chemistry proceedings

MDPI

Proceeding Paper

In Silico Evaluation of Novel 2-Pyrazoline Carboxamide Derivatives as Potential Protease Inhibitors Against *Plasmodium* Parasites †

Yusuf Jimoh, Idris Yunusa Abdullah, Asmau Nasir Hamza, Maryam Abdullahi, Jibril Ahmadu, Lukman Ali Hassan, Muhammad Shamsudeen Yakubu and Zukhruf Salami

Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria 810107, Nigeria; ndagiginda59@gmail.com (I.Y.A.); hamza.kyauta.asmau@gmail.com (A.N.H.); maryamabkif3@gmail.com (M.A.); darahmadu@gmail.com (J.A.); hlukmanali@gmail.com (L.A.H.); muhammadshamsudeenyakubu@gmail.com (M.S.Y.); beensalam2@gmail.com (Z.S.)

* Correspondence: jimyus1@gmail.com

† Presented at the 28th International Electronic Conference on Synthetic Organic Chemistry (ECSOC 2024), 15–30 November 2024; Available online: https://sciforum.net/event/ecsoc-28.

Abstract: Malaria, a devastating disease caused by *Plasmodium* parasites, continues to pose a significant threat to global health, with increasing resistance to current antimalarial drugs. In this study, we employed an in silico approach to design and evaluate novel 2-pyrazoline carboxamide derivatives as potential protease inhibitors against *Plasmodium falciparum*. Our results show that all designed ligands exhibit good drug-like properties, satisfying Lipinski's rule of five, and demonstrate low toxicity profiles. Molecular docking studies revealed that five newly designed ligands (P5, P6, P7, P11, and P13) exhibit promising binding affinities and interactions with key protease enzymes involved in the hemoglobin degradation pathway, including Falcipain-2, Falcipain-3, and Plasmepsin-2 with PDB (Protein Data Bank) codes; 6JW9, 3BWK and 1LF3 respectively. Notably, ligand P13 showed the strongest binding affinity with Falcipain-2, forming an additional hydrogen bond with CYS42, a residue essential for the enzyme's catalytic activity. The interactions between the ligands and the enzymes suggest a competitive inhibition mechanism, with the potential to disrupt the hemoglobin degradation pathway and halt the parasite's lifecycle. The biological implications of these findings are significant, as they suggest that these novel ligands could be effective against *Plasmodium* parasites, particularly in the context of increasing resistance to current antimalarial drugs. Overall, this study provides valuable insights into the potential of novel 2-pyrazoline carboxamide derivatives as protease inhibitors against *Plasmodium* parasites and highlights their potential as a promising strategy for antimalarial drug development and the importance of in silico approaches in the discovery of novel therapeutics.

Keywords: antimalarial; computational chemistry; in silico; *Plasmodium falciparum*; protease inhibitors; pyrazoline

Citation: Jimoh, Y.; Abdullah, I.Y.; Hamza, A.N.; Abdullahi, M.; Ahmadu, J.; Hassan, L.A.; Yakubu, M.S.; Salami, Z. In Silico Evaluation of Novel 2-Pyrazoline Carboxamide Derivatives as Potential Protease Inhibitors Against *Plasmodium* Parasites. *Chem. Proc.* **2024**, *6*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2024

1. Introduction

Malaria, caused by Plasmodium parasites and transmitted via Anopheles mosquitoes (Ravindar et al., 2023). It remains a global health challenge with 249 million cases and 608,000 fatalities in 2022 (WHO, 2023). The emergence of drug-resistant strains of *P. falciparum* underscores the urgency for novel antimalarial therapies (Adigun et al., 2023). Targeting parasite-specific pathways, such as the protease enzymes, which is crucial for hemoglobin degradation and amino acid supply, has emerged as a promising strategy (Aggarwal et al., 2019; Goud et al., 2005). Pyrazoline derivatives, recognized for their

broad biological activities, have shown potential in inhibiting FP-2, offering a pathway for new antimalarial drug development (Himangini et al., 2018; Wiratama et al., 2022).

