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Original Article

NATURAL ISOTHIOCYANATE ANTI-MALARIA: MOLECULAR DOCKING, PHYSICOCHEMICAL, ADME, TOXICITY AND SYNTHETIC ACCESSIBILITY STUDY OF EUGENOL AND CINNAMALDEHYDE

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ABSTRACT

Objective: This study aims to evaluate novel compounds of isothiocyanate (ITC) based on eugenol and cinnamaldehyde derivatives as the drug candidate of *Plasmodium falciparum* anti-malaria using *in silico* method, physicochemical, pharmacokinetics, toxicity, and synthetic accessibility prediction. This present study also describes molecular docking and pharmacoinformatics of natural ITC in *Moringa oleifera* leaves.

Methods: A series of novel ITC compounds (3, 5, and 6) were designed and analyzed with a series of natural ITC compounds (7, 8, 9, 10) for *P. falciparum* anti-malaria. This research is descriptive qualitative and uses the reverse molecular docking method, proving the biological activity of compounds theoretically using software and database information.

Results: Molecular docking study showed that compound 6 exhibits binding affinity (-5.3 Kcal/mol) on Van der Waals interaction with the residual active site (His159, Cys25) of cysteine protease. All designed ITC compounds are obeyed the Lipinski and Veber Rule, have a well-brain penetrant character and have a medium risk for mutagenic, tumorigenic, and reproductive prediction. They are also in the simple rate of synthetic accessibility (SA) estimation. In regards to natural ITCs, they all have better assay characteristics except the SA.

Conclusion: Molecular docking, physicochemical, pharmacokinetic, and toxicity studies show that methyl eugenol isothiocyanate and cinnamaldehyde isothiocyanate are promising anti-malaria compounds. Substituents of hydroxy, acetate and tetrahydropyran groups in the building block ring are suggested for better *in silico* profiles enhancement.

Keywords: Isothiocyanate, Eugenol, Cinnamaldehyde, *Plasmodium falciparum*, Cysteine protease

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INTRODUCTION

Plasmodium falciparum malaria is a mosquito infection disease in erythrocytes that demands public attention [1–3]. Malaria long-term treatment using multidrug such as chloroquine (C) and sulfadoxine-pyrimethamine causes drug resistance [4–8], increased morbidity, mortality, and health care costs [9]. The discovery of new, better bioactivity and lower toxicities drugs is focused on the abundant and renewable natural building blocks.

Active anti-malaria compound discovery has been carried out from natural sources, for instance, *Eleutherine bulbosa* [10] and *Andrographis paniculate* [11]. Eugenol and cinnamaldehyde are major components in clove essential oil (EO) and cinnamon EO. Clove (*Syzygium aromaticum (L) Merr. Et Perry*) and Cinnamon (*Cinnamomum burmanii*) plants can thrive in Indonesia and other tropical countries. The clove EO has various bioactivity potentials including antimicrobial [12–14], antifungals [15–17], anticancer [18], antiprotozoal [19], analgesic [20] and anti-inflammatory [21–23]. At the same time, cinnamaldehyde derived from cinnamon EO has antiproliferative, antibacterial, and antimicrobial activity [24–26]. This bioactivity predicts influenced by eugenol ($C_{10}H_{12}O_2$) and cinnamaldehyde (C_9H_8O) content as the major compound.

Molecular drug discovery is also focused on the functional group (FG) that has better bioactivity prediction. In some areas, people have used *Moringa oleifera* leaves for malaria treatment. It is known as a pearl of local wisdom on malaria therapy. Rhamnosyloxy benzyl isothiocyanates, the natural isothiocyanate found in *M. oleifera* leaves, are predicted responsible FG of anti-plasmodial [27]. Natural ITCs are known to have several bioactivities [28–38]. The double bond FG has partially positive and negative properties, and it is possible to carry out additional reactions into other FG. Eugenol and cinnamaldehyde have a double bond and aldehyde FG that building blocks potentially for ITC compounds. The bioactivity of eugenol-ITC and cinnamaldehyde-ITC derivatives as an anti-malaria is not known.

In the past, drug discovery was made with these categories such as time-consuming, trial and error process, high cost, and petroleum-based. Molecular docking and other bioinformatics techniques speed up the process of drug finding with better efficacy [39–41]. This recent study attempted to design ITC compounds from eugenol and cinnamaldehyde, identify its *falciparum* anti-malaria activities using *in silico*, and compare its prediction with chloroquine and natural ITC from *Moringa oleifera* leaves. This study will also briefly discuss the synthetic accessibility of ITC compounds.

