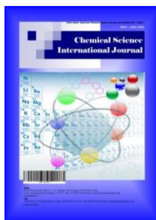

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Evaluation of Cytotoxic Profile of Some Chalcone Derivatives with Antimalarial Properties

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Authors' contributions

This work was carried out in collaboration among all authors. Author MTD contributed in the conception and design, data collection, analysis and interpretation of data, draft article and final version approval. Author MO contributed in to writing and final version approval of manuscript. Author EVA contributed in the conception and study design, contribution to writing and approval of final version. Author KDT contributed in design, interpretation of data and contribution in writing and final version approval. Authors AJK and NBC contributed of analysis and interpretation of data. Author WY contributed in correction of draft and final version approval. All authors read and approved the final manuscript.

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ABSTRACT

Malaria continues to kill thousands of people worldwide. People South of the Sahara are paying the heaviest price for this epidemic. In addition, the resistance of *Plasmodium falciparum* which spreads to artemisinin derivatives becomes worrying. To respond to this emergency, the search for new molecules effective against the parasite is essential. Otherwise, chalcones have shown their potential as an effective pharmaceutical agent in numerous studies. However, despite their mainly antiparasitic efficacy, several compounds have been shown to be cytotoxic. This study aimed to assess the cytotoxic profile of chalcone derivatives. Cytotoxicity tests were carried out according to the method described by Taylor and collaborators on Vero cells. The 96-well plates were used in carrying out this study, 100 µl of the compounds were added with concentrations ranging from 7.5 to 1000 µg/ml in DMEM. The results obtained report three compounds derivatives (Chal_B14, Chal_B17 & Chal_SCA03) no-cytotoxic with LDH product values between 129 - 132.5 U/L and ATP ranging from 8.55 to 13.6 RLU. The IC_{50s} for no-cytotoxic compounds ranged from 102 to 236 µM. On the other hand, the cytotoxic compounds had IC_{50s} of less than 30 µM.

The results of this study show that the derivatives Chal_B14, Chal_B17 & Chal_SCA03 are candidate compounds with a view to finding new molecules.

Keywords: Cell cytoxicity; malaria; Plasmodium falciparum; vero cells; chalcone derivatives.

1. BACKGROUND

Despite the control methods, malaria remains a major public health problem in the world and particularly in south-Saharan Africa. In 2018, the World Health Organization (WHO) estimated the number of reported malaria cases to be around 228 million worldwide. In the same year, malaria caused nearly 405,000 deaths, the majority (85 %) of which occurred in sub-Saharan Africa [1]. In addition, the resistance of *Plasmodium falciparum* to most available antimalarial drugs raise a strong concern about the therapeutic management of the disease in endemic countries [2]. The urgency of the current situation, and the need to remedy it by improving existing therapies, paves the way for the search new molecules that are effective against *P. falciparum*. At the base of many active ingredients of several drugs, chalcones take an important place in the field of organic chemistry as a synthetic intermediary and therapeutically as a biologically active product [3]. The origin of these compounds can be natural, plant extracts, semi synthetic or totally synthetic. Chalcones also possess a wide range of pharmacological activities including antiparasitic (antileishmanial, antitrypanosomal, antimalarial), antibacterial (antituberculosis), antifungal and antiviral [4,5,6]. Chalcones are also one of the most important intermediate classes in the process of synthesizing certain drugs [7]. Some compounds led by Lichochalcone A have been proposed as antimalarial drug candidates [8]. However, the establishment of a drug cannot be done without the exploration of its toxic or cytotoxic profile [9]. In this line, the use of animal cell culture method, in the context of evaluation of active molecules for the treatment of various pathologies is widely expanding [10]. The production of drugs or effective compounds with cytotoxic profile is known remain, to this day, a major issue both pharmacologically and in the industrial production of drugs [11]. Previous studies carried out on chalconic derivatives have made it possible to demonstrate a good to very good activity on *P. falciparum* [12] (Table 1); which makes these compounds promising molecules against *P. falciparum* malaria. However, several compounds active on *Plasmodium* can be cytotoxic [13]. It was therefore important to explore the cytotoxic profile of pharmacoactive compounds. It is within this framework that this study was set up, which aimed to evaluate the cytotoxic profile of some synthetic chalcone derivatives.

