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Molecular Docking Study of *Nigella sativa* Alkaloid Compounds as the Inhibitor of Papain-Like Protease SARS-CoV-2

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Abstract

SARS-CoV-2 causes about 66% of China's Wuhan market workers to experience fever, dry cough, and fatigue. Black cumin (*Nigella sativa*) is a plant with many benefits to cure many illnesses like hypertension, headache, infection, and inflammation. This study aimed to investigate the inhibition by compounds belonging to the Alkaloid group from Black Cumin Seed to inhibit PLpro activity as a target for SARS-CoV-2. The study used five alkaloid compounds ((2E,4E)-Decadienal, (2E,4Z)-Decadienal, Nigellicine, Nigellidine, and Nigellimine) from the Black cumin seed and a PLpro SARS-CoV-2 receptor (PDB ID: 6WX4). The methods used are ligand and receptor preparation, grid box validation, molecular docking, 2D and 3D visualisation, and data analysis using Gibbs free energy, type of interaction, and contact of amino acid residues data. This study used YASARA structure and BIOVIA Discovery Studio. The results showed that Nigellidine has the highest Gibbs free energy with a -2.67 kcal/mol score, higher than VIR251. PLpro has a catalytic triad at Cys111, His272, and Asp286 residues, the compound that binds to the active site is Nigellicine found at amino acid Cys111 with Pi-Sulfur.

Keywords: Alkaloid, SARS-CoV-2, PLpro, Molecular docking, *Nigella sativa*

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1 Introduction

At the end of 2019, a mysterious disease broke out in China, precisely in the Wuhan market. This disease causes about 66% of workers there to experience fever, dry cough, and fatigue symptoms. After an investigation, it was found that the cause of this disease was Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which was later known as COVID-19 [1], [31]. Coronavirus is a single-stranded RNA virus with a 65-125 nm diameter. The word "*Corona*" in coronavirus represents the shape of a crown located on the outermost structure of the virus. Coronavirus can cause Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS), which will result in respiratory failure and eventually lead to death [2]. SARS-CoV-2 is a virus with a genome size of 29.9 kb consisting of 16 non-structural proteins (Nsp1-16) and 4 structural proteins, which are spike protein, envelope protein, membrane protein, and nucleocapsid protein [3]. To date, WHO has recorded 318,648,834 cases and 5,518,343 people died due to SARS-CoV-2 on January 16, 2022, worldwide [4].

SARS-CoV-2 has 2 large polyproteins, Pp1a and Pp1ab, which can be processed by 2 cysteine proteases, papain-like protease (PLpro) and chymotrypsin-like main protease (3CLpro), also known as main protease (Mpro). Mpro cleaves at 11 polyprotein sites with a consensus "X-(L/F/M)-Q↓(G/A/S)-X" sequence, while PLpro cleaves at 3 sites with a consensus "LXGG↓XX" sequence [5]. PLpro also has a role as a deUbiquitinating (DUB) and deISGylating agent, which is essential to suppress the innate immune system by acting on the IFN-β and Nf-κB signalling pathways [6]. Due to its varied and vital functions, PLpro is a desirable target for drug development.

Black cumin (*Nigella sativa*) is a famous plant with many benefits and rich historical background. Black cumin seeds are essential in Indian medical systems like Ayurveda and Unani. Black cumin seeds are also quite popular among Muslims because, in a hadith of the prophet Muhammad PBUH, black cumin seeds is a cure for all diseases except death [7]. Black cumin is widely used because it has broad functions such as treating diabetes, hypertension, headache, infection, and inflammation. Phytochemical analysis of black cumin shows that black cumin contains hundreds of phytoconstituents, mainly alkaloids, saponins, sterols, and essential oils [8]. There are several alkaloid compounds in black cumin, such as Nigellicine, Nigellimine, Nigellidine and Decadienal. Alkaloid compounds have anti-microbial, anti-diabetic, anti-malarial, anti-diarrhoea, and suppression of Nf-κB gene expression [9], [30].

In silico approach is a popular method to help find drug candidates and is used as a preliminary test through molecular docking [10]. The purpose of this study is to analyse the interaction between receptors and ligands from black cumin seed alkaloid compounds by analysing the possible formation of Gibbs free energy, interaction types, and amino acid residue contact at the catalytic triad of the PLpro target receptor.

