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Short Communication

In Vitro Antiplasmodial Activity and Cytotoxic Effect of (Z)-2-Benzylidene-4, 6-Dimethoxybenzofuran-3(2H)-One Derivatives

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Abstract

Background: Aurones are naturally occurring compounds that belong to flavonoids family and have antiplasmodial effects. This study investigated some new aurones derivatives against chloroquine sensitive *Plasmodium falciparum*. Here we report the synthesis, in vitro antiplasmodial activity and cytotoxic evaluation of 11 compound from derivatives of (Z)-2-benzylidene-4, 6-dimethoxybenzofuran-3(2H)-one.

Methods: The cytotoxic evaluations of active compounds were performed with MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide) assay on human breast cancer cell lines; MCF7 and T47D.

Results: From 11 compounds M3, M6 and M7 compounds showed good antiplasmodial effect against chloroquine-sensitive 3D strain of *P. falciparum* with IC₅₀ (50% inhibitory concentration) values of 7.82, 7.27 and 2.3 μM respectively. No noticeable toxicity was observed with these compounds when tested against tested cell lines.

Conclusion: The replacement of the 4 and 5 positions at ring B of aurone derivatives, with propoxy and bromide (Br) respectively was revealed highly advantageous for their antiplasmodial effect.

Introduction

Malaria is an infectious disease that caused by *Plasmodium* species (1). *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* have been known as causes of human malaria (2-3). The most severe and deadly form of disease is caused by *P. falciparum* subspecies that causes the death of 1% of patients (2). According to WHO Malaria Report 2012, there was an approximately 219 million cases of malaria all around the world and 660,000 people died from this disease (4).

Nowadays after AIDS and tuberculosis, malaria is the most important human diseases. Unfortunately, despite using different methods for eradication or control of malaria, this disease is endemic in many tropical and subtropical countries (about 104 countries worldwide). Developing and spreading multidrug-resistant strains of *P. falciparum*, developing resistance in malaria vectors to the usual effective insecticides and unsuccessfulness in developing an effective and inexpensive vaccine are the most important reasons of failure in malaria eradication or control programs (4).

Today artemisinin-based combination therapy is the last defense against the malaria but there are some reports about the resistance against atremisinin and its derivatives (5). Furthermore, these drugs are too expensive for widespread use (6). Therefore, we need effective and inexpensive drugs to replace chloroquine, which was the main stay of chemotherapy of malaria for more than 50 years.

Aurones (2-benzylidenbenzofuran-3(2H)-ones), are secondary metabolite belonging to the flavonoids family and have an important role for the pigmentation of flowers in which they are found (7). They are structural isomers of flavones and there are reports about their inhibitory effects on erythrocytic stages of *P. falciparum* strains in vitro (8-10). Other effects of aurones are antileishmanial (11-12), trypanocidal (13), antiviral, and antifungal effects (7).

The aim of this study was to investigate aurones analogs in order to find new drug candidates against malaria.

Materials and Methods

Chemicals

Reagents and materials obtained from Merck (Darmstadt, Germany) and Sigma Aldrich (Steinheim, Germany), and DMSO was purchased from Fluka (Steinheim, Germany) and RPMI 1640 medium from Gibco-Invitrogen (Paisley, Scotland, UK).

Tested compounds

Synthesis of these compounds has been described previously (10, 14-15). The basic structure of aurones derivatives is indicated in Fig. 1. The structures of synthesized aurones have been shown in Table 1.

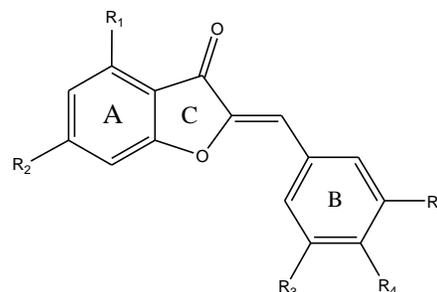


Fig. 1: Basic structures of aurones derivatives

Parasite Culture

The *P. falciparum* strain used in this study was 3D7 chloroquine (CQ) sensitive. In vitro culture of *P. falciparum* was carried out according to the method described by Trager and Jensen with some modifications (16-18). Parasites were maintained in continuous culture on human erythrocytes (blood group O⁺), provided by the Blood Transfusion Organization (Zanjan, Iran), in RPMI 1640 medium with 5% of human AB⁺ serum, 0.3 g/100 mL Albumax I, 25 mM HEPES, 19 mM sodium

carbonate and 30 µg/mL gentamicin sulfate at pH 7.2. The growth medium was replaced daily, and cultures were gassed with a mixture of

91% N₂, 6% CO₂, and 3% O₂. Synchronization to the ring stage was achieved by sorbitol method (19).

