Synthesis of [(7-Chloroquinolin-4-yl)amino]chalcones: Potential Antimalarial and Anticancer Agents

Rosa FERRER 1, Gricela LOBO 1, Neira GAMBOA 2, Juan RODRIGUES 2,3, Claudia ABRAMJUK 3, Klaus JUNG 3, Michael LEIN 3,4, Jaime E. CHARRIS * 1

1 Laboratorio de Síntesis Orgánica, Facultad de Farmacia, Universidad Central de Venezuela, Apartado 47206, Los Chaguaramos, 1041-A Caracas, Venezuela.
2 Laboratorio de Bioquímica, Facultad de Farmacia, Universidad Central de Venezuela, Apartado 47206, Los Chaguaramos, 1041-A Caracas, Venezuela.
3 Department of Urology, University Hospital Charité, Humboldt University, Berlin, Germany.
4 Berlin Institute for Urologic Research, Berlin, Germany.

* Corresponding author. E-mail: jaime.charris@ucv.ve (J. E. Charris)

Sci Pharm. 2009; 77: 725–741    doi:10.3797/scipharm.0905-07
Published:  October 15th 2009   Received:  May 11th 2009
Accepted:  October 14th 2009

This article is available from: http://dx.doi.org/10.3797/scipharm.0905-07

© Ferrer et al.; licensee Österreichische Apotheker-Verlagsgesellschaft m. b. H., Vienna, Austria.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

[(7-Chloroquinolin-4-yl)amino]chalcone derivatives derived from the corresponding 3- or 4-[(7-chloroquinolin-4-yl)amino]acetophenone were synthesized and evaluated for in vitro antimalarial and anticancer activity. The most active compounds 12, 13, 15, 17 and 19 from the 3-substituted series displayed inhibitory values against heme crystallization in the range of 93.14 ± 1.74 – 94.93 ± 1.50 % as an antimalarial mechanism and cytotoxic effect with IC50 values of 7.93 ± 2.05, 7.11 ± 2.06 and 6.95 ± 1.62 µg/mL for 13, 17 and 19 respectively against humane prostate LNCaP tumor cells.

Keywords

Malaria • CQ • β-Hematin • Cancer • Prostate

Introduction

Malaria is believed to affect between 300 to 500 million people worldwide, and to cause one to three million deaths each year [1]. In the past, quinoline-derived compounds were extensively studied for the development of new therapeutic agents that led to the development of some molecules, namely, pamaquine [2] and mepacrine [3]. One of the
first drugs to be prepared was the potent and inexpensive chloroquine (CQ), which is a 7-chloroquinoline with an amino substituent in position 4. Chloroquine’s antimalarial activity appears to be linked to the parasite’s heme metabolism [4]. Development of drug resistance to chloroquine in malaria has become a major health concern in endemic areas, which has prompted a search for alternative antimalarials against the CQ-resistant strains [5]. As a result, several new class of antimalarial drugs has been developed; none of them has reached the same status of recognition as the drug of choice in malaria therapy as CQ. Amodiaquine (AQ), a Mannich base 4-aminoquinoline, is effective against CQ-resistant strains of P. falciparum [6]; however, the clinical use of this drug has been severely restricted because of the hepatotoxicity and agranulocytosis side effects associated with its long term use; lysosomal accumulation and bioactivation of reactive quinoneimine metabolite are implicated to be cause of the observed AQ in vivo toxicity [7]. Isoquine (IQ) is an analogue of AQ, in which the 4'-hydroxy group on the aniline ring of AQ is interchanged with a 3'-Mannich base side chain. IQ was demonstrated to possess higher antimalarial activity against P. yoelii than AQ. In contrast to AQ, IQ was excreted primarily as a glucuronide, instead of a glutathione conjugate [8]. Another promising compound from the class of the 4-aminoquinolines is tebuquine (TQ), which is a biaryl analogue of AQ. TQ is significantly more active than AQ and CQ in both \textit{in vitro} and \textit{in vivo} tests [9, 10]. Similar to AQ, TQ forms an active quinoneimine metabolite and consequently develops the same toxic side effects as AQ in prolonged use. On the other hand, substituted quinolines possess medicinal properties for effective control of malaria and cancer. Unfortunately the design and subsequent synthesis of new antimalarials are hindered by the fact that the mechanism of resistance is not fully understood [11].

Hemoglobin degradation in intraerythrocytic malaria parasites is a biochemical event which takes place in an acidic digestive vacuole by different proteases to provide free amino acids necessary to protein synthesis. In this process, the oxidant heme group is separated from the globin chains and parasite protects itself by crystallizing the heme moieties into a non-soluble and non-toxic pigment called hemozoin or β-hematin [12]. In this context, compounds which are able to inhibit hemoglobin proteolysis and/or β-hematin synthesis could be considered as potential antimalarials.

On the other hand, different evidence has demonstrated the ability of quinolines as potential antitumor agents. Recently the antitumour potential of quinolines against MCF-7 human breast cancer cells, with chloroquine being the most apoptosis-inducing agent, has been reported [13]. All differentiation-inducing quinolines caused growth suppression in MCF-7 and MCF10A cells. The mechanism of action of the differentiation-inducing quinolines has been proposed to involve strong suppression of E2F1 that inhibits growth by preventing cell cycle progression and fosters differentiation by creating a permissive environment for cell differentiation. A series of new thioquinolines were tested for antiproliferative activity \textit{in vitro} against human [SW707 (colorectal adenocarcinoma), CCRF/CEM (leukemia)] and murine [P388 (leukemia), B16 (melanoma)] cancer lines. All the compounds exhibited antiproliferative activity comparable to cisplatin [14]. Indeed Lukevic et al., showed that another thioquinoline analog was active on human fibrosarcoma HT-1080 cell line [15]. A novel intercalating compound of a pyrimido[4',5':4,5]selenopheno[2,3-b]quinoline series also showed cytotoxic effect in a concentration and time-dependent manner. Cell cycle analysis and tritiated thymidine assays revealed that this compound affects the cell cycle progression by arresting at S
phase. DNA fragmentation, nuclear condensation and changes in the expression levels of BCL2/BAD confirmed the activation of apoptosis [16]. Other evidence has demonstrated that these kinds of structures, like the well known quinoline analog MT477, suppress cell signaling through Ras molecular pathway, inhibiting PKC activity. The effect of this compound is dose-dependent on H226, MCF-7, U87, LNCaP, A431 and A549 cancer cell lines [17]. Two murine xenograft models of human A431 and H226 lung carcinoma were used also to evaluate tumor response to intraperitoneal administration of MT477. Tumor growth was inhibited significantly in H226 xenografts following this treatment, compared to vehicle controls. As we could notice, the effects of the quinoline structures are not specific to one type of cells, for example, a dose dependent decrease in cell viability was observed also in tumor bladder cancer cells treated with another quinoline derivate, an imidazoquinoline. This type of structure induced apoptosis and cytokine production significantly. In \textit{in vivo} experiments most mice treated with this therapy showed only an intense inflammatory response with no evidence of tumor, while control mice showed tumor growth [18]. There is also evidence of the potential antitumor activity of quinoline derivates in prostate cancer (PCa). Linomide, a quinoline-3-carboxamide derivate, has a dose-response ability to inhibit the growth of a series of four additional human and rodent prostate cancer models in mice and this efficacy is correlated with inhibition of angiogenesis [19]. Our ongoing efforts in the direction of identifying new classes of 4-aminoquinolines with antimalarial and anticancer properties prompted us to undertake the synthesis of a variety of 7-chloroquinolinyl-4-aminophenylchalcones.