2 Experimental section

2.1 Equipment and Materials

The equipment used in this research is a computer with a Windows 10 Professional 64-bit operating system, x64-based processor, and an Intel ® Core TM i5-6400T @ 2.20GHz 2.21 GHz processor specification. The software used is Yet Another Scientific Artificial Reality Application (YASARA) structure, BIOVIA Discovery Studio, and PyMOL.

The material used in this study is the PLpro SARS-CoV-2 receptor downloaded from the RCSB PDB (https://www.rcsb.org/) with the PDB ID code "6WX4". All test ligands are (2E,4E)-Decadienal (CID_5283349), (2E,4Z)-Decadienal (CID_6427087), Nigellicine (CID_11402337), Nigellidine (CID_136828302), and Nigellimine (CID_20725) were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Table 1).

2.2 Ligands and Receptor Preparation

The receptor and ligand preparation protocol follows the research of [11] and [12]. Receptor preparation removes unnecessary chains, removes water molecules, removes crystallographic ligand, and add hydrogen atoms. Hydrogen atoms were added because the resolution of the crystal structure could not predict the presence of hydrogen. Only 1 chain is used as the target receptor [12], [23]. All ligands were energy-optimized using the YASARA structure [13].

2.3 Gridbox Validation and Molecular Docking

Validation was done by redocking the crystallographic ligand on the receptor [11]. Redocking uses the command file dock_runlocal.mcr with runs=250, Amber14 force field and X= 24.11, Y= 24.11, Z= 24.11 for define simulation cell. The parameter used is RMSD with a value of <2 Å [14]. Molecular docking follows the same steps as the validation protocol but uses a pre-prepared test ligand [32], [33].

2.4 Data Analysis and Visualisation

The types of interactions that are formed can be seen using the BIOVIA Discovery Studio software in 2D [15]. PyMOL is used to visualise the docking results in 3D.

Table 1 Black cumin seed alkaloid compounds used in the study [16]

CID	Name	Molecular Formula
5283349	(2E,4E)-Decadienal	$C_{10}H_{16}O$
6427087	(2E,4Z)-Decadienal	$C_{10}H_{16}O$
11402337	Nigellicine	$C_{13}H_{14}N_2O_3$
136828302	Nigellidine	$C_{18}H_{18}N_2O_2$
20725	Nigellimine	$C_{12}H_{13}NO_2$

3 Results and Discussion

The entire structure of the test ligand to be simulated was from PubChem, which was downloaded in .sdf format, then prepared and saved in .pdb format using the YASARA structure. YASARA structure is excellent for molecular modelling, virtual screening, molecular docking, and molecular dynamics [18], [19], [24], [26] – [28]. Molecular docking was done using five test ligands from the black cumin seed alkaloid compounds against PLpro SARS-CoV-2 as a target to inhibit its activity. PLpro is inhibited because it has a vital role in forming functional replicase complexes in SARS-CoV-2 [20].

The parameters used in molecular docking are the interacting amino acids, the types of interactions, and the Gibbs free energy [15].

Validation using VIR251 as the native ligand by redocking shows the Gibbs free energy of -2.52 kcal/mol, while the RMSD has a value of 0.9709 Å. The RMSD value is declared valid because the Root Mean Square Deviation (RMSD) is a parameter used to view and compare the position of atoms between the crystallographic structure (native) and the redocked. RMSD can be used if it has a value of <2 Å [14]. If the RMSD value is close to 0 Å (smaller), then the prediction shows that the pose of the ligand has a good level of similarity according to the native pose [14].

The Gridbox is an area that can limit the ligand's interaction with the target enzyme so that when a ligand is docked, it can be fixed to the active site area of the target enzyme. Papain-Like Protease (PLpro) SARS-CoV-2 has a catalytic triad, which are Cysteine 111 (Cys111), Histidine 272 (His272), and Aspartic acid (Asp286). Zinc ions are located at the finger sub-domain. This Zinc ion is coordinated by residues Cys189, Cys192, Cys224, and Cys226. Zinc binding is considered vital because it has a role in structural integrity and protease activity [5].