Table 1: the structure of synthesized auronones derivatives

Compound	R ₁	R ₂	R ₃	R ₄	R ₅
M1	OMe	OMe	OMe	OMe	Br
M2	OMe	OMe	OMe	OMe	OMe
M3	OMe	OMe	-	OMe	OMe
M4	OMe	OMe	OMe	OH	Cl
M5	OMe	OMe	OMe	OH	Br
M6	OMe	OMe	OMe	OEt	Br
M7	OMe	OMe	OMe	OPr	Br
M8	-	-	OMe	OMe	Cl
M9	-	-	OMe	OMe	Br
M10	-	-	OMe	OH	Cl
M11	-	-	OMe	OH	Br

In vitro antimalarial tests

Compounds were prepared in DMSO at concentration of 10 mg/mL and serially diluted with culture medium to reach 1mg/mL before use. Twenty µL of 2-fold dilution series (50 - 0.3906 µg/mL) of compounds prepared in assay medium and added to each well of 96-well plates in triplicate. One hundred eighty µL of synchronous *P. falciparum* culture (1% parasitemia and 2% hematocrit) added to each well reaching a final volume of 200 µL per well. Plates were incubated at 37° C for 24 h. Chloroquine was used as positive control and parasitized erythrocytes without drug were used as negative control. After 24 h incubation, Giemsa stained thin smears were made and parasitemia was confirmed by the numeration of 1000 erythrocytes (20). Data were imported in SPSS 16.0 software and IC₅₀ values were calculated by means of Finney's Probit analysis (21).

In vitro cytotoxicity assay

The toxic effects of active compounds against *P. falciparum* were assessed on human breast cancer cell lines (MCF7 and T47D) by using MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide) assay (22-23) and results were compared with untreated

control. The cells were cultured in RPMI 1640 medium containing 10% FBS (Fetal Bovine Serum) and incubated at 37° C with 5% CO₂ and 96% humidity. After several subcultures, cells were distributed in 96-well plates at 1,000 cells in 100 µL of culture medium and incubated for 24 h at same condition to allow attachment of cells to the bottom of wells. Then culture medium removed and 100 µL of the same medium containing the drugs at various concentrations (100, 30, 10, 3, 1, 0.1 µM) added to each well in triplicate. Plates further incubated for 5 days in same condition. The last column of plate containing 1000 cells in 100 µL of culture medium was regarded as control. After 5 days incubation the drug-containing medium discharged. For evaluation of cell survival, 25 µL of MTT solution (4 mg/mL in PBS) added to each well and plates incubated for 3 h (in same condition). Then 100 µL of DMSO added to each well and plates were gently shaken to dissolve the formed formazan crystals. The absorbance of each well measured at 540 nm using an ELISA plate reader (Infinite M200, Tecan). The GI% (Growth Inhibition percent) was calculated using the formula; %Growth Inhibition = 100 - (OD_{test} - OD_{control}) × 100, where OD_{test} is the mean absorbance of treated cells and OD_{control}

is the mean absorbance of a negative control. The cell survival of control assumed 100% and IC_{50} values generated from dose-response curves for each cell line.

Results

In vitro antiplasmodial activity

All the 11 compounds screened for in vitro antiplasmodial activity against the CQ-sensitive (3D7) *P. falciparum* strain (Table 2). Of 11 compounds tested, 3 compounds had better antiplasmodial activity. Compound M7 showed significant antiplasmodial activity with IC_{50} value of 2.3 μ M. Compounds M3 and M6 also showed good antiplasmodial activity with

IC_{50} values of 7.82 and 7.27 μ M respectively. The rest of compounds did not show noticeable antiplasmodial activity.