\textbf{Results and Discussion}

\begin{center}
\includegraphics[width=\textwidth]{sch1.png}
\end{center}

\textbf{Sch. 1.} Synthesis of (E)-1-[3 or 4-(7-chloroquinolin-4-ylamino)phenyl]-3-(phenylsubstituted)prop-2-en-1-ones 5–19
The target compounds 5–19 were prepared as outlined in Scheme 1. The key intermediates 3, 4 were obtained by aromatic nucleophilic substitution of 4,7-dichloroquinoline with the appropriate aniline derivatives in ethanol and refluxing. Attachment of the lateral α,β-unsaturated side chain of compounds 5–19 was accomplished in one step: reaction of compounds 3, 4 with substituted benzaldehydes in methanol, and one pellet of KOH (catalytic) at room temperature (Claisen–Schmidt condensation). After completion of the reaction (usually 96 h), the desired products were obtained in excellent yields and purity. Compounds were characterized by spectroscopic means and their purity established by elemental analysis. Theoretically, E and Z geometric isomers can be equally formed during the reaction. However, Z configuration is highly unfavorable. By 1H NMR we assigned the configuration (E) for the C–C double bond based on the vicinal proton-proton coupling J: 15 Hz. The selectivity in configuration may derive from the repulsion between the substituted phenyl groups, which are two bulky moieties bonding on C–C double bond.

Indeed, a previous study has shown that quinolines and chalcones exhibited potent antimalarial activities, inhibiting heme crystallization, globin proteolysis and showing also in vivo effects in a malaria murine model [20], representing new alternatives which needs to be taken on consideration in further studies.

Fig. 1. Effects on globin proteolysis of 3'- or 4'-[(7-chloroquinolin-4-yl)amino]-3- or 4-substituted-chalcones. The samples were solubilized in SDS-sample buffer containing β-mercaptoethanol and boiled before electrophoresis in 15 % SDS-PAGE gels. The gels were stained with Coomassie blue. Undegraded globin bands appear at 14.4 kDa; A: control hemoglobin; B: control hemoglobin in presence of parasite extract; 5–19: hemoglobin in presence of parasite extracts and compounds.

In this work we tested compounds 5–19 for their effects as inhibitors of β-hematin formation and inhibitors of hemoglobin proteolysis in vitro. To evaluate the potential antimalarial activity of 5–19, we tested the ability of these compounds to inhibit heme crystallization, considering that heme can crystallize spontaneously under acid and low oxygen conditions found in the vacuole of the parasite [21]. Results that showed more than 90% of inhibition of heme crystallization were considered significant (compounds 12, 13, 15, 17, 19, Table 1). The substitution of ketone α,β-unsaturated group on position 3 and the presence of a hydrogen, halogen or N-dimethyl groups as substituent in the aromatic ring appeared to be favorable for the potential antimalarial activity, since most of the compounds possessing these groups showed measurable levels of inhibition of β-hematin formation. Results reveal that the compounds were as active as chloroquine (93.61 ±
0.26 %) inducing the inhibition of heme crystallization. Consequently all compounds were tested for their capacity of inhibiting hemoglobin proteolysis, in an *in vitro* assay which uses trophozoite-rich extract to digest the native hemoglobin of mice. The electrophoretic analysis indicated that only compound 10 was partially effective inhibiting the proteolysis of hemoglobin 44.62 ± 1.23 % (Figure 1 and Table 1).

**Tab. 1.** Biological activity of 3'- or 4'-[(7-chloroquinolin-4-yl)amino]-3- or 4-substituted-chalcone derivatives. The results are expressed by the mean ± standard error of the mean. *p>0.05 compared to chloroquine. IβHS: inhibition of β-hematin synthesis; IGP: inhibition of globin proteolysis. †Cytotoxic effect of compounds on LNCaP tumor cells. n=3.

| Cpd.* | R          | %IβHS       | %IGP       | IC₅₀ (µg/mL) † |
|-------|------------|-------------|------------|--------------|
| 5     | 4-OCH₃     | 0           | <10        |
| 6     | 4-N(CH₃)₂  | 23.71 ± 2.67| <10        |
| 7     | 4-Cl       | 23.54 ± 5.11| <10        |
| 8     | 4-F        | 38.77 ± 2.28| <10        |
| 9     | 4-OCH₃     | 71.75 ± 4.33| <10        |
| 10    | 3-OCH₃     | 42.54 ± 2.52| 44.62 ± 1.23|
| 11    | 2-OCH₃     | <10         | <10        |
| 12    | 4-N(CH₃)₂  | 94.55 ± 0.25*| <10        | 23.66 ± 1.24 |
| 13    | 4-Cl       | 94.34 ± 0.99*| 12.22 ± 0.76 | 7.93 ± 2.05  |
| 14    | 3-Cl       | 36.76 ± 3.70| <10        |
| 15    | 2-Cl       | 94.42 ± 1.89*| <10        | 10.09 ± 2.29 |
| 16    | 4-F        | 39.50 ± 6.10| <10        |
| 17    | 3-F        | 94.93 ± 1.50*| <10        | 7.11 ± 2.06  |
| 18    | 2-F        | 78.93 ± 2.34| <10        | 47.83 ± 0.71 |
| 19    | H          | 93.14 ± 1.74*| <10        | 6.95 ± 1.62  |
| (LEP) | Leupeptin   | 89.06 ± 0.69|            |
| (PEP) | Pepstatin   | 92.94 ± 0.67|            |
| CQ    | Chloroquine | 96.61 ± 0.26|            |

*5–8: 4'-substituted; 9–19: 3'-substituted.

On the other hand, prostate cancer (PCa) represents the most common solid tumor and the second leading cause of cancer death among men [22]. The antiandrogen hormone therapy is the standard treatment for advanced prostate cancer, but it is only effective for a limited period of time and after that, the hormone refractory phase, which involves the majority of cases of the PCa, will grow in a more aggressive and progressive fashion. In this stage of the disease, PCa increases in a primary tumor which leads to metastases and
the options of medication are restricted. Therefore the development of new compounds targeting human hormone refractory PCa represents a key in order to have more alternatives to fight against this disease.

In this context, recent evidences have shown the effect of different quinolines and chalcones in PCa. The quinoline analog, clioquinol can inhibit the proteasomal chymotrypsin-like activity, repress androgen receptor (AR) protein expression, and induce apoptotic cell death in human prostate cancer LNCaP and C4-2B cells and animal studies showed that clioquinol treatment significantly inhibited the growth of human prostate tumor C4-2B xenografts (by 66%), associated with in vivo proteasome inhibition, angiogenesis suppression, and apoptosis induction [23]. Also, ionone-based chalcones have demonstrated substantial in vitro anti-proliferative activities in LNCaP and PC-3 prostate cancer cell lines as antagonists of androgen receptor [24].

