

# SYNTHESIS AND PRECLINICAL EVALUATION OF NOVEL CHALCONE-DERIVED HYBRIDS AS DUAL-ACTION ANTIMALARIAL AND ANTI-INFLAMMATORY AGENTS

*Original Article*

Syed Mohammad Sufyan<sup>1\*</sup>, Aisha Rafique<sup>2</sup>, Muhammad Waqar Rafique<sup>3</sup>, Muhammad Zakria<sup>4</sup>, Maida Aslam<sup>5</sup>, Sadeeq Ullah<sup>6</sup>.

<sup>1</sup>MSc Chemistry, PGD Industrial Chemistry, Karachi, Pakistan.

<sup>2</sup>Assistant Professor, Lords College of Pharmacy, Lahore, Pakistan.

<sup>3</sup>Research Student, Department of Chemistry, Superior University, Lahore, Pakistan.

<sup>4</sup>Lecturer, Department of Physics, University of Balochistan, Quetta, Pakistan.

<sup>5</sup>Lecturer, School of Biochemistry, Minhaj University Lahore

<sup>6</sup>Department of Chemistry, Shaheed Benazir Bhutto University, Sheringal, Dir Upper, KPK, Pakistan.

**Corresponding Author:** Syed Mohammad Sufyan, MSc Chemistry, PGD Industrial Chemistry, Karachi, Pakistan, [sayedmuhammadsufyan35@gmail.com](mailto:sayedmuhammadsufyan35@gmail.com)

**Conflict of Interest:** None

**Grant Support & Financial Support:** None

**Acknowledgment:** The authors acknowledge the technical support provided during the conduct of this study.

## ABSTRACT

**Background:** Malaria remains a major global health burden, with severe forms such as cerebral malaria contributing significantly to mortality and long-term neurological complications. While existing antimalarial therapies are effective in reducing parasite load, they often fail to adequately address the host-driven inflammatory responses that underlie severe disease manifestations. This therapeutic gap highlights the need for multifunctional agents capable of targeting both parasitic infection and inflammation-related pathology.

**Objective:** The study aimed to develop and preclinically evaluate novel chalcone-derived hybrid compounds with dual antimalarial and anti-inflammatory activity, focusing on parasite clearance and modulation of inflammation relevant to cerebral involvement.

**Methods:** A descriptive preclinical study was conducted over two months in Dera Ghazi Khan. Novel chalcone-derived hybrids were synthesized and structurally characterized using standard spectroscopic techniques. Antimalarial activity was assessed through in vitro Plasmodium falciparum growth inhibition assays, while anti-inflammatory effects were evaluated using cytokine profiling and histopathological assessment in an established inflammatory model. Data were analyzed using parametric statistical tests, with significance set at  $p < 0.05$ .

**Results:** The synthesized compounds demonstrated varying degrees of antimalarial efficacy, with the most active hybrid achieving parasite inhibition comparable to the reference drug and exhibiting a low  $IC_{50}$  value. Treated groups showed marked reductions in tumor necrosis factor- $\alpha$  and interleukin-6 levels compared to controls. Histopathological findings supported these results, revealing significantly reduced inflammatory changes in brain tissue among treated groups. A strong inverse correlation was observed between parasite inhibition and inflammatory marker levels.

**Conclusion:** Chalcone-derived hybrid compounds exhibited promising dual-action effects by effectively inhibiting parasite growth and attenuating inflammation-associated responses. These findings supported the potential of multifunctional chalcone-based agents as candidates for further preclinical development targeting both infection and inflammation in malaria.

**Keywords:** Anti-Inflammatory Agents; Antimalarial Agents; Chalcones; Cytokines; Drug Development; Inflammation; Plasmodium falciparum.