2. MATERIALS AND METHODS

2.1 Molecules

These are six chalcone synthesis derivatives including derivatives of 1,3-diphenylpropenone chalcone variously modulated by groups of chlorine, hydroxyl, methoxyl, nitro and dimethylamine type. The compounds used were synthesized by the department of therapeutic and organic chemistry of the training and research unit of the pharmaceutical and biological sciences of Felix Houphouët Boigny University, Abidjan – Côte d'Ivoire.

Table 1. Molecular activity according to the type of strain and isolate [12]

Molécules	Strain						Isolates					
	NF54			K1			CQ-S			CQ-R		
	IC_{50}(µg/ml)	IC_{50}(µM)	A	IC_{50}(µg/ml)	IC_{50}(µM)	A	IC_{50}(µg/ml	IC_{50}(µM)	A	IC_{50}(µg/ml)	IC_{50}(µM)	A
Chal_B1	10.03	41.47	C	8.38	34.65	C	5.8	23.99	C	10.73	44.36	C
Chal_B11_B11	6.37	23.42	C	1.04	3.82	A	6.47	23.79	C	12.31	45.26	C
Chal_B14	2.93	10.59	B	2.73	9.87	A	4.53	16.40	B	10.28	37.19	C
Chal_B17	3.29	11.55	B	1.71	6.00	A	2.69	9.45	A	4.1	14.41	B
Chal_SCA02	4.24	12.66	B	3.36	10.03	B	7.33	21.89	C	16.77	50.09	D
Chal_SCA03	2.04	9.09	A	1.08	4.83	A	10.01	44.50	C	15.38	68.35	D

** A: activity (A = very good activity; B = good activity; C = moderate activity; D = low activity; E = very low activity)*
** CQ-S: Chloroquinosensitiveisolates ; CQ-R: Chloroquinoresistantisolates*

2.2 Study Site and Population

African monkey green kidney (Vero Cell) [14] ATCC cells were used for this study. The department of epidemic viruses of the Institute Pasteur (I.P.CI) of Côte d'Ivoire by its continuous cell culture cell line was the donor of the Vero cells and served as a site for the realization of this study.

2.3 Cytotoxicity Test

2.3.1 Vero cells culture

Vero cells were cultured in DMEM (Thermo Scientific Lot # SLBL6087V) supplemented with 10% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37°C. The trypsin digestion method was adopted after reaching 90% confluence of the cells [15]. All experiments were performed under conditions of hygiene and sterility to minimize contamination by bacteria or fungi that may interfere with the toxicity profile. The cells were cultured for 48 hours in multiwell plates (96 wells). At withdrawal the different tests were applied.

2.3.2 Preparation of solutions

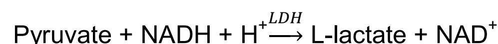
The various stock solutions (1000 µg/mL) of the chalcone derivatives were obtained by dilution in dimethyl sulphoxide (DMSO) and filtered using Millipore filters (0.2 µm). Then, the other concentrations (500 - 7.8 µg/ml) were obtained by dichotomous dilution with the culture medium. Hundred microliters (100 µL) of the derivatives at different concentrations were added to each well except the control wells according to the periods defined in the protocol. Three different reagents were used in the context of the cytotoxicity tests: CellTiter-Glo, LDH and MTT.

2.3.3 Cell Titer-glo test

The CellTiter-Glo test is a reaction that uses ATP. The latter is involved in the Luciferase reaction as an indicator of metabolically active cells [16]. The enzyme acts in the presence of Magnesium and ATP to produce Oxyluciferin and releases energy in the form of light. The intensity of light is proportional to the amount of ATP present in the medium [17]. CellTiter-Glo Luminescent reagent "Cell Viability Assay" is manufactured by Promega (Buffer and Freeze-Dried Substrate) REF: G7570 Lot: 0000262953. The preparation of the substrate was made according to the manufacturer's recommendations. After 48 hours of incubation at 37°C cells, 100 µL of the derivatives at different concentrations were added to each well except the control wells and the plate was incubated for 24 hours at 37°C. At the withdrawal of the oven plates, 100 µL of the reagent were introduced into each well including control wells. The reading was done using a Thermo-Scientific 355 type reader Ref: 51118170 RS-232C series, at a wavelength of 460 nm.