MATERIALS AND METHODS

Preparation compound and receptor

The common structure of compounds (1, 2, 4, and C) was collected in fig. 1. Novel designed-ITC (3, 5, 6) compounds (fig. 1) and ITC derived from *Moringa oleifera* leaves (7, 8, 9, and 10) were constructed and converted to 3D by MarvinSketch (fig. 2). Receptor PDB ID 1YVB was obtained from the RCSB protein databank. All compounds were prepared into ligands by adding charge and hydrogen using the Chimera 1.13.1 program with the AMBERff12SB standard and Gesteiger method. The receptor was prepared and purified from water, native ligands, and its optimization was carried out by adding charge AM1-BCC and hydrogen. Molecular docking was done by PyRx and the grid dimension set as a center at x = 83.731069686; y =-36.3469508554, z =-92.6668557437 then size point of x, y and z as 25Å, 25 Å, and 25 Å respectively. Interaction of ligand with receptor was determined using affinity binding (Kcal/mol). The 3D complex structures were visualized through Discovery Studio Visualizer 2019 client.

Physicochemical, pharmacokinetics, and bioactivities analysis

Physicochemical, pharmacokinetics, and toxicities of ligands were examined respectively by Swiss ADME, Molinspiration, and Osiris.

RESULTS AND DISCUSSION

Preparation compound and receptor for molecular docking

Docking system validation was done by redocking the native ligand of the 1YVB chain A. The precision of the docking process was determined by RMSD *(Root Mean Standard Deviation)* value less than 2.0. All ligands have good validation at 0.0 . The molecular docking principle is based on ligand binding interactions with active amino acids in the receptors via the presence of hydrogen bonds, Van der Waals, and electrostatic interactions. Ligand and amino acids bond distance will affect the affinity energy (ΔG) or complex stability between ligand and receptor [42]. The smaller the bond distance, the better the value of the ligand-receptor complex affinity.

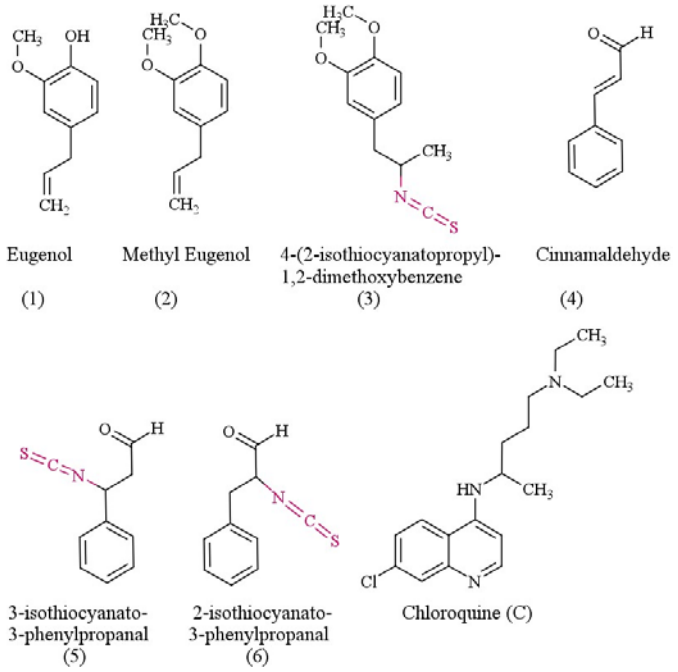
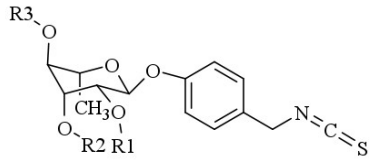


Fig. 1: Series of the building blocks (1, 2, 4), novel ITC (3, 5, 6), and chloroquine



(7): 4-(α-L-rhamnosyloxy)benzyl isothiocyanate (R1, R2, R3 = H)
(8): 4-(2'-O-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (R2, R3 = H; R1 = Acetate)
(9): 4-(3'-O-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (R1, R3 = H; R2 = Acetate)
(10): 4-(4'-O-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (R1, R2 = H; R3 = Acetate)