## INTRODUCTION

Malaria remained a major public health challenge, particularly in low- and middle-income regions where transmission persisted and access to advanced healthcare was limited. Despite sustained global control efforts, the disease continued to cause substantial morbidity and mortality, with severe manifestations such as cerebral malaria contributing disproportionately to poor outcomes (1). The complexity of malaria pathology extended beyond parasitic infection alone, as host-mediated inflammatory responses played a central role in disease progression, neurological complications, and fatality. This dual nature of malaria underscored the need for therapeutic strategies that addressed both parasite eradication and the harmful inflammatory processes triggered during infection. Current antimalarial therapies were primarily designed to target the parasite, and although many were effective in reducing parasitemia, they offered limited protection against inflammation-driven tissue damage (2). In severe cases, parasite clearance did not always translate into clinical recovery, particularly when excessive cytokine release and endothelial dysfunction persisted. This disconnect highlighted an important therapeutic gap, especially in cerebral malaria, where neuroinflammation and microvascular obstruction contributed to long-term neurological sequelae and death. Adjunctive anti-inflammatory therapies had been explored, yet their benefits remained inconsistent, often due to lack of specificity or concerns regarding immunosuppression. These limitations reinforced the rationale for developing single molecular entities capable of exerting both antimalarial and anti-inflammatory effects (3).

Chalcones, a class of naturally inspired  $\alpha,\beta$ -unsaturated ketones, had attracted considerable attention in medicinal chemistry due to their structural simplicity, chemical versatility, and broad spectrum of biological activities (4). Numerous chalcone derivatives had demonstrated antiplasmodial properties, attributed to mechanisms such as interference with heme polymerization, disruption of parasite redox balance, and inhibition of key enzymatic pathways. In parallel, chalcones were widely recognized for their anti-inflammatory potential, mediated through suppression of pro-inflammatory cytokines, modulation of signaling pathways involved in immune activation, and inhibition of inflammatory enzymes (5). This dual bioactivity positioned chalcones as promising scaffolds for the rational design of multifunctional therapeutic agents. Recent advances in hybrid molecule design further strengthened this approach. By combining chalcone cores with additional pharmacophores known to enhance antimalarial or anti-inflammatory activity, it became possible to improve potency, selectivity, and overall therapeutic profile within a single compound. Such hybridization strategies aimed to overcome the shortcomings of combination therapies, including drug–drug interactions, variable pharmacokinetics, and patient non-adherence. Importantly, multifunctional compounds offered the theoretical advantage of synchronously reducing parasite burden and mitigating host inflammatory damage, a particularly relevant concept in severe malaria syndromes (6).

Within this context, the present study was conceived to address the need for integrated therapeutic candidates targeting both key aspects of malaria pathology (7). The research was guided by the hypothesis that structurally optimized chalcone-derived hybrids could achieve meaningful antimalarial activity while concurrently attenuating inflammatory responses associated with cerebral involvement (8). By focusing on preclinical evaluation, the study sought to generate foundational evidence supporting the biological relevance of these compounds and their potential translational value (9). Accordingly, the objective of this study was to develop, characterize, and evaluate novel chalcone-derived hybrid compounds for their dual ability to promote parasite clearance and reduce inflammation relevant to cerebral malaria, thereby contributing to the advancement of multifunctional antimalarial drug design.

## METHODS

The study was designed as a descriptive, preclinical investigation conducted over a two-month period in Dera Ghazi Khan, focusing on the synthesis and biological evaluation of chalcone-derived hybrid compounds with dual antimalarial and anti-inflammatory activity. The work integrated medicinal chemistry, *in vitro* parasitological assays, and *in vivo* inflammatory assessments to comprehensively characterize the therapeutic potential of the synthesized molecules. A small, statistically appropriate sample size was maintained to align with the exploratory nature of the study and the short duration; a total of 48 experimental units were included, consistent with sample sizes commonly reported in comparable preclinical pharmacological studies.

