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EVALUATION OF ANTIMALARIAL ACTIVITY OF 4-METHOXYBENZOYL NEOLIGNAN DERIVATIVES THROUGH A MOLECULAR DOCKING STUDY AGAINST CHLOROQUINE-SENSITIVE AND -RESISTANT PROTEINS

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ABSTRACT
In the present work, a molecular docking approach was conducted to investigate the antimalarial activity of neolignan derivatives with 4-methoxybenzoyl substituent (N1-N10) against chloroquine-sensitive and -resistant proteins. It was found that the neolignan derivatives were able to interact with the amino acid residues of Ala16, and Ser167/Tyr170 in the active site of the chloroquine-sensitive protein. On the other hand, the interactions of the neolignan derivatives with Arg59, Arg122, and Phe116/Ser120 residues were observed in the active site of the chloroquine-resistant protein. Most neolignan derivatives had stronger interaction than the native ligands in the active sites of both proteins. Among the evaluated neolignan derivatives, N9 and N8 compounds gave the strongest binding energy against the active sites of chloroquine-sensitive and -resistant proteins, respectively. These findings demonstrate that the presence of the nitro group in the neolignan structure is crucial for its inhibitory activity against chloroquine-sensitive and -resistant proteins.
Keywords: Neolignan, Molecular Docking, Antimalarial, Chloroquine, Protein.
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INTRODUCTION

Currently, malaria is still considered one of the most harmful parasitic diseases in Indonesia.[1-4] Andrews *et al.* stated that around 0.8–1.2 million annual deaths were caused by malaria disease; thus, highly efficient antimalarial drugs are crucially needed in Indonesia.[5] Chloroquine is an approved antimalarial drug by the World Health Organization, unfortunately, several cases of chloroquine resistance have been reported making the world's health condition worse.[6-9] These serious problems in malaria treatment motivate the researchers to design and develop more active antimalarial agents either from natural product isolation or an organic synthesis process. Researchers have put their attention on the identification of molecular targets for the development of antimalarial agents. One of the most representative targets is the cysteine protease of *Plasmodium falciparum* (*P. falciparum*), falcipain-2 (FP2), which is reported as an important enzyme for the *P. falciparum* replication.[10] Several studies reported that chalcones inhibited FP2 protein.[11] However, chalcone derivatives are very toxic for normal cells; thus, a real application of chalcones for malarial therapy is not available.[12,13] In contrast, neolignans are highly active as antimalarial agents even at nanomolar concentrations, which is remarkable. The neolignans are commonly derived from the oxidative homo- or cross-coupling reaction of C6-C3 units.[14] The natural neolignans with methoxy groups are not toxic to normal cells; therefore, they have been considered promising antimalarial prototypes.[15-18] In an attempt to identify that the non-toxic FP2 inhibitors retain high antimalarial activity, novel neolignan derivatives with methoxy groups were designed based on their structural similarity with chalcones in this study. A computational study is an efficient preliminary assay for antimalarial drug development.[19-21] Molecular docking is an efficient, effective, and rapid process; thus, repeated trials and errors in the laboratory could be greatly minimized.[22-27] The molecular docking study can predict the preferred orientation of ligands as well as their interactions with the amino acid residues in the active site proteins.[28]

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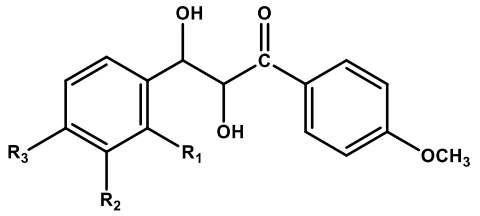
The obtained data from the molecular docking study are useful to determine the free binding energy (ΔG) between the ligand and the targeted protein. A lower ΔG value between ligand and protein receptor demonstrates higher antimalarial activity.[29,30] Molecular docking is also preferable to predict the antimalarial activity of ligands with sensitive- and resistant-dihydrofolate reductases–thymidylate synthase (PfDHFR-TS) proteins. However, a molecular docking study has not yet been performed for neolignans with methoxy substituents against chloroquine-sensitive and -resistant proteins. Therefore, in this work, we reported a molecular docking study of the neolignan derivatives with a 4-methoxybenzoyl substituent as a preliminary stage of the antimalarial drug development process. Ten neolignan derivatives (N1-N10) were evaluated against chloroquine-sensitive and -resistant protein active sites to understand the role of neolignan structure and its substituents on their antimalarial activity.



