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Original article

# Synthesis of novel analogs of 2-pyrazoline obtained from [(7-chloroquinolin-4-yl)amino]chalcones and hydrazine as potential antitumor and antimalarial agents

Braulio Insuasty [a,*], Alba Montoya [a], Diana Becerra [a], Jairo Quiroga [a], Rodrigo Abonia [a], Sara Robledo [b], Ivan Darío Vélez [b], Yulieth Upegui [b], Manuel Nogueras [c], Justo Cobo [c]

[a] Heterocyclic Compounds Research Group, Department of Chemistry, Universidad del Valle, A. A. 25360 Cali, Colombia
[b] PECET-SIU, Universidad de Antioquia, PO Box 1226, Medellín, Colombia
[c] Department of Inorganic and Organic Chemistry, Universidad de Jaén, 23071 Jaén, Spain

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ABSTRACT

A new series of *N*-acetyl and *N*-formyl-pyrazoline derivatives **6** and **7**–**8** were synthesized by cyclocondensation reaction of [(7-chloroquinolin-4-yl)amino]chalcones with hydrazine hydrate in acetic acid and hydrazine hydrate in formic acid respectively. These compounds were evaluated *in vitro* as antitumor and as antimalarial agents. Compounds **7b** and **8b**–**e** showed remarkable antitumor activity against cancer cell lines, with the most important $GI_{50}$ values ranging from 0.13 to 0.99 μM. The best antimalarial response was observed for compound **7a** with an inhibition percentage of 50.8% for *Plasmodium falciparum*, a hemolytic capacity of 3.2% and an $IC_{50}$ of 14.1 μg/mL.



## 1. Introduction

The quinoline nucleus is an important class of heterocyclic structure found in many synthetic and natural occurring products with a wide range of pharmacological activities [1], such as antiviral [2], anticancer [3], antibacterial [4], antifungal [5], antiobesity [6] and anti-inflammatory [7]. These properties are well illustrated by the large number of commercially available drugs containing this heterocyclic system.

The research work on aminoquinolines could be justified on the basis of several considerations: (1) aminoquinolines have proved to be the most highly successful class of compounds for the treatment and prophylaxis of malaria; (2) 4-aminoquinolines are easily synthesized and inexpensive to produce; (3) 4-aminoquinolines are generally well tolerated, with acceptable toxicity profiles for treatment of acute infection; (4) the 4-aminoquinolines has resulted be effective against chloroquine-resistant strains of the parasite [8]. The structure–activity relationship studies on 4-aminoquinolines as antimalarial agents suggested that the 7-chloro-4-aminoquinoline nucleus is obligatory for antimalarial activity, particularly, due to its accumulation into the vacuole of the parasite, the inhibition of β-hematin formation and for instance, permitting the accumulation of FPIX-4-aminoquinoline complex, a highly toxic substance for the parasite which causes its dead [9–16].

Chalcones have extensively been studied for their broad spectrum of biological activities as antifungal [17], antiparasitic [18], antimitotic [19], antitumor and antioxidant [20], immunomodulatory [21], antileishmanial [22], antimalarial [23,24], anti-invasive [25,26] and anti-inflammatory [27–30].

Reports on pyrazolines, reflects the biological activity that this kind of compound have displayed as anti-inflammatory [31,32], antitumor [33], antimycobacterial [34], antidepressants and anticonvulsants [35], antimicrobial [36,37] and antifungal [38], among others.

Continuing with our current studies directed toward the synthesis of pyrazoline derivates with biological activity [39,40], in this paper we describe the synthesis of new pyrazoline derivatives, containing the 7-chloro-4-aminoquinoline moiety in their structures, and the evaluation of their cytotoxic and antimalarial activities.

* Corresponding author. Fax: +57 2 33392440.
*E-mail addresses:* braulio.insuasty@correounivalle.edu.co, brainsu@univalle.edu.co (B. Insuasty).

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## 2. Results and discussion

### 2.1. Chemistry

In order to obtain the key α,β-unsaturated carbonyl compounds (chalcones **4** and **5**), as starting materials for the synthesis of the target products **6**–**8**, precursors 4-(7-chloroquinolin-4-ylamino) acetophenone **2** [41] and 3-(7-chloroquinolin-4-ylamino)acetophenone **3** [41] were obtained through a selective nucleophilic aromatic substitution ($S_NAr$) of the 4-chlorine atom on the 4,7-dichloroquinoline **1** with 3- and 4-aminoacetophenones respectively (Scheme 1). Then the Claisen–Schmidt condensation of ketones **2** and **3** with different aromatic aldehydes led to the formation of chalcones **4** and **5**, respectively, in good yields (Scheme 1).

The IR spectrum of compound **4a** taken in KBr pellet showed an absorption band at 1656 $cm^{-1}$ corresponding to the stretching vibration of the carbonyl group. In the $^1H$ NMR spectrum of compound **4a** is observed two doublets at 7.66 and 7.87 ppm corresponding to protons H-2″ and H-3″ with $J$ = 16.3 Hz, confirming the *E*-configuration for the C=C bond.

Reaction of the synthesized chalcones **4** and **5** with hydrazine hydrate in acetic acid or in formic acid by stirring at room temperature or under reflux led to the formation of the *N*-acetyl and *N*-formyl-pyrazolines **6** and **7**,**8** respectively (Scheme 2). Products **6**–**8** were obtained in acceptable to excellent yields. The new pyrazolines **6**–**8**, were fully characterized by means of spectroscopic techniques such as FT-IR and 1D and 2D-NMR (see Experimental section).

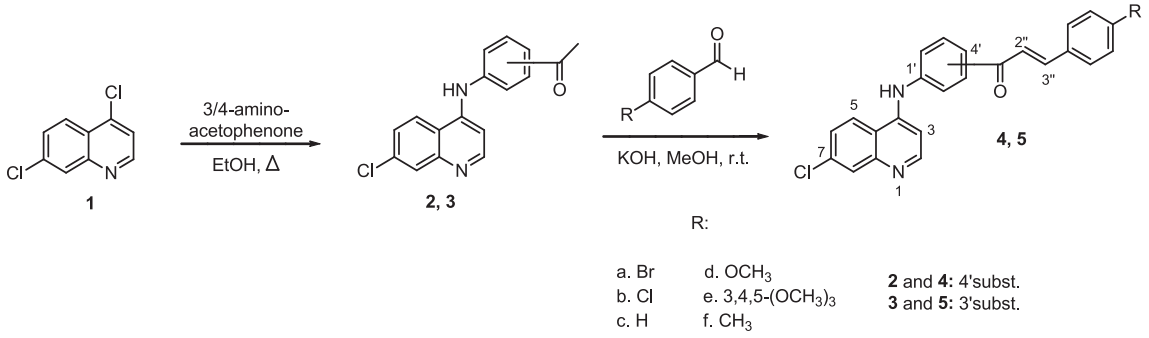
As an example, in the IR spectrum for compound **6a**, an absorption band is observed at 1646 $cm^{-1}$ which corresponds to the stretching vibration of the C=O amide functionality. At 1640 and 1579 $cm^{-1}$ are observed the stretching bands of C=N and C=C respectively. In the $^1H$ NMR spectrum for compound **6a**, the protons on the diastereotopic center C-4′, of the pyrazoline ring appears as two double-doublets at $\delta$ = 3.14 and 3.85 ppm with $^2J_{AM}$ = 17.9, $^3J_{AX}$ = 4.3 and $^3J_{MX}$ = 11.9 Hz, while the H-5′ proton is observed as a double-doublet at 5.53 ppm with $^3J_{MX}$ = 11.9 and $^3J_{AX}$ = 4.3 Hz. All types of carbon atoms were completely assigned by using DEPT-135, HSQC and HMBC techniques.