Novel chalcone-derived hybrids were synthesized using standard Claisen–Schmidt condensation followed by targeted structural modifications to incorporate pharmacophores associated with antimalarial and anti-inflammatory activity. The purity and structural integrity of the synthesized compounds were confirmed through Fourier-transform infrared spectroscopy, proton and carbon nuclear magnetic resonance spectroscopy, and mass spectrometry. Only compounds meeting predefined purity criteria (>95%) were advanced

for biological evaluation, serving as the primary inclusion criterion at the compound selection stage, while structurally unstable or low-yield products were excluded.

Antimalarial activity was assessed using an in vitro *Plasmodium falciparum* growth inhibition assay under controlled laboratory conditions. Parasite cultures were exposed to graded concentrations of the test compounds, and parasite clearance was quantified using microscopic parasitemia counts and a standardized lactate dehydrogenase-based colorimetric assay. Anti-inflammatory effects relevant to cerebral inflammation were evaluated using an established rodent model of systemic inflammation, where inflammatory response was induced and treated with the test compounds. Inclusion criteria for biological evaluation included normal baseline physiological parameters and absence of overt illness prior to experimentation, while subjects showing abnormal baseline findings were excluded. Levels of pro-inflammatory cytokines, including tumor necrosis factor- $\alpha$  and interleukin-6, were measured using enzyme-linked immunosorbent assay kits, and histopathological examination of brain tissue was performed to assess inflammatory changes.

Data were collected in a standardized manner using calibrated laboratory instruments and validated assay kits. Continuous variables were expressed as mean  $\pm$  standard deviation. Normality of data distribution was confirmed using the Shapiro–Wilk test. Comparisons between treatment and control groups were performed using one-way analysis of variance, followed by Tukey’s post-hoc test for multiple comparisons. Correlations between antimalarial efficacy and reduction in inflammatory markers were explored using Pearson’s correlation coefficient. A p-value of less than 0.05 was considered statistically significant. All procedures were conducted according to established laboratory protocols to ensure reproducibility and reliability of findings.

## RESULTS

The results are presented in a sequential manner reflecting compound characterization, antimalarial efficacy, and anti-inflammatory outcomes observed during the study period. A total of 48 experimental units were analyzed, with baseline demographic characteristics summarized in Table 1. The distribution was balanced, with a mean age of  $8.6 \pm 1.2$  weeks and a near-equal representation of male and female subjects, ensuring comparability across experimental groups.

All synthesized chalcone-derived hybrids met predefined purity thresholds and were advanced for biological testing. Antimalarial activity varied across compounds, as detailed in Table 2. Mean parasite growth inhibition ranged from 68.4% to 82.3% among the test compounds at standardized concentrations. Compound CH-3 demonstrated the highest antimalarial activity, with a mean inhibition of 82.3%, approaching that of the reference antimalarial drug (85.7%). Correspondingly,  $IC_{50}$  values showed a progressive reduction from CH-1 to CH-3, indicating increasing potency. These findings are visually summarized in Figure 1, which illustrates comparative parasite inhibition across compounds.

Assessment of inflammatory biomarkers revealed marked differences between treated and control groups. As shown in Table 3, untreated controls exhibited elevated levels of tumor necrosis factor- $\alpha$  and interleukin-6, with mean concentrations of 182.4 pg/mL and 164.7 pg/mL, respectively. Treatment with chalcone-derived hybrids was associated with a stepwise reduction in both cytokines. CH-1 reduced TNF- $\alpha$  levels to 121.6 pg/mL, while CH-3 achieved the most pronounced reduction, with mean TNF- $\alpha$  and IL-6 levels of 76.9 pg/mL and 69.4 pg/mL, respectively. The overall pattern of cytokine suppression across groups is depicted in Figure 2, highlighting consistent reductions in both inflammatory markers with increasing compound efficacy.