![Graph showing dose-response curves of selected compounds on LNCaP tumor cells treated with compounds.](image)

**Fig. 2.** Dose-response curves of selected compounds on LNCaP tumor cells treated with compounds. Compounds: ♦ 13, ■ 17, ▲ 19. Results are expressed as the mean ± SEM of three different experiments. Each experiment was performed in five different wells. ***p<0.001 compared to the same treatment at 15 and 25 µg/mL n=3.

In this study, we also evaluated the effect of the most active inhibitors of β-hematin formation compounds on an androgen-sensitive human prostate tumor cell line (LNCaP) according on the reactivity of a tretazolium salt (sodium 3,3'-[1-(phenylamino)carbonyl]-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate, XTT), as described by Denizot and Lang [25]. The IC₅₀ value in the XTT assay was defined as the concentration of the tested compound leading to a 50% of inhibition of cell viability compared to untreated cells. The results demonstrated the potential antitumor activity of each compound tested (Table 1). Special attention should be paid to compounds 12, 17, and 19.
which showed the stronger cytotoxic results (IC$_{50}$ <10 μg/mL). The effect of these compounds is dose-dependent, showing a lower viable cell number as the drug concentration increase (Figure 2) and also, this effect is time-dependent, which is evident from 24 hours to 96 hours after drug exposure (Figure 3).

![Graph showing time-dependent inhibition of selected compounds on cell growth (ICG) at IC$_{50}$ concentrations. Results are expressed as the mean ± SEM. *p<0.05; **p<0.01; ***p<0.001. n=3.](image)

The observed activities of these 4-aminoquinolines against the β-hematin synthesis may be speculated from the result of several factors. The 4-amino-7-chloroquinoline subunit is an antimalarial pharmacophore that inhibits heme dimerization into the non-toxic hemozoin [26]. The presence of the substitution of a α,β-unsaturated ketone group on position 3' and the presence of a hydrogen, halogen or N,N-dimethylamino groups as substituent in the aromatic ring may be resulting in a secondary interaction. These results clearly indicate that an increase of the lipophilic property, with appropriate groups on the phenyl substituent, produces good inhibitory activity of the heme dimerization into non-toxic hemozoin. The fact that the activity is markedly affected by altering the substituents on 4-aminophenyl ring, suggests that this aromatic ring make a specific contribution to the binding via an aromatic ring orientation. On the other hand, these compounds were also active as antitumor agents in human prostate cancer cells, confirming a diverse biological response of these 4-aminoquinolines. It is important to note that the presence of a hydrogen or a halogen on position 3 or 4 as substituent groups in the aromatic ring improved the anticancer activity of these structures.
Conclusions

The present study describes the synthesis and biological activity of a series of 4-aminoquinoline derivatives with a non-basic side chain nitrogen. In summary, some of them showed good selectivity index between the parasite and tumor cells. Compounds 12, 13, 15, 17, 19 exhibited potential effects as inhibitors of β-hemin formation and inhibitors of human prostate cancer cell proliferation. The studies confirm that the antimalarial mechanism of action could be similar to that of chloroquine, as most of the compounds form an association complex with hematin and thereby inhibit hemozoin formation. The results provide basic information to establish that the basicity of the side chain nitrogen is not very essential for an inhibitory activity of heme dimerization of 4-aminoquinolines and opens new vistas for the design of new antimalarial agents. Rationally, such a combination of antimalarial pharmacophores and other functionalities offers many attractive features for accelerating antimalarial drug discovery. On the other hand, the same structures were also active as inhibitors of human prostate tumor cell proliferation. In this context, further studies are needed to elucidate the antitumor mechanism of action.

Experimental

Melting points were determined on a Thomas micro hot stage apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. The $^1$H NMR and $^{13}$C NMR spectra were recorded using a Jeol Eclipse 270 (270 MHz/67.9 MHz), spectrometers using CDCl$_3$ or DMSO-d$_6$, and are reported in ppm downfield from the residual CHCl$_3$ or DMSO respectively. Elemental analyses were performed on a Perkin Elmer 2400 CHN analyser, results were within ± 0.4% of the predicted values for all compounds. Chemical reagents were obtained from Aldrich Chemical Co, USA. All solvents were distilled and dried in the usual manner.

Chemistry

General procedure for the synthesis of [(7-chloroquinolin-4-yl)amino]-acetophenones 3, 4

A mixture of 4,7-dichloroquinoline 0.5g (2.5 mmol) and 3- or 4-aminoacetophenone 0.37g (2.75 mmol) in ethanol (10 mL) was refluxed (80-85 °C) over night. The solid formed was filtered washed with water, diethyl ether and recrystallized from ethanol.

1-{4-[(7-Chloroquinolin-4-yl)amino]phenyl}ethanone
(4-[(7-chloroquinolin-4-yl)amino]acetophenone, 3)

Yield: 93%; mp: 209–210 °C; IR: 3440, 1676, 1622, 1587 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): δ 2.63 (s, 3H, CH$_3$), 7.08 (d, 1H, H$_3$, J: 6.9Hz), 7.65 (d, 2H, H$_{3',5'}$, J:8.4Hz), 7.92 (dd, 1H, H$_6$, J:9.4, 1.7Hz), 8.12 (d, 2H, H$_{2',6'}$, J:8.4Hz), 8.16 (d, 1H, H$_8$, J:1.7Hz), 8.62 (d, 1H, H$_2$, J:6.9Hz), 8.86 (d, 1H, H$_5$, J:9.4Hz), 11.22 (brs, 1H, NH). $^{13}$C NMR: 27.3, 101.8, 117.1, 119.9, 124.9, 126.9, 128.1, 130.5, 135.5, 139.1, 139.8, 142.2, 144.4, 154.7, 197.4. Anal. Calcd for C$_{17}$H$_{13}$N$_2$OCl: C 68.81%, H 4.41%, N 9.44%. Found C 68.85%, H 4.43%, N 9.52%.
Synthesis of [(7-Chloroquinolin-4-yl)-amino]chalcones: Potential Antimalarial and Anticancer ...