Based on table 2 shows the results of the bonding energy between five test ligands and PLpro. Nigellidine (CID 136828302) has a Gibbs free energy of -2.67 kcal/mol, thus making the ligand has the largest free energy value than the other four ligands. When compared with the Gibbs free energy value of the native ligand (VIR251), -2.52 kcal/mol, Nigellidine still has a higher Gibbs free energy. This shows good

results. In addition, 2E,4Z-Decadienal (CID 6427087), which was second-best, has a close Gibbs free energy value with the native ligand (VIR251). Nigellicine (CID 11402337) was the ligand with the lowest Gibbs free energy value of 1.55 kcal/mol.

Table 2 Docking results of alkaloid compounds against SARS-CoV-2 PLpro

CID	Ligand	Gibbs free energy (kcal/mol)
5283349	(2E,4E)-Decadienal	-0.65
6427087	(2E,4Z)-Decadienal	-2.35
11402337	Nigellicine	1.55
136828302	Nigellidine	-2.67
20725	Nigellimine	-1.09

Docking score (Gibbs free energy) is a parameter to determine the strength of the ligand-receptor bond. If the value gets smaller (negative), the stronger the bond between the test ligand and the receptor [21]. The results from the molecular docking of alkaloid compounds against PLpro showed that Nigellidine had great strength to interact with the SARS-CoV-2 PLpro target receptor. This is parallel with the research of [17], which states that the selected Gibbs free energy is the one that has the most negative value because it has a strong interaction. Binding affinity measures a drug's ability to bind to a receptor. Suppose the value obtained in the simulation is getting smaller (negative). In that case, the affinity between the receptor and the ligand is higher, and vice versa; if the value leads to a positive result, the affinity is lower [17]. The molecular docking results were 3D visualised using PyMOL, and BIOVIA Discovery Studio for 2D. The 2D and 3D visualisation can be seen in Figure 1-5.

All ligands are expected to bind to the catalytic triad of PLpro (Cys111, His272, and Asp286). Therefore, an analysis is needed through visualisation using BIOVIA Discovery Studio software to display the types of interactions and the contacting amino acid residues involved in the ligand-receptor binding [15], [25].

The simulation results visualised using BIOVIA Discovery Studio are shown in Table 3, showing the types of interactions, contacting residue and the distances. The test ligand with the highest Gibbs free energy, Nigellidine, has a van der Waals interaction type on the amino acid residues Lys157, Glu161, Tyr264, Gly163, Gln269, Tyr268 and an alkyl interaction type on the amino acid residue Leu162 (Figure 4). However, the ligands do not have direct contacting residue with the catalytic triad. The test ligand that has contact with the catalytic triad is Nigellicine. Nigellicine has various interactions, including van der Waals at the active His272, conventional hydrogen bonds, carbon hydrogen bonds, Pi-Sulfur, Pi-Lone Pair, Alkyl and Pi-Alkyl (Figure 3). The contact residue on the active site is found in Pi-Sulfur, Cys111. (2E,4E)-Decadienal only has a van der Waals type of interaction with the Leu162 and Gln269 residue (Figure 1). (2E,4Z)-Decadienal has a van der Waals type of bond with residues Gly163, Asp164, Glu167, Tyr264, Tyr268, Gln269, conventional hydrogen bonds with residues Arg166, and Alkyl with residues Leu163 (Figure 2). Nigellidine has two types of interactions: van der Waals and Alkyl, with residues contacting Lys 157, Glu161 and Leu162, respectively. Nigellimine has three types of interactions: Van der Waals, alkyl, and pi-Alkyl, with residues contacting Asn267 and Cys270 (Figure 5).

The interactions formed between the test ligands and the PLpro target receptor were van der Waals, conventional hydrogen bonds, hydrogen carbon bonds, Pi-Sulfur, Pi-Lone Pair, Alkyl and Pi Alkyl. The van der Waals interactions bind to the remaining residues [19].

The presence of ligand binding residue to the active site of the amino acid residue of the target molecule indicates the possibility that the ligand can deliver functional modulation to the target molecule. Other studies have also shown that the test ligand bound to the active site can cause functional changes in the target molecule [22], [29], [32], [34].