In vitro cytotoxicity assay

Cytotoxicity of compounds with IC_{50} value less than 50 μ M assessed on MCF7 and T47D cell lines. Results of toxicity activity of the tested compounds and selectivity index (SI) are shown in Table 2. The SI is defined as the ratio of the toxicity to the antiplasmodial activity and the higher selectivity should offer the potential of safer therapy.

The M7 compound had high selectivity for *P. falciparum* than studied cell lines in comparison with other compounds ($P = 0.008$).

Table 2: Antiplasmodial activity and toxicity assessment of tested compounds

Compound	<i>P. falciparum</i> IC_{50} (μ M)	MCF7 IC_{50} (μ M)	T47D IC_{50} (μ M)	SI for MCF7	SI for T47D
M1	22.29	36.34	28.85	1.63	1.29
M2	≥ 50	-	-	-	-
M3	7.82	20.80	3.15	2.65	0.4
M4	≥ 50	-	-	-	-
M5	28.64	34.75	19.39	1.21	1.21
M6	7.27	29.90	9.35	4.11	1.28
M7	2.3	17.19	11.65	7.47	5.06
M8	22.04	20.60	9.07	0.93	0.41
M9	32.32	34.99	29.47	1.08	0.91
M10	36.38	20.27	11.72	0.55	0.55
M11	≥ 50	-	-	-	-
CQ	0.775	-	-	-	-

Discussion

Several studies have been done concerning the aurones as chemotherapeutic antimalarial agents (8-10). Aurones [2-benzylidene-nebenzofuran-3(2H)-ones] are natural compounds belong to the flavonoids family. The natural entity of aurones, their inhibitory activity on *Leishmania* and malaria parasites (8, 24-25), has prompted us to investigate aurones analogs in order to gather more structural elements required for the antiplasmodial activity.

Three out of 11 compounds (M3, M6 and M7) showed good antiplasmodial activity with IC_{50} values of 7.82, 7.27 and 2.3 μ M against 3D7 strain of *P. falciparum* respectively. The M7 compound had better selectivity index than other two active compound (with SI value of 7.47 and 5.06 for MCF7 and T47D cell lines respectively). This let us believe that activity obtained with this compound is not due to general toxicity against *P. falciparum* but can be explained by specific anti-plasmodial activity and should offer potential for safer therapy.

In one study a library comprising 44 different substituted aurones derivatives was synthesized and screened against the chloroquine resistant *P. falciparum* W2 strain and demonstrated that some compounds showed antiplasmodial activity in micromolar range (10). "A series of naturally occurring aurones was synthesized and evaluated for their ability to inhibit erythrocytic stages of *P. falciparum* strains in vitro" (8). The most active compound was 4,6,4'-triacetyl-3',5'-dimethoxy-2-aurone with IC₅₀ values of 0.007 μM and 0.18 μM for the *P. falciparum* strains K1 and NF54, respectively.

According to previous investigations, the structure activity relationship confirmed that methoxylation at 4 and 6 carbons were beneficial for antiplasmodial activity of aurones derivatives (9). In our study, these substitutions were confirmed (compound M1 versus M9 and M5 versus M11). This substitution pattern involved in binding of aurones derivatives to protozoal target protein ATP-binding cassette (ABC) transporters like P-glycoprotein (26-27). At the ring B, presence of a hydrophobic group was highly beneficial for antiplasmodial effect as illustrated by comparing derivatives M6 versus M5 and M9 versus M11. The elongation of chain from methoxy to ethoxy and then to propoxy leads to an increase in antiplasmodial effect (compound M1 versus M6 and M7). The presence of a hydrophobic halogen atom at the ring B at 5 positions was quite beneficial as illustrated by comparing derivatives M1 and M2. In conclusion, throughout the present study, we report the preliminary results regarding the structural requirements for the antiplasmodial activity of aurones. We have found that the replacement of the 4 and 5 positions at ring B with propoxy and bromide (Br) respectively was revealed to be highly advantageous for the activity. We also optimized (at least in part) the nature of substituents to be present at the A and B-rings.

Conclusion

The present results bring essential elements, which will be used, for the synthesis of more active aurones against malaria parasite. Further studies need to confirm the in vivo antiplasmodial properties of active aurones derivatives against rodent malaria.

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