Fig. 2: Natural ITC compounds in *Moringa oleifera* leaves [27]

As starting material, eugenol has the same affinity with the cinnamaldehyde complex (table 1). However, OH-eugenol is predicted to inhibit the ITC group's entry, and methylation is required to form methyl eugenol (ME). It was showed that ME increased the stability complex and has a better affinity (AG =-5.2 Kcal/mol) than eugenol. Methyl eugenol isothiocyanate (ME-ITC) or 4-(2-isothiocyanatopropyl)-1,2-dimethoxybenzene has affinity -4.9 Kcal/mol. Inserting the ITC group into cinnamaldehyde resulted in 2 types of ITC prediction, namely 3-isothiocyanato-3-phenylpropanal (ligand 5) and 2-isothiocyanato-3-phenylpropanal (ligand 6). The affinity score of ligand 6 is better than its derivatives. However, both ME-ITC (ligand 3) and cinnamaldehyde-ITCs (ligand 5 and 6) could not achieve chloroquine affinity and natural ITC from *Moringa oleifera* leaves (ligands 7, 8. 9, and 10). In general, ligands 8 and 10 have better affinity than chloroquine. It is in accordance with the local tradition of using *Moringa oleifera* leaves as a traditional malaria medicine. Ligand 8 has the best energy affinity compared to chloroquine and other ligands (table 1).

Table 1: Binding affinities of complex 1YVB chain A with ligand

Ligand	Affinity ΔG (kcal/mol)	Ligand	Affinity ΔG (kcal/mol)	Ligand	Affinity ΔG (kcal/mol)
1	-5.1	5	-4.8	9	-5.8
2	-5.2	6	-5.3	10	-6.4
3	-4.9	7	-6.2	C	-6.3
4	-5.1	8	-6.6		

