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Short communication

Antiplasmodial activity of harmirins – novel harmine–coumarin hybrids

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Introduction

Malaria still poses a major global health burden due to the lack of an effective vaccine and the emergence of resistant strains of *Plasmodium* parasites to the existing antimalarial drugs. Despite years of continuous progress, World Malaria Report 2021 estimates that there were 241 million new malaria cases in 2020 alone, leading to 627 thousand malaria-related deaths. Compared to 2019, this represents an increase of 6% in malaria cases and 12% in malaria deaths mainly due to the disruption to services during the COVID-19 pandemic.

Since malaria, a mosquito-borne parasitic disease, is characterized by an asymptomatic liver and a symptomatic blood stage in humans, an effective and safe antimalarial drug active against both stages of the parasite would be a valuable tool in the fight against malaria. The molecular hybridization (MH) concept is one of the widely applied approaches in rational drug design where two or more biologically active molecules or their pharmacophores are combined to form a new hybrid compound (Zhang et al., 2017). The MH approach is particularly useful for targeting complex diseases, such as malaria.

In our previous paper, we employed the MH concept in the design and synthesis of harmirins and investigated their antiproliferative activities. Harmirins are the hybrid compounds comprising both harmine and coumarin pharmacophores linked by 1,2,3-triazole (Pavić et al., 2021).

Harmine, the most notable representative of the β-carboline alkaloids, possesses a wide range of biological activities such as anticancer, antiviral, anti-inflammatory or antimalarial. It has been shown that harmine is a selective inhibitor of *P. falciparum* heat shock protein 90, a molecular chaperone that plays a critical role in parasitic signaling and cell division (Shahinas et al., 2012). Similarly, coumarins, naturally occurring compounds, also possess a wide variety of biological activities, including antimalarial. The coumarins are thought to act through the inhibition of DNA gyrase, an enzyme present in the apicoplast of the *Plasmodium* (Yadav et al., 2018).

Since harmirins combine two biologically active molecules (harmine and coumarin) with different modes of action, the combined chemotherapy-like effect of such hybrids might be achieved. To that end, we have decided to investigate the antiplasmodial activity of harmirins against the erythrocytic stage of chloroquine (CQ)-sensitive and CQ-resistant strains of *P. falciparum*, *Pf*Dd2 and *Pf*Dd2, respectively.

Materials and methods

Antiplasmodial activity of harmirins was evaluated against two laboratory *P. falciparum* strains (*Pf*3D7 and *Pf*Dd2), as previously described, using the histidine-rich protein 2 assay (Marinović et al., 2021). Briefly, 96-well plates were pre-coated with the tested compounds in a three-fold dilution before ring-stage parasites were added to the complete culture medium at a hematocrit of 1.5% and parasitemia of 0.05%. After three days of incubation at 37 °C, 5% CO_2 and 5% oxygen, plates were frozen until analyzed by HRP2-ELISA. All compounds were evaluated in duplicate in at least two independent experiments. The IC_{50} was determined by nonlinear regression analysis of log concentration-response curves using the drc-package v0.9.0 of R v2.6.1.

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Results and discussion

Five series of harmirins (**4a-d**, **5a-d**, **11a-d**, **12a-d**, and **13a-d**), which differ by the position of the coumarin-based substituents on the β-carboline core (C-1, C-3, O-6, O-7 or N-9 position, respectively), were previously described by our research group. In each series, four different substituents at position 6 of the coumarin ring (-H, -CH_3, -Cl, -F) were varied (Pavić et al., 2021).

For the *in vitro* antiplasmodial screening against the erythrocytic stages of the *Plasmodium* life cycle, we selected two strains of *P. falciparum* – CQ-sensitive (*Pf*3D7) and CQ-resistant (*Pf*Dd2) strain. The commonly used antimalarial drugs CQ and primaquine (PQ) were used as positive controls. The results were compared with the parent compound harmine.

To assess the selectivity of harmirins, we used the results of the cytotoxicity evaluation of harmirins against the human non-cancer cell line Hek293 from our previous paper (i.e., the selectivity index (SI) was calculated as a fractional ratio between the IC_{50} values for Hek293 and the IC_{50} values for *Pf*3D7 or *Pf*Dd2) (Pavić et al., 2021).

Generally, the antiplasmodial activity against erythrocytic stages of *Plasmodium* parasite decreased as follows: **11** (O-6) > **12** (O-7) > **13** (N-9) > **5** (C-3) > **4** (C-1). Interestingly, this pattern is in complete agreement with the pattern of antiproliferative activity against four human cancer cell lines reported in our previous paper (Pavić et al., 2021). The most active compounds **11b**,**c**, **12b** and **5c**, bearing the largest substituents on the coumarin ring, methyl or chlorine, showed the highest antiplasmodial activity at one-digit micromolar concentrations (IC_{50} = 3.5–7.5 μM) which were the same order of magnitude as the standard antimalarial drug PQ (IC_{50} = 1.5 μM). The only active compound bearing a small substituent (fluorine) on the coumarin ring was N-9 substituted derivative **13d**. However, this compound was non-selective. All the above-mentioned compounds exhibited higher antiplasmodial activity than the parent compound harmine, especially against the CQ-resistant strain *Pf*Dd2.

To our delight, we discovered that O-7 substituted harmirin with a methyl group on the coumarin ring, **12b**, was again the most promising hybrid due to the most favorable SI (SI > 11.4), as was the case in the antiproliferative assay. This finding could further support the hidden connections between malaria and cancer. Harmirin **12b** was followed by O-6 substituted derivative **11b** (SI ~ 5.5). The two compounds are structurally very similar as both bear substituents at the neighboring positions of the β-carboline core (6 and 7), bridged by an ether, and a bulky methyl group on the coumarin ring. It is worth noting that, in the case of harmirins, the IC_{50} values for both tested strains were comparable, while the IC_{50} of CQ against the CQ-resistant strain *Pf*Dd2 was one order of magnitude larger than the IC_{50} against the CQ-sensitive strain *Pf*3D7. However, all harmirins were still significantly less active than CQ against the tested strains.

Conclusion

We have evaluated *in vitro* antiplasmodial activity against the erythrocytic stage of *P. falciparum* of five series of harmirins, hybrid molecules of alkaloid harmine and coumarin derivatives connected *via* triazole linker. Compound **12b** showed the highest activity against the tested *P. falciparum* strains and the lowest cytotoxicity against non-cancer cell line Hek293. However, all harmirins were significantly less active than CQ against both tested strains. To further evaluate their potential as antimalarial agents, the activity against hepatic stages of the *Plasmodium* parasite needs to be investigated.

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