1-{3-[(7-Chloroquinolin-4-yl)amino]phenyl}ethanone
(3-[(7-chloroquinolin-4-yl)amino]acetophenone, 4)
Yield: 96%; mp: 223–225 °C, (Lit [27]: 250 °C); IR: 3440, 1683, 1625, 1609 cm⁻¹; ¹H NMR (DMSO-d₆): δ 2.63 (s, 3H, CH₃), 6.88 (d, 1H, H₃, J: 6.9Hz), 7.77 (m, 1H, H₂), 7.89 (dd, 1H, H₆, J:9.2, 1.9Hz), 8.00 (t, 1H, H₅, J:7.4Hz), 8.04 (m, 1H, H₂, J:1.6Hz), 8.18 (d, 1H, H₈, J:1.9Hz), 8.56 (d, 1H, H₅, J:6.9Hz), 8.88 (d, 1H, H₆, J:9.2Hz), 11.27 (brs, 1H, NH).  ¹³C NMR: 27.4, 100.9, 116.7, 119.8, 125.3, 126.9, 127.7, 128.0, 130.4, 131.0, 138.2, 138.9, 139.0, 139.7, 144.1, 155.3, 197.8 Anal. Calcd for C₁₇H₁₃N₂OCl: C 68.81%, H 4.41%, N 9.44%. Found C 68.93%, H 4.50%, N 9.63%.

General procedure for the synthesis of [(7-chloroquinolin-4-yl)amino]chalcones 5–19
A mixture of [(7-chloroquinolin-4-yl)amino]acetophenone 3 or 4 100 mg (0.36 mmol), the respective benzaldehydes (0.40 mmol), and potassium hydroxide (one pellet) in methanol (8 mL) was stirred at room temperature by 96 h. Water was added, the resulting precipitate was collected by filtration, washed with water, diethyl ether and recrystallized from ethanol-water (1:0.5).

(2E)-1-{4-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-(4-methoxyphenyl)prop-2-en-1-one
(4'-[(7-chloroquinolin-4-yl)amino]-4-methoxychalcone, 5)
Yield: 85%; mp: 201–203 °C; IR: 3336, 1676, 1622, 1587 cm⁻¹; ¹H NMR (DMSO-d₆): δ 3.83 (s, 3H, OCH₃), 7.03 (d, 2H, H₃'',5'', J:8.9Hz), 7.32 (d, 1H, H₃, J: 5.2Hz), 7.50 (d, 2H, H₃',5', J:8.7Hz), 7.63 (dd, 1H, H₆, J:9.2, 2.2Hz), 7.71 (d, 1H, HC=, J:15.6Hz), 7.84 (d, 1H, HC=, J:15.6Hz), 7.89 (d, 2H, H₂, J:8.9Hz), 8.41 (d, 1H, H₅, J:9.2Hz), 9.48 (brs, 1H, NH).  ¹³C NMR: 55.9, 105.6, 115.0, 119.8, 119.9, 120.0, 125.3, 126.1, 128.0, 128.4, 130.9, 131.2, 132.5, 134.8, 143.8, 146.2, 146.6, 150.3, 152.7, 161.8, 187.7. Anal. Calcd for C₂₅H₁₉N₂O₂Cl: C 72.38%, H 4.62%, N 6.75%. Found C 72.23%, H 4.84%, N 6.87%.

(2E)-1-{4-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-[4-(dimethylamino)phenyl]prop-2-en-1-one
(4'-[(7-chloroquinolin-4-yl)amino]-4-(dimethylamino)chalcone, 6)
Yield: 93%; mp: 204–206 °C; IR: 3404, 1676, 1622, 1584 cm⁻¹; ¹H NMR (CDCl₃): δ 6.81 (s, 1H, NH), 7.29 (d, 1H, J:5.2Hz), 7.36 (d, 2H, H₃'',5'', J:6.9Hz), 7.01 (d, 1H, H₃, J: 3.7Hz), 7.57–7.75 (m, 7H), 7.93 (d, 2H, H₂, J:6.8Hz, J:8.7Hz), 8.43 (d, 1H, H₅, J:9.2Hz), 8.51 (d, 1H, H₃, J:3.7Hz), 9.31(brs, 1H, NH). ¹³C NMR: 39.8, 105.3, 112.3, 116.6, 119.6, 119.8, 119.9, 122.6, 125.2, 126.0, 126.1, 128.3, 130.6, 131.2, 133.0, 134.7, 144.8, 150.1, 152.4, 152.6, 187.4. Anal. Calcd for C₂₆H₂₂N₃OCl: C 72.98%, H 5.18%, N 9.82%. Found C 73.09%, H 5.36%, N 9.77%.

(2E)-3-(4-Chlorophenyl)-1-{4-[(7-chloroquinolin-4-yl)amino]phenyl}prop-2-en-1-one
(4-chloro-4'-[(7-chloroquinolin-4-yl)amino]chalcone, 7)
Yield: 83%; mp: 185–187 °C; IR: 3390, 1651, 1609, 1564 cm⁻¹; ¹H NMR (CDCl₃): δ 6.81 (s, 1H, NH), 7.29 (d, 1H, J:5.2Hz), 7.36 (d, 2H, H₃'',5'', J:8.7Hz), 7.43 (d, 2H, H₃',5', J:8.5Hz), 7.53 (dd, 1H, H₆, J:9.2, 2.1Hz), 7.56 (d, 1H, HC=, J:15.7Hz), 7.60 (d, 2H, H₂, J:6.6, J:8.7Hz), 7.82 (d, 1H, HC=, J:15.7Hz), 7.92 (d, 1H, H₅, J:9.2Hz), 8.10 (d, 1H, H₆, J:2.1Hz), 8.12 (d, 2H, H₂, J:6.6, J:8.5Hz), 8.72 (d, 1H, H₃, J:5.2Hz). ¹³C NMR: 105.7, 119.6, 120.0, 123.3, 125.3, 126.0, 128.2, 129.5, 131.0, 131.1, 131.8, 134.4, 134.7, 135.4, 142.2, 146.7, 146.9, 150.2,
(2E)-1-{4-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-(4-fluorophenyl)prop-2-en-1-one
(4'-[(7-chloroquinolin-4-yl)amino]-4-fluorochalcone, 8)

Yield: 75%; mp: 217–219 °C; IR: 3401, 1654, 1603, 1561 cm⁻¹; ¹H NMR (CDCl₃): δ 6.77 (s, 1H, NH), 7.29 (d, 1H, H₃, J:5.1Hz), 7.36 (d, 2H, H₃',₅', J:8.5Hz), 7.51 (d, 1H, HC=, J:15.7Hz), 7.54 (dd, 1H, H₆, J:8.9, 2.3Hz), 7.67 (d, 2H, H₂',₆', J:8.5Hz), 7.86 (d, 1H, HC=, J:15.7Hz), 7.92 (d, 1H, H₅, J:8.9Hz), 8.10 (d, 1H, H₈, J:2.1Hz), 8.12 (d, 2H, H₂',₆', J:8.5Hz), 8.74 (d, 1H, H₃, J:5.1Hz). ¹³C NMR: 105.7, 116.4 (J:22 Hz), 119.6, 119.7, 119.8, 122.4, 125.2, 126.1, 128.3, 130.9, 131.2, 131.6 (J:9.0 Hz), 132.0, 134.7, 142.5, 146.4 (J:6.0 Hz), 150.2, 152.6, 163.7 (J: 252 Hz), 187.6. Anal. Calcd for C₂₄H₁₆N₂OFCl: C 71.56%, H 4.00%, N 6.95%. Found C 71.37%, H 3.96%, N 7.17%.