Compound	-R_1	-R_2	-R_3
N1	-H	-H	-H
N2	-H	-OCH_3	-OH
N3	-H	-OCH_3	-OCH_3
N4	-H	-H	-OCH_3
N5	-H	-H	-OH
N6	-H	-H	-Cl
N7	-H	-H	-F
N8	-H	-NO_2	-H
N9	-H	-H	-NO_2
N10	-Cl	-H	-Cl

Fig.-1: The Chemical Structure of Ten 4-Methoxybenzoyl Neolignan Derivatives in this Study

EXPERIMENTAL

The chemical structures of ten neolignan compounds were designed using Chemdraw Professional 15.0 software as shown in Fig.-1. The molecular docking study was performed using GaussView 5.0.8 and Gaussian 09W software to build and optimize the three-dimensional (3D) structure of neolignan derivatives. On the other hand, the preparation of native ligands and target proteins and the visualization of their interactions were conducted using UCSF Chimera and AutoDock 4.2 software, respectively. Molecular docking was conducted against dihydrofolate reductases–thymidylate synthase (PfDHFR-TS) protein with Protein Data Bank ID of 1J3I.pdb and 4DP3.pdb as chloroquine-sensitive and -resistant proteins, respectively. The 3D structures of neolignan derivatives were drawn and optimized by using Gaussian 09W software with DFT/B3LYP method using 6-31+G(d,p) as the basis set. Meanwhile, the preparations of native ligands and proteins were conducted using UCSF Chimera software. In this work, 6,6-dimethyl-1-(3-(2,4,5-trichlorophenoxy)propoxy)-1,6-dihydro-1,3,5-triazine-2,4-diamine and 3-(2-(3-((2,4-diamino-6-ethylpyrimidin-5-yl)oxy)propoxy)phenyl)propanoic acid were employed as the native ligands in the active site of chloroquine-sensitive and -resistant proteins, respectively. The native ligands and proteins were separately saved in the .pdb format. The 3D grid box with a 40 Å grid size (x,y,z) and spacing of 0.375 Å were created on the macromolecule Cartesian coordinates. The grid maps were utilized to represent the intact ligand in the actual docking target site. During the molecular docking study, the position of the ligand was flexibly maintained while the protein was kept in its rigid form. The root means square deviation (RMSD) value was maintained to be less than 2 Å in the molecular docking analysis.[31] The ΔG values of the native ligands and neolignans after being docked in the active site of the protein target were determined using AutoDock 4.2 software. One hundred independent docking conformations were set for each analysis and then Discovery Studio Visualizer 2019 software (Accelrys, Inc., San Diego, USA) was used to visualize the chemical interactions between the ligands and proteins.

RESULTS AND DISCUSSION

Molecular Docking Study

Neolignan derivatives were designed through two consecutive chemical reactions of 4-methoxychalcones, *i.e.*, epoxidation and hydrolysis reactions. The chemical structure of ten neolignan derivates (Fig.-1) was designed to evaluate the effect of the substituents in R_1, R_2, and/or R_3 position on their ΔG values in two

types of proteins, *i.e.*, chloroquine-sensitive and -resistant proteins. The investigation of the interactions of the neolignans in the active site of the targeted proteins was performed to understand the potential application of neolignan derivatives as potential antimalarial agents. Computational results in ΔG values and interactions of neolignan derivatives are listed in Table-1 for chloroquine-sensitive protein. Based on the binding energy value in Table-1, all neolignans exhibit a lower ΔG value than 6,6-dimethyl-1-(3-(2,4,5-trichlorophenoxy)propoxy)-1,6-dihydro-1,3,5-triazine-2,4-diamine as the native ligand for chloroquine-sensitive protein. The main reason was due to the high antimalarial activity of the neolignan framework which was in agreement with the reported experimental data.[15-18] The addition of a fluoro substituent as an electron-withdrawing group in compound N7 did not change the ΔG value of neolignan N1. However, the presence of other substituents such as hydroxyl (N2 and N5) and methoxy (N2-N4) as electron-donating groups, as well as chloro (N6 and N10) and nitro (N9) as electron-withdrawing groups decreased the ΔG value of neolignan N1; thus, consequently increased the antimalarial activity of neolignans. The N9 compound (ΔG = -7.55 kcal/mol) exhibited the lowest ΔG value in comparison to the native ligand (ΔG = -5.44 kcal/mol) and other neolignan derivatives (ΔG = -6.47 to -7.41 kcal/mol). It means that the presence of nitro substituent as an electron-withdrawing group is pivotal for the antimalarial activity of neolignan against the chloroquine-sensitive protein.