2. Materials and Methods

2.1. In Silico Drug-Likeness and Toxicity Predictions

The SwissADME platform (http://www.swissadme.ch/index.php) was used to provide insights into ADME (Absorption, Distribution, Metabolism, and Excretion) parameters, pharmacokinetic properties, drug-likeness attributes, and the suitability of compounds from a medicinal chemistry perspective (Boudou et al., 2023). The assessment of toxicity was carried out using the ProTox 3.0 web tool (https://comptox.charite.de/protox3/), which identified potential toxicities by analyzing various components within the drug.

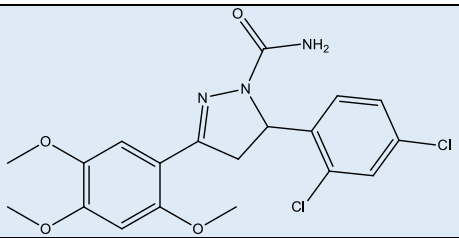
2.2. Molecular Docking Analysis

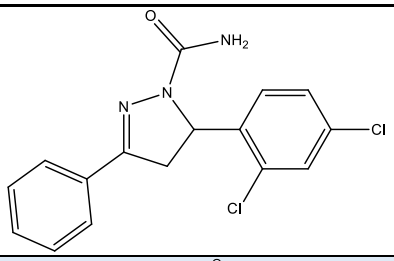
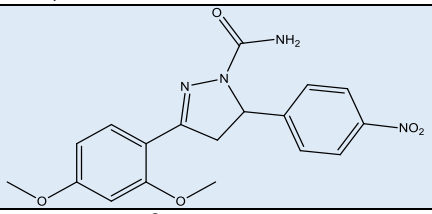
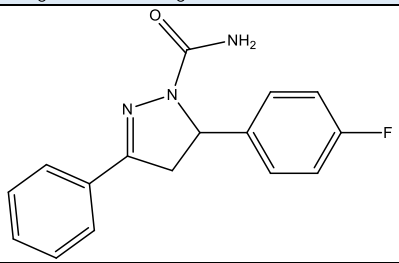
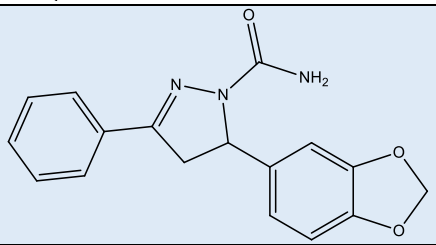
The ligand pyrazoline carboxamide was designed using Chem Draw Ultra 12.0 which was later subjected to Spartan14 for Energy Minimization and optimization and saved in mol2 format. These files were then processed with AutoDock tools to generate pdbqt files for molecular docking using AutoDock vina (Trott & Olson, 2010). The crystal structures of the target proteins, Falcipain-2 (PDB ID: 6JW9) and Falcipain-3 (PDB ID: 3BWK), and Plasmepsin-2 (PDB ID: 1LF3) were obtained from the web-site www.rcsb.org. Water molecules were removed, and hydrogen atoms were added to the protein structure using UCSF Chimera. Molecular docking was performed using AutoDock Tools and Autodock Vina in the grid box size for falcipain-2 28Å × 24Å × 20Å centered at −8.889, 15.368, −38.694 (X, Y, Z coordinates), for falcipain-3 16Å × 16Å × 12Å centered at 5.96, −22.35, 50.07 (X, Y, Z coordinates) and for plasmepsin-2 12Å × 20Å × 18Å centred at 16.22, 6.85, 27.61 (X, Y, Z coordinates). BIOVIA Discovery studio 2020 Client was used to analyze the obtained conformations of the each docked complex ligand interactions.

3. Discussion

From Table 1, all the designed ligands satisfy Lipinski's rule of five, indicating good drug-like properties. Their high gastrointestinal absorption (GI) potential suggests efficient oral bioavailability and their moderate lipophilicity (MLogP) suggests good solubility while their hydrogen bond acceptors (HbA) may enhance their binding Interaction. Also the toxicity evaluation, the predicted LD50 values of all the designed ligands fall within the low toxicity range.