2.3.4 LDH test

Lactate dehydrogenase [18] is a cytological enzyme found in most eukaryotic cells. The presence of LDH in the culture medium determines the death of the cell or the destruction of the cytoplasm of the membrane. The increase in LDH activity in the supernatant is proportional to the number of lysed cells. The LDH test is a colorimetric method that uses a reaction cocktail [19]. LDH catalyzes the reduction of pyruvate by means of NADH, according to the following reaction:



$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{LDH} \text{L-lactate} + \text{NAD}^+$$

The LDH reagent that was used for the test was manufactured by SPINREACT ref: 1001260, lot: 2306T. The preparation of the reagent and the test procedure were made according to the manufacturer's recommendations. After 24 and 48 hours of incubation at 37°C. In the presence of the compounds, 100 µL of the substrate were introduced into each well, including the controls. The reading was done using a Thermo-Scientific 355 type reader Ref: 51118170 RS-232C series, at a wavelength of 340 nm.

2.3.5 MTT test

Traditionally, *in vitro* determination of the toxic effects of compounds has been performed by cell counting after vital staining. Alternative methods used include measuring the incorporation of a radioisotope to estimate DNA synthesis, enumeration by automata, and colorimetric methods to evaluate cell activity. For this study, the MTT test {3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide} manufactured by Sigma Aldrich lot # MKBX8173V, was used. This test uses a chromogenic substrate reduced by the mitochondrial dehydrogenases of living cells [20,21]. The tests were made in triplicate and the percentage of viability [22] was calculated according to the following formula:

$$\% V = \frac{meanODofA}{AverageODofB}$$

with A: given concentration of a compound, B: control and OD: optical density. After 24 h, 48 h and 72 h of incubations at 37°C., 10 µl of the substrate were introduced into each well, including the controls. The reading was done using a Thermo-Scientific reader type 355 Ref: 51118170 RS-232C series, at a wavelength of 470 nm.

2.3.6 Statistical analysis

After incubation times (24, 48 and 72 hours) in the presence of the compounds at different concentrations, and the addition of the different reagents, the optical densities were read using a Thermo-Scientific 355 type reader Ref.: 51118170 RS-232C series at different wavelengths by changing the filters. Then, the ODs obtained made it possible to estimate the cell viability, the quantity of LDH produced and calculate IC_{50s} of toxicity of the compounds. The Excel and Graph pad prism software version 5.01 was used to calculate averages, standard deviations and confidence intervals r^2 by nonlinear regression. The interpretation of the statistical tests is made with a risk α = 5%. These IC_{50s} are coupled with the production of ATP and LDH. The cytotoxic classification of our derivatives is made according to the model of Xia M and collaborators [23].

3. RESULTS

Preliminary results of sensitivity activity of our compounds are shown in Table 1. The results obtained are shown in Table 2. These data allow us to classify the derivatives into two groups.

- Non-toxic derivatives at the concentrations used are compounds B14, B17 and SCA03
- Cytotoxic derivatives at 72 h of incubation, in particular compounds B1, B11 and SCA02

Table 2 also shows the production of various molecules such as ATP, LDH of Vero cells in the presence of derivatives. Inhibitory concentrations in µM of the compounds were recorded.