Table 3 Types of interaction

CID	Ligand	Types of interaction	Contact Residue Amino Acid	Description
5283349	(2E,4E)-Decadienal	Van der waals	Leu162, Gln269	Figure 1
6427087	(2E,4Z)-Decadienal	Van der waals	Gly163, Asp164, Glu167, Tyr264, Tyr268 , Gln269	Figure 2
		Conventional hydrogen	Arg166	
		Alkyl	Leu163	
11402337	Nigellicine	Van der waals	Asn110, Ala107, **His272**, Tyr112, Gln269 Cys270	Figure 3
		Conventional hydrogen	Gly271	
		Hydrogen carbon	Cys270 .	
		Pi-Sulfur	**Cys111**	
		Pi-Lone Pair	Asn109	
		Alkyl	Trp106	
		Pi-Alkyl	Trp106	
136828302	Nigellidine	Van der waals	Lys157, Glu161, Tyr264, Gly163, Gln269, Tyr268	Figure 4
		Alkyl	Leu162	
20725	Nigellimine	Van der waals	Asn267	Figure 5
		Alkyl	Cys270	
		Pi-Alkyl	Cys270	

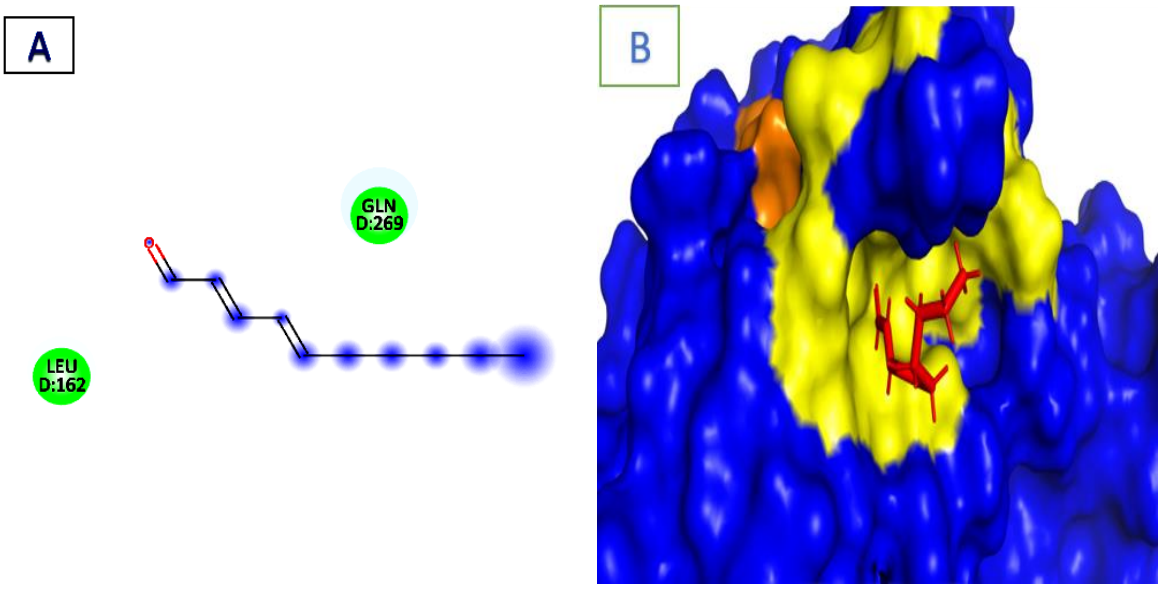

Figure 1. (A). (2E,4E)-Decadienal 2D Visualisation. (B) (2E,4E)-Decadienal 3D Visualisation.