Table 1. Chemical description P5, P6, P7, P11 and P13.

Compound ID	Molecular Formula	2D- Representation	IUPAC Name
P5	$C_{19}H_{19}Cl_2N_3O_4$		5-(2,4-dichlorophenyl)-3-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide

P6	$C_{16}H_{13}Cl_2N_3O$		5-(2,4-dichlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide
P7	$C_{18}H_{18}N_4O_5$		3-(2,4-dimethoxyphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide
P11	$C_{16}H_{14}FN_3O$		5-(4-fluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide
P13	$C_{17}H_{15}N_3O_3$		5-(benzo[d][1,3]dioxol-5-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide

Furthermore, the molecular docking results presented in this study offer valuable insights into the potential inhibitory effects of newly designed ligands (P5, P6, P7, P11 and P13) against key proteases of *Plasmodium falciparum*. These enzymes, Falcipain-2 is a crucial cysteine protease involved in the hemoglobin degradation pathway of Plasmodium falciparum, the parasite responsible for malaria. By cleaving hemoglobin within the food vacuole, falcipain-2 provides free amino acids necessary for parasite protein synthesis (Rosenthal, 2020). Falcipain-3 is also implicated in hemoglobin degradation. However, gene disruption studies suggest that falcipain-3 might have an indispensable function, possibly due to its role in erythrocyte invasion (Molina-Franky et al., 2022). And Plasmepsin-2 is another vital enzyme in the hemoglobin degradation pathway. Its inhibition is directly linked to preventing the supply of amino acids to the parasite, thereby halting its proliferation (Burns et al., 2019). The inhibition of these enzymes is a promising strategy for antimalarial drug development, especially in the face of increasing drug resistance.

The native ligands (E64, C1P, and EH5) serve as benchmarks for the docking study, providing a reference for the binding affinities and interactions expected of effective inhibitors. The newly designed ligands, P5, P6, P7, P11, and P13, were evaluated for their binding affinities and interactions with the active site residues of the target enzymes.

Falcipain-2 (PDB ID: 6JW9): The native ligand E64 showed moderate binding affinity, forming key hydrogen bonds with residues such as GLN36 and HIS174 (Table 3). These interactions are crucial for the stability of the enzyme-ligand complex and suggest a competitive inhibition mechanism. Among the newly designed ligands, P13 exhibited the strongest binding affinity (Table 2), which can be attributed to its additional hydrogen bond with CYS42 as presented in (Table 3), a residue essential for the catalytic activity of falcipain-2. This interaction not only enhances the binding affinity but also suggests a

specificity that could translate to a potent inhibitory effect on the enzyme's function in hemoglobin degradation.

Table 2. Analysis of theoretical oral bioavailability of the designed compounds based on Lipinski's rule of five, GI absorption, predicted LD50 and Toxicity Class.

Compound ID	Lipinski's rule of five[b]							
	Mol.Wt[a]	HbA	HbD	MLogP	GI	Inference	LD50	Toxicity Class
P5	424.28	5	1	2.73	High	Pass	1000 mg/kg	4
P6	334.20	2	1	3.68	High	Pass	1000 mg/kg	4
P7	370.36	6	1	1.16	High	Pass	1000 mg/kg	4
P11	283.30	3	1	3.06	High	Pass	1880 mg/kg	4
P13	309.32	4	1	2.21	High	Pass	1000 mg/kg	4

(a) Molecular weight in g/mol, (b) Lipinski et al., 2001 (Mwt ≤ 500, MLogP ≤ 4.15, N or O ≤ 10, NH or OH ≤ 5 and number of rotatable bonds≤ 1.

Table 3. Binding Energies of Designed Pyrazolines Carbaxomides and respective co-crystallized ligands.

