reported in human following rotenone intoxication (Wood et al., 2005). Therefore, rotenone can cause unprecedented alterations in various organs.

Exposure to rotenone occurs through nasal, oral, and dermal routes. People spraying rotenone at farms, gardens, residences, schools, and offices are regularly exposed to this chemical through inhalation. Unfortunately respiratory protective equipments including surgical masks most frequently worn by farmers may not provide adequate protection from pesticide inhalation (Sapbamrer et al., 2021). In such cases, lungs and its biomolecules are regularly exposed to rotenone and other pesticides. Till date studies investigating the impacts of inhaled rotenone in lungs and its biomolecular components are lacking.

Respiratory cycle of lungs is precisely controlled by pulmonary surfactant. It is a lipoprotein complex secreted by type II alveolar cells. The main function of pulmonary surfactant includes reduction of surface tension at the air/liquid interface within the alveoli. Indeed, they also participate in host defense system. Almost 90% of this multimolecular complex is composed of phospholipids whereas remaining 10% consists of surfactant proteins (SPs) (Fig. 1). SPs include high molecular weight hydrophilic proteins (SP-A and SP-D) and low molecular weight extremely hydrophobic proteins (SP-B and SP-C).

SP-A is the first identified and most abundant surfactant protein in lungs (Weaver and Whitsett, 1991). It is a large octadecameric protein having a molecular mass of about 650 kDa. Eighteen monomers of SP-A in the form of six trimeric units are organized in a bouquet-like structure. A human SP-A trimer is composed of two SP-A1 and one SP-A2 monomers (Voss et al., 1991). Each monomer made up of a Calcium dependent C-terminal carbohydrate recognition domain (CRD), a hydrophobic neck region, a collagenous domain, and an amino-terminal cysteine-rich domain. CRD is vital for most of the physiological functions of SP-A. CRD binds with lipopolysaccharide (LPS) of microbes and accumulate around them to block infection. SP-A helps surfactant phospholipids to lower surface tension in the alveolus and counteracts the inhibitory effects of plasma proteins released during lung injury on surfactant function (Weaver and Whitsett, 1991). SP-A is copious in alveoli although a little is found in respiratory pseudostratified epithelium that lines the conducting airways. It is also found in human tracheal submucosal glands.

SP-B is a hydrophobic membrane protein having molecular mass of about 8 kDa and composed of 79 amino acids. Majority of amino acids in SP-B include leucine, isoleucine, phenylalanine, valine, alanine, and tryptophan. They are positively charged and highly hydrophobic in nature. Two monomers of SP-B constitute homodimers in alveolar membranes and other lipid structures (Weaver and Conkright, 2001). SP-B has been heralded for proper biophysical function of the lungs. It enhances adsorption potential of phospholipids at the air–water interface and it is involved in the synthesis of tubular myelin (Hawgood, 2004). Moreover, SP-B protects from Oxygen-induced lung injuries and has anti-inflammatory property (Tokieda et al., 1999).

SP-C is an extremely hydrophobic, 35–amino acid lipopeptide having two palmitates attached at amino acids C5 and C6. The segment between residues 13 and 28 is a hydrophobic α-helix comprising of aliphatic amino acids, especially valine. SP-C is exclusively produced by type II pneumocytes and released into alveolar spaces (Ten Brinke et al., 2002). The α-helix of SP-C is extremely stable as transmembrane structure. Similar to SP-B, SP-C increases adsorption rate of phospholipids and stabilizes alveolar surfactant film (Qanbar et al., 1996). It also participates in host defense mechanism. A 12-residue nontransmembrane domain of SP-C interacts with bacterial LPS and promotes microbial recognition by phagocytes (Augusto et al., 2002).

SP-D is a 43 kDa protein belonging to the collectin family. SP-D has sequence homology to SP-A. Mature SP-D is a tetrameric complex composed of four homotrimers. Each homotrimer is made up of three identical subunits. Each SP-D subunit contains an N-terminal domain, a nucleating neck region, a collagenous region, and a C-terminal lectin domain. SP-D is the collagenous family of proteins comprised of CRD. CRD helps SP-D to agglutinate microbes and apoptotic cells, and enhances their removal from the lungs.

Therefore surfactant proteins are critical to proper lung function. The present in silico study investigates the binding potential of rotenone for pulmonary surfactant proteins. The study will help to understand the impact of rotenone inhalation on lung physiology (Fig. 2). In silico study is important to investigate the impacts of various xenobiotics on different biomolecules of the body (Tripathi et al., 2016; Tripathi, et al., 2015) and suggests a potential adverse outcome pathway (AOP). The present in silico study suggests an AOP of rotenone inhalation. AOP starts when inhaled single or mixtures of chemical (s) reach the alveolar environment and inhibit the function of the lung surfactant proteins. Inhibition of lung surfactant proteins leads to alveolar collapse at the end of expiration and reduced lung function (Da Silva et al., 2021). AOP was initially introduced in the context of ecotoxicology (Ankley et al., 2010) nevertheless it is now endorsed by Organisation for Economic Cooperation and Development (OECD). AOP aims to facilitate mechanism-based hazard identification of chemicals and particles relying on non-animal methods.

2. Materials and methods

2.1. Retrieval of pesticides and preparation for molecular docking

The three dimensional structure of rotenone (PubChem CID: 6758) having molecular formula $C_{23}H_{22}O_6$ was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Ligand was prepared for docking analysis using AutoDock 4.2.

2.2. Retrieval of protein structures and preparation for molecular docking

The three dimensional structure of four lung surfactant proteins viz. SP-A (PDB ID: 1R13), SP-B (PDB ID for N-terminal segment: 1DFW; PDB ID for C-terminal peptide: 1RG4), SP-C (PDB ID: 2ESY) and SP-D (PDB ID: 3IKP) were retrieved in .pdb format from RCSB Protein Data Bank (https://www.rcsb.org/). Each crystal confirmation of