(2E)-1-{3-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-(4-methoxyphenyl)prop-2-en-1-one
(3'-[(7-chloroquinolin-4-yl)amino]-4-methoxychalcone, 9)

Yield: 95%; mp: 211–212 °C; IR: 3312, 1651, 1600, 1564 cm -1; ¹H NMR (DMSO-d₆): δ 3.82 (s, 3H, OCH₃), 7.01 (d, 2H, H₃'',₅'', J:8.9Hz), 7.59 (m, 4H), 7.77 (d, 1H, H₂', J:2.2Hz), 7.86 (d, 2H, H₂',₆', J:8.9Hz), 7.91 (d, 1H, HC=, J:15.8Hz), 8.01 (d, 1H, HC=, J:15.7Hz), 8.05 (s, 2H, H₈), 8.44 (d, 1H, H₅, J:9.2Hz), 8.51 (d, 1H, H₃, J:5.4Hz), 9.30 (s, 1H, NH). ¹³C NMR: 55.9, 102.7, 114.9, 119.1, 120.1, 122.4, 124.4, 125.1, 125.6, 126.9, 127.8, 128.2, 130.4, 131.4, 134.5, 139.7, 141.6, 144.8, 148.2, 150.1, 152.5, 161.9, 189.3. C₂₅H₁₉N₂O₂Cl: C 72.38%, H 4.62%, N 6.75%. Found C 72.41%, H 4.65%, N 6.91%.

(2E)-1-{3-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-(3-methoxyphenyl)prop-2-en-1-one
(3'-[(7-chloroquinolin-4-yl)amino]-3-methoxychalcone, 10)

Yield: 71%; mp: 135–137 °C; IR: 3329, 1651, 1602, 1565 cm⁻¹; ¹H NMR (CDCl₃): δ 3.89 (s, 3H, OCH₃), 6.81(brs, 1H, NH), 6.92 (t, 1H, H₅', J:7.8Hz), 7.06 (d, 1H, H₃, J:5.1Hz), 7.45 (t, 1H, H₅', J:7.0Hz), 7.56 (m, 2H, H₆',₄'), 7.57 (d, 1H, HC=, J:15.5Hz), 7.59 (d, 1H, H₄', J:7.7Hz), 7.68 (d, 1H, H₆', J:7.6Hz), 7.91 (m, 2H, H₂',₆', J:8.5Hz), 8.03 (d, 1H, H₈, J:1.9Hz), 8.12 (d, 1H, HC=, J:15.6Hz), 8.59 (d, 1H, H₂', J:5.0Hz). ¹³C NMR: 56.3, 102.7, 112.4, 119.1, 121.2, 122.2, 122.4, 123.4, 124.3, 125.0, 125.6, 126.9, 128.2, 129.1, 130.4, 132.9, 134.5, 139.4, 139.5, 141.7, 148.1, 150.2, 152.5, 158.8, 189.6. C₂₅H₁₉N₂O₂Cl: C 72.38%, H 4.62%, N 6.75%. Found C 72.05%, H 4.64%, N 6.55%.

(2E)-1-{3-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-(2-methoxyphenyl)prop-2-en-1-one
(3'-[(7-chloroquinolin-4-yl)amino]-2-methoxychalcone, 11)

Yield: 83%; mp: 181–183 °C; IR: 3376, 1651, 1603, 1568 cm⁻¹; ¹H NMR (CDCl₃): δ 3.94 (s, 3H, OCH₃), 6.73 (brs, 1H, NH), 6.98 (t, 1H, H₅', J:8.5Hz), 7.06 (d, 1H, H₃, J:5.3Hz), 7.41 (t, 1H, H₅', J:7.2Hz), 7.56 (m, 2H, H₆',₄'), 7.60 (d, 1H, HC=, J:15.8Hz), 7.65 (d, 1H, H₄', J:7.2Hz), 7.84 (d, 1H, H₆', J:7.2Hz), 7.91 (m, 2H, H₂',₆', J:8.5Hz), 8.08 (d, 1H, H₈, J:2.2Hz), 8.17 (d, 1H, HC=, J:15.7Hz), 8.62 (d, 1H, H₂', J:5.3Hz). ¹³C NMR: 55.8, 102.5, 113.0, 119.1, 121.2, 122.2, 122.4, 124.0, 124.9, 125.3, 125.9, 127.3, 128.2, 130.1, 131.2, 132.9, 134.5, 139.4, 139.5, 142.7, 148.9, 149.8, 152.7, 161.8, 189.4. C₂₅H₁₉N₂O₂Cl: C 72.38%, H 4.62%, N 6.75%. Found C 72.17%, H 4.83%, N 7.03%.
Yield: 91%; mp: 169–171 °C; IR: 3328, 1670, 1609, 1566 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)): \(\delta\) 3.01 (s, 6H, N(CH\(_3\))\(_2\)), 6.75 (d, 2H, H\(_{3''},5''\), J:5.9Hz), 7.27 (m, 2H, H\(_2,4\)), 7.45 (m, 2H, HCl=, H\(_6\)), 7.67 (m, 3H, HCl=, H\(_{6'},2\)'), 7.98 (m, 2H, H\(_8,2\)'), 8.15 (d, 2H, H\(_2'',6''\), J:5.9Hz), 8.39 (t, 1H, Hz, J:5.9Hz), 8.62 (d, 1H, Hz, J: 3.6Hz), 9.41 (brs, 1H, NH). \(^{13}\)C NMR: 39.7, 102.6, 110.6, 112.3, 116.5, 118.9, 122.3, 122.6, 124.1, 125.1, 125.6, 126.5, 128.2, 130.2, 131.3, 134.4, 140.2, 141.3, 145.7, 148.1, 150.1, 152.6, 188.8. Anal. Calcd for C\(_{26}H_{22}N_3OCl\): C 72.98%, H 5.18%, N 9.82%. Found C 72.95%, H 4.89%, N 9.93%.

Yield: 72%; mp: 204–205 °C; IR: 3312, 1654, 1606, 1564 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 6.71 (brs, 1H, NH), 7.05 (d, 1H, Hz, J:5.2Hz), 7.42 (d, 2H, H\(_{3''},5''\), J:8.3Hz), 7.50 (d, 1H, HCl=, J:15.7Hz), 7.52 (dd, 1H, H\(_6\), J:9.1, 2.2Hz), 7.58 (m, 3H, H\(_{5''},2'',6''\)), 7.80 (d, 1H, HCl=, J:15.7Hz), 7.84 (d, 1H, Hz, J:7.1Hz), 7.91 (d, 1H, Hz, J:9.1Hz), 8.09 (d, 1H, Hz, J:2.2Hz), 8.63 (d, 1H, Hz, J:5.3Hz). \(^{13}\)C NMR: 102.7, 119.0, 122.4, 123.3, 124.6, 124.9, 125.7, 127.2, 128.3, 129.5, 130.4, 131.1, 134.1, 134.5, 135.7, 139.2, 141.5, 143.3, 148.1, 150.0, 152.6, 189.3. Anal. Calcd for C\(_{24}H_{16}N_2OCl_2\): C 68.75%, H 3.85%, N 6.68%. Found C 69.03%, H 3.86%, N 6.85%.