PDB ID	Ligands	Binding Affinity (kcal/mol)	Residues Involved in Bonded Interaction
6JW9	E64	−5.1	H-Bond: GLN36, CYS42, GLY83, HIS174, ASN81 Pi-Donor H-Bond: TYR78 Pi-Alkyl: LEU84 Van der Waals: GLY82, GLY40, SER41, TRP43, ASN173, ALA175, ILE85, SER149, LEU172
	P5	−6.4	Carbon H-Bond: GLY83 Alkyl: ALA175, LEU172, LEU84, ILE85, CYS42, Pi-Alkyl: TRP43, ALA175 Amide-Pi Stacked: ASN173, Pi-Sulfur: CYS42 Van der Waals: SER149, HIS174, GLY82, GLY40, ASN81, CYS80
	P6	−6.9	H-Bond: GLY83 Carbon H-Bond: TRP43 Pi-Alkyl: LEU84, ALA175 Van der Waals: ILE85, HIS174, GLY40, CYS42, GLY82, SER41, ASN81, ASN173, LEU172, ASP234, SER149
	P7	−6.5	H-Bond: GLN36 Carbon H-Bond: GLY40, GLY83 Alkyl: ALA175, LEU84, ILE85 Pi-Alkyl: ALA175 Amide-Pi Stacked: ASN173 Pi-Sulfur: CYS42 Van der Waals: HIS174
	P11	−6.6	H-Bond: GLY83 Carbon H-Bond: TRP43 Pi-Alkyl: LEU84, ALA175 Van der Waals: SER149, ILE85, GLY40, CY42, SER41, GLY82, ASN81, HIS174, ASN173, LEU172, ASP234
	P13	−7.0	H-Bond: CYS42 Pi-Alkyl: LEU84, ILE85, ALA175

			Van der Waals: HIS174, ASN81, ASN173, TYR78, GLY82, GLY83, LEU172, ASP234, SER149, TRP43
3BWK	C1P	−7.1	H-Bond: GLN45, TRP215, GLY92, ASN182 Carbon H-Bond: TRP52, GLY91, GLY49, TYR90 Alkyl: Pi-Alkyl: TYR93, ALA161 Amide-Pi Stacked: GLY91 Pi-Sulfur: TRP215 Van der Waals: ASN87, PRO181, SER158, ILE94, ALA184, ALA166, HIS183, CYS51, CYS89
	P5	−6.4	H-Bond: TYR90 Carbon H-Bond: GLY91, SER158 Alkyl: ALA184, CYS51, ILE94 Pi-Donor H-Bond: TYR93 Amide-Pi Stacked: ASN182, HIS183 Pi-Sulfur: CYS51 Van der Waals: HIS183, TRP52, GLY92, ASN87, GLN45, GLY49, CYS89, PRO181
	P6	−6.4	H-Bond: TYR90 Alkyl: CYS51 Pi-Alkyl: ALA184 Amide-Pi Stacked: ASN182, HIS183 Van der Waals: ILE94, TRP52, GLY92, TYR93, GLY91, GLY49,HIS183, PRO181, GLU243, SER158
	P7	−6.6	H-Bond: ILE94, TYR90, Carbon H-Bond: GLY91, TYR93, SER158 Pi-Sulfur: CYS51 Pi-Alkyl: ALA175 Van der Waals: GLU243, TYR93, TRP52, ASN182, ALA184, GLY49, GLY92, HIS183
	P11	−6.4	H-Bond: ASN87, TYR90 Pi-Alkyl: ALA184 Amide-Pi Stacked: ASN182 Van der Waals: SER158, HIS183, TRP52, CYS51, CYS89, GLY49, GLY91, GLY92, PRO181
	P13	−6.7	H-Bond: ASN87, TYR90 Carbon H-Bond: TYR77, ASP214 Pi-Alkyl: ALA184 Amide-Pi Stacked: ASN182 Van der Waals: GLY49, GLY91, GLY92, PRO181, HIS183, SER158, ILE94, TRP52, CYS51
1LF3	EH5	−10.0	H-Bond: VAL78, SER79, SER218, GLY216, ASP34, ASP214 Carbon H-Bond: GLY36, ILE14, GLY216, Alkyl: VAL78, ILE290, PHE294 Pi-Sigma: VAL78 Pi-Donor H-Bond: TYR192 Pi-Alkyl: ILE32, ILE123 Pi-Pi T-shaped: TYR77 Pi-Sulfur: MET15 Van der Waals: LEU29, ILE300, PHE111, THR114, PHE120, SER37, LEU131, MET75, ASN76, ILE212, ALA219, THR217