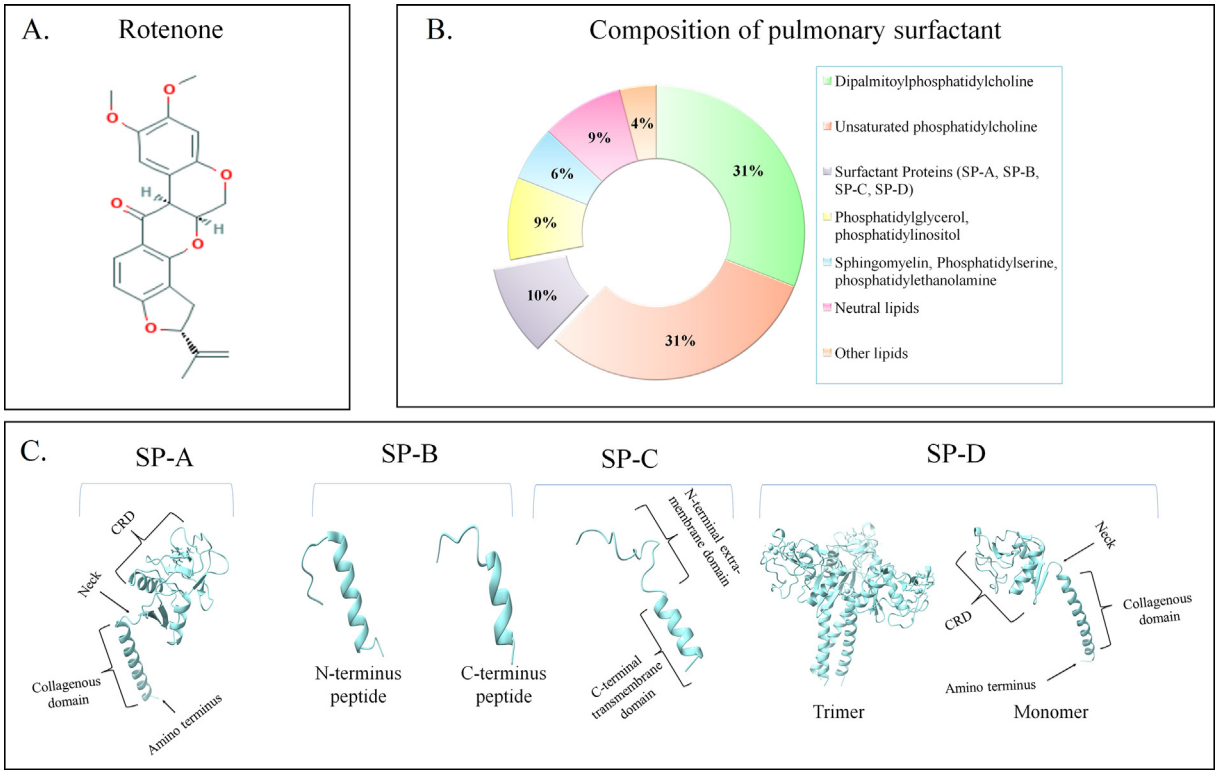

Fig. 1. The figure represents (A) molecular structure of rotenone, (B) bio-molecular composition of human pulmonary surfactants and (C) the three-dimensional structures of human surfactant proteins (SP-A, SP-B, SP-C, SP-D) as retrieved from RCSB protein data bank.

protein was processed for docking analysis by removing water moieties and ligands that were bound to the SPs during purification.

2.3. Molecular docking analysis

Molecular docking was performed using rotenone as ligand and pulmonary surfactant proteins as target molecules. AutoDock 4.2 employing Lamarckian genetic algorithm was used for the analysis. Further water molecules were removed from crystal structure of surfactant proteins to minimize free energy and facilitate protein–ligand docking. Moreover, polar Hydrogen and Kollman charges were added to analyze binding affinity of rotenone. Grid box was added to each target protein and configured to cover the major binding sites. Finally, molecular docking was conducted using AutoDock Vina (Trott and Olson, 2010) and results were saved in log.txt file. In addition, rotenone_out.pdbqt was also exported and saved for each docking analysis to visualize binding interactions between rotenone and target surfactant protein. Parameters like polar interactions, non-polar interactions, Hydrogen bond length, and interacting amino acids with rotenone were studied using Chimera X and Discovery Studio Visualizer.

2.4. Comparison of binding affinity

Three regularly used pesticides such as dichlorvos, acephate and ethion with a nasal route of human exposure were chosen at random to compare their binding affinity for SPs with respect to rotenone. For this purpose, three dimensional confirmations of these chemicals were retrieved from the PubChem (compound CID: 3039; CID: 1982 and CID: 3286) and processed for the study. Molecular docking was performed as stated above.

3. Results

3.1. Rotenone ~ SP-A interaction

Molecular docking between rotenone and SP-A has revealed binding potential of rotenone at four sites of SP-A (Fig. 3). At site 1 of CRD, rotenone forms three H-bonds with TYR208 (bond length: 2.4 Å), ARG216 (bond length: 2.632 Å), and ARG222 (bond length: 2.327 Å). Van der Waals interaction between rotenone and SP-A involved four amino acid residues viz. ASN162, GLU206, THR209, and ASN214. Pi-Pi interaction was established by TYR164. The affinity for this site has been recorded as −6.8 kcal/mol.

Binding of rotenone at site 2 of CRD involved three H-bonds. H-bond length between rotenone and amino acid residues such as ASN163, TYR164, and TYR208 were measured as 2.444 Å, 3.44 Å, and 2.407 Å respectively. Other interactions such as Van der Waals (ASN 162, TYR 221, ASN214, GLN199) and Pi interaction (GLU206, ARG222) were also noted. The affinity for binding site 2 was recorded as −6.6 kcal/mol.

Rotenone interacted with SP-A at binding site 3 (CRD) with an affinity of −6.4 kcal/mol. Two H-bonds were shared between rotenone and SP-A (PHE178) with bond lengths of 2.281 Å and 2.460 Å. Other interactions included Van der Waals with eight amino acids.

Binding affinity between rotenone and SP-A at binding site 4 was recorded as −6.2 kcal/mol. Conventional H-bond was formed between rotenone and amino acid residue TYR161. Van der Waals interaction was established using six amino acids. C–H bonds were visible between rotenone and aminoacids GLY109 and SER110. Amino acids such as Leu105, MET111, and VAL118 established other non-covalent interactions.