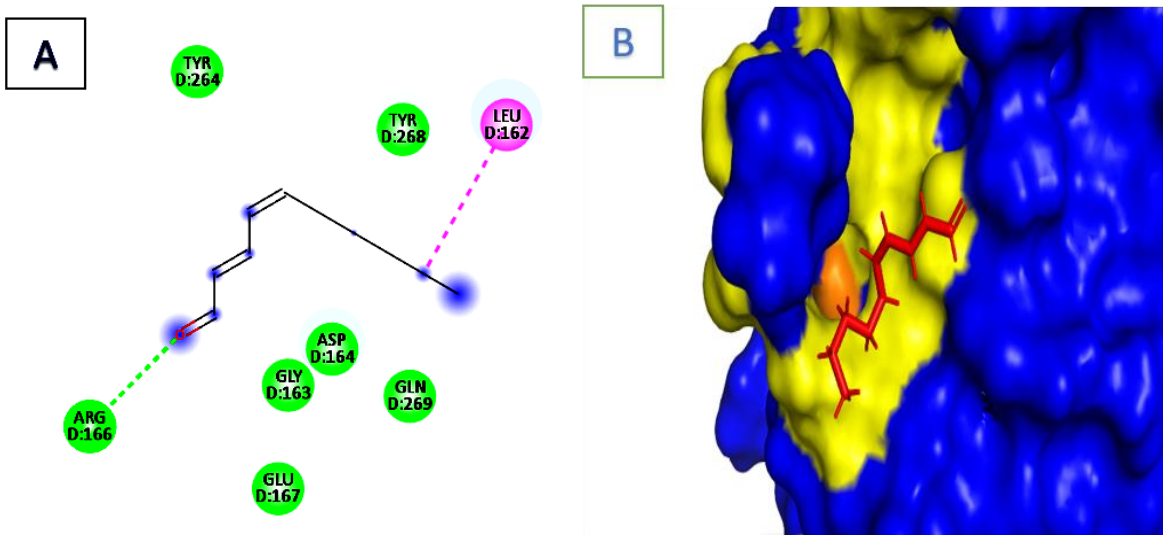

Figure 2. (A). (2E,4Z)-Decadienal 2D Visualisation. (B) (2E,4Z)-Decadienal 3D Visualisation.

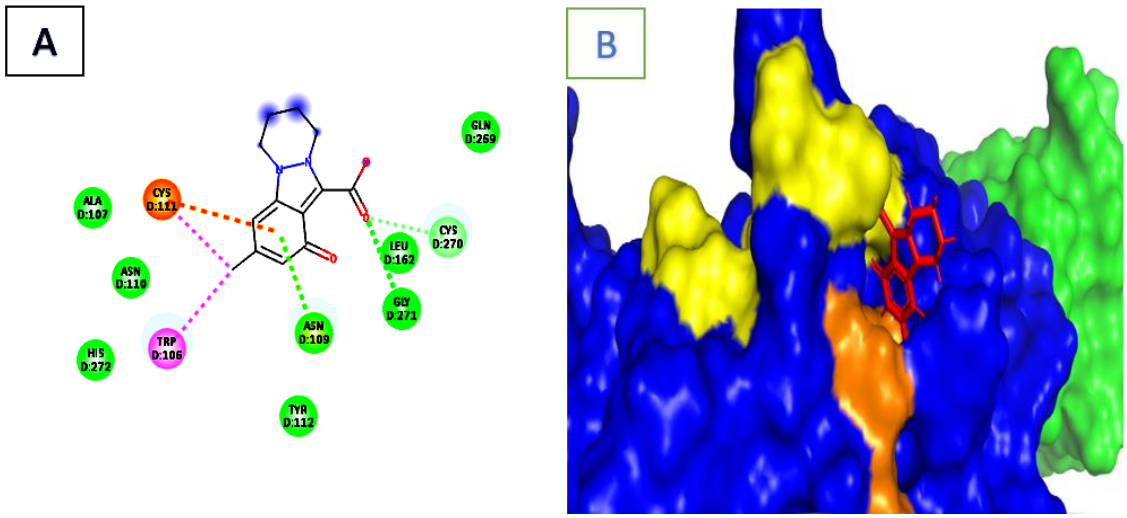

Figure 3. (A). Nigellicine 2D Visualisation. (B) Nigellicine 3D Visualisation.