	P5	−7.3	H-Bond: SER79, ASP214 Carbon H-Bond: GLY36 Alkyl: VAL78, ILE123, LEU292 Pi-Sigma: TYR77 Pi-Anion: ASP214 Pi-Pi Stacked: PHE294 Pi-Alkyl: TYR77, PHE111, PHE294, VAL78 Van der Waals: ILE300, ILE212, THR217, ASP34, SER37, TYR192
	P6	−8.1	H-Bond: ASP214 Pi-Alkyl: VAL78, ILE32, ILE123 Pi-Pi T-shaped: TYR77 Van der Waals: PHE111, PHE120, ASP34, TYR192, GLY36, THR217, ILE300, GLY216, SER79
	P7	−8.0	H-Bond: SER79, GLY36, ASP214 Alkyl: ILE133 Pi-Sigma: TYR77 Pi-Alkyl: VAL78 Pi-Pi T-shaped: TYR77 Van der Waals: PHE111, SER37, MET75, LEU131, TYR92, ILE300, THR217, ILE212, ILE32, ASP34, GLY216, ILE123
	P11	−8.1	H-Bond: THR217 Carbon H-Bond: THR217 Alkyl: VAL78 Halogen (Fluorine): ASP34 Pi-Pi Stacked: TYR77, TYR192 Pi-Alkyl: ILE212, ILE300 Van der Waals: PHE294, SER79, GLY216, ILE32, ILE123, ASP214
	P13	−7.9	H-Bond: VAL78, TYR192 Carbon H-Bond: TYR77, ASP214 Pi-Sigma: VAL78 Pi-Alkyl: ILE32, ILE123, VAL78 Pi-Pi T-shaped: TYR77 Van der Waals: ASP34, ASP214, GLY36, THR217, ILE300, SER79, PHE111, PHE120

* E64, C1P and EH5 are the co-crystallized ligands for the respective enzyme.

Falcipain-3 (PDB ID: 3BWK): C1P, the native ligand, demonstrated a high binding affinity (Table 2) engaging in hydrogen bonds with residues like GLN45 and TRP215 (Table 3). These interactions are indicative of a natural regulatory mechanism of the enzyme's activity. The newly designed ligands showed comparable binding affinities (Table 3), with interactions involving key residues such as TYR90 (Table3). The consistent involvement of this residue across several ligands as shown in (Table 3) highlights its importance in the binding process and suggests a potential for effective inhibition that could impact the parasite's ability to invade red blood cells.

Plasmepsin-2 (PDB ID: 1LF3): EH5, the native ligand, exhibited a notably high binding affinity, forming extensive interactions with the enzyme (Table 2), including hydrogen bonds with VAL78 and SER79 (Table 3). Such interactions are indicative of a potent natural inhibition mechanism. The newly designed ligands, particularly P13, also demonstrated promising binding affinities and interactions. The hydrogen bond with ASP214, a residue critical for the enzyme's activity, suggests that these ligands could effectively disrupt the hemoglobin degradation pathway, depriving the parasite of essential nutrients.

The biological implications of these findings are significant. By inhibiting the activity of these enzymes, the newly designed ligands could effectively halt the parasite's lifecycle, leading to a cessation of the disease progression. This is particularly crucial in the context of increasing resistance to current antimalarial drugs.

4. Conclusions

In conclusion, the molecular docking study presents compelling evidence that the newly designed ligands have the potential to serve as effective inhibitors of crucial malaria parasite proteases. Their strong binding affinities and specific interactions with key active site residues make them promising candidates for the development of new antimalarial drugs. Future work will involve experimental validation of these in silico predictions to assess the therapeutic potential of these ligands.