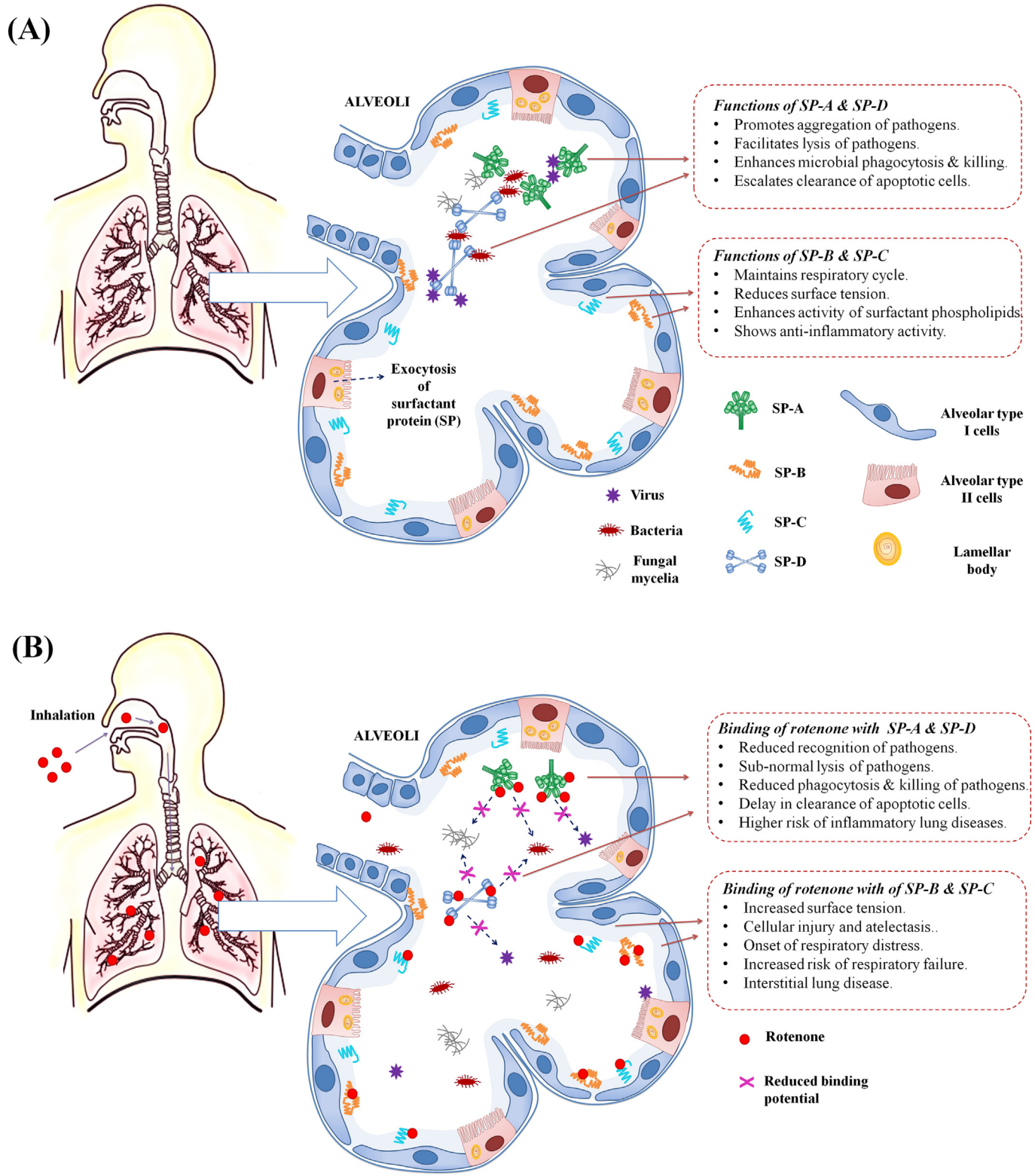

Fig. 2. Putative impacts of rotenone inhalation on lung physiology. Figure (A) demonstrates the major surfactant proteins (SPs) and their functions. SPs are synthesized and stored in alveolar type II cells in the form of lamellar bodies until they are released into the alveolar lumen through exocytosis. CRD domain of SP-A and SP-D recognizes various pathogens like virus, bacteria, and fungi to facilitate microbial aggregation. This enhances opsonization and phagocytosis of microbes by macrophages. SP-A and SP-B also escalate removal of apoptotic cells from alveoli. SP-B and SP-C are involved in maintenance of biophysical functions of lungs and are vital for respiratory cycle. They can also impart anti-inflammatory function. Figure (B) demonstrates that, inhalation of rotenone can expose lung surfactant proteins to this pesticide. Rotenone has potential to bind SPs at multiple sites. Such interaction can disrupt various functions of alveolar SPs. Binding of rotenone with SP-A and SP-D can subvert recognition and opsonisation of microbes in lungs. This may increase the risk of severe pathogenic infection/sepsis and inflammatory lung diseases. Binding of rotenone with SP-B and SP-C can increase the surface tension at air/water interface in alveoli. This may fuel the onset of respiratory distress syndrome, forced breathing, risk of respiratory failure and development of interstitial lung disease.

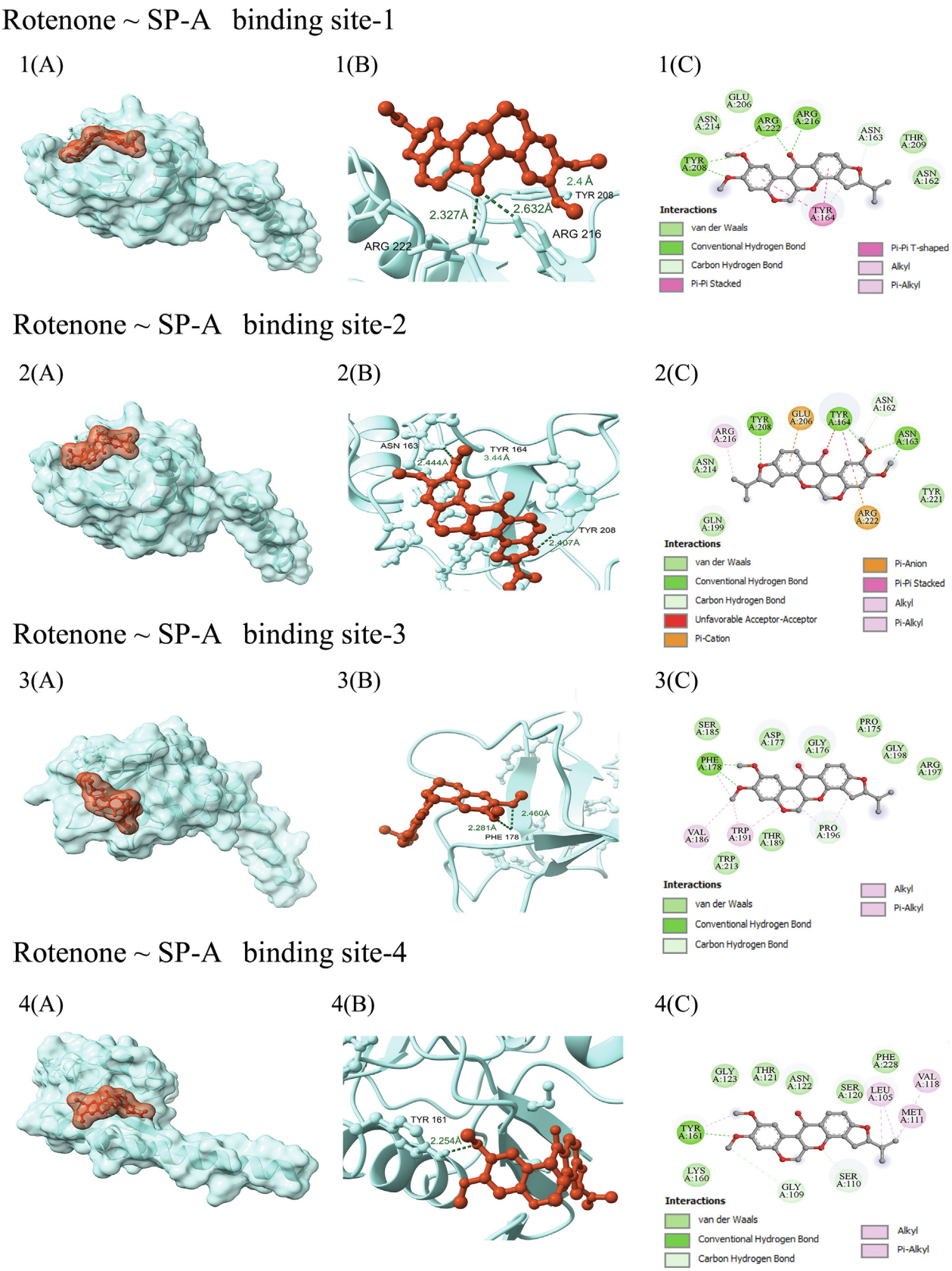

Fig. 3. The figure demonstrates binding of rotenone at multiple sites of SP-A. Figure in first column (A) provides information regarding the binding of rotenone at a particular site of the protein. Column (B) represents H-bonds with bond length between the amino acid residues of protein and ligand. Column (C) represents all the polar and non-polar interactions between various amino acid residues of protein and ligand.

3.2. Rotenone ~ SP-B interaction

Rotenone interacted with binding site 1 of N-terminal peptide of SP-B with affinity of −6.8 kcal/mol. ARG12 formed two conventional H-bonds of 2.099 Å and 2.208 Å length with rotenone. Other covalent interactions were also evident between rotenone and several amino acid residues.