### 2.2. Anticancer activity

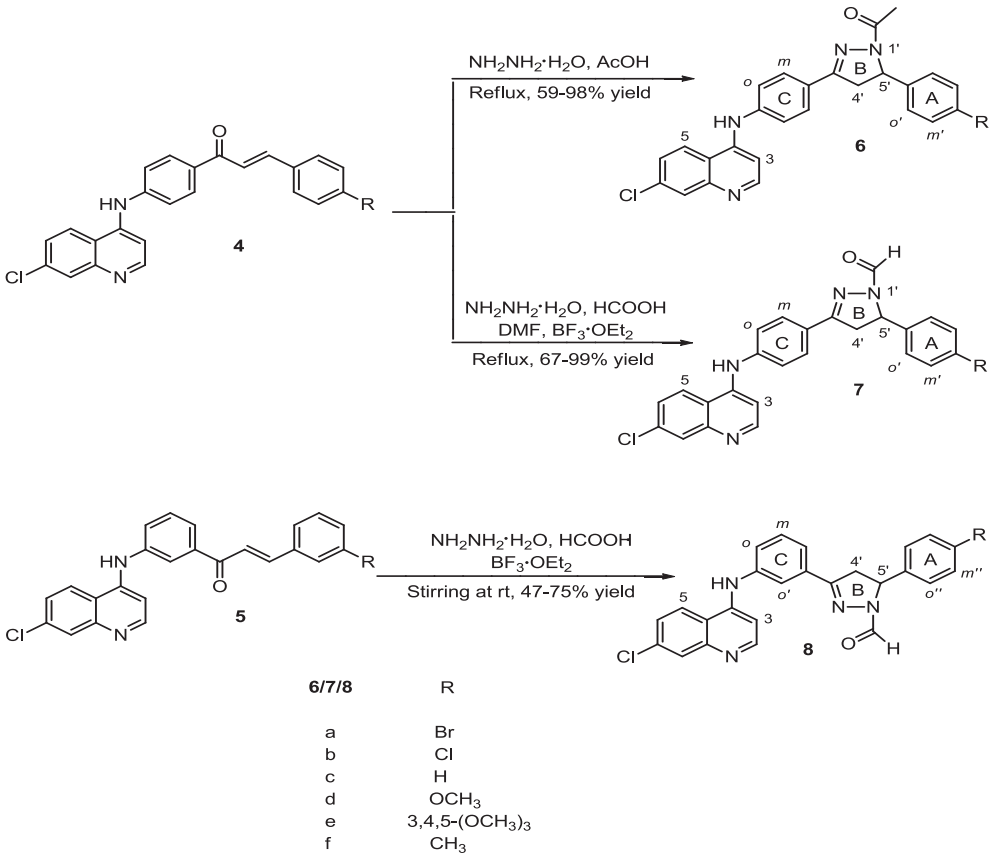
As a preliminary screening, structures of all new compounds (i.e. **2**–**3**, **4**–**5**, and **6**–**8** series) were submitted to the Developmental Therapeutics Program (DTP) at National Cancer Institute (NCI) for evaluation of their anticancer activity against different human cell lines. Twelve (i.e. **6b**,**d**,**e**; **7b**–**f** and **8b**–**e**) of the submitted structures were selected and subjected to the preliminary evaluation against the 60 tumor cell lines at a single dose of 10 μM after 48 h of incubation. The output from the single dose screening was reported as a mean graph available for analysis by the COMPARE program (data are not shown). The results of this first assay showed that compounds **6b**, **7b**–**f** and **8b**–**e** were active. A structural feature which worth be highlighted is the fact that none of the pyrazolines bearing the Br-substituent, i.e. **6a**, **7a** and **8a** were chosen to examine their antitumor activity.

Then, active compounds passed to a second stage in order to determine their cytostatic activity against the 60 tumor cell lines represented in *leukemia*, *melanoma*, *lung*, *colon*, *brain*, *breast*, *ovary*, *kidney* and *prostate* panels; where the testing results were expressed according to the following three parameters: $GI_{50}$ which is the molar concentration of the compounds required to inhibit the growing of the cell lines to 50% (relative to untreated cells). TGI as the molar concentration that causes total growth inhibition, and $LC_{50}$ which is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells [42]. The active compounds were evaluated at five concentration levels (100, 10, 1.0, 0.1, and 0.01 μM) and the test consisted of a 48 h continuous drug exposure protocol using sulforhodamine B (SRB) protein assay to estimate cell growth. Details of this evaluation method, and the complementary information related with the activity pattern over all cell lines, have been published [43–47].

As an outstanding result, compounds **6b**, **7b**,**c** and **8b**–**e** exhibited significant activities, with $GI_{50}$ values lower than $1.0 \times 10^{-6}$ M against several cell lines. Particularly, compounds **8** were relatively more actives than compounds **6** and **7**. Probably the *meta* substitution of the ring C in compounds **8** could be related with this finding. A raw comparison of the activities of our obtained compounds **6**–**8** with respect to the activity reported for the standard drug Adriamycin, used by NCI as control, reflects that the activities displayed for our compounds against several cell lines were comparable or even higher than those for the standard drug control. In this sense, it is remarkable that compounds **7b**, **8b**, **8c**, **8d** and **8e** displayed activities with $GI_{50}$ values of 0.58, 0.90, 0.52, 0.44 and $0.55 \times 10^{-6}$ M, respectively, against the HCT-15 cell line (*Colon cancer* panel), while this value was $6.46 \times 10^{-6}$ M for the standard drug Adriamycin; compounds **7b**, **8c**, **8d** and **8e** displayed activities with $GI_{50}$ values of 0.20, 0.13, 0.14 and $0.22 \times 10^{-6}$ M, respectively, against the MDA-MB-435 cell line (*Melanoma* panel), while this value was $0.25 \times 10^{-6}$ M for Adriamycin; as well as, compound **8b** had a $GI_{50}$ value of $0.29 \times 10^{-6}$ M against this cell line. Compounds **7b** and **8c** displayed activities with $GI_{50}$ values of 0.35 and $0.28 \times 10^{-6}$ M, respectively, against the OVCAR-3 cell line (*Ovarian cancer* panel), while this value was $0.39 \times 10^{-6}$ M for Adriamycin; compounds **7b**, **8b**, **8c** and **8d** displayed activities with $GI_{50}$ values of 0.36, 0.76, 0.29 and $0.35 \times 10^{-6}$ M, respectively, against the NCI/ADR-RES cell line (*Ovarian cancer* panel), while this value was




**Scheme 1.** Synthesis of [(7-chloroquinolin-4-yl)amino]chalcones **4** and **5**.




**Scheme 2.** Synthesis of new *N*-acetyl and *N*-formylpyrazolines **6** and **7**–**8**.

7.16 × $10^{-6}$ M for Adriamycin. Compounds **7b**, **8b**, **8c**, **8d** and **8e** displayed activities with $GI_{50}$ values of 0.41, 0.95, 0.33, 0.33 and 0.55 × $10^{-6}$ M, respectively, against the CAKI-1 cell line (*Renal cancer* panel), while this value was 0.95 × $10^{-6}$ M for Adriamycin. Compound **8c** displayed $GI_{50}$ values of 0.41, 0.29 and 0.21 × $10^{-6}$ M against UO-31 (*Renal cancer* panel), HS 578T and BT-549 (*Breast cancer* panel) cell lines, respectively, while the values against the same cell lines for Adriamycin were 0.49, 0.33 and 0.23 × $10^{-6}$ M, respectively. Additionally comparable activities were found for compounds **8c** and **8d** displaying $GI_{50}$ values of 0.29 and 0.36 × $10^{-6}$ M against KM12 (*Colon cancer* panel) and HS 578T (*Breast cancer* panel) cell lines, respectively, while the corresponding values for Adriamycin were 0.27 and 0.33 × $10^{-6}$ M, respectively. These findings make compounds **7b** and **8c**–**e**, promising targets to perform structural modifications pursuing to improve their activities and hence to develop possible new leader antitumor molecules based on their structures. Moreover, the values of cytotoxicity of the evaluated compounds **6**–**8**, measured as $LC_{50}$, were higher than 100 μM, for the most cell lines, indicating a low toxicity of such compounds for normal human cell lines, as required for developing of potential antitumor agents (see Tables 1 and 2).

## 2.3. Antimalarial activity

### 2.3.1. Antiplasmodial activity

The antiplasmodial activity was evaluated *in vitro* on asynchronic cultures of *Plasmodium falciparum* (NF54 strain), see Experimental section. It have been stated that an inhibition of growing higher than 20% represents a significant antimalarial activity for the compound evaluated. After our essays, only 5 (i.e. **6e**, **7d**, **7f**, **8c** and **8f**) of the 18 screened compounds (i.e. **6**–**8** series), at concentrations of 20 μg/mL, do not showed any growth inhibition of *P. falciparum*. The remaining compounds showed changes in the rate of growth and reproduction of the parasite.

The best dose–response observed was by compound **7a** with a percentage growth inhibition of 50.8%, followed by compounds **6b**–**d** with percentages growth inhibitions >30% and compounds **8e** and **7c** with percentage of inhibition of 24.6 ± 3.2 and 20.2 ± 4.4, respectively (see Table 3). Although the percentages growth inhibitions for all assayed compounds were lower than that for the standard (CQ), it is worth mention that compound **7a** shown the closer value (i.e. 50.8%) compared with reference drug (CQ) which had a percentage growth inhibition of 68.4%, making compound **7a** as the more promising structure, of the obtained compounds, for our future drug developing antimalarial studies.

### 2.3.2. Hemolytic activity

For the most active compounds (i.e. **6b**, **6c**, **6d** and **7a**), it was determined their hemolytic activities to confirm that the action was directed against the parasite and not host cells destruction occurred. None of the compounds screened showed a marked hemolytic capacity, i.e. ≥5%. Indeed, all of them displayed values lower than that for the standard drug (CQ) (see Table 3).