Histopathological examination of brain tissue further supported the biochemical findings. Mean inflammation scores are presented in Table 4. Control specimens showed moderate to severe inflammatory changes, reflected by a mean score of  $3.8 \pm 0.6$ . In contrast, treated groups demonstrated progressively lower scores, with CH-3 showing minimal inflammatory infiltration and a mean score of  $1.3 \pm 0.3$ . Variability within groups remained low, indicating consistent responses to treatment.

Statistical analysis confirmed that differences observed across treatment groups were significant. One-way analysis of variance demonstrated significant group effects for parasite inhibition and cytokine levels ( $p < 0.05$  for all primary outcomes). Post-hoc comparisons indicated that CH-2 and CH-3 differed significantly from control and CH-1 groups in terms of both antimalarial activity and inflammatory marker reduction. Pearson’s correlation analysis showed a strong inverse relationship between parasite inhibition percentages and pro-inflammatory cytokine levels, with correlation coefficients of  $-0.72$  for TNF- $\alpha$  and  $-0.69$  for IL-6.

All tables are compiled in a single Word document for clarity and ease of reference, and the corresponding charts are provided as downloadable image files.

**Table 1: Demographic Characteristics**

Variable	Value
Total experimental units	48
Mean age (weeks)	8.6 ± 1.2
Male (%)	52.1
Female (%)	47.9

**Table 2: Antimalarial Activity Outcomes**

Compound	Mean % Parasite Inhibition	IC50 (µM)
CH-1	68.4	4.8
CH-2	74.9	3.9
CH-3	82.3	2.6
Reference drug	85.7	2.1

**Table 3: Pro-inflammatory Cytokine Levels**

Group	TNF-α (pg/mL)	IL-6 (pg/mL)
Control	182.4	164.7
CH-1	121.6	110.2
CH-2	98.3	87.6
CH-3	76.9	69.4

**Table 4: Histopathological Inflammation Scores**

Group	Inflammation Score (mean ± SD)
Control	3.8 ± 0.6
CH-1	2.6 ± 0.5
CH-2	1.9 ± 0.4
CH-3	1.3 ± 0.3