Rotenone interacted with N-terminal peptide (binding site 2) of SP-B with affinity of −5.6 kcal/mol. Two H-bonds between ligand and ARG17 were noticed with bond length of 2.382 Å and 2.421 Å. ILE15 established Van der Waals interaction with the ligand. Further, Pi interactions were also recorded between rotenone and PHE1, LEU14, ILE18, and MET21 of SP-B.

Rotenone binds with C-terminal peptide of SP-B with various bonds and interactions. Amino acids such as MET3 and LEU4 formed H-bonds with rotenone. Bond lengths were measured as 2.567 Å for MET3 and 3.01 Å for LEU4. Pi interaction was established between ligand and ARG10 & LEU13 of SP-B. Affinity for C-terminal peptide of SP-B was recorded as −5.6 kcal/mol (Fig. 4).

3.3. Rotenone ~ SP-C interaction

Rotenone interacted with binding site 1 of SP-C with affinity of −5.8 kcal/mol. Three H-bonds were established between rotenone and ARG10 with bond length of 2.339 Å, 2.402 Å, and 2.434 Å. Alkyl and Pi-alkyl interactions were contributed by PRO9 and ARG12. Ligand established Van der Waals interaction and C–H bonds with SER1 and PRO3 respectively.

Interaction between binding site 2 of SP-C and rotenone was stabilized by conventional H-bond (bond length: 2.155 Å) shared by LYS23. Other interactions involved Pi interaction (ILE15, PRO19, VAL20, LEU22, LEU26) and Van der Waals interaction (PHE17). Affinity for binding site 3 was recorded as −5.5 kcal/mol.

Rotenone interacted with binding site 3 of SP-C using conventional H bond between ligand and ALA8 (bond length: 2.420 Å and 2.438 Å). C–H bond was shared between ligand and ALA7. Van der Waals interaction was stabilized by amino acid residues viz. PRO9, ARG10, and PRO16. ILE15 and PHE17 established other non-covalent interactions with the rotenone. Affinity for binding site 3 was recorded as −5.3 kcal/mol (Fig. 5).

3.4. Rotenone ~ SP-D interaction

Interaction between rotenone and binding site 1 (CRD) of SP-D was stabilized by three conventional H bonds shared by ASN288, ALA290, and ARG343. Other interactions included Van der Waals (GLU289, THR336, ARG349) and Pi interaction (GLU333, PHE335). Affinity was measured as −6.3 kcal/mol.

Rotenone can bind CRD (binding site 2) through conventional H bond (GLN258; bond length: 2.047 Å) and C–H bonds (SER294, SER298). Van der Waals interaction was stabilized between ligand and amino acids such as PHE254, MET295, THR296, TYR306, PRO307, THR305, and GLY309. Rotenone interacted with binding site 2 of SP-D with affinity of −6.3 kcal/mol.

Rotenone binds at neck region (binding site 3) of SP-D with two conversional H bonds shared between the ligand and amino acid ARG272 (bond length: 2.745 Å) and SER273 (bond length: 2.047 Å). Other interactions included Van der Waals (GLY241, GLU242, GLU354, PHE355) and Pi-interactions (GLU276, VAL240, LYS243, ALA275). Affinity for binding site 3 was recorded as −6.2 kcal/mol (Fig. 6).

Binding between rotenone and CRD domain (binding site 4) of SP-D was stabilized by one H-bond (ARG343), several Van der Waals interactions (GLU289, THR336, ARG349, GLU321, GLU329, ASN323, ASP325, ASN341), Pi-Pi interaction (PHE335) and other non-covalent interactions (GLU333, ALA-290). Affinity was measured as −6.0 kcal/mol.

Interaction of rotenone with CRD of SP-D at binding site 5 was mediated by two conventional H bonds. Amino acids ARG-272 and SER-273 participated in convensional H bond formation between SP-D and rotenone. GLY241, GLU242, and GLU354 were involved in Van der Waals interaction between protein and ligand. Binding of ligand was further stabilized by Pi-interactions stabilized by VAL240 and ALA275. Other non-covalent interactions were established by LYS243 and GLU276. Affinity for binding site 5 was recorded as −5.9 kcal/mol (Fig. 7). Table 1 represents hydrogen and hydrophobic interactions between SPs and ligand.

3.5. Affinity of rotenone and other pesticides for SPs

Molecular docking analyses have shown that rotenone has higher affinity for SPs compared to other pesticides (Table 2; Fig. 8). Rotenone showed 2–2.19 fold higher affinity than the dichlorvos, acephate, and ethion for SP-A. Affinity of rotenone for SP-B was 1.94–2.06 fold higher than the other three pesticides. Rotenone had 1.70, 1.81, and 1.87 fold greater affinity for SP-C when compared to dichlorvos, acephate, and ethion respectively. Rotenone showed higher affinity (1.70–1.96 fold) than dichlorvos, acephate, and ethion for SP-D.

4. Discussion

The present in silico study has revealed potential of rotenone to bind pulmonary surfactant proteins at multiple sites.

Rotenone was detected to interact with multiples sites of CRD of monomeric SP-A. Interactions were stabilized by conventional H bonds, C–H bonds, alkyl/pi-alkyl contacts and Van der Waals interaction between the ligand and the several amino acid residues of SP-A. CRD is critical to most SP-A mediated biophysical functions and is needed for maintenance of proper respiratory cycle. In addition, globular domain of CRD interacts with carbohydrate or other ligands of microbial pathogens in lungs and blocks further infection. For instance, CRD of SP-A binds with surface glycoprotein of *Pneumocystis carinii*, a common cause of life-threatening pneumonia and enhances adherence to alveolar macrophages (McCormack et al., 1997). It helps in neutralization, agglutination, and clearance of *Pneumocystis carinii*. SP-A acts as an important modulator of alveolar macrophage function that is required for enhanced capacity of phagocytosis of *Mycobacterium tuberculosis* (Gaynor et al, 1995). Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis in developing world. Trimeric units of SP-A effectively neutralize RSV in human bronchial epithelial cells and reduce the level of infection (Watson et al., 2017). Moreover, SP-A via its Sialic acid residues functions as an opsonin in the phagocytosis of influenza A virus (H1N1 and H3N2) by alveolar macrophages (Benne et al., 1997; Benne et al., 1995). *Aspergillus fumigates* is an opportunistic fungal pathogen that causes allergic bronchopulmonary aspergillosis. Studies have clearly indicated that, SP-A interacts with the glycosylated antigens and allergens of *Aspergillus fumigates* and thereafter lowers risk of allergic reactions like high levels of IgG, IgE, blood eosinophilia, and extensive infiltration of lymphocytes (Madan et al., 2001). In another study, SP-A null mice was observed to be more susceptible to pulmonary fungal infection with *Histoplasma capsulatum* than age-matched wild-type control mice (McCormack et al., 2003). The increased susceptibility was associated with reduced number of $CD8^+$ cells in lungs of SP-A null mice. Carboxyl-terminal domain of SP-A containing C-type lectin CRD has antioxidant property. It directly protects surfactant phospholipids and macrophages from lipid peroxidation and oxidative cellular injury (Bridges et al., 2000). Thus, binding of rotenone to CRD at multiple sites can disrupt the interaction between SP-A and several microbial pathogens that can further result in exacerbated infection in alveoli