Yield: 62%; mp: 166–168 °C; IR: 3346, 1660, 1606, 1564 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 6.77 (brs, 1H, NH), 7.05 (d, 1H, Hz, J:5.2Hz), 7.41 (d, 2H, H\(_{3''},5''\), J:7.6Hz), 7.50 (d, 1H, HCl=, J:15.7Hz), 7.52 (dd, 1H, H\(_6\), J:9.1Hz), 7.65 (d, 1H, Hz, J:15.7Hz), 7.67 (d, 1H, Hz, J:5.3Hz), 7.81 (d, 1H, Hz, J:15.7Hz), 7.83 (d, 1H, Hz, J:7.0Hz), 7.92 (d, 1H, Hz, J:5.3Hz). \(^{13}\)C NMR: 102.8, 119.1, 122.6, 124.3, 124.6, 124.9, 125.7, 127.2, 128.3, 129.5, 130.4, 131.1, 134.1, 134.5, 135.7, 139.2, 141.5, 143.3, 148.1, 150.0, 152.6, 189.3. Anal. Calcd for C\(_{24}H_{16}N_2OCl_2\): C 68.75%, H 3.85%, N 6.68%. Found C 68.79%, H 4.07%, N 7.10%.

Yield: 73%; mp: 180–181 °C; IR: 3.312, 1638, 1609, 1568 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 6.70 (brs, 1H, NH), 7.05 (d, 1H, Hz, J:5.2Hz), 7.41 (d, 2H, Hz, J:5.7Hz), 7.44–7.65 (m, 6H), 7.65 (d, 1H, HCl=, J:15.7Hz), 7.83 (d, 1H, Hz, J:7.1Hz), 7.87 (d, 1H, Hz, J:8.9Hz), 8.09 (d, 1H, Hz, J:2.0Hz), 8.63 (d, 1H, Hz, J:5.2Hz). \(^{13}\)C NMR: 102.8, 119.1, 122.6, 124.1, 124.7, 125.0, 125.7, 127.4, 128.3, 128.5, 128.6, 130.5, 130.8, 131.3, 133.4, 134.6, 137.5, 139.2, 141.6, 143.1, 148.1, 150.2, 152.6, 189.3. Anal. Calcd for C\(_{24}H_{16}N_2OCl_2\): C 68.75%, H 3.85%, N 6.68%. Found C 68.79%, H 4.07%, N 7.10%.
8.09 (d, 1H, H8, J:2.0Hz), 8.63 (d, 1H, H2, J:5.3Hz). 13C NMR: 102.7, 116.5 (J:22 Hz), 119.0, 122.4, 124.5, 124.9, 125.6, 127.2, 128.2, 130.4, 131.0, 131.8 (J:9.0 Hz), 134.5, 139.3, 141.5, 143.5, 148.1, 150.1, 152.5, 163.9 (J: 251 Hz), 189.3. Anal. Calcd for C24H16N2OFCl: C 71.56%, H 4.00%, N 6.95%. Found C 71.60%, H 4.23%, N 7.24%.

(2E)-1-{3-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-(3-fluorophenyl)prop-2-en-1-one (3’-[7-chloroquinolin-4-yl]amino)-3-fluorochalcone, 17)

Yield: 53%; mp: 163–164 °C; IR: 3.328, 1657, 1606, 1564 cm-1; 1H NMR (CDCl3): δ 6.74 (brs, 1H, NH), 7.05 (d, 1H, H3, J:5.3Hz), 7.15 (m, 2H, H5,5’), 7.35–7.42 (m, 2H ), 7.49-7.62 (m, 4H), 7.80 (d, 1H, H2, J:15.5Hz), 8.10 (d, 1H, H2, J:5.1Hz). 13C NMR: 102.8, 115.3 (J:22 Hz), 117.6 (J:22 Hz), 119.1, 122.5, 124.8, 125.0, 125.7, 126.2, 127.4, 128.3, 130.5, 131.4, (J:8.0 Hz), 134.6, 137.8 (J:8.0 Hz), 139.2, 141.6, 143.4, 148.1, 150.2, 152.6, 163.1 (J: 243 Hz), 189.4. Anal. Calcd for C24H16N2OFCl: C 71.56%, H 4.00%, N 6.95%. Found C 71.74%, H 4.12%, N 7.19%.

(2E)-1-{3-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-(2-fluorophenyl)prop-2-en-1-one (3’-[7-chloroquinolin-4-yl]amino)-2-fluorochalcone, 18)

Yield: 62%; mp: 119–120 °C; IR: 3.360, 1657, 1606, 1564 cm-1; 1H NMR (CDCl3): δ 6.72 (brs, 1H, NH), 7.06 (d, 1H, H3, J:5.5Hz), 7.20 (m, 2H, H6, H6’), 7.41–7.66 (m, 6H), 7.82 (d, 1H, H6’, J:7.1Hz), 7.91 (d, 1H, H5, J:8.5Hz), 8.09 (d, 1H, H8, J:2.4Hz), 8.19 (d, 1H, H2, J:15.6Hz), 8.63 (d, 1H, H2, J:5.4Hz). 13C NMR: 102.7, 115.2 (J:24 Hz), 117.8 (J:19 Hz), 118.9, 122.5, 123.9, 124.7, 124.9, 125.7, 126.1, 127.4, 128.2, 130.4, 131.3, (J:6.0 Hz), 134.5, 137.7 (J:10.0 Hz), 139.1, 141.5, 143.3, 148.1, 150.1, 152.6, 163.1 (J: 250 Hz), 189.3. Anal. Calcd for C24H16N2OFCl: C 71.56%, H 4.00%, N 6.95%. Found C 71.60%, H 4.09%, N 7.13%.

(2E)-1-{3-[(7-Chloroquinolin-4-yl)amino]phenyl}-3-phenylprop-2-en-1-one (3’-[7-chloroquinolin-4-yl]amino)chalcone, 19)

Yield: 93%; mp: 190–192 °C; IR: 3.348, 1654, 1596, 1570 cm-1; 1H NMR (CDCl3): δ 6.74 (brs, 1H, NH), 7.05 (d, 1H, H3, J:5.3Hz), 7.44–7.56 (m, 7H), 7.65-7.68 (m, 3H), 7.83 (d, 1H, H8, J:6.9Hz), 7.87 (d, 1H, H2, J:15.8Hz), 7.91 (d, 1H, H5, J:9.1Hz), 8.08 (d, 1H, H8, J:1.9Hz), 8.64 (d, 1H, H2, J:5.3Hz). 13C NMR: 102.8, 119.1, 122.4, 122.6, 124.6, 125.0, 125.7, 127.2, 128.3, 129.5, 130.5, 131.3, 134.6, 135.2, 139.4, 141.5, 144.9, 148.1, 150.2, 152.6, 189.5. Anal. Calcd for C24H17N2OCl: C 74.90%, H 4.45%, N 7.28%. Found C 75.01%, H 4.52%, N 7.55%.