### 2.3.3. Determination of the $IC_{50}$

$IC_{50}$ analysis was performed only for compounds **6b**, **6c**, **6d** and **7a**, since they showed percentages growth inhibitions higher than 30%. The results indicated that compound **7a** displayed the lower $IC_{50}$ value (i.e. 14.1 μg/mL) of the assayed compounds, although it is still too far in comparing with that for the standard drug (CQ) (i.e. 0.02 μg/mL) (see Table 3).

**Table 1**
*In vitro* testing expressed as growth inhibition of cancer cell lines for compounds **6b** and **7b**–**f**.[a]

| Panel/cell line | Compounds | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **6b** | | **7b** | | **7c** | | **7d** | | **7e** | | **7f** | |
| | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) |
| *Leukemia* | | | | | | | | | | | | |
| CCRF-CEM | – | – | – | – | 2.51 | >100 | 2.71 | >100 | 1.82 | >100 | 2.25 | >100 |
| HL-60(TB) | 3.56 | >100 | 0.38 | >100 | 2.66 | 72.5 | 2.23 | >100 | 2.53 | 75.8 | 2.27 | >100 |
| MOLT-4 | 2.42 | >100 | 0.53 | >100 | 2.28 | >100 | 2.54 | >100 | 3.36 | >100 | 5.13 | >100 |
| RPMI-8226 | – | – | – | – | 2.55 | >100 | 2.71 | >100 | 2.56 | >100 | 2.18 | >100 |
| SR | **0.61** | >100 | **0.32** | >100 | 1.77 | >100 | 2.83 | >100 | 2.58 | >100 | 3.18 | >100 |
| *Non-small cell lung* | | | | | | | | | | | | |
| A549/ATCC | 8.81 | >100 | **0.99** | >100 | 4.05 | >100 | 50.3 | >100 | 6.90 | >100 | 9.86 | >100 |
| EKVX | | >100 | | >100 | 4.94 | >100 | >100 | >100 | 11.7 | >100 | | >100 |
| HOP-62 | >100 | >100 | **0.86** | >100 | 5.26 | >100 | 43.7 | >100 | 4.13 | 96.1 | >100 | >100 |
| HOP-92 | >100 | >100 | >100 | >100 | 4.28 | >100 | | >100 | 1.83 | >100 | 2.14 | >100 |
| NCI-H226 | >100 | >100 | 4.66 | >100 | 5.22 | >100 | >100 | >100 | 34.5 | >100 | >100 | >100 |
| NCI-H23 | >100 | >100 | 2.77 | >100 | 3.64 | >100 | 5.06 | >100 | 20.4 | >100 | 6.93 | >100 |
| NCI-H322M | >100 | >100 | >100 | >100 | – | – | – | – | – | – | – | – |
| NCI-H460 | >100 | >100 | **0.59** | >100 | 3.96 | >100 | >100 | >100 | 4.42 | >100 | >100 | >100 |
| NCI-H522 | 5.05 | >100 | **0.39** | >100 | – | – | – | – | – | – | – | – |
| *Colon cancer* | | | | | | | | | | | | |
| COLO 205 | 5.93 | >100 | 2.40 | >100 | 2.21 | >100 | 3.56 | >100 | 3.73 | >100 | 4.59 | >100 |
| HCC-2998 | >100 | >100 | 1.61 | >100 | 3.50 | >100 | 18.1 | >100 | 5.52 | >100 | 7.44 | >100 |
| HCT-116 | 38.8 | >100 | **0.50** | >100 | 3.58 | >100 | 9.97 | >100 | 3.21 | >100 | 25.2 | >100 |
| HCT-15 | 3.33 | >100 | **0.58** | >100 | 2.54 | >100 | 2.97 | >100 | 3.56 | >100 | 2.78 | >100 |
| HT29 | 5.40 | >100 | **0.41** | >100 | 3.21 | >100 | 3.81 | >100 | 2.83 | >100 | 6.09 | >100 |
| KM12 | 6.59 | >100 | **0.54** | >100 | 3.41 | >100 | 16.5 | >100 | 5.87 | >100 | 6.64 | >100 |
| SW-620 | >100 | >100 | **0.48** | >100 | 2.73 | 68.7 | 35.8 | >100 | 3.49 | >100 | >100 | >100 |
| *CNS cancer* | | | | | | | | | | | | |
| SF-268 | 9.10 | >100 | **0.85** | >100 | 3.96 | >100 | >100 | >100 | 28.0 | >100 | 8.61 | >100 |
| SF-295 | 3.58 | >100 | **0.27** | >100 | 2.41 | >100 | 24.8 | >100 | 5.63 | >100 | 4.00 | >100 |
| SF-539 | >100 | >100 | **0.58** | >100 | 4.57 | 99.2 | >100 | >100 | 2.75 | 37.6 | >100 | >100 |
| SNB-19 | >100 | >100 | 1.92 | >100 | 5.13 | >100 | 5.65 | >100 | 18.2 | >100 | >100 | >100 |
| SNB-75 | | >100 | **0.42** | >100 | 5.03 | >100 | >100 | >100 | 5.19 | >100 | >100 | >100 |
| U251 | >100 | >100 | **0.51** | >100 | 3.12 | >100 | 1.50 | >100 | 2.06 | 19.4 | >100 | >100 |
| *Melanoma* | | | | | | | | | | | | |
| LOX IMVI | 3.15 | >100 | **0.60** | >100 | 1.61 | 6.88 | 2.32 | >100 | 1.89 | 7.83 | 4.11 | >100 |
| MALME-3M | | >100 | | >100 | 4.21 | >100 | >100 | >100 | 3.20 | >100 | >100 | >100 |
| M14 | 5.24 | >100 | **0.62** | >100 | 4.12 | >100 | 7.67 | >100 | 3.75 | 48.3 | 6.88 | >100 |
| MDA-MB-435 | **0.57** | >100 | **0.20** | >100 | 1.59 | >100 | 1.72 | >100 | 2.10 | >100 | 1.95 | >100 |
| SK-MEL-2 | >100 | >100 | 7.86 | >100 | – | – | – | – | – | – | – | – |
| SK-MEL-28 | 4.88 | >100 | 0.76 | >100 | 8.35 | >100 | 20.9 | >100 | 2.22 | 14.0 | >100 | >100 |
| SK-MEL-5 | 2.20 | >100 | **0.81** | >100 | 1.38 | 11.2 | **0.62** | >100 | 1.98 | 35.5 | 1.74 | >100 |
| UACC-257 | >100 | >100 | >100 | >100 | 10.1 | >100 | 4.39 | >100 | 2.84 | 55.4 | >100 | >100 |
| UACC-62 | 2.95 | >100 | **0.57** | >100 | 1.55 | 17.4 | 5.99 | >100 | 2.20 | 59.3 | 4.13 | >100 |
| *Ovarian cancer* | | | | | | | | | | | | |
| IGROV1 | >100 | >100 | 5.01 | >100 | 9.36 | >100 | 43.1 | >100 | 22.3 | >100 | 26.6 | >100 |
| OVCAR-3 | 7.19 | >100 | **0.35** | >100 | 2.78 | 43.2 | 14.5 | >100 | 1.91 | 6.03 | 6.82 | >100 |
| OVCAR-4 | 6.92 | >100 | 6.48 | >100 | 3.88 | >100 | 13.4 | >100 | 3.19 | >100 | 5.15 | >100 |
| OVCAR-5 | >100 | >100 | 6.46 | >100 | 19.8 | >100 | >100 | >100 | 69.2 | >100 | >100 | >100 |
| OVCAR-8 | >100 | >100 | 3.22 | >100 | 4.53 | >100 | 69.8 | >100 | 9.98 | >100 | >100 | >100 |
| NCI/ADR-RES | 3.04 | >100 | **0.36** | >100 | 2.69 | >100 | 7.04 | >100 | 7.88 | >100 | 5.22 | >100 |
| SK-OV-3 | >100 | >100 | **0.57** | >100 | 11.3 | >100 | >100 | >100 | 3.36 | 55.8 | >100 | >100 |
| *Renal cancer* | | | | | | | | | | | | |
| 786-0 | >100 | >100 | **0.91** | >100 | 3.36 | 98.4 | 32.0 | >100 | 2.31 | 9.93 | 50.9 | >100 |
| A498 | >100 | >100 | >100 | >100 | 1.30 | >100 | >100 | >100 | 15.3 | >100 | | >100 |
| ACHN | 3.96 | >100 | 1.00 | >100 | 4.21 | >100 | 7.02 | >100 | 3.24 | 47.0 | 3.27 | >100 |
| CAKI-1 | >100 | >100 | **0.41** | >100 | 3.82 | >100 | >100 | >100 | 5.47 | >100 | >100 | >100 |
| RXF 393 | 5.07 | >100 | 1.88 | >100 | 2.20 | >100 | 11.6 | >100 | 2.22 | >100 | 4.89 | >100 |
| SN12C | 30.7 | >100 | **0.98** | >100 | 3.80 | >100 | 12.6 | >100 | 4.37 | >100 | 7.23 | >100 |
| TK-10 | >100 | >100 | 9.57 | >100 | 9.51 | >100 | 20.9 | >100 | 3.09 | 38.6 | >100 | >100 |
| UO-31 | 3.87 | >100 | 1.30 | >100 | 3.81 | >100 | 19.7 | >100 | 4.96 | 67.5 | 5.49 | >100 |
| *Prostate cancer* | | | | | | | | | | | | |
| PC-3 | 3.02 | >100 | 0.48 | >100 | 4.76 | >100 | 9.43 | >100 | 11.7 | >100 | 4.61 | >100 |
| DU-145 | >100 | >100 | 1.94 | >100 | 3.80 | >100 | 38.0 | >100 | 6.63 | >100 | >100 | >100 |
| *Breast cancer* | | | | | | | | | | | | |
| MCF7 | 4.04 | >100 | **0.48** | >100 | 3.42 | >100 | 8.78 | >100 | 3.23 | >100 | 8.39 | >100 |
| MDA-MB-231/ATCC | >100 | >100 | 1.43 | >100 | 2.43 | >100 | 20.1 | >100 | 4.39 | >100 | 6.09 | >100 |
| HS 578T | >100 | >100 | 3.93 | >100 | 3.77 | >100 | >100 | >100 | 3.24 | >100 | | >100 |
| BT-549 | 5.40 | >100 | 4.77 | >100 | 3.81 | 53.8 | 10.9 | >100 | 1.91 | 8.23 | 5.64 | >100 |
| T-47D | 1.57 | >100 | 1.56 | >100 | 2.18 | >100 | 1.38 | >100 | 2.33 | >100 | 1.65 | >100 |
| MDA-MB-468 | 2.41 | >100 | **0.32** | >100 | **0.98** | 10.0 | 1.00 | >100 | 1.83 | 40.9 | 1.51 | >100 |