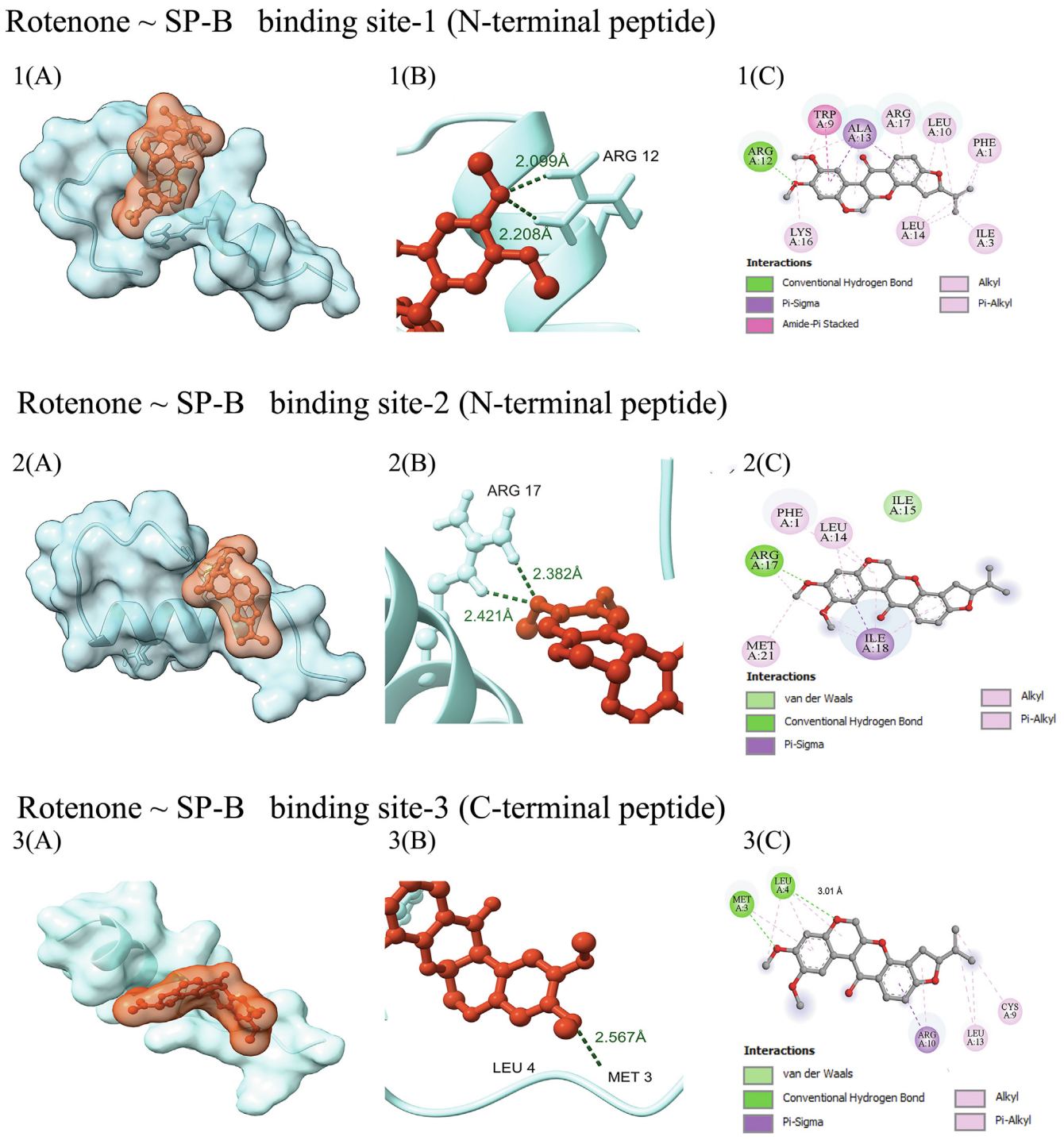

Fig. 4. The figure demonstrates binding of rotenone at N- and C- terminal peptide of SP-B. Figure in first column (A) provides information regarding the binding of rotenone at a particular site of the protein. Column (B) represents H-bonds with bond length between the amino acid residues of protein and ligand. Column (C) represents all the polar and non-polar interactions between various amino acid residues of protein and ligand.

and bronchial epithelial cells. Moreover, formation of rotenone ~ SP-A complex can impede antioxidant activity of SP-A which could enhance oxidative lung injury in rotenone exposed individuals. Additionally, lung transcriptome analysis of a COVID-19 patient has revealed impaired surfactant production in lungs (Gonzalez-Andrades et al., 2016).

Rotenone interacted with SP-B at N-terminal and C-terminal peptides. Protein-ligand binding was stabilized by conventional H-bonds and Van der Waals interaction. Such protein–ligand interaction may impede proper function of SP-B. SP-B is mainly responsible for reduction of surface tension in alveolar walls by increasing lateral stability of the phospholipid layer and prevention of collapse of pulmonary alveoli (Cochrane and Revak, 1991). SP-B dysfunction is linked to severe respiratory failure (Thompson, 2001). It has been seen that, the concentration of SP-B becomes markedly reduced in patients with acute respiratory distress syndrome (Simonato et al., 2011). SP-B is involved in formation of highly structured lamellar bodies. Lamellar bodies act as the storage site of surfactant phospholipids and proteins,