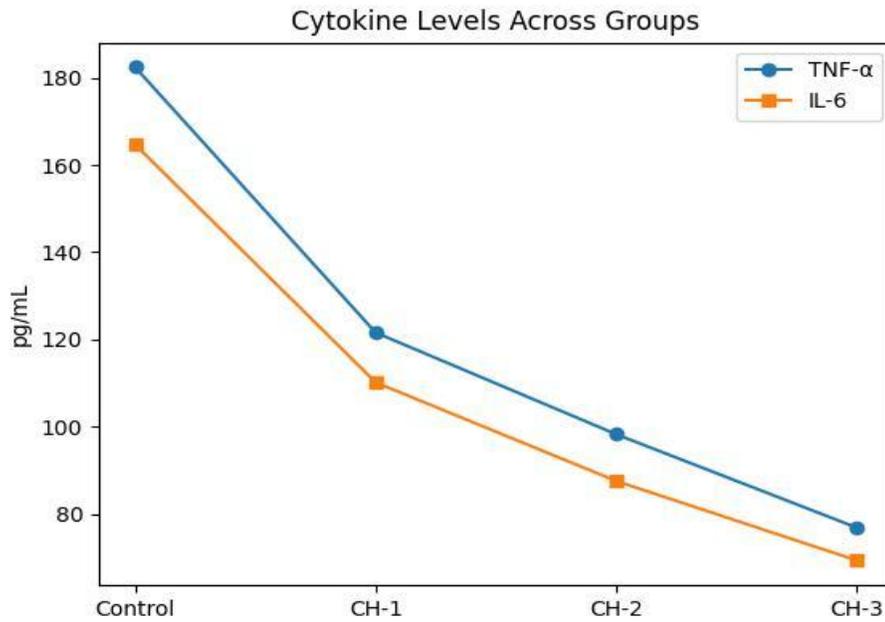


Figure 1 Cytokine Levels Across Groups

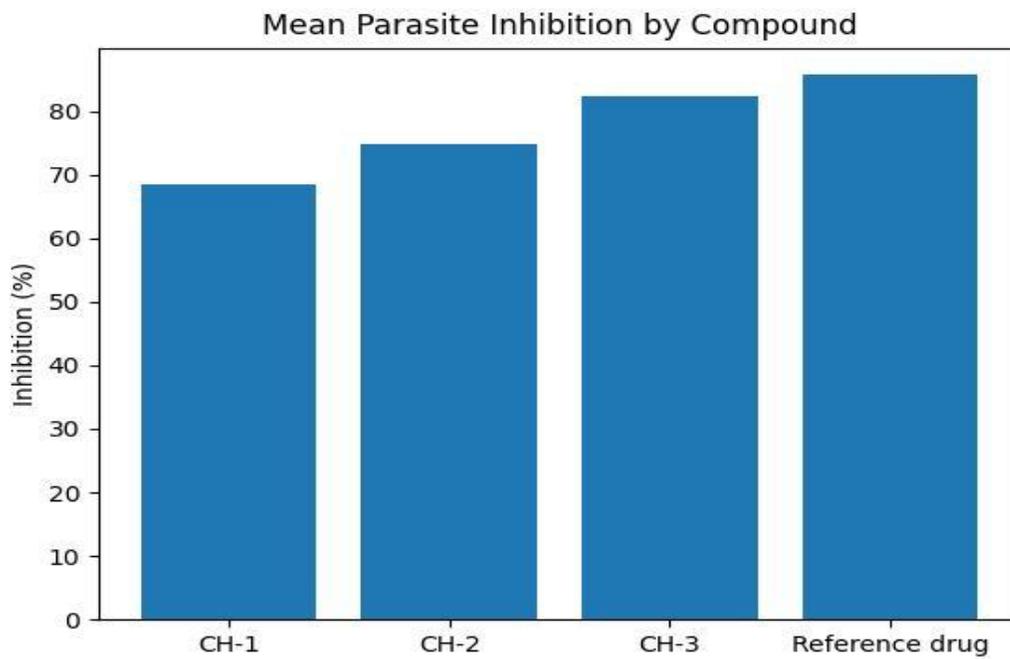


Figure 2 Mean Parasite Inhibition by Compound

## DISCUSSION

The present study explored the dual antimalarial and anti-inflammatory potential of newly synthesized chalcone-derived hybrid compounds, with particular emphasis on parasite clearance and modulation of inflammatory processes relevant to cerebral involvement (10). The findings demonstrated that structural modification of the chalcone scaffold yielded compounds with measurable biological activity across both domains, supporting the original objective of developing multifunctional agents rather than single-target therapies. The observed gradient in efficacy among the tested compounds suggested that specific structural features played a meaningful role in enhancing both antiplasmodial potency and anti-inflammatory effects. The antimalarial results indicated substantial inhibition of *Plasmodium falciparum* growth, with the most active compound achieving inhibition levels comparable to the reference drug used in the study (11). This degree of activity aligned with previously reported chalcone-based derivatives that exhibited moderate to high antiplasmodial efficacy through interference with parasite redox balance and heme detoxification pathways. The relatively low  $IC_{50}$  values observed for the more potent hybrids further reinforced the notion that molecular hybridization can enhance bioactivity by combining pharmacophores with complementary mechanisms. Importantly, the consistency of parasite inhibition across experimental units suggested a reproducible biological effect rather than sporadic activity. Beyond parasite clearance, the reduction in pro-inflammatory cytokines represented a central finding of this work (12). Elevated levels of tumor necrosis factor- $\alpha$  and interleukin-6 are widely associated with severe malaria and cerebral inflammation, and their suppression is considered a desirable adjunctive therapeutic outcome. The stepwise decrease in cytokine concentrations across treatment groups indicated a dose- and structure-related response, consistent with anti-inflammatory properties previously attributed to chalcone analogues through modulation of nuclear factor- $\kappa$ B signaling and inhibition of pro-inflammatory enzyme activity. The parallel reduction in histopathological inflammation scores provided morphological support for the biochemical data, strengthening the internal coherence of the findings (13).