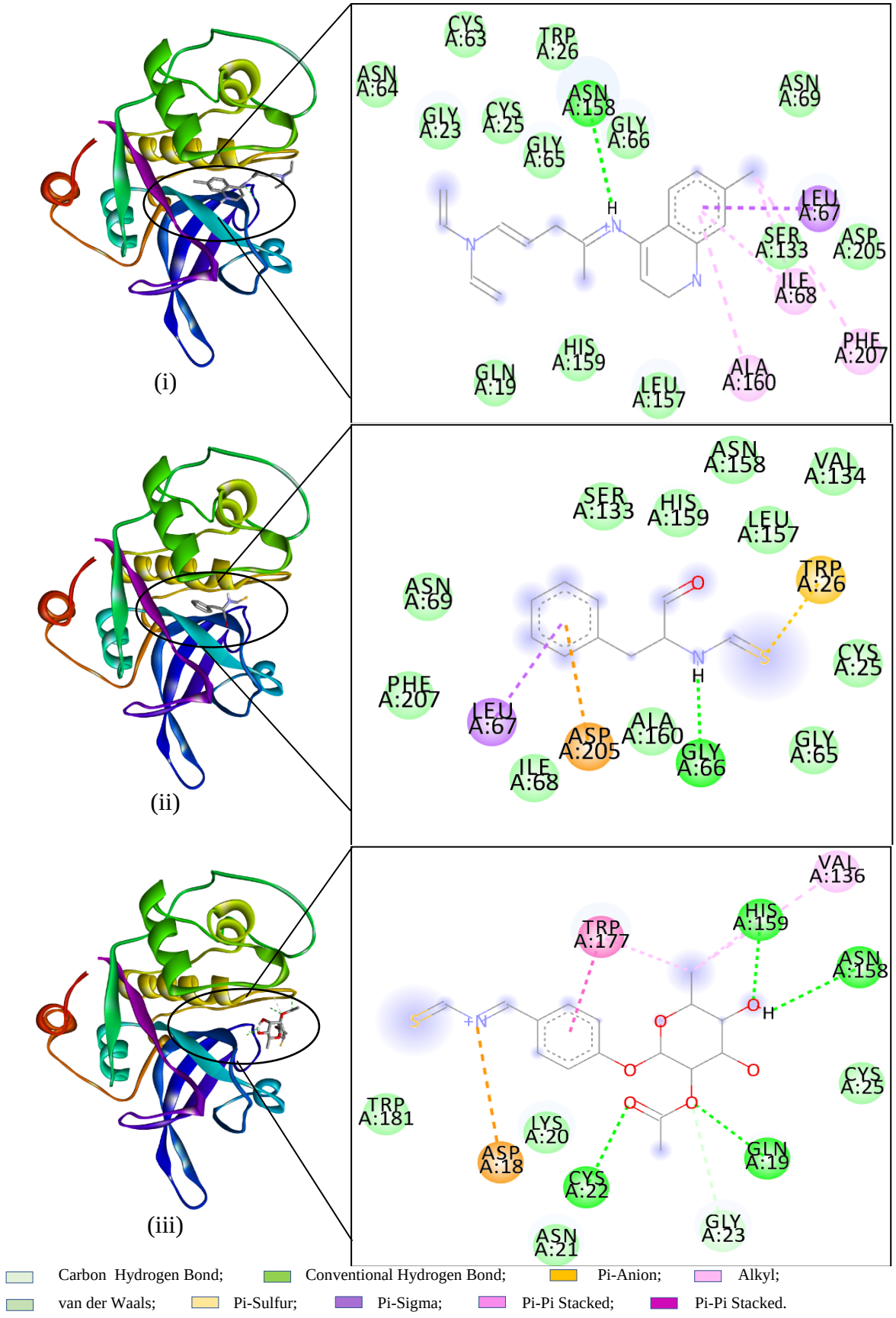


Fig. 3: The complex structure and 2D interaction of 1YVB chain A with: (i) chloroquine; (ii) ligand 6; (iii) ligand 8

Result docking analysis is should also notice the interaction between ligand with active site residue. His159 and Cys25 residues are the active sites in the surface layer of cysteine protease responsible for the proliferation of *falciparum* erythrocyte [43]. Ligand 6 and C make Van der Waals link at His159 and Cys25 with its affinities -5.3 Kcal/mol and -6.3 kcal/mol, respectively. Ligand 8, with the lowest affinity (-6.6 Kcal/mol), has hydrogen bond interaction between His159 and the hydroxy group of tetrahydropyran. The Cys25 forms. Van der Waals bonds around the sulfur double bond (fig. 3). Hydrogen bonds in the complex's residue ligand are much stronger than the Van der Waals; they stabilize the complex bonds and reduce affinity energy.

Physicochemical, pharmacokinetics, and bioactivities of compound

The physicochemical of drug candidates was measured by its properties covered by the Lipinski Rule of Five (RO5) and Veber Rule [44–46]. The n-octanol/water partition coefficient (Log P) is a parameter that determines the hydrophobicity of a compound. Drug compounds' hydrophilic/lipophilic properties affect drug absorption, drug-receptor interactions, molecular metabolism, and toxicity [47]. Topological Polar Surface Area (TPSA) is a predictor of drug transport properties such as intestinal absorption and penetration of the blood-brain barrier. TPSA deals with hydrogen bonds in compounds. The number of rotatable bonds (RB) measures the flexibility of the compound related to drug absorption and bioavailability. All the ligands obeyed Lipinski and Veber Rule (table 2).

Drug candidates should have a pharmacokinetics character such as ADMET (Absorption, Distribution, Metabolism, Elimination, and Toxicological) as an integral part of screening to get the promising drug candidates [48]. ADMET is covered in drug-likeness (properties and bioactivities). The bioactivity of a drug candidate can be determined by calculating the G-Protein-Coupled Receptor (GPCR) ligand score, ion channel modulator, nuclear receptor ligand, a kinase inhibitor, protease inhibitor, enzyme inhibitor [47]. Ligands' biological activity scores of more than 0.00 are recognized as active, and less than-0.50 are inactive [49]. The potential bioactivity of building block compounds (ligands 1, 2, and 4) and ITC designed ligands 3, 5, and 6 are moderately active. Furthermore, the native ITC in M. oleifera has a variation score around active and moderate (table 3).