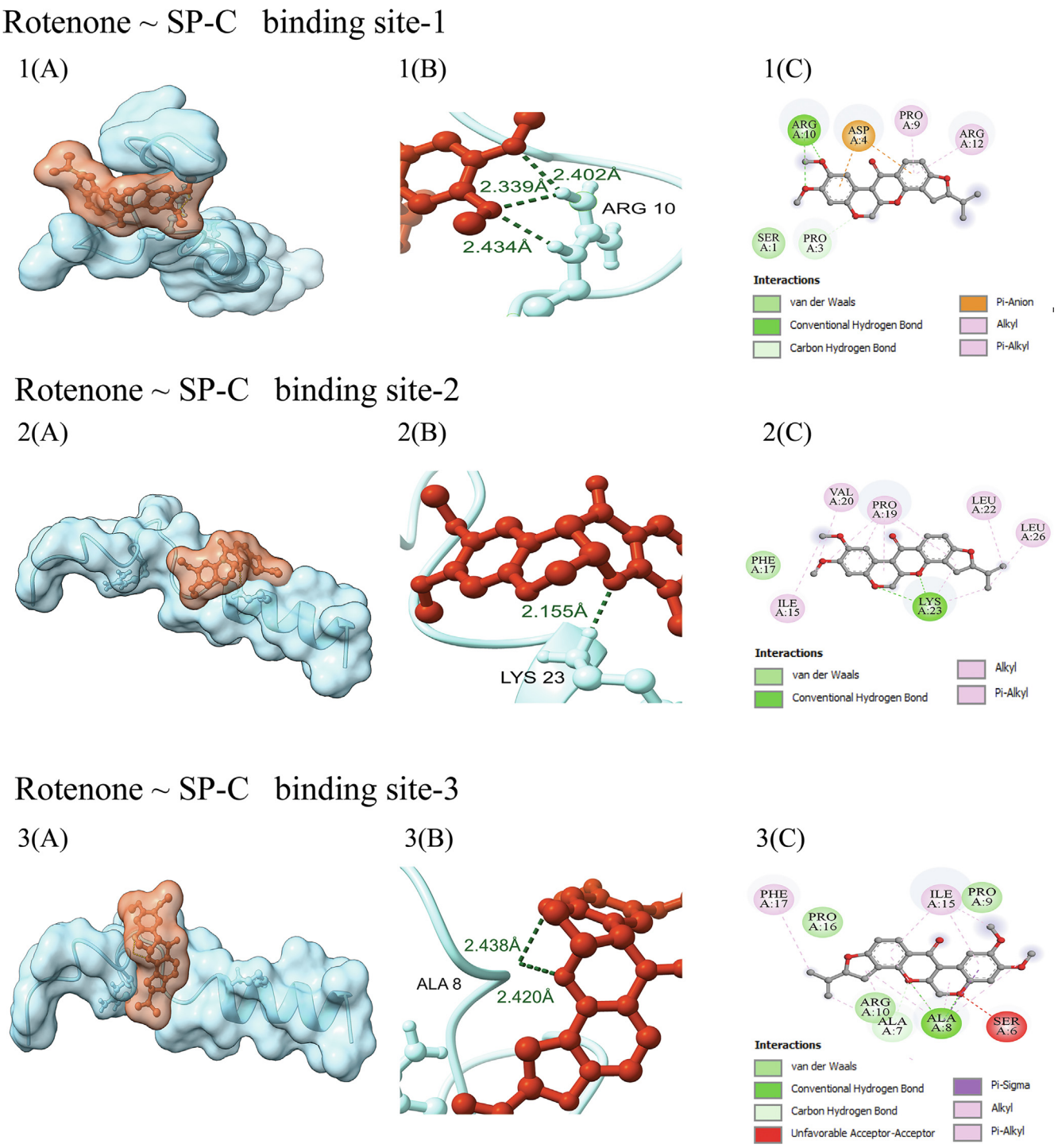

Fig. 5. The figure demonstrates binding of rotenone at multiple sites of SP-C. Figure in first column (A) provides information regarding the binding of rotenone at a particular site of the protein. Column (B) represents H-bonds with bond length between the amino acid residues of protein and ligand. Column (C) represents all the polar and non-polar interactions between various amino acid residues of protein and ligand.

and involve in exocytosis of lung surfactant. Interaction of rotenone with SP-B might hinder the formation lamellar bodies. Reduced level of functional lamellar bodies in type II alveolar cells is associated with fatal respiratory diseases (Cutz et al., 2000).

SP-C is a strong hydrophobic peptide that can form biomolecular complex with inhaled rotenone. In the present study, rotenone was observed to interact with SP-C at multiple sites and the resultant ligand–protein complex was stabilized by conventional H bonds and Van der Waals interactions. SP-C organizes phospholipids during respiratory cycle. It stabilizes the alveolar surface film and minimizes the film collapse. In addition, combined action of SP-B and SP-C regulates permeability and dynamics of phospholipid membranes that may be critical to proper lung physiology (Parra et al., 2011). Deficiency of alveolar SP-C has been linked to inflammatory lung diseases. Lack of SP-C function contributes to enhanced pSTAT3 signaling in alveolar type 2 cells that may lead to hyperinflammation with severe diffuse alveolar injury and sometimes death (Jin et al., 2018). Notably, recombinant form of SP-C is used in surfactant replacement therapy to treat

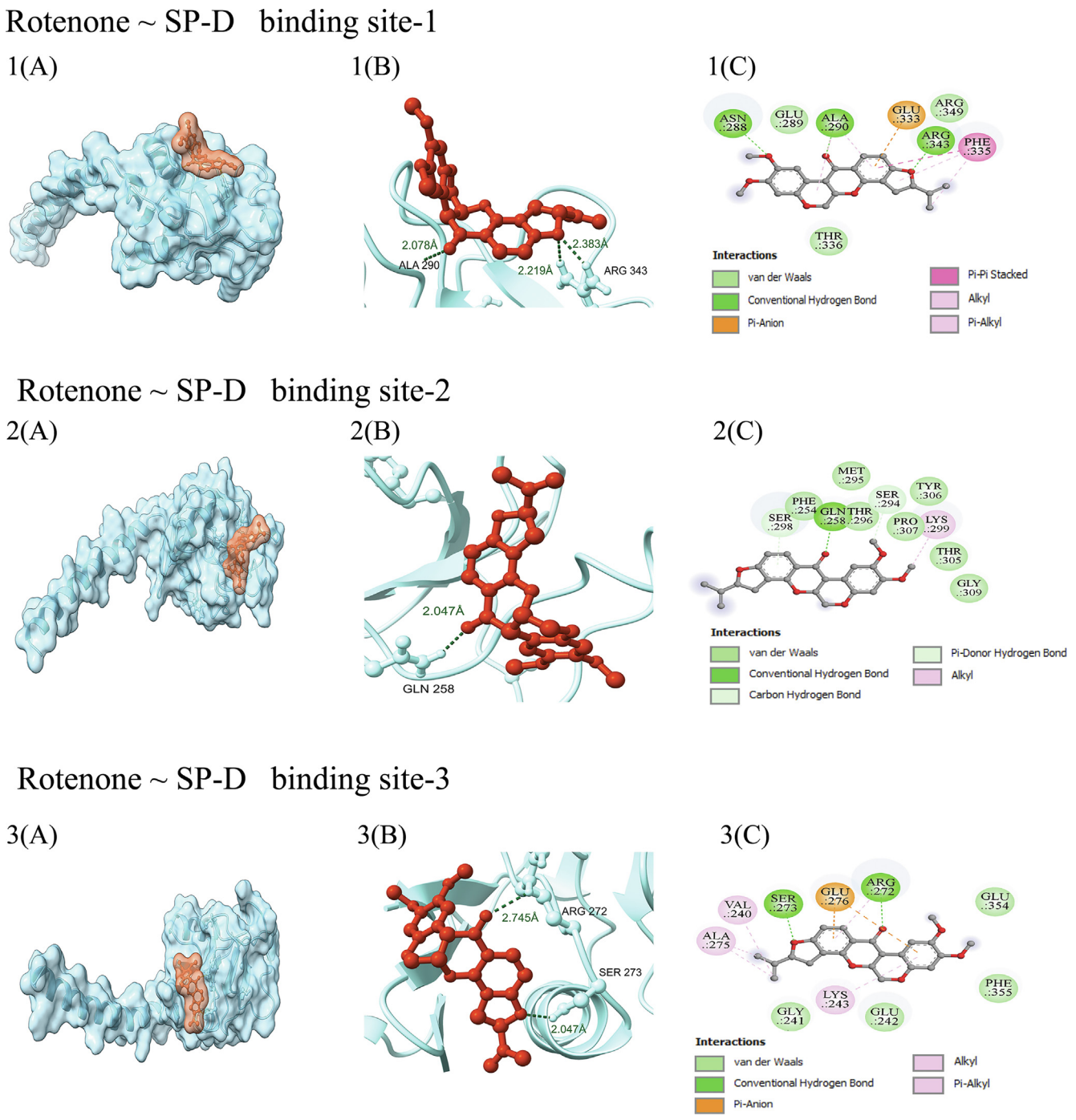

Fig. 6. The figure demonstrates binding of rotenone at binding site 1, 2 and 3 of SP-D. Figure in first column (A) provides information regarding the binding of rotenone at a particular site of the protein. Column (B) represents H-bonds with bond length between the amino acid residues of protein and ligand. Column (C) represents all the polar and non-polar interactions between various amino acid residues of protein and ligand.