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell lines screen [42–47].
[b] $GI_{50}$ was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Determined at five concentration levels (100, 10, 1.0, 0.1, and 0.01 μM).
[c] $LC_{50}$ is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells.

## 3. Conclusions

Starting from compounds **4** and **5** were synthesized three novel series of acetyl **6a**–**f** and *N*-formyl **7a**–**f** and **8a**–**f** pyrazoline derivates through a simple and easy synthetic methodology, conducting to the obtained products in acceptable to excellent yields and in short reaction times. The anticancer evaluation data against 60 cancer cell lines revealed that derivatives **7b** and **8b**–**e** exhibited the highest activities with $GI_{50}$ values lower than 1.0 μM for the most of the cell lines tested. Indeed, such compounds displayed higher activity against several cell lines than the standard drug Adriamycin. Regarding to the antimalarial activity, derivative **7a** was the most active compound inhibiting the growth of *P. falciparum* parasites in 50.8%. Derivatives **6d**, **6c** and **6b** were moderately active inhibiting the growth of *P. falciparum* in 38.3, 33.0 and 32.9%, respectively. None of the evaluated compounds showed marked hemolytic activity, moreover, their values were lower than that for the standard drug chloroquine (CQ). Owing to the significant obtained results, chemical studies are currently being conducted pursuing for an improvement of the antitumor and antimalarial activities of such compounds.

## 4. Experimental

Commercially available starting materials, reagents and solvents were used as supplied. TLC analyses were performed on Merck silica gel 60 F254 aluminum plates. Melting points were determined in a Büchi melting point apparatus and are uncorrected. IR spectra were performed on a Shimadzu FTIR 8400 spectrophotometer in KBr disks. The $^{1}H$ and $^{13}C$ NMR spectra were run on a Bruker DPX 400 spectrophotometer operating at 400 MHz and 100 MHz, respectively, using dimethyl sulfoxide-$d_6$ as solvent and tetramethylsilane as internal reference. The mass spectra were obtained on a Hewlett Packard HP Engine-5989 spectrometer (equipped with a direct inlet probe) operating at 70 eV. The elemental analyses were obtained using a Thermo Finnigan Flash EA1112 CHN (STIUJA) elemental analyzer.

### 4.1. Chemistry

#### 4.1.1. General procedure for the synthesis of the precursors

Using a similar methodology like reported by Ferrer and coworkers [41], it was carried out the synthesis of the precursors **2**, **3**, **4** and **5**.

*4.1.1.1. General procedure for the synthesis of [(7-chloroquinolin-4-yl) amino]acetophenones **2** and **3**.* A mixture of 4,7-dichloroquinoline (2.5 mmol) and 3- or 4-aminoacetophenone (2.75 mmol) in ethanol (10 mL) was heated under reflux for 8 h. The solid formed was filtered and recrystallized from ethanol.

*4.1.1.2. General procedure for the synthesis of [(7-chloroquinolin-4-yl)amino]chalcones **4** and **5**.* A mixture of [(7-chloroquinolin-4-yl) amino]acetophenone **2** or **3** (0.36 mmol), the respective

**Table 2**
*In vitro* testing expressed as growth inhibition of cancer cell lines for compounds **8b**–**e** and the standard drug Adriamycin.[a]

| Panel/cell line | Compounds | | | | | | | | Doxorubicin (Adriamycin), NSC 123127 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **8b** | | **8c** | | **8d** | | **8e** | | 100 μM[d] | |
| | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) |
| *Leukemia* | | | | | | | | | | |
| CCRF-CEM | 2.34 | >100 | **0.37** | >100 | **0.68** | >100 | **0.65** | >100 | 0.08 | 100.00 |
| HL-60(TB) | 1.10 | 40.2 | **0.20** | 4.56 | **0.25** | 5.28 | **0.26** | 3.97 | 0.12 | 89.33 |
| K-562 | **0.41** | >100 | **0.26** | >100 | **0.29** | >100 | **0.43** | >100 | 0.19 | 100.00 |
| MOLT-4 | 1.92 | >100 | **0.39** | >100 | **0.49** | >100 | **0.59** | >100 | 0.03 | 100.00 |
| RPMI-8226 | 2.45 | >100 | **0.49** | >100 | 16.7 | >100 | 8.83 | >100 | 0.08 | 100.00 |
| SR | **0.37** | >100 | **0.20** | >100 | **0.24** | >100 | **0.34** | >100 | 0.03 | 100.00 |
| *Non-small cell lung* | | | | | | | | | | |
| A549/ATCC | **0.94** | >100 | **0.54** | >100 | **0.57** | >100 | **0.66** | >100 | 0.06 | 100.00 |
| EKVX | >100 | >100 | **0.95** | >100 | **0.64** | >100 | **0.98** | >100 | 0.41 | 47.97 |
| HOP-62 | 1.91 | >100 | **0.51** | >100 | **0.66** | >100 | 1.12 | 66.5 | 0.07 | 67.61 |
| HOP-92 | **0.65** | >100 | **0.21** | >100 | >100 | >100 | 2.75 | >100 | 0.10 | 42.27 |
| NCI–H226 | >100 | >100 | 6.91 | >100 | 41.6 | >100 | 2.20 | >100 | 0.05 | 6.40 |
| NCI–H23 | 2.48 | >100 | **0.61** | >100 | **0.95** | >100 | 1.38 | >100 | 0.15 | 13.15 |
| NCI–H322M | – | – | – | – | – | – | – | – | 0.54 | 67.76 |
| NCI–H460 | **0.79** | >100 | **0.41** | >100 | **0.40** | >100 | **0.42** | >100 | 0.02 | 51.29 |
| NCI–H522 | **0.80** | >100 | **0.21** | >100 | **0.30** | >100 | **0.75** | >100 | 0.03 | 2.80 |
| *Colon cancer* | | | | | | | | | | |
| COLO 205 | 1.39 | >100 | **0.36** | 7.92 | **0.45** | 13.9 | **0.64** | >100 | 0.18 | 4.33 |
| HCC-2998 | 3.36 | >100 | 1.22 | >100 | 1.32 | >100 | 2.16 | >100 | 0.26 | 21.68 |
| HCT-116 | **0.60** | >100 | **0.39** | 4.75 | **0.38** | 38.1 | **0.40** | 6.71 | 0.08 | 54.58 |
| HCT-15 | **0.90** | >100 | **0.52** | >100 | **0.44** | >100 | **0.55** | >100 | 6.46 | 100.00 |
| HT29 | 0.48 | >100 | 0.32 | 4.32 | **0.34** | >100 | **0.40** | 4.99 | 0.12 | 67.45 |
| KM12 | **0.59** | >100 | **0.29** | >100 | **0.44** | >100 | **0.50** | >100 | 0.27 | 92.68 |
| SW-620 | **0.56** | >100 | **0.35** | >100 | **0.36** | >100 | **0.47** | >100 | 0.09 | 58.61 |
| *CNS cancer* | | | | | | | | | | |
| SF-268 | 4.37 | >100 | 1.04 | >100 | 2.56 | >100 | 2.55 | >100 | 0.10 | 30.48 |
| SF-295 | 1.06 | >100 | **0.37** | 41.2 | **0.32** | >100 | **0.38** | >100 | 0.10 | 69.98 |
| SF-539 | 3.78 | >100 | **0.31** | 35.7 | **0.61** | 66.2 | **0.81** | 55.5 | 0.12 | 27.23 |
| SNB-19 | 3.48 | >100 | **0.60** | >100 | **0.87** | >100 | 3.42 | >100 | 0.04 | 49.77 |
| SNB-75 | 1.58 | >100 | **0.22** | >100 | **0.33** | >100 | **0.38** | 51.0 | 0.07 | 3.30 |
| U251 | 1.29 | >100 | **0.37** | 5.07 | **0.45** | 41.8 | **0.45** | >100 | 0.04 | 30.62 |
| *Melanoma* | | | | | | | | | | |