Table 2: Physicochemical properties of ligands

Ligand	Lipinski rule*				Veber rule**	
	MW	HBA	HBD	LogP	RB	TPSA
1	164.20	2	1	2.37	3	29.46
2	178.23	2	0	2.65	4	18.46
3	237.32	3	0	2.95	5	62.91
4	132.16	1	0	1.65	2	17.07
5	191.25	2	0	2.14	4	61.52
6	191.25	2	0	2.06	4	61.52
7	311.36	6	3	2.71	4	123.60
8	353.40	7	2	2.99	6	129.67
9	353.40	7	2	2.50	6	129.67
10	353.40	7	2	1.85	6	129.67
C	319.88	2	1	3.95	8	28.16

*Lipinski rule: MW: Molecular weight ≤500g/mol, HBA: Hydrogen Bond Acceptors ≤10, HBD: hydrogen bond donors ≤5, LogP ≤5

**Veber rule: RB: Rotatable Bonds ≤10, TPSA ≤140

All ligands are equipped with gastrointestinal absorption and brain access assays via the Brain or IntestinaL EstimateD (Boiled-Egg) permeation method for predicting lipophilicity and polarity of the drug candidate. The white egg illustrates the physicochemical space of compounds with the highest absorbed probability by the gastrointestinal tract (GI absorption), and the yellow region (yolk) is the highest permeate space to the brain (BBB permeant). Well-absorbed compound (blue point), well-brain penetrant compound symbolized as pink point [50]. Analysis of all ligands shows that the building blocks (molecule 1, 2, and 4) have a well-brain penetrant character and are distributed in egg yolk (table 4). It also occurred to the ITC-designed ligands and chloroquine (molecule 11). However, natural ITC (molecules 7, 8, 9, and 10) are stacked at the egg white border and assumed to be absorbed by the gastrointestinal tract (fig. 4). Ligands 1 and 2 were observed in high-risk mutagenic, tumorigenic, and irritant categories, but ligand 4 was only tumorigenic toxic. Ligands 3 and 6 dominantly have medium-risk toxicity properties, and it is a promising drug for anti-malaria. Chloroquine has a high risk of mutagenicity and irritant. Natural ITC from *M. oleifera* leaves showed mutagenic, tumorigenic, and reproductive effects at medium risk (table 5).

Table 3: Bioactivities score of ligands

Ligand	GPCR	Ion channel modulator	Kinase inhibitor	Nuclear receptor	Protease inhibitor	Enzyme inhibitor
1	-0.86	-0.36	-1.14	-0.78	-1.29	-0.41
2	-0.81	-0.38	-1.06	-0.80	-1.14	-0.43
3	-0.19	-0.17	-0.84	-0.54	-0.58	0.10
4	-1.09	-0.39	-1.24	0.96	-0.79	-0.46
5	-0.56	0.01	-1.37	-0.91	-0.69	-0.04
6	-0.37	-0.10	-1.18	-0.55	-0.17	0.22
7	0.03	0.05	-0.40	-0.20	-0.11	0.49
8	0.07	-0.03	-0.48	-0.12	0.01	0.46
9	0.07	0.06	-0.40	0.05	0.00	0.51
10	0.07	0.06	-0.40	-0.05	0.00	0.51
C	0.32	0.32	0.38	-0.19	0.05	0.11

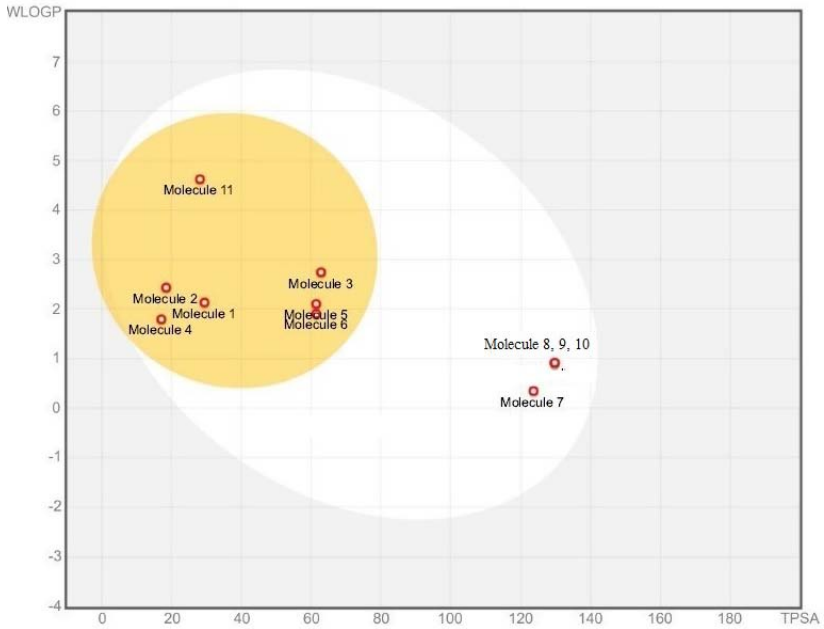


Fig. 4: Boiled-Egg model of all ligands: white area = GI absorption, yolk area = BBB