acute respiratory distress syndrome (Spragg et al., 2003). Therefore, alteration of SP-C activity upon binding with rotenone may fuel diffuse lung disease with variable prognosis and severity. SP-C has antimicrobial and immuno-protective actions in the alveolar and upper airways. SP-C deficiency has been associated with reduced phagocytic activity of alveolar macrophages and enhanced proinflammatory responses in the lungs following *Pseudomonas aeruginosa* infection (Glasser et al., 2008). In a study, SP-C deficient mice were susceptible to RSV infection (Glasser et al., 2009). In such cases, RSV aggravated severe interstitial thickening, air space consolidation, and goblet cell hyperplasia. Viral clearance was also decreased. Hence, interaction of rotenone with SP-C may reduce or destroy its antimicrobial activity rendering lungs vulnerable to viral and bacterial pathogens.

In the present study, rotenone established conventional H bonds, C–H bonds, and Van der Waals interactions with multiple sites of SP-D that can interfere with its biological functions. Similar to this study, SP-D was also inhibited by methyl isocyanate, alphanaphthol, butylated hydroxytoluene, and carbaryl (Shrivastava et al., 2016; Tripathi et al., 2020). SP-D is associated with innate immunity, regulation of pulmonary surfactants and maintenance of lipid homeostasis. SP-D also escalates allergen removal and modulates allergic inflammation (Hawgood et al., 2004). It down-regulates proinflammatory

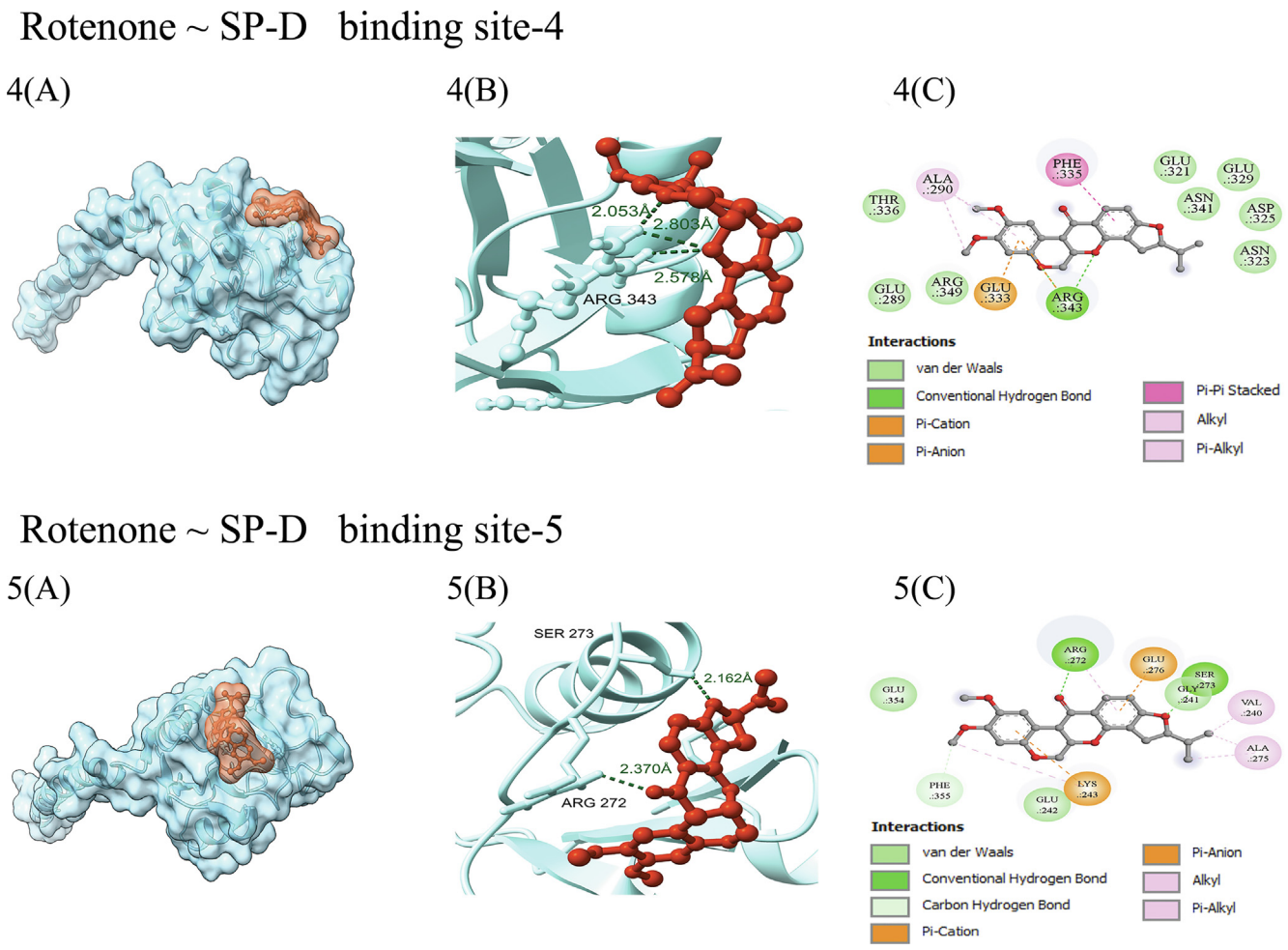

Fig. 7. The figure demonstrates binding of rotenone at binding site 4 and 5 of SP-D. Figure in first column (A) provides information regarding the binding of rotenone at a particular site of the protein. Column (B) represents H-bonds with bond length between the amino acid residues of protein and ligand. Column (C) represents all the polar and non-polar interactions between various amino acid residues of protein and ligand.

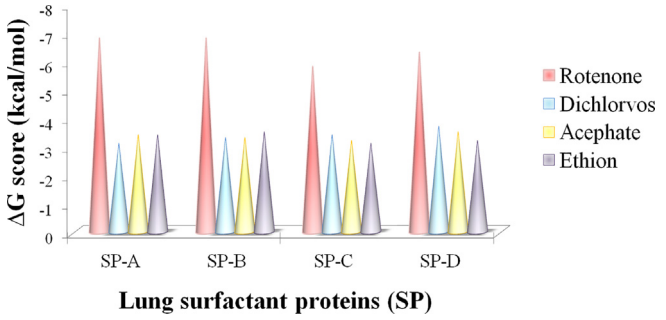

Fig. 8. Clustered cone diagram showing binding affinity of pesticides such as rotenone, dichlorvos, acephate, and ethion for surfactant proteins (SP-A, SP-B, SP-C, and SP-D). Cones indicate greater binding affinity (lower ΔG scores) of rotenone for SPs where as other pesticides show comparatively lower binding affinity (higher ΔG scores) for SPs.