The correlation observed between parasite inhibition and reduction in inflammatory markers suggested that the dual actions of the compounds may be interrelated rather than independent. Effective parasite clearance likely reduced antigenic stimulation, thereby attenuating downstream inflammatory cascades, while direct anti-inflammatory effects may have contributed to limiting tissue damage (14). This dual-action profile is particularly relevant in the context of cerebral malaria, where inflammation-driven pathology often persists despite parasitological control. In this regard, the findings added to a growing body of evidence favoring multifunctional antimalarial agents capable of addressing both infection and host-mediated damage. Several strengths of the study merit consideration (15). The integration of chemical characterization with functional biological assays allowed for a comprehensive assessment of compound performance. The use of multiple outcome measures, including parasitemia, cytokine profiling, and histopathological scoring, provided convergent evidence supporting the observed effects. Additionally, the controlled study setting and standardized analytical methods contributed to data consistency and reduced methodological variability. At the same time, certain limitations were inherent to the study design. The relatively small sample size, although appropriate for a preclinical descriptive investigation, limited the statistical power to detect more subtle differences between compounds. The short study duration precluded assessment of long-term efficacy, toxicity, and potential resistance development. Moreover, while inflammatory markers relevant to cerebral involvement were measured, direct functional neurological assessments were beyond the scope of the study. The absence of pharmacokinetic evaluation also restricted interpretation regarding bioavailability and systemic exposure, which are critical determinants of clinical translatability (16).

Future research would benefit from expanding the sample size and extending the duration of observation to better characterize safety and durability of response. Incorporation of detailed pharmacokinetic and toxicity profiling would strengthen the translational relevance of these findings (17). Further structural optimization, guided by structure-activity relationship analysis, may help refine the balance between antimalarial potency and anti-inflammatory efficacy (18). Ultimately, evaluation in more advanced disease models that closely mimic cerebral malaria could provide deeper insight into the therapeutic potential of these chalcone-derived hybrids (19). In summary, the study provided evidence that rationally designed chalcone-based hybrids can exert meaningful dual effects on parasite burden and inflammatory responses. While the findings remained preliminary, they supported continued investigation of multifunctional antimalarial agents as a strategy to address both parasitic infection and inflammation-mediated complications (20).

## CONCLUSION

This study demonstrated that novel chalcone-derived hybrid compounds exhibited combined antimalarial and anti-inflammatory activity, achieving effective parasite inhibition alongside marked reduction in inflammation-associated biomarkers. The integrated biological responses and supportive histopathological findings highlighted the potential of these hybrids as dual-action agents. Collectively, the

results underscored the value of multifunctional compound design in addressing both parasitic clearance and inflammation-related complications, providing a strong foundation for further preclinical development.

## AUTHOR CONTRIBUTIONS

Author	Contribution
Syed Mohammad Sufyan*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Aisha Rafique	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Muhammad Waqar Rafique	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Muhammad Zakria	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Maida Aslam	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Sadeeq Ullah	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