**Table 2** (*continued*)

| Panel/cell line | Compounds | | | | | | | | Doxorubicin (Adriamycin), NSC 123127 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **8b** | | **8c** | | **8d** | | **8e** | | 100 μM[d] | |
| | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) | GI50[b] (μM) | LC50[c] (μM) |
| LOX IMVI | 1.56 | 50.4 | **0.45** | 4.23 | **0.58** | 6.70 | **0.71** | >100 | 0.07 | 50.35 |
| MALME-3M | 1.63 | >100 | **0.37** | >100 | **0.74** | >100 | **0.81** | >100 | 0.12 | 3.97 |
| M14 | 1.04 | >100 | **0.33** | >100 | **0.40** | >100 | **0.35** | 57.5 | 0.18 | 4.05 |
| MDA-MB-435 | **0.29** | >100 | **0.13** | >100 | **0.14** | >100 | **0.22** | >100 | 0.25 | 9.57 |
| SK-MEL-2 | 2.05 | >100 | 0.26 | >100 | **0.49** | >100 | **0.73** | 50.1 | 0.17 | 1.06 |
| SK-MEL-28 | 5.63 | >100 | **0.76** | >100 | 0.96 | >100 | 1.02 | 97.1 | 0.21 | 15.92 |
| SK-MEL-5 | 1.86 | >100 | **0.29** | 64.2 | **0.41** | >100 | **0.42** | 23.5 | 0.08 | 0.49 |
| UACC-257 | | >100 | **0.33** | >100 | | >100 | 1.40 | >100 | 0.14 | 8.15 |
| UACC-62 | 1.63 | >100 | **0.53** | >100 | **0.67** | >100 | **0.72** | 84.0 | 0.12 | 0.74 |
| *Ovarian cancer* | | | | | | | | | | |
| IGROV1 | **0.76** | >100 | **0.82** | >100 | 1.44 | >100 | 3.29 | >100 | 0.17 | 100.00 |
| OVCAR-3 | 1.20 | >100 | **0.28** | 7.38 | **0.47** | 77.4 | **0.52** | 6.06 | 0.39 | 84.33 |
| OVCAR-4 | 4.13 | >100 | **0.56** | >100 | **0.89** | >100 | 1.13 | >100 | 0.37 | 74.30 |
| OVCAR-5 | >100 | >100 | 1.38 | >100 | 3.51 | >100 | 4.32 | >100 | 0.41 | 100.00 |
| OVCAR-8 | 3.80 | >100 | **0.51** | 56.0 | **0.95** | >100 | 2.06 | >100 | 0.10 | 43.25 |
| NCI/ADR-RES | **0.76** | >100 | **0.29** | >100 | **0.35** | >100 | 1.36 | >100 | 7.16 | 100.00 |
| SK-OV-3 | 3.48 | >100 | **0.33** | >100 | **0.70** | >100 | 1.40 | >100 | 0.22 | 100.00 |
| *Renal cancer* | | | | | | | | | | |
| 786-0 | 4.73 | >100 | **0.59** | >100 | **0.53** | >100 | **0.84** | 76.3 | 0.13 | 51.64 |
| A498 | **0.83** | >100 | **0.23** | >100 | **0.26** | >100 | **0.29** | >100 | 0.10 | 1.90 |
| ACHN | 5.18 | >100 | **0.86** | >100 | **0.87** | >100 | 1.27 | >100 | 0.08 | 100.00 |
| CAKI-1 | **0.95** | >100 | **0.33** | 35.3 | **0.33** | >100 | **0.55** | 12.1 | 0.95 | 100.00 |
| RXF 393 | 3.08 | >100 | **0.39** | >100 | **0.85** | >100 | 1.54 | 73.4 | 0.10 | 4.69 |
| SN12C | 3.86 | >100 | **0.63** | >100 | **0.78** | >100 | **0.78** | >100 | 0.07 | 72.44 |
| TK-10 | – | – | – | – | – | – | – | – | 0.18 | 86.70 |
| UO-31 | **0.88** | >100 | **0.41** | >100 | **0.85** | >100 | 1.30 | >100 | 0.49 | 26.18 |
| *Prostate cancer* | | | | | | | | | | |
| PC-3 | – | – | – | – | **0.85** | >100 | 1.46 | >100 | 0.32 | 87.10 |
| DU-145 | 4.17 | >100 | **0.44** | >100 | 1.35 | >100 | 2.68 | >100 | 0.11 | 100.00 |
| *Breast cancer* | | | | | | | | | | |
| MCF7 | **0.81** | >100 | **0.34** | >100 | **0.37** | >100 | **0.37** | >100 | 0.03 | 51.29 |
| MDA-MB-231/ATCC | 2.12 | >100 | 1.15 | >100 | 3.19 | >100 | 1.60 | >100 | 0.51 | 34.75 |
| HS 578T | 1.97 | >100 | **0.29** | >100 | **0.36** | >100 | **0.68** | >100 | 0.33 | 85.70 |
| BT-549 | 1.36 | >100 | **0.21** | 30.9 | **0.45** | 55.6 | **0.39** | 4.61 | 0.23 | 21.33 |
| T-47D | 2.63 | >100 | **0.34** | >100 | **0.53** | >100 | **0.65** | 69.3 | 0.06 | 85.70 |
| MDA-MB-468 | **0.42** | >100 | **0.22** | >100 | **0.21** | >100 | **0.30** | 23.3 | 0.05 | 2.52 |

[a] Data obtained from NCI's *in vitro* disease-oriented human tumor cell lines screen [42–47].
[b] $GI_{50}$ was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Determined at five concentration levels (100, 10, 1.0, 0.1, and 0.01 μM).
[c] $LC_{50}$ is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells.
[d] The values of activity against human tumor cell lines displayed by Adriamycin correspond to the reported by NCI at highest concentration of $10^{-4}$ M. Please visit: http://dtp.nci.nih.gov/dtpstandard/cancerscreeningdata/index.jsp.

benzaldehyde (0.40 mmol), and potassium hydroxide (one pellet) in methanol (8 mL) was stirred at room temperature for 12 h. Then, water (2 mL) was added and the resulting precipitate was collected by filtration, washed with water and recrystallized from a mixture ethanol/water (1:0.5).

Analytical data for compounds **2**, **3**, **4b–f** and **5b–d** were consistent with those reported for the same compounds in Ref. [41].

*4.1.1.2.1. (E)-3-(4-Bromophenyl)-1-[4-(7-chloro-quinolin-4-ylamino)phenyl]prop-2-en-1-one* ***4a****.* Yellow solid; 81% yield; mp: 225–226 °C. FTIR (KBr) υ ($cm^{-1}$): 3388 (NH), 3059 (=C–H), 1656 (C=O), 1608 and 1573 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 7.27 (d, $J$ = 3.7 Hz, 1H, H-3), 7.46 (d, $J$ = 8.5 Hz, 2H, H$o'$), 7.57 (d, $J$ = 9.1 Hz, 1H, H-6), 7.63 (d, $J$ = 8.5 Hz, 2H, H$o$), 7.66 (d, $J$ = 16.3 Hz, 1H, H-2″), 7.78 (d, $J$ = 8.5 Hz, 2H, H$m'$), 7.87 (d, $J$ = 16.3 Hz, 1H, H-3″), 7.96 (s, 1H, H-8), 8.15 (d, $J$ = 8.5 Hz, 2H, H$m$), 8.37 (d, $J$ = 9.1 Hz, 1H, H-5), 8.64 (d, $J$ = 3.7 Hz, 1H, H-2), 9.26 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 119.0, 121.9, 122.9, 123.7, 124.7, 125.6, 127.8, 128.3, 130.6, 131.1, 131.4, 131.8, 134.2, 134.6, 136.6, 141.8, 146.0, 150.0, 152.1, 187.1. Anal. Calcd. For $C_{24}H_{16}BrClN_2O$: C, 62.16; H, 3.48; N, 6.04. Found: C, 62.22; H, 3.51; N, 5.97.