cytokine release and subsequent lung tissue injury through different signaling pathways such as TLR4 (Arroyo and Kingma, 2021). SP-D inactivity in severe asthma is involved in disease persistence. SP-D enhances binding and internalization of allergen-containing sub-pollen particles with primary bronchial epithelial cells and facilitates their clearance (Schleh et al., 2010). Therefore, lack of functional SP-D may result in allergen-mediated breathing ailments. Importantly, phagocytosis and pulmonary clearance of RSV in airways are enhanced by SP-D as CRD recognizes RSV glycoproteins in a Calcium-dependent fashion (LeVine et al., 2004). Influenza virus is also targeted and opsonized by SP-D (White et al., 2008). Interestingly, recombinant fragment of human SP-D has been shown to interact with spike protein of SARS-CoV-2: the causative agent of ongoing pandemic disease coronavirus disease-19 (COVID-19) (Madan et al., 2021). In this study, recombinant fragment of human SP-D inhibited SARS-CoV-2 replication more efficiently than antiviral drug, Remdesivir. In another study, SP-D showed a dose-responsive binding to receptor binding domain and acted as entry inhibitor of SARS-CoV-2 pseudo-typed viral particles (Hsieh et al., 2021). These findings have suggested that, depletion in functional SP-D might result in increased susceptibility to COVID-19. SP-D also inhibits bacterial LPS-triggered inflammatory cell response (Atochina-Vasserman et al., 2010). Various ligands such as 1,3-β-D-glucan, 1,6-β-D-glucan, galactosaminogalactan galactomannan, glucuronoxylomannan, and mannoprotein 1 of pathogenic fungi are recognized by CRD that aids to fungicidal activity of SP-D (Madan and Kishore, 2020). It agglutinates *Aspergillus fumigatus conidia* and escalates uptake of opsonized conidia by alveolar macrophages and neutrophils (Madan et al., 1997). SP-D also binds with acapsular

Table 1
Amino acid residues of four surfactant proteins interacting with rotenone through Hydrogen bonds and hydrophobic interaction.

Type of surfactant protein (SP)	Site of interaction	Conventional hydrogen bond	Hydrophobic interaction
SP-A	Binding site-1	TYR208, ARG222, ARG216	ASN214, GLU206, THR209, ASN162
	Binding site-2	TYR208, TYR164, ASN163	ASN214, GLN199, TYR221
	Binding site-3	PHE178	THR189, TRP213, SER185, ASP117, GLY176, PRO175, GLY198, ARG197
	Binding site-4	TYR161	LYS160, GLY123, THR121, ASN122, SER120, PHE228
SP-B	Binding site-1 (N-terminal peptide)	ARG12	
	Binding site-2 (N-terminal peptide)	ARG17	ILE15
	Binding site-3 (C-terminal peptide)	MET3, LEU4	
SP-C	Binding site-1	ARG10	SER1
	Binding site-2	LYS23	PHE17
	Binding site-3	ALA8	ARG10, PRO16, PRO9
SP-D	Binding site-1	ASN288, ALA290, ARG343	THR336, GLU289, ARG349
	Binding site-2	GLN258	PHE254, MET295, THR296, PRO307, TYR306, THR305, GLY309
	Binding site-3	SER273, ARG272	GLY241, GLU242, GLU354, PHE355
	Binding site-4	ARG343	ARG349, GLU289, THR336, GLU321, GLU329, ASN341, ASP325, ASN323
	Binding site-5	ARG272, SER273	GLY241, GLU242, GLU354

form of cryptococci leading to their aggregation (van de Wetering et al., 2004). Notably, growth of *Candida albicans* and its adherence to the mucosal epithelial cells are directly inhibited by SP-D (Madan and Kishore, 2020). SP-D also promotes neutrophil mediated clearance of opsonized *Candida* (Ordonez et al., 2019). *Pneumocystis carinii* is a yeast-like fungus that causes severe pneumonia in immunocompromised patients. Studies have indicated that, CRD region of SP-D strongly binds with surface glycoprotein A of *Pneumocystis carinii* and activates defense mechanism against the pathogen (Vuk-Pavlovic et al., 2001). Mucormycosis is a deadly fungal infection in lungs, brain, and sinus that causes mortality rate of at least 50% despite of first line of treatment. Glucose-regulated protein 78 (GRP78) acts as a host receptor for *Rhizopus oryzae* and facilitates invasion and damage of human endothelial cells by the fungal pathogen (Liu et al., 2010). Interestingly, SP-D binds with the GRP78 (Thakur et al., 2021) and has potential to disrupt such infection. Rotenone exposure can increase susceptibility to the mucormycosis by lowering the binding affinity of SP-D for GRP78. Hence, interaction of rotenone with CRD of SP-D might result in reduced fungicidal activity. SP-D is able to activate P53 and Fas-mediated apoptotic pathways in cancerous cells to execute its anti-cancer function (Kaur et al., 2018; Mahajan et al., 2013). Therefore, the results of the current in silico study have demonstrated the potential of rotenone to bind with SP-D. This binding can subvert diverse biological activities of SP-D in human body.

5. Conclusion

The present in silico study has revealed that inhaled rotenone can bind at multiple sites of human lung surfactant proteins. These surfactant proteins are known to control the respiratory cycle of lungs by reducing surface tension at air–water interface. Surfactant proteins like SP-A and SP-D are anti-allergic and provide first line of defense against an array of bacterial, fungal, and viral pathogens. It is worth mentioning that, SP-D acts as an entry inhibitor for SARS-CoV-2, the causative agent for ongoing coronavirus pandemic disease. Rotenone upon binding with surfactant proteins could impair biophysical functions of pulmonary surfactant leading to disturbed respiratory cycle and enhanced breathing troubles. In addition, interaction between rotenone and surfactant proteins might dwarf host-defense system in lungs resulting to increased susceptibility towards a range of pathogenic diseases including the coronavirus disease-19.

CRediT authorship contribution statement

Prem Rajak: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Sumedha Roy:** Conceptualization, Writing – review & editing, Supervision. **Achintya Kumar Pal:** Writing – original draft. **Manas Paramanik:** Writing – original draft. **Moumita Dutta:** Writing – review & editing. **Sayanti Podder:** Writing – review & editing. **Saurabh Sarkar:** Writing – review & editing. **Abhratanu Ganguly:** Writing – original draft. **Moutushi Mandi:** Writing – review & editing. **Anik Dutta:** Writing – review & editing. **Kanchana Das:** Writing – review & editing. **Siddhartha Ghanty:** Writing – review & editing. **Salma Khatun:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 2
Comparison of binding affinity of rotenone and three other insecticides towards SPs.

Lung surfactant protein (SP)	Rotenone (maximum affinity)	Control Dichlorvos (maximum affinity)	Acephate (maximum affinity)	Ethion (maximum affinity)
SP-A	−6.8 kcal/mol	−3.1 kcal/mol	−3.4 kcal/mol	−3.4 kcal/mol
SP-B	−6.8 kcal/mol	−3.3 kcal/mol	−3.3 kcal/mol	−3.5 kcal/mol
SP-C	−5.8 kcal/mol	−3.4 kcal/mol	−3.2 kcal/mol	−3.1 kcal/mol
SP-D	−6.3 kcal/mol	−3.7 kcal/mol	−3.5 kcal/mol	−3.2 kcal/mol

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crtox.2021.11.003.

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