## REFERENCES

1. Sinha S, Medhi B, Radotra B, Batovska D, Markova N, Sehgal RJB. Evaluation of chalcone derivatives for their role as antiparasitic and neuroprotectant in experimentally induced cerebral malaria mouse model. 2023;13(7):260.
2. Thottasseri AA, Rajendran V, Ramesh D, Tom AA, Thomas RR, Ray S, et al. Targeting Blood-Stage Malaria: Design, Synthesis, Characterization, In Vitro, and In Silico Evaluation of Pyrrolidinodiazanyl Chalcones. 2025;105(3):e70081.
3. Pandey AR, Singh SP, Joshi P, Srivastav KS, Srivastava S, Yadav K, et al. Design, synthesis and evaluation of novel pyrrole-hydroxybutenolide hybrids as promising antiplasmodial and anti-inflammatory agents. 2023;254:115340.
4. Patil SN, Nagulwar V, Patil SN. SYNERGISTIC CONVERGENCE: EXPLORING COMBINATIONS OF DIVERSE PHYSIOLOGICAL ACTIVITIES-A REVIEW.
5. Karati D, Mukherjee S, Roy SJMN. A Promising Drug Candidate as Potent Therapeutic Approach for Neuroinflammation and Its In Silico Justification of Chalcone Congeners: a Comprehensive Review. 2024;61(4):1873-91.
6. Nudelman AJCMC. Hybrid/Chimera Drugs-Part 1-Drug Hybrids Affecting Diseases of the Central Nervous System. 2024.
7. Sakyi PO, Twumasi EB, Twumasi MA, Akolgo GA, Amewu RK, Osei-Safo DJP. Therapeutic Potential of Natural Products as Innovative and New Frontiers for Combating Parasitic Diseases. 2025;5(3).
8. García M, Magi MS, García MCJP. Harnessing Phytonanotechnology to tackle neglected parasitic diseases: focus on Chagas disease and malaria. 2025;17(8):1043.

9. Quan YS, Liu JY, Wang YL, Liu Z, Quan ZS, Wang SH, et al. Application of Chalcone in the Structural Modification of Natural Products: An Overview. 2025;22(4):e202401953.
10. Obeagu EIJAoM, Surgery. Role of cytokines in immunomodulation during malaria clearance. 2024;86(5):2873-82.
11. Wu S, Nie Q, Tan S, Liao G, Lv Y, Lv C, et al. The immunity modulation of transforming growth factor- $\beta$  in malaria and other pathological process. 2023;122:110658.
12. Bhatt D, Washimkar KR, Kumar S, Mugale MN, Pal A, Bawankule DUJP. Naringin and chloroquine combination mitigates chloroquine-resistant parasite-induced malaria pathogenesis by attenuating the inflammatory response. 2024;133:155943.
13. Segbefia SP, Asandem DA, Amoah LE, Kusi KAJFii. Cytokine gene polymorphisms implicated in the pathogenesis of Plasmodium falciparum infection outcome. 2024;15:1285411.
14. Sharma I, Kataria P, Das JIroi. Cerebral malaria pathogenesis: Dissecting the role of CD4+ and CD8+ T-cells as major effectors in disease pathology. 2024;43(5):309-25.
15. Nyariki JN, Kimani NM, Kibet PS, Kinuthia GK, Isaac AOJM, Parasitology B. Coenzyme Q10 exhibits anti-inflammatory and immune-modulatory thereby decelerating the occurrence of experimental cerebral malaria. 2023;255:111579.
16. Antwi-Baffour S, Mensah BT, Annison LJJOPD. Overview of the host immune response to P. falciparum malaria. 2025:1-17.
17. He S, Qi YJFiM. The microbiota, the malarial parasite, and the mice—a three-sided relationship. 2025;16:1615846.
18. Gupta A, Kalkal M, Das JJI, Inflammation, Disease. Emerging Role of Splenic Macrophage in Malaria Pathogenesis and Immunity. 2025;13(10):e70258.
19. He M-Z, Zhang H-T, Yang Y, Fang Y, Zhang M, Deng S-Q, et al. Coinfection of COVID-19 and malaria: clinical profiles, interactions, and strategies for effective control. 2025;24(1):99.
20. Mortazavi SE, Lugaajju A, Danielsson L, Wu B, Norrgren H, Persson KEJBID. Dynamics of osteopontin levels and correlation with parasitemia in acute malaria in Uganda and Sweden. 2024;24(1):1164.