Table-1: ΔG Value and Interaction of Ten 4-Methoxybenzoyl Neolignans and Native Ligand in the Active Site of Chloroquine-Sensitive Protein

Compound	RMSD (Å)	ΔG value (kcal/mol)	Hydrogen bond interaction	Other binding interactions with amino acid residue
N1	1.99	-6.74	Ala16; Tyr170	π-alkyl: Ala16; Leu40
N2	1.43	-7.09	Ala16; Tyr170	π-alkyl: Leu40; Val195 π-σ: Ala16
N3	1.50	-7.30	Ala16; Ser167; Tyr170	π-alkyl: Leu40; Leu46 π-π T-shaped: Phe58 π-σ: Ala16
N4	0.94	-7.05	Ala16; Tyr170	π-alkyl: Leu40 π-σ: Ala16
N5	1.72	-7.00	Ala16; Tyr170	π-σ: Ala16
N6	0.45	-7.25	Ala16; Tyr170	π-alkyl: Ile14; Ala16; Val195 Amide-π stacked: Cys15; Leu40
N7	0.52	-6.74	Ala16; Tyr170	π-alkyl: Ile14; Ala16 π-π T-shaped / Amide-π stacked: Cys15; Leu40; Phe58 Halogen: Val45
N8	1.25	-6.47	Ile14; Ile164; Ser167	π-alkyl: Ala16; Leu46 π-π T-shaped: Phe58 π-sulfur: Met55 π-anion: Asp54
N10	1.41	-7.41	Ala16; Ile164; Tyr170	π-alkyl: Ile14; Ala16; Leu46; Trp48; Tyr57 π-π T-shaped / Amide-π stacked: Cys15; Phe58

Additionally, molecular docking results in ΔG values and interactions of neolignan derivatives are shown in Table-2 for chloroquine-resistant protein. Different from the results of chloroquine-sensitive protein, only four neolignans, *i.e.*, N1 (ΔG = -7.17 kcal/mol), N6 (ΔG = -7.08 kcal/mol), N8 (ΔG = -9.24 kcal/mol), and N9 (ΔG = -7.93 kcal/mol) gave a lower ΔG value than 3-(2-(3-((2,4-diamino-6-ethylpyrimidin-5-yl)oxy)propoxy)phenyl)propanoic acid (ΔG = -6.99 kcal/mol) as the native ligand (Table-2) against chloroquine-resistant protein. This result was observed due to the higher stability of the chloroquine-resistant protein in comparison to the chloroquine-sensitive protein.[9] The presence of hydroxyl (N2 and N5) and methoxy (N3-N4) substituents as an electron-donating group, as well as fluoro (N7) substituent as an electron-withdrawing group in the left aromatic ring, increased the ΔG value of neolignan. In contrast, either chloro (N6, ΔG = -7.08 kcal/mol) or nitro (N9, ΔG = -7.93 kcal/mol) substituent as an electron-

withdrawing group at the para-position increased the binding ability of neolignan derivatives in the active sites of chloroquine-resistant protein, which was remarkable. The presence of nitro substituent at the meta-position (N8, ΔG = -9.24 kcal/mol) exhibited the lowest ΔG values among the other evaluated neolignans (ΔG = -5.74 to -7.93 kcal/mol) in this work. Furthermore, the ΔG value of the N8 compound (ΔG = -9.24 kcal/mol) was 1.32 times lower than the native ligand (ΔG = -6.99 kcal/mol), which was remarkable.