4. DISCUSSION

The CellTiter-Glo test is designed for use with the multiwell plate format. This test, in its implementation leads to cell lysis and the generation of a luminescent signal proportional to the amount of ATP present in the medium. The amount of ATP present in the medium is also proportional the number of cells present in culture and viable. The data obtained show that with the cell line used there was a significant correlation between the increase in cell number and ATP. The increase of ATP in the extracellular medium may be an event subsequent to mitochondrial involvement [17]. This attack of the mitochondria could lead to cessation of most functions of the cell and its collapse. Glutathione levels and certainly other enzymatic activities could be reduced. Modification of the enzymatic mechanisms of the cell would be the basis of the dislocation of the integrity of the cell membrane. Chal_SCA02 was toxic at 24 h incubation. However, at 48 h this toxicity was reduced, this could be due to instability of the compound or a reversibility of the cellular toxicity of this compound. Compounds Chal_B1, Chal_B11 and Chal_SCA02 would be toxic on cell mitochondria. Lactate dehydrogenase is a stable enzyme in the cytoplasm. It is released into the extracellular environment once the cell membrane is broken. Thus, the release of LDH in the medium demonstrates a toxic action on the cytoplasmic membrane. The process of necrosis could cause a massive production of lactate and this could be explained by different phenomena. At first, the need to rebalance the redox potential NAD +/ NADH of the cell. Indeed, NAD + is an important element for the proper functioning of the cell and is located at the level of the mitochondria [19,24]. In addition, the exposure of Vero cells to the different concentrations of the compounds and their toxic profiles could justify the variation of the amount of LDH produced. The compounds with a toxic profile would act directly on the cytoplasmic membrane of the cell. Abundant production of LDH was observed with compounds Chal_B1, Chal_B11 and Chal_SCA02. On the other hand, this abundant production of LDH in the extracellular medium was not observed with the compounds Chal_B14, Chal_B17 and Chal_SCA03. These results corroborate with those of Sriphana et al. [25] which evaluated the cytotoxicity of two chalcones isolated for the first time from fruits of *Millettia leucantha* on Vero cells. Similarly, Rizvi et al. evaluated the anti-HIV1 and cytotoxic activities of piperidyl-thienyl chalcones and their 2-pyrazoline derivatives on PBM, CEM and Vero cells. Eleven compounds were non-toxic with IC_{50s} between 50 and 100 µM and thirteen cytotoxic on the Vero cells [26]. Pande et al. also obtained a relative cytotoxicity

Table 2. *In vitro* cytotoxic profile of chalcone derivatives

Tests		Compounds					
		Chal_B1	Chal_B11	Chal_B14	Chal_B17	Chal_SCA02	Chal_SCA03
CellTiter-Glo(RLU)		6.4	6.55	13.6	8.85	5.5	8.55
LDH (U/L)	48 h	257.5	223.7	-	202.4	138	129
	72 h	239.5	205.2	232.2	202.8	159.5	137.9
MTT(CI_{50}) µM		29.49	24.9	236	102	24	212
Cytotoxic profile		C	C	NC	NC	C	NC

**reference values LDH: 120-240 U/L*
**C: cytotoxic; NC: not-cytotoxic*

of the 2'hydroxy chalcone derivatives. The results obtained indicated a variation in toxicity ranging from reduced to elevated [27]. For these authors the activity tests should take into account the toxic concentrations of the compounds studied; and allow the reduction of inputs. The different experimental steps to determine the cytotoxic profile of compounds or drugs at different concentrations through the use of the MTT test is a reference method [28]. It is shown that decreasing absorbance for treated cells with increasing concentrations of compounds compared to control cells. This decrease in absorbance would express a good application of the experimental procedures. The results of this work show that our compounds with cytotoxic profiles could induce necrosis and death of the cell; the values of our IC_{50s} prove it. Our results are contrary to those obtained by Aponte & al which analyzed the cytotoxic profile of 2'4'-diallyloxy-6'methoxy-chalcone derivatives. The results they obtained show a severe cytotoxicity on Vero cells [29]. This study reveals that compounds Chal_B14, Chal_B17 and Chal_SCA03 are not dangerous for Vero cells, in terms of therapeutic activity and non-cytotoxic pair make them good drug candidates.

5. CONCLUSION

The cytotoxicity tests of various compounds in the context of the evaluation of their toxic profile remain decisive for the continuation of the work. However, laboratories in developing countries still do not have the necessary means for a good evaluation of the compounds that are natural, semisynthetic or synthetic. The acquisition of the different cell lines needed for the evaluation of new bioactive molecules remains a challenge. The North - South and South - South collaborations would make it possible to remedy this handicap. The results of the evaluation of the cytotoxic profile of chalcone derivatives on Vero cells show that three compounds expressed toxicity on the cells used; they are compounds Chal_B1, Chal_B11 and Chal_SCA02. On the other hand, compounds Chal_B14, Chal_B17 and Chal_SCA03 did not express any toxicity with respect to Vero cells. These could be some promising antimalarial drugs candidates.

ETHICAL APPROVAL

The protocol of the study was approved by the National Committee of Ethics and Research of Côte d'Ivoire (reference 043/MSHP/CNER-Kp).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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