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest:

References

1. Adigun, R.A.; Malan, F.P.; Balogun, M.O.; October, N. Design, synthesis, and in silico-in vitro antimalarial evaluation of 1,2,3-triazole-linked dihydropyrimidinone quinoline hybrids. *Struct. Chem.* **2023**, *34*, 2065–2082. https://doi.org/10.1007/s11224-023-02142-y.
2. Aggarwal, S.; Paliwal, D.; Kaushik, D.; Gupta, G.K.; Kumar, A. Synthesis, Antimalarial Evaluation and SAR Study of Some 1,3,5-Trisubstituted Pyrazoline Derivatives. *Lett. Org. Chem.* **2019**, *16*, 807–817. https://doi.org/10.2174/1570178616666190212145754.
3. Boudou, F.; Sehmi, A.; Belakredar, A.; Zaoui, O. Synthesis, characterization, antimicrobial activity, and in silico assessment of a novel pyrazoline carboxamide heterocyclic compound. *Bangladesh J. Pharmacol.* **2023**, *18*, 152–161. https://doi.org/10.3329/bjp.v18i4.69267.
4. Burns, A.L.; Dans, M.G.; Balbin, J.M.; De, T.F.; Gilson, P.R.; Beeson, J.G.; Boyle, M.J.; Wilson, D.W. Targeting malaria parasite invasion of red blood cells as an antimalarial strategy. *FEMS Microbiol. Rev.* **2019**, *43*, 223–238. https://doi.org/10.1093/femsre/fuz005.
5. Goud, P.M.; Sheri, A.; Desai, P.V.; Watkins, E.B.; Tekwani, B.; Sabnis, Y.; Avery, M.A. Design, synthesis and evaluation of trisubstituted thiazoles targeting Plasmodium falciparum cysteine proteases. *Med. Chem. Res.* **2005**, *14*, 74–105.
6. Himangini Pathak, D.P.; Sharma, V.; Kumar, S. Designing novel inhibitors against falcipain-2 of Plasmodium falciparum. *Bioorganic Med. Chem. Lett.* **2018**, *28*, 1566–1569. https://doi.org/10.1016/j.bmcl.2018.03.058.
7. Molina-Franky, J.; Patarroyo, M.E.; Kalkum, M.; Patarroyo, M.A. The Cellular and Molecular Interaction Between Erythrocytes and Plasmodium falciparum Merozoites. *Front. Cell. Infect. Microbiol.* **2022**, *12*, 1–21. https://doi.org/10.3389/fcimb.2022.816574.
8. Ravindar, L.; Hasbullah, S.A.; Rakesh, K.P.; Hassan, N.I. Pyrazole and pyrazoline derivatives as antimalarial agents: A key review. *Eur. J. Pharm. Sci.* **2023**, *183*, 106365. https://doi.org/10.1016/j.ejps.2022.106365.
9. Rosenthal, P.J. Falcipain cysteine proteases of malaria parasites: An update. *Biochim. Et Biophys. Acta Proteins Proteom.* **2020**, *1868*, 140362. https://doi.org/10.1016/j.bbapap.2020.140362.
10. Trott, O.; Olson, A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J. Comput. Chem.* **2010**, *31*, 455–461. https://doi.org/10.1002/jcc.21334.
11. WHO. *The 2023 WHO World Malaria Report*; WHO: Geneva, Switzerland, 2023. https://doi.org/https://doi.org/10.1016/S2666-5247(24)00016-8World.
12. Wiratama, M.; Satria, S.; Waskitha, W.; Haryadi, W.; Wahyuningsih, T.D. Synthesis, antimalarial activity assay and molecular docking study of N-substituted chloro-pyrazolines. *Trop. J. Pharm. Res.* **2022**, *21*, 1255–1261. https://doi.org/10.4314/tjpr.v21i6.18.