Synthetic Accessibility (SA) score is an estimation parameter of a drug designed to be synthesized on a laboratory scale. It was measured based on complexity, starting materials, or retrosynthetic-based [51]. SA score of ITC-designed ligands is between 2.14-2.51 for easily synthesized and natural-ITCs in 4.22–4.38 for moderately categories (table 4). The 8 compound has an OR group in the para position where R is a tetrahydropyran molecule containing an acetate group. The therapeutic activity of ITC is also influenced by aromatic and aliphatic side chains [28]. In this regard, in the design of eugenol-ITC and cinnamaldehyde-ITC for malaria drug purposes, the substitution of tetrahydropyran, hydroxy, or acetate groups in the building block rings should be recommended to determine better anti-malaria potential.

Table 4: Boiled-egg permeation and synthetic accessibility properties of ligands

Ligand	Pharmacokinetics			SA	Ligand	Pharmacokinetics			SA
	GIA	BBBp	P-gs			GIA	BBBp	P-gs	
1	H	Y	N	1.58	7	H	N	N	4.22
2	H	Y	N	1.71	8	H	N	N	4.22
3	H	Y	N	2.51	9	H	N	N	4.32
4	H	Y	N	1.65	10	H	N	N	4.38
5	H	Y	N	2.24	C	H	Y	N	2.76
6	H	Y	N	2.14					

*GIA: Gastrointestinal absorption, BBBp: Blood-brain barrier permeant, P-gs: P-*glycoprotein* substrate, SA: Synthetics accessibility, H: High, Y: Yes, N: No

Table 5: Toxicity prediction of ligands

Ligand	OSIRIS prediction				Ligand	OSIRIS prediction			
	MP	TP	IP	RP		MP	TP	IP	RP
1	HR	HR	HR	NI	7	MR	MR	NI	MR
2	HR	HR	HR	NI	8**	MR	MR	NI	MR
3**	MR	MR	NI	MR	9	MR	MR	NI	MR
4	MR	HR	MR	NI	10	MR	MR	NI	MR
5	MR	MR	HR	MR	C	HR	*NI*	HR	*NI*
6**	MR	MR	NI	MR					

*HR: High Risk, MR: Medium Risk, NI: No Indication, MP: Mutagenic Prediction, TP: Tumorigenic Prediction, IP: Irritant Prediction, RP: Reproductive Prediction, **Good indication

Eugenol and cinnamaldehyde have a methylene FG, allowing addition reactions to be carried out into various other ones. The double bond addition using thiocyanic acid will produce two isothiocyanate and thiocyanate with varying yields. Isothiocyanate compounds have been synthesized from *Brazilianflora* limonene by transforming terminal methylene groups using potassium thiocyanate (in chloroform) for 24 h reaction time [52].

Synthesis of ITC in several natural compounds using amine groups has also been successfully formed with raw materials noscapine, bile acids, amino acids, and several aromatic compounds performed by transforming the-NH2 group into an ITC group [53]. The natural product that has a triple bond group, -8,15-diisocyano-11(20)-amphilectene, has been isolated from the *Caribbean sponge Svenzea flava* and used as a building block to form isothiocyanate derivatives [54]. Various degrees of commercial amine (primary, secondary or tertiary), cyclic, aromatic,

and all amine positions (ortho, meta, or para) have been used to synthesize the ITC group by one-pot method and water-based at room temperature [55]. Separation and purification of ITC compounds are generally done by column chromatography or preparative Thin Layer Chromatography. Its identification is implemented mainly by Infrared, Liquid Chromatography-Mass Spectrometry (LCMS), Gas Chromatography-Mass Spectroscopy (GCMS), and High-Performance Liquid Chromatography-Mass Spectrometry [55–57] because these compounds are unstable.

CONCLUSION

Eugenol and cinnamaldehyde availability allow them to be the raw materials and building block for isothiocyanate compounds. *In silico* studies show that methyl eugenol isothiocyanate and cinnamaldehyde isothiocyanate are promising antimalarial compounds in terms of substituents variation such as natural isothiocyanates. This research is an invaluable essential reference for the synthesis of isothiocyanates as anti-malaria.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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