**Biological assays**

**Inhibition of heme crystallization**

The heme crystallization assay was performed according to [21], briefly, a solution of hemin chloride (50 µL, 4mM), dissolved in DMSO (5.2 mg/mL), was distributed in 96-well micro plates. The compounds dissolved in DMSO (100 µM) were added in triplicate in test wells (50 µL). Controls contained either water, DMSO or chloroquine (50 µL). β-hemin formation was initiated by the addition of acetate buffer (100 µL 0.2 M, pH 4.4). The plates were incubated at 37 °C for 48 hours to allow for completion of the reaction and centrifuged (4000 RPM x 15 minutes, IEC-CENTRA, MP4R). After discarding the supernatant, the pellet was washed twice with DMSO (200 µL) and finally, dissolved in...
Synthesis of [(7-Chloroquinolin-4-yl)-amino]chalcones: Potential Antimalarial and Anticancer …

NaOH (200 µL, 0.2 N). The dissolved aggregates were further diluted 1:2 with NaOH (0.1N) and absorbances recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as a percentage of inhibition of β-hematin synthesis.

**Parasite, experimental host and strain maintenance.**

Male Balb-C mice, weighing 18–22 g were maintained on a commercial pellet diet and housed under international standard conditions approved by the Ethics Committee, School of Pharmacy, Central University of Venezuela. *Plasmodium berghei* (ANKA strain), a murine malaria parasite, was used for infection. Mice were infected intraperitoneally with 1 x 10⁶ infected erythrocytes diluted in phosphate buffered saline solution (PBS, 10 mM, pH 7.4, 0.1 mL). Parasitemia was monitored by microscopic examination of Giemsa stained smears.

**Parasite extracts**

Blood of infected animals, at a high level of parasitemia (30–50%), was collected by cardiac puncture with an heparinized syringe and the blood pool was centrifuged (500g x 10 minutes, 4 °C). Plasma and buffy coat were removed and the red blood cells (RBCs) pellet was washed twice with chilled PBS-Glucose (5.4 %). The washed RBC pellet was centrifuged on a discontinuous percoll gradient (80–70% percoll in PBS-Glucose, 20000g x 30 min x 4 °C) [28]. The upper band (RBCs with mature forms of parasites) was removed by aspiration, collected and washed twice with chilled PBS-Glucose and the infected erythrocytes were lysed with the non-ionic detergent saponin (0.1% in PBS x 10 min). 1 mL of cold PBS was added and the samples were centrifuged (13000g x 5 minutes, 4°C) to remove erythrocyte cytoplasm content (including erythrocyte haemoglobin). The free parasites were suspended in PBS-Glucose (5.4 %), and subjected to three freeze-thaw cycles (−70°C / +37°C). The final homogenate was used in the inhibition of hemoglobin proteolysis assay [28].

**Mice native hemoglobin**

Native hemoglobin from non-infected mice was obtained by mixing one volume of pellet erythrocytes with two volumes of water. The resulting solution was used as the substrate in the inhibition of the hemoglobin proteolysis assay.

**Inhibition of hemoglobin proteolysis**

The proteolytic effect of the parasite extract on the native mice hemoglobin was assayed according to [29], using 96-wells tissue culture plate. The assay mixture contained: mice native hemoglobin (10 µL), parasite extract (50 µL), GSH (10 µL, 10 µM), compounds dissolved in DMSO (10µL, 10µM) and acetate buffer (0.2 M, pH 5.4) to a final volume of 200µL. The incubations were carried out at 37 °C for 18 hours and the reaction were stopped by the addition of reduced sample buffer. The degree of hemoglobin digestion was evaluated electrophoretically by SDS-PAGE 15% and the globin bands were analysed by densitometry. A DMSO control was electrophoresed at the same time.

**In vitro cytotoxicity**

A 96-well microtiter plate (tissue culture grade) containing 0.1 ml of growth medium/per well (RPMI) was seeded with 1.2x10⁴ human prostate LNCaP tumor cells. After 24h of culture, cells were exposed to the compounds for 72 h at concentrations ranging from 5 to
100 µg/ml, and then evaluated for cytotoxicity. In all cases, although the compounds were dissolved in dimethylsulfoxide (DMSO), the final concentration of this solvent in the culture medium was lower than 0.2%, a concentration that has neither cytotoxic effect nor causes any interference with the colorimetric detection method. Cytotoxicity assays were carried out by colorimetry following the reduction of a tretazolium salt (sodium 3,3’-[1-(phenyl-amino)carbonyl]-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate, XTT) (Roche Applied Science, Mannheim, Germany) [25]. After 72h of compound incubations, cells grown in microtiter plates were incubated with XTT at 37°C for 4h and the colorimetric detection of the orange product formazan was registered at 492nm (SpectraFluor-Tecan). The IC50 value in the XTT assay was defined as the concentration of the tested compound leading to a 50% of inhibition of cell viability compared to untreated cells. All experiments were done in triplicates.

Effect of compounds on human prostate tumor cell growth

LNCaP cells (2.4x10^5) were plated in 10-cm² tissue culture dishes at 37°C in RPMI containing 10% FBS and the compounds at their IC50 values. Cells were collected from culture dishes after trypsin-EDTA treatment for 7 min at 37°C. The number of viable cells per dish was counted with a hemocytometer at 24-h intervals for a period of 96 h. All experiments were done in triplicates.

Acknowledgements

We thank the IIF-FF and CDCH-UCV (grant PI. 06-00-7078-2007), and CYTED-RIDIMEDCHAG programe for financial support.

Supporting Information

The scanned 13C NMR spectra of compounds 3, 4, 7–9, 13, 15, 16 and 19 are available in the online version (Format: PDF, Size: ca. 0.2 MB): http://dx.doi.org/10.3797/scipharm.0905-07.

Authors’ Statements

Competing Interests

The authors declare no conflict of interest.

Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details.

References

[1] WHO World Malaria Report. 2008 (http://rbm.who.int/wmr2008).