*4.1.1.2.2. (E)-3-(4-Bromophenyl)-1-[3-(7-chloroquinolin-4-ylamino)phenyl]prop-2-en-1-one* ***5a****.* Yellow solid; 94% yield; mp: 201–203 °C. FTIR (KBr) υ ($cm^{-1}$): 3347 (NH), 3059 (=C–H), 1660 (C=O) and 1576 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 7.01 (d, $J$ = 5.3 Hz, 1H, H-3), 7.57–7.98 (m, 11H, H-6, H-2″, H-3″, H$o$, H$o'$, H$m$, H$p$, H$o''$, H$m''$), 8.08 (s, 1H, H-8), 8.47 (d, $J$ = 9.0 Hz, 1H, H-5), 8.51 (d, $J$ = 5.3 Hz, 1H, H-2), Not observed (br s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 78.9, 101.9, 118.3, 121.6, 122.6, 123.7, 124.2, 124.8, 126.4, 127.4, 129.6, 130.5, 131.6, 133.6, 133.7, 138.5, 140.8, 142.5, 147.3, 149.4, 151.7, 188.6. Anal. Calcd. For $C_{24}H_{16}BrClN_2O$: C, 62.16; H, 3.48; N, 6.04. Found: C, 62.10; H, 3.56; N, 6.10.

*4.1.1.2.3. (E)-1-[3-(7-Chloroquinolin-4-ylamino)phenyl]-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one* ***5e****.* Yellow solid; 71% yield; mp: 256–258 °C. FTIR (KBr) υ ($cm^{-1}$): 3307 (NH), 3062 (=C–H), 1647 (C=O) and 1575 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 3.73 (s, 3H, $OCH_3$), 3.87 (s, 6H, $OCH_3$), 7.04 (d, $J$ = 5.3 Hz, 1H, H-3), 7.24 (s, 2H, H$o''$), 7.56–8.00 (m, 7H, H-6, H-2″, H-3″, H$o$, H$m$, H$p$, H$o'$), 8.06 (s, 1H, H-8), 8.48 (d, $J$ = 9.0 Hz, 1H, H-5), 8.52 (d, $J$ = 5.3 Hz, 1H, H-2), Not observed (br s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$)

**Table 3**
*In vitro* testing antimalarial and hemolytic activity for compounds **6a**–**f**, **7a**–**f** and **8a**–**f** and the standard drug chloroquine (CQ).[a]

| Compound | Inhibition (%) ± SD | Coefficient of variation | Hemolysis (%) | $IC_{50}$[b] (μg/mL) ± SD |
|---|---|---|---|---|
| **6a** | 6.8 ± 0 | 0 | n.e. | n.e. |
| **6b** | 32.9 ± 1.8 | 5.5 | 3.4 ± 0.05 | 54.6 ± 1.5 |
| **6c** | 33.0 ± 8.0 | 24.0 | 2.8 ± 0.6 | 38.0 ± 1.9 |
| **6d** | 38.3 ± 3.0 | 7.8 | 3.1 ± 0.2 | >100 |
| **6e** | 0 ± 0 | 0 | n.e. | n.e. |
| **6f** | 10.6 ± 2.8 | 26.5 | n.e. | n.e. |
| **7a** | 50.8 ± 4.6 | 9.0 | 3.2 ± 0.1 | 14.1 ± 1.7 |
| **7b** | 1.1 | 0 | n.e. | n.e. |
| **7c** | 20.2 ± 4.4 | 22.2 | 3.8 ± 0.6 | n.e. |
| **7d** | 0 ± 0 | 0 | n.e. | n.e. |
| **7e** | 5.8 ± 0 | 0 | n.e. | n.e. |
| **7f** | 0 ± 0 | 0 | n.e. | n.e. |
| **8a** | 12.5 ± 2.0 | 16.0 | n.e. | n.e. |
| **8b** | 7.0 ± 1.2 | 16.6 | n.e. | n.e. |
| **8c** | 0 ± 0 | 0 | n.e. | n.e. |
| **8d** | 4.1 ± 0 | 0 | n.e. | n.e. |
| **8e** | 24.6 ± 3.2 | 13.1 | 3.4 ± 0.2 | n.e. |
| **8f** | 0 ± 0 | 0 | n.e. | n.e. |
| CQ[c] | 68.4 ± 3.6 | 5.5 | 17.2 ± 0.6 | 0.02 ± 0.01 |

SD: Standard deviation.
n.e.: not evaluated.
[a] Data obtained from PECET *in vitro* for the antimalarial activity [48–52].
[b] $IC_{50}$ (μg/mL): concentration corresponding to 50% growth inhibition of the parasite.
[c] CQ: chloroquine diphosphate salt.

δ ppm 55.7, 59.6, 78.7, 101.7, 106.2, 118.7, 120.9, 121.2, 121.9, 123.5, 124.0, 124.6, 125.9, 126.6, 127.2, 129.3, 130.3, 134.2, 139.2, 140.1, 141.1, 144.3, 151.5, 153.3, 189.1. Anal. Calcd. For $C_{27}H_{23}ClN_2O_4$: C, 68.28; H, 4.88; N, 5.90. Found: C, 68.17; H, 4.96; N, 5.98.

*4.1.1.2.4. (E)-1-[3-(7-Chloroquinolin-4-ylamino)phenyl]3-(p-tolyl)-prop-2-en-1-one* ***5f***. Yellow solid; 86% yield; mp: 216–217 °C. FTIR (KBr) υ ($cm^{-1}$): 3392 (NH), 3061 (=C–H), 1661 (C=O), 1595 and 1570 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.35 (s, 3H, $CH_3$), 7.01 (d, *J* = 5.4 Hz, 1H, H-3), 7.27 (d, *J* = 8.0 Hz, 2H, Ho″), 7.53–7.97 (m, 9H, H-6, H-2″, H-3″, Ho, H*m*, H*p*, Ho′, H*m*″), 8.06 (s, 1H, H-8), 8.46 (d, *J* = 8.8 Hz, 1H, H-5), 8.51 (d, *J* = 5.4 Hz, 1H, H-2), Not observed (br s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 21.1, 79.1, 102.2, 118.5, 121.0, 121.8, 123.9, 124.5, 125.1, 126.5, 127.7, 128.9, 129.5, 129.8, 131.9, 134.0, 138.9, 140.8, 144.3, 147.6, 149.6, 152.0, 188.9. Anal. Calcd. For $C_{25}H_{19}ClN_2O$: C, 75.28; H, 4.80; N, 7.02. Found: C, 75.19; H, 4.86; N, 6.97.

### *4.1.2. General procedure for the synthesis of 1-(3-[4-((7-chloroquinolin-4-yl)amino)phenyl]-5-substitutedphenyl-4,5-dihydro-1H-pyrazole-1-yl)ethanones* ***6a–f***

A mixture of 4-(7-chloroquinolin-4-yl)aminochalcone **4** (0.67 mmol), hydrazine hydrate (0.87 mmol) and acetic acid (2.0 mL) was heated under reflux for 6 h until complete consumption of the chalcone (TLC control). After cooling, the resulting solution was neutralized with concentrate ammonium hydroxide. Then, the adding of crushed ice to the solution precipitated a solid which was filtered and washed with water. Pure compounds **6** were obtained by crystallization from ethanol.

*4.1.2.1. 1-(5-(4-Bromophenyl)-3-[4-(7-chloroquinolin-4-ylamino) phenyl]-4,5-dihydro-1H-pyrazole-1-yl)ethanone* ***6a***. White solid; 95% yield; mp: 140–142 °C. FTIR (KBr) υ ($cm^{-1}$): 3320 (NH), 3060 (=C–H), 1646 (C=O), 1640 and 1579 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.30 (s, 3H, $CH_3$), 3.14 (dd, *J* = 17.9, 4.3 Hz, 1H, H-4′a), 3.85 (dd, *J* = 17.9, 11.9 Hz, 1H, H-4′b), 5.53 (dd, *J* = 11.9, 4.3 Hz, 1H, H-5′), 7.13 (d, *J* = 4.9 Hz, 1H, H-3), 7.17 (d, *J* = 8.3 Hz, 2H, Ho′), 7.43 (d, *J* = 8.3 Hz, 2H, Ho), 7.52 (d, *J* = 8.3 Hz, 2H, H*m*′), 7.60 (d, *J* = 8.4 Hz 1H, H-6), 7.79 (d, *J* = 8.3 Hz, 2H, H*m*), 7.93 (s, 1H, H-8), 8.41 (d, *J* = 8.4 Hz, 1H, H-5), 8.54 (d, *J* = 4.9 Hz, 1H, H-2), 9.31 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 22.2, 42.4, 59.4, 103.9, 119.4, 120.7, 121.4, 125.1, 125.8, 126.1, 128.2, 128.3, 128.4, 132.0, 134.6, 142.3, 143.1, 147.3, 150.1, 152.5, 154.3, 167.8. MS (70 eV) *m*/*z* (%): 518/520 [$M^+$] (100/75), 478 (63), 480 (45), 43 (64). Anal. Calcd. For $C_{26}H_{20}BrClN_4O$: C, 60.07; H, 3.88; N, 10.78. Found: C, 60.20; H, 3.95; N, 10.82.