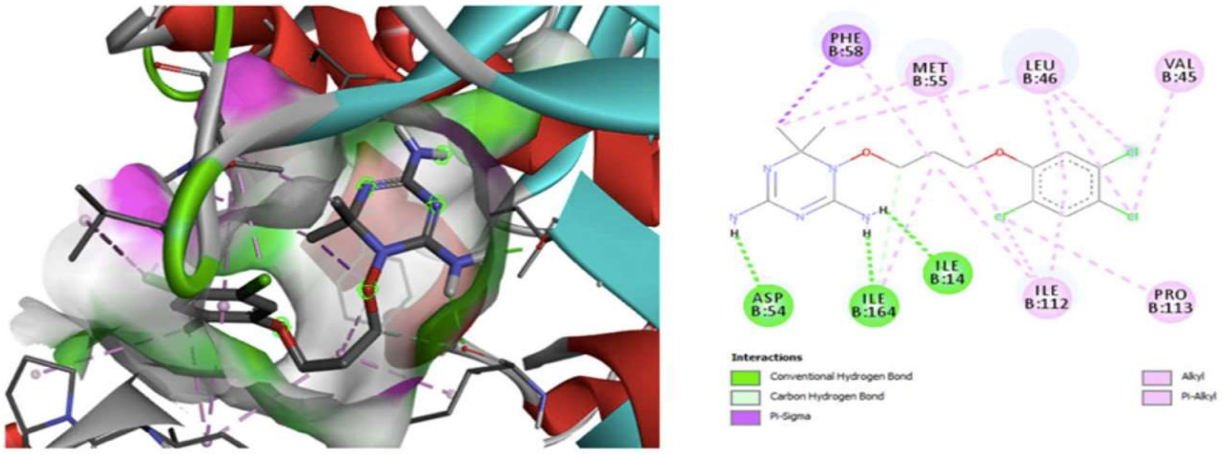
Table-2: ΔG Value and Interaction of Ten 4-Methoxybenzoyl Neolignans and Native Ligand in the Active Site of Chloroquine-Resistant Protein

Compound	RMSD (Å)	ΔG value (kcal/mol)	Hydrogen bond interaction	Other binding interactions with amino acid residue
N1	1.24	-7.17	Arg59; Phe116; Ser120; Arg122	π-alkyl: Ile112 π-sulfur: Met55 π-σ: Leu119
N2	1.33	-6.60	Arg59; Lys115; Phe116; Arg122	π-alkyl: Ile112 π-sulfur: Met55 π-σ: Leu119
N3	1.67	-5.74	Arg59; Ser120; Arg122	π-alkyl: Met55; Ile112 π-π stacked: Phe116
N4	1.15	-6.02	Arg59; Ser120; Arg122	π-alkyl: Ile112 π-σ: Met55; Phe116; Leu119
N5	1.63	-5.89	Arg59; Lys115; Phe116; Ser120; Arg122	π-σ: Met55; Ile112; Leu119
N6	0.53	-7.08	Arg59; Ser120; Arg122	π-σ: Met55; Ile112; Leu119
N7	1.28	-5.75	Arg59; Ser120; Arg122	π-alkyl: Met55; Ile112 π-π stacked: Phe116 π-σ: Ser120
N9	0.50	-7.93	Asn108; Arg122	π-alkyl: Ile14; Ile112; Leu164 π- π stacked: Phe58 π-sulfur: Met55 π-σ: Leu119
N10	1.32	-6.48	Arg59; Phe116; Ser120; Arg122	π-alkyl: Ile112; Phe116 π-σ: Met55; Leu119