[2] Coatney G, Cooper W, Eddy N, Grennberg J. Survey of antimalarial agents. Public Health Monogr. 1953; 15: 1–322.
[3] Greenwood D. 
Conflicts of interest: The genesis of synthetic antimalarial agents in peace and war. 
J Antimicrob Chemother. 1995; 36: 857–872. 
doi:10.1093/jac/36.5.857

[4] Fitch C. 
Ferriprotoporphyrin IX, phospholipids, and the antimalarial actions of quinoline drugs. 
Life Sci. 2004; 74: 1957–1972. 
doi:10.1016/j.lfs.2003.10.003

[5] White N. 
Drug resistance in malaria. 
Br Med Bull. 1998; 54: 703–715. 
PMid:10326925

[6] Watkins W, Sixsmith D, Spencer H, Boriga D, Karjuki D, Kipingor T, Koech D. 
Effectiveness of amodiaquine as a treatment for chloroquine resistant Plasmodium falciparum. 
Lancet. 1984; 1: 357–359. 
doi:10.1016/S0140-6736(84)90410-0

[7] Ruscoe J, Tingle M, O’Neill P, Magg J, Ward S, Park B. 
Effect of disposition of Manich antimalarial agents on the pharmacology and toxicology. 
Antimicrob Agents Chemother. 1998; 42: 2410–2416. 
PMid:9736572

[8] O’Neill P, Mukhtar A, Stocks P, Randle L, Hindley S, Ward S, Storr R, Bickley J, O’Neill I, Maggs J, 
Hughes R, Winstanley P, Bray P, Park B. 
Isoquine and related amodiaquine analogs: A new generation of improved 4-aminquinoline antimalarials. 
J Med Chem. 2003; 46: 4933–4945. 
doi:10.1021/jm030796n

[9] Werbel L, Cook P, Elslager E, Hung J, Johnson J, Kesten S, McNamara D, Ortwine D, Worth D. 
Synthesis, antimalarial activity, and quantitative structure-activity relationships of tebuquine and a series of related 5-[(7-chloro-4-quinolinyl)amino]-3-[alkylamino)methyl][1,1’-biphenyl]2-ols and N”-oxides. 
J Med Chem. 1986; 29: 924–939. 
doi:10.1021/jm00156a009

[10] Neill P, Willock D, Hawley S, Bray P, Storr R, Ward S, Park B. 
Synthesis, antimalarial activity, and molecular modelling of tebuquine analogues. 
J Med Chem. 1997; 40: 437–448. 
doi:10.1021/jm960370r

[11] Biot C, Chibale K. 
Novel approaches to antimalarial drug discovery. 
Infect Disord Drug Targets. 2006; 6: 173–204. 
doi:10.2174/187152606784112155

[12] Goldberg D, Slater A, Cerami A, Henderson G. 
Hemoglobin degradation in the malaria parasite Plasmodium falciparum: an ordered process in a unique organelle. 
Proc Natl Acad Sci U S A. 1990; 87: 2931–2935. 
doi:10.1073/pnas.87.8.2931

[13] Martirosyan A, Rahim-Bata R, Freeman A, Clarke C, Howard R, Strobl J. 
Differentiation-inducing quinolines as experimental breast cancer agents in the MCF-7 human cancer cell model. 
Biochem Pharmacol. 2004; 68: 1729–1738. 
doi:10.1016/j.bcp.2004.05.003
[14] Mól W, Matyja M, Filip B, Wietrzyk S.
Synthesis and antiproliferative activity in vitro of novel (2-buthynyl)thioquinolines.
Bioorg Med Chem. 2008; 16: 8136–8141.
doi:10.1016/j.bmc.2008.07.047

[15] Lukevics E, Abele E, Arsenyan P, Abele R, Rubina K, Shestakova I, Domracheva I, Vologdina V.
Synthesis and citotoxicity of Silicon containing pyridine and quinoline sulfides.
Met Based Drugs. 2002; 9: 45–51.
doi:10.1155/MBD.2002.45

[16] Shahabuddin M, Nambiar M, Choudhary B, Advirao G, Raghavan S.
A novel DNA intercalator, butylamino-pyrimido[4',5':4,5]selenolo(2,3-b)quinoline, induces cell cycle
arrest and apoptosis in leukemic cell.
Invest New Drugs. 2009; in press.
doi:10.1007/s10637-008-9212-6

[17] Jasinki P, Welsh B, Galvez J, Land D, Zwolak P, Ghandi L, Terai K, Dudek A.
A novel quinoline, MT477: suppresses cell signaling through Ras molecular patway, inhibits PKC
activity, and demonstrates in vivo anti-tumor activity against human carcinoma cell lines.
Invest New Drugs. 2008; 26: 223–232.
doi:10.1007/s10637-007-9096-x

[18] Smith E, Schwartz M, Kawamoto H, You X, Hwang D, Liu H, Scherr D.
Antitumor effects of imidazoquinolines in urothelial cell carcinoma of the bladder.
J Urol. 2007; 177: 2347–2351.
doi:10.1016/j.juro.2007.01.112

[19] Isaacs J, Pili R, Qian D, Dalrymple S, Garrison J, Kyprianou N, Björk A, Olsson A, Leanderson T.
Identification of ABR-215050 as lead second generation quinoline-3-carboxamide anti-angiogenic
agent for the treatment of prostate cancer.
Prostate. 2006; 66: 1768–1778.
doi:10.1002/pros.20509

[20] Charris J, Domínguez J, Gamboa N, Rodrigues J, Angel J.
Synthesis and antimalarial activity of E-2-quinolinylbenzocycloalcanones.
Eur J Med Chem. 2005; 40: 875–881.
doi:10.1016/j.ejmech.2005.03.013

[21] Baelmans R, Deharo E, Muñoz V, Sauvain M, Ginsburg H.
Experimental conditions for testing the inhibitory activity of chloroquine on the formation of β-hematin.
J Exp Parasitol. 2000; 4: 243–248.
doi:10.1006/expr.2000.4558

[22] Jemal A, Thomas A, Murray T, Thun M.
Cancer statistics, 2002.
CA Cancer J. Clin. 2002; 52: 23–47.
doi:10.3322/canjclin.52.1.23

[23] Chen D, Cui Q, Yang H, Barrea R, Sarkar F, Sheng S, Yan B, Reddy G, Dou Q.
Clioquinol, a therapeutic agent for Alzheimer's disease, has proteasome-inhibitory, androgen receptor-
suppressing, apoptosis-inducing, and antitumor activities in human prostate cancer cells and
xenografts.
Cancer Res. 2007; 67: 1636–1644.
doi:10.1158/0008-5472.CAN-06-3546

[24] Zhou J, Geng G, Batist G, Wu J.
Syntheses and potential anti-prostate cancer activities of ionone-based chalcones.
Bioorg Med Chem Lett. 2009; 19: 1183–1186.
doi:10.1016/j.bmcl.2008.12.089
[25] Denizot F, Lang R. Rapid colorimetric assay for cell grow and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Meth. 1986; 89: 271–277. doi:10.1016/0022-1759(86)90368-6

[26] Egan T. Haemozoin formation as a target for the rational design of new antimalarials. Drug Des Rev. 2004; 1: 93–110. doi:10.2174/1567269043480744

[27] Chauhan P, Pratap R, Sharma S. Synthesis and antiparasitic activity of 4-(aryl/heteroarylamino)-7-chloroquinolines. Indian J Chem. 1985; 24B: 1154–1157.

[28] Deharo E, Gautret P, Ginsburg H, Chabaud A, Landau I. Synchronization of Plasmodium yoelii nigeriensis and P. killicki infection in the mouse by means of Percoll-glucose gradient stage fractionation: Determination of the duration of the schizogonic cycle. Parasitol Res. 1994; 80: 159–164. doi:10.1007/BF00933785

[29] Rosenthal P. Plasmodium falciparum: effects of proteinase inhibitors on globin hydrolysis by cultured malaria parasites. Exp Parasitol. 1995; 80: 272–281. doi:10.1006 экспр.1995.1033