*4.1.2.2. 1-(5-(4-Chlorophenyl)-3-[4-(7-chloroquinolin-4-ylamino) phenyl]-4,5-dihydro-1H-pyrazole-1-yl)ethanone* ***6b***. White solid; 96% yield; mp: 139–142 °C. FTIR (KBr) υ ($cm^{-1}$): 3316 (NH), 3068 (=C–H), 1645 (C=O), 1614 and 1577 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.30 (s, 3H, $CH_3$), 3.11 (dd, *J* = 17.8, 3.9 Hz, 1H, H-4′a), 3.83 (dd, *J* = 17.8, 12.0 Hz, 1H, H-4′b), 5.53 (dd, *J* = 12.0, 3.9 Hz, 1H, H-5′), 7.13 (d, *J* = 5.3 Hz, 1H, H-3), 7.22 (d, *J* = 8.3 Hz, 2H, Ho′), 7.39 (d, *J* = 8.3 Hz, 2H, H*m*′), 7.44 (d, *J* = 8.0 Hz, 2H, Ho), 7.59 (d, *J* = 9.7 Hz, 1H, H-6), 7.80 (d, *J* = 8.0 Hz, 2H, H*m*), 7.93 (s, 1H, H-8), 8.40 (d, *J* = 9.7 Hz, 1H, H-5), 8.54 (d, *J* = 5.3 Hz, 1H, H-2), 9.29 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 22.2, 42.4, 59.4, 103.9, 119.4, 120.7, 121.4, 125.1, 125.8, 126.1, 128.2, 128.3, 128.4, 132.0, 134.6, 142.3, 143.1, 147.2, 150.1, 152.5, 154.3, 167.8. MS (70 eV) *m*/*z* (%): 474/476 [$M^+$] (100/71), 432 (73), 434 (49), 321 (14), 43 (25). Anal. Calcd. For $C_{26}H_{20}Cl_2N_4O$: C, 65.69; H, 4.24; N, 11.79. Found: C, 65.59; H, 4.29; N, 11.88.

*4.1.2.3. 1-(3-[4-(7-Chloroquinolin-4-ylamino)phenyl]-5-phenyl-4,5-dihydro-1H-pyrazole-1-yl)ethanone* ***6c***. White solid; 89% yield; mp: 141–143 °C. FTIR (KBr) υ ($cm^{-1}$): 3276 (NH), 3172 (=C–H), 1642 (C=O), 1614 and 1573 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.30 (s, 3H, $CH_3$), 3.10 (dd, *J* = 18.0, 4.4 Hz, 1H, H-4′a), 3.84 (dd, *J* = 18.0, 11.7 Hz, 1H, H-4′b), 5.53 (dd, *J* = 11.7, 4.4 Hz, 1H, H-5′), 7.13 (d, *J* = 5.3 Hz, 1H, H-3), 7.19 (d, *J* = 7.3 Hz, 2H, Ho′), 7.25 (m, 1H, H*p*), 7.32 (d, *J* = 7.3 Hz, 2H, H*m*′), 7.43 (d, *J* = 8.8 Hz, 2H, Ho), 7.58 (dd, *J* = 9.1, 2.2 Hz 1H, H-6), 7.80 (d, *J* = 8.8 Hz, 2H, H*m*), 7.93 (d, *J* = 2.2, 1H, H-8), 8.40 (d, *J* = 9.1 Hz, 1H, H-5), 8.54 (d, *J* = 5.3 Hz, 1H, H-2), 9.29 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 21.7, 42.2, 58.8, 103.4, 118.5, 120.9, 124.6, 125.4, 125.7, 127.9, 128.7, 131.3, 132.0, 134.1, 134.6, 139.5, 142.5, 146.3, 149.6, 152.0, 153.8, 167.2. MS (70 eV) *m*/*z* (%): 440/442 [$M^+$] (100/38), 398 (99), 400 (34), 321 (18), 43 (23). Anal. Calcd. For $C_{26}H_{21}ClN_4O$: C, 70.82; H, 4.80; N, 12.71. Found: C, 70.73; H, 4.91; N, 12.73.

*4.1.2.4. 1-(3-[4-(7-Chloroquinolin-4-ylamino)phenyl]-5-(4-methoxy phenyl)-4,5-dihydro-1H-pyrazole-1-yl)ethanone* ***6d***. Yellow solid; 62% yield; mp: 163–165 °C. FTIR (KBr) υ ($cm^{-1}$): 3261 (NH), 3075 (=C–H), 1640 (C=O), 1612 and 1573 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.28 (s, 3H, $CH_3$), 3.48 (dd, *J* = 20.4, 5.6 Hz, 1H, H-4′a), 3.74 (s, 3H, $OCH_3$), 3.81 (dd, *J* = 20.4, 9.3 Hz, 1H, H-4′b), 5.49 (dd, *J* = 9.3, 5.6 Hz, 1H, H-5′), 6.87 (d, *J* = 8.4 Hz, 2H, Ho′), 7.07 (d, *J* = 5.2 Hz, 1H, H-3), 7.13 (d, *J* = 8.3 Hz, 2H, Ho), 7.40 (d, *J* = 8.4 Hz, 2H, H*m*′), 7.52 (d, *J* = 8.0 Hz, 1H, H-6), 7.78 (d, *J* = 8.3 Hz, 2H, H*m*), 7.91 (s, 1H, H-8), 8.38 (d, *J* = 8.0 Hz, 1H, H-5), 8.53 (d, *J* = 5.2 Hz, 1H, H-2), Not observed (br s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 21.1, 41.7, 54.8, 58.7, 103.4, 118.6, 120.7, 124.0, 124.7, 125.8, 126.0, 126.3, 127.1, 132.0, 133.7, 134.6, 141.4, 142.5, 146.9, 150.0, 153.2, 158.2, 166.8. MS (70 eV) *m*/*z* (%): 470/472 [$M^+$] (100/30), 428 (66), 426 (33), 321 (13), 295 (19), 280 (22), 43 (75). Anal. Calcd. For $C_{27}H_{23}ClN_4O_2$: C, 68.86; H, 4.92; N, 11.90. Found: C, 68.79; H, 4.96; N, 11.99.

*4.1.2.5. 1-(3-[4-(7-Chloroquinolin-4-ylamino) phenyl]-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-yl)ethanone* ***6e***. Yellow solid; 59% yield; mp: 120–123 °C. FTIR (KBr) υ ($cm^{-1}$): 3274 (NH), 3002 (=C–H), 1639 (C=O) and 1589 (C=N and C=C). $^1$H NMR (400 MHz,

DMSO-$d_6$) $\delta$ ppm 2.03 (s, 3H, $CH_3$), 2.87 (dd, $J$ = 16.1, 11.2 Hz, 1H, H-4′a), 3.43 (dd, $J$ = 16.1, 10.5 Hz, 1H, H-4′b), 3.64 (s, 3H, $OCH_3$), 3.77 (s, 6H, $OCH_3$), 4.80 (t, $J$ = 10.92, 1H, H-5′), 6.72 (s, 2H, Ho′), 7.00 (d, $J$ = 5.3 Hz, 1H, H-3), 7.37 (d, $J$ = 8.4 Hz, 2H, Ho), 7.57 (dd, $J$ = 9.0, 2.0 Hz 1H, H-6), 7.66 (d, $J$ = 8.4 Hz, 2H, H*m*), 7.90 (d, $J$ = 2.0, 1H, H-8), 8.42 (d, $J$ = 9.0 Hz, 1H, H-5), 8.49 (d, $J$ = 5.3 Hz, 1H, H-2), Not observed (br s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ ppm 21.3, 41.9, 55.3, 58.7, 59.6, 103.5, 119.6, 121.7, 123.0, 124.9, 126.1, 126.6, 127.7, 132.3, 133.9, 134.1, 134.5, 141.3, 142.2, 146.1, 151.8, 153.7, 158.5, 167.0. MS (70 eV) *m*/*z* (%): 530/532 [$M^+$] (66/14), 488 (34), 43 (100). Anal. Calcd. For $C_{29}H_{27}ClN_4O_4$: C, 65.59; H, 5.13; N, 10.55. Found: C, 65.49; H, 5.14; N, 10.63.