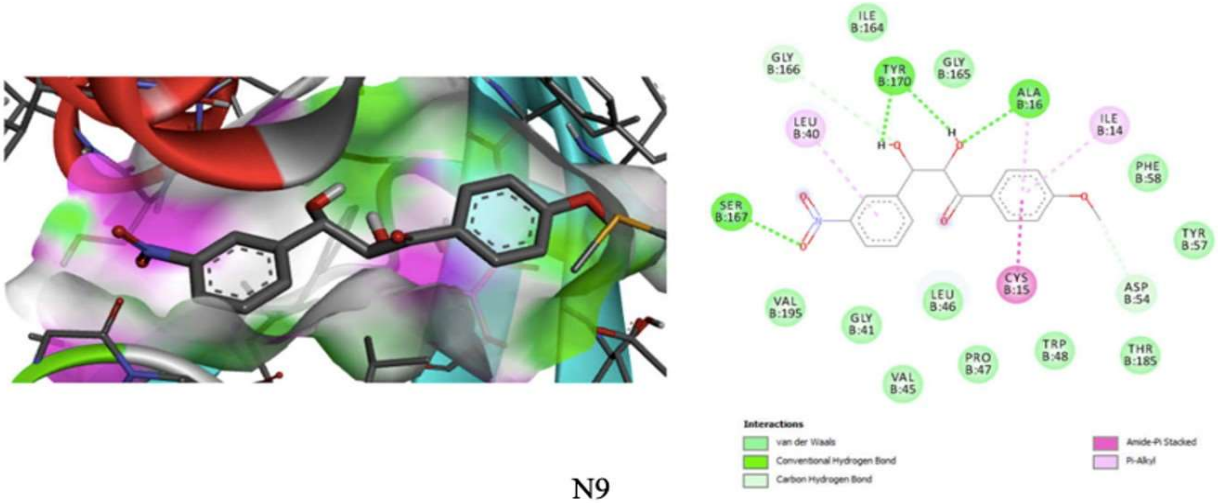
Visualization of the interactions between nine neolignan derivatives in the active sites of chloroquine-sensitive protein is shown in Fig.-1. Meanwhile, the visualization of the intermolecular interactions between the most active neolignans and native ligands in the active sites of chloroquine-sensitive protein is shown in Fig.-2. All neolignans exhibited a similar binding interaction with amino acid residues in the active site of the chloroquine-sensitive protein, such as Ala16, and Ser167/Tyr170 except for the N8 compound. Figure-2 shows that the N9 compound interacts with Ala16, Ser167, and Tyr170 residues. Yuvaniyama *et al*. reported that Tyr170 amino acid residue was critical for antimalarial activity.[32] Therefore, it is reasonable that the N9 compound exhibited the highest antimalarial activity in the present work as reflected by the lowest ΔG value due to the hydrogen bond with Tyr170 residue which is absent for the native ligand.

On the other hand, visualization of the interactions between nine neolignan compounds with the active site of chloroquine-resistant protein is shown in Fig.-2. Meanwhile, the visualization of the formed interactions between the most active neolignan compounds, as well as native ligand, with the active site of chloroquine-resistant protein is shown in Fig.-3. All neolignans showed a similar binding interaction with amino acid residues in the active site of the chloroquine-resistant protein, such as Arg 59, Arg122, and Phe116/Ser120. Figure-3 shows the N8 compound exhibits the best antimalarial agent due to the lowest ΔG value. The N8 compound formed hydrogen bonds with Arg122, Arg59, and Ile51 residues. This result agreed with the previous report stating that the hydrogen bond with Arg122 was responsible for the inhibition of PfDHFR.[33] Also, the hydrogen bonding interaction with Ile51 was only found in the N8 compound and not found in the other neolignans and the native ligand. Therefore, the hydrogen bonding interaction with Ile51 seems to play a pivotal role in lowering the ΔG value of neolignan derivatives in the active site of the chloroquine-resistant protein. Further investigation is still required to elucidate this phenomenon through molecular

dynamics simulation. Finally, it is worthy to note that 4-methoxybenzoyl neolignans are promising antimalarial candidates against both chloroquine-sensitive and -resistant proteins.