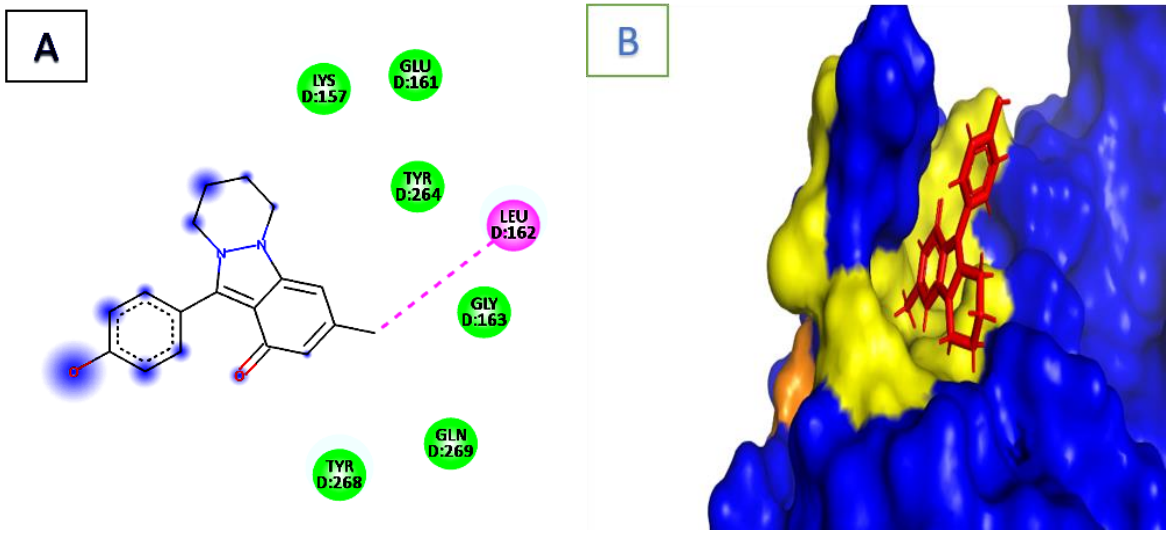

Figure 4. (A). Nigellidine 2D Visualisation. (B) Nigellidine 3D Visualisation.

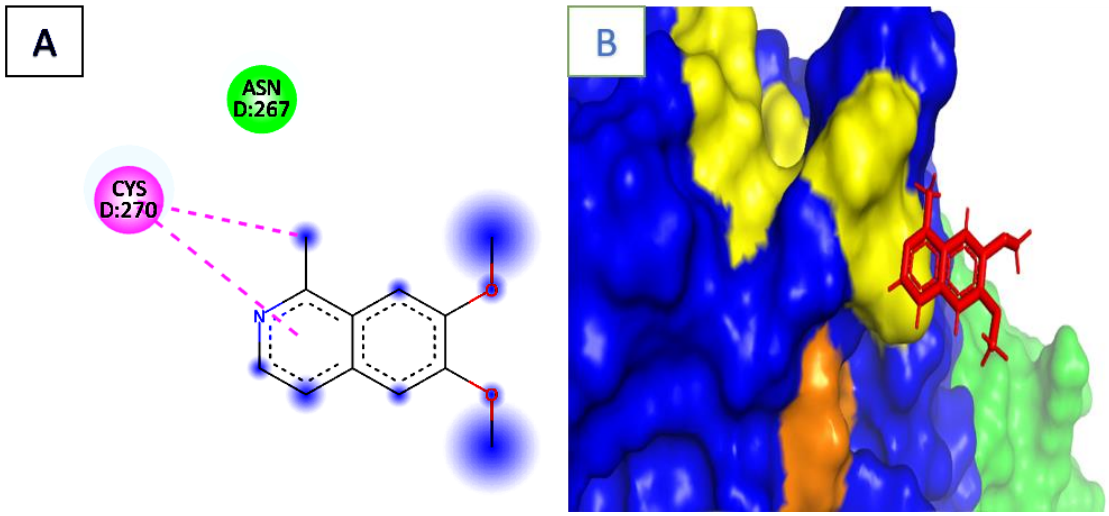

Figure 5. (A). Nigellimine 2D Visualisation. (B) Nigellimine 3D Visualisation.

4 Conclusions

Based on the results of the research that has been carried out, the largest Gibbs free energy is found in the Nigellidine test ligand at -2.67 kcal/mol. These results even beat the native ligand Gibbs free energy (VIR251) of -2.52 kcal/mol, but the test ligand that interacts directly with the active site is Nigellicine. All ligands form multiple interaction types, including van der Waals, conventional hydrogen bonds, carbon hydrogen bonds, Pi-Sulfur, Pi-Lone Pair, Alkyl and Pi-Alkyl.

5 Declarations

5.1 Author Contributions

Writing-original draft, review, editing, concept: GMG, IAF., experimental: GMG, IAF., data collection, analysis: GMG., interpretation, literature research: GMG, IAF., All authors have read and approved the final manuscript.

5.2 Ethical Clearance

None.

5.3 Conflict of Interest

The authors declare no conflict of interest.

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