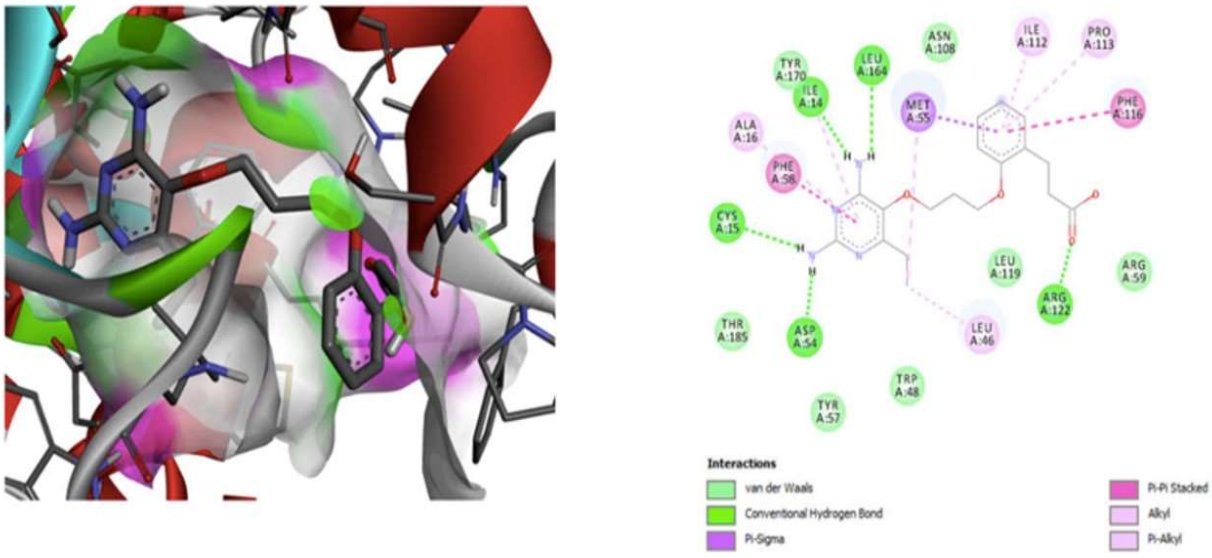


Native Ligand

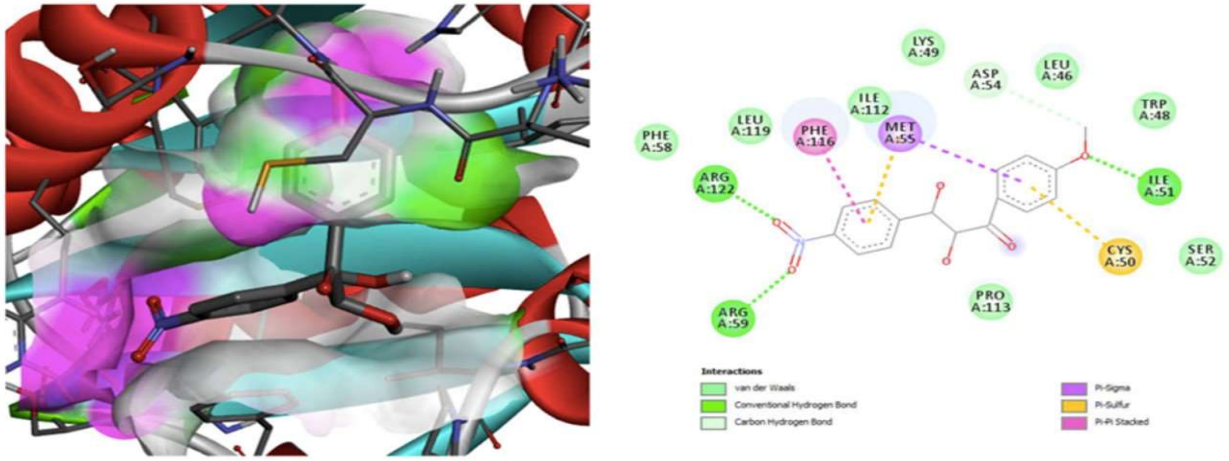


N9

Fig.-2: Visualization of the Formed Interactions between Native Ligand and N9 Neolignan Derivative in the Active Site of Chloroquine-Sensitive Protein



Native Ligand



N8

Fig.-3: Visualization of the Formed Interactions Between Native Ligand and N8 Neolignan Derivative in the Active Site of Chloroquine-Resistant Protein

CONCLUSION

A molecular docking study of ten neolignans with 4-methoxybenzoyl moiety against chloroquine-sensitive and resistant proteins has been carried out in this work. Most of the neolignans exhibited a stronger interaction in the proteins' active sites as shown by their lower binding energy value than the native ligands. It is interesting to note that all neolignan derivatives in this work are able to interact with Ala16 and Ser167/Tyr170 residues through hydrogen bonds in the active sites of the chloroquine-sensitive protein. The lower binding energy was observed because all neolignans exhibited a similar chemical interaction with Arg 59, Arg122, and Phe116/Ser120 residues in the active sites of the chloroquine-resistant protein. Compounds N8 and N9 with a nitro substituent exhibited the most active antimalarial activity demonstrating that these neolignans are promising antimalarial candidates to be evaluated through molecular dynamics simulation and further experimental assays.

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