*4.1.2.6. 1-(3-[4-(7-Chloroquinolin-4-ylamino)phenyl]-5-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-yl)ethanone* ***6f***. Yellow solid; 85% yield; mp: 162–164 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3314 (NH), 3086 (=C–H), 1642 (C=O) and 1585 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.87 (s, 3H, $CH_3$), 2.26 (s, 3H, $CH_3$), 3.16 (dd, $J$ = 18.0, 4.7 Hz, 1H, H-4′a), 3.89 (dd, $J$ = 18.0, 11.7 Hz, 1H, H-4′b), 5.48 (dd, $J$ = 11.7, 4.7 Hz, 1H, H-5′), 7.04–7.16 (m, 5H, H-3, Ho′, H*m*′), 7.44 (d, $J$ = 8.5 Hz, 2H, Ho), 7.62 (dd, $J$ = 9.0, 2.1 Hz, 1H, H-6), 7.81 (d, $J$ = 8.5 Hz, 2H, H*m*), 7.93 (d, $J$ = 2.1, 1H, H-8), 8.42 (d, $J$ = 9.0 Hz 1H, H-5), 8.54 (d, $J$ = 5.5 Hz, 1H, H-2), 8.87 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ ppm 21.1, 23.3, 41.9, 55.1, 56.7, 103.2, 118.1, 120.7, 122.0, 124.7, 125.8, 126.3, 127.1, 132.0, 133.9, 134.6, 141.4, 142.5, 147.9, 150.0, 152.2, 158.2, 166.8. MS (70 eV) *m*/*z* (%): 454/456 [$M^+$] (15/5), 440 (100), 442 (39), 411 (32), 321 (37), 295 (45), 280 (45), 43 (16). Anal. Calcd. For $C_{27}H_{23}ClN_4O$: C, 71.28; H, 5.10; N, 12.31. Found: C, 71.19; H, 5.16; N, 12.41.

*4.1.3. General procedure for the synthesis of 1-(3-[4-(7-chloroquinolin-4-yl)amino)phenyl]-5-substitutedphenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***7a–f***

A mixture of 4-(7-chloroquinolin-4-yl)aminochalcone **4** (0.38 mmol), DMF (1.0 mL), boron trifluoride diethyl etherate (3 drops) and hydrazine hydrate (0.49 mmol) was heated to reflux for 6 h until complete consumption of the chalcone **4** (TLC control). Then, formic acid (2.0 mL) was added to the reaction mixture and the refluxing was continued by 2 h further. After cooling, the adding of crushed ice precipitated a solid which was filtered and washed thoroughly with water and dried at ambient temperature to afford compounds **7**.

*4.1.3.1. 5-(4-Bromophenyl)-3-[4-(7-chloroquinolin-4-ylamino)phenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***7a***. Yellow solid; 80% yield; mp: 201–203 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3472 (NH), 3062 (=C–H), 1674 (C=O), 1618 and 1593 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 3.21 (dd, $J$ = 18.1, 4.8 Hz, 1H, H-4′a), 3.91 (dd, $J$ = 18.1, 11.7 Hz, 1H, H-4′b), 5.53 (dd, $J$ = 11.7, 4.8 Hz, 1H, H-5′), 7.12 (d, $J$ = 5.4 Hz, 1H, H-3), 7.21 (d, $J$ = 8.4 Hz, 2H, Ho′), 7.44 (d, $J$ = 8.8 Hz, 2H, Ho), 7.54 (d, $J$ = 8.4 Hz, 2H, H*m*′), 7.62 (dd, $J$ = 9.0, 2.1 Hz, 1H, H-6), 7.81 (d, $J$ = 8.8 Hz, 2H, H*m*), 7.94 (d, $J$ = 2.1 Hz, 1H, H-8), 8.12 (s, 1H, NH), 8.42 (d, $J$ = 9.0 Hz, 1H, H-5), 8.55 (d, $J$ = 5.4 Hz, 1H, H-2), 8.88 (s, 1H, CHO). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ ppm 42.1, 57.9, 103.4, 118.8, 120.5, 121.1, 124.7, 125.4, 125.5, 128.1, 128.2, 131.6, 133.5, 140.7, 151.2, 155.7, 159.0, 159.5, 161.5, 163.0, 166.8. MS (70 eV) *m*/*z* (%): 504/506 [$M^+$] (76/100), 475 (15), 477 (21), 321 (47), 323 (15), 280 (40), 282 (13). Anal. Calcd. For $C_{25}H_{18}BrClN_4O$: C, 59.37; H, 3.59; N, 11.08. Found: C, 59.44; H, 3.66; N, 10.17.

*4.1.3.2. 5-(4-Chlorophenyl)-3-[4-(7-chloroquinolin-4-ylamino)phenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***7b***. Yellow solid; 87% yield; mp: 216–218 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3472 (NH), 3065 (=C–H), 1676 (C=O), 1618 and 1594 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 3.21 (dd, $J$ = 18.1, 4.8 Hz, 1H, H-4′a), 3.91 (dd, $J$ = 18.1, 11.7 Hz, 1H, H-4′b), 5.54 (dd, $J$ = 11.7, 4.8 Hz, 1H, H-5′), 7.12 (d, $J$ = 5.5 Hz, 1H, H-3), 7.27 (d, $J$ = 8.5 Hz, 2H, Ho′), 7.41 (d, $J$ = 8.5 Hz, 2H, Ho), 7.44 (d, $J$ = 8.5 Hz, 2H, H*m*′), 7.60 (dd, $J$ = 9.0, 2.1 Hz, 1H, H-6), 7.80 (d, $J$ = 8.5 Hz, 2H, H*m*), 7.93 (d, $J$ = 2.1 Hz, 1H, H-8), 8.13 (s, 1H, NH), 8.41 (d, $J$ = 9.0 Hz, 1H, H-5), 8.55 (d, $J$ = 5.5 Hz, 1H, H-2), 8.88 (s, 1H, CHO). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ ppm 42.1, 57.9, 103.5, 114.1, 118.8, 121.0, 124.7, 125.4, 125.5, 127.8, 128.1, 128.7, 132.0, 134.3, 140.3, 142.7, 155.6, 155.7, 159.0, 159.4, 159.5. MS (70 eV) *m*/*z* (%): 460/462 [$M^+$] (100/68), 431 (25), 433 (18), 321 (47), 323 (16), 280 (44), 282 (14). Anal. Calcd. For $C_{25}H_{18}Cl_2N_4O$: C, 65.09; H, 3.93; N, 12.14. Found: C, 65.00; H, 3.99; N, 12.15.

*4.1.3.3. 3-[4-(7-Chloroquinolin-4-ylamino)phenyl]-5-phenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***7c***. Yellow solid; 73% yield; mp: 120–123 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3473 (NH), 3048 (=C–H), 1675 (C=O), 1615 and 1593 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 3.24 (dd, $J$ = 18.1, 4.8 Hz, 1H, H-4′a), 3.96 (dd, $J$ = 18.1, 11.7 Hz, 1H, H-4′b), 5.57 (dd, $J$ = 11.7, 4.8 Hz, 1H, H-5′), 7.02 (d, $J$ = 6.5 Hz, 1H, H-3), 7.25 (d, $J$ = 7.3 Hz, 2H, Ho′), 7.35 (d, $J$ = 7.3 Hz, 2H, H*m*′), 7.54 (d, $J$ = 8.5 Hz, 2H, Ho), 7.82 (dd, $J$ = 9.0, 2.0 Hz, 1H, H-6), 7.92 (d, $J$ = 8.5 Hz, 2H, H*m*), 7.89 (d, $J$ = 2.0 Hz, 1H, H-8), 8.13 (d, $J$ = 5.8 Hz, 1H, H*p*), 8.38 (s, 1H, NH), 8.57 (d, $J$ = 6.5 Hz, 1H, H-2), 8.63 (d, $J$ = 9.0 Hz, 1H, H-5), 8.92 (s, 1H, CHO). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ ppm 42.3, 58.6, 101.5, 116.8, 119.7, 121.4, 124.1, 125.5, 125.6, 127.1, 127.5, 128.3, 128.5, 128.8, 137.6, 139.8, 141.3, 149.0, 155.4, 159.0, 159.7. MS (70 eV) *m*/*z* (%): 426/428 [$M^+$] (100/36), 397 (28), 399 (10), 321 (46), 323 (15), 280 (29), 282 (10). Anal. Calcd. For $C_{25}H_{19}ClN_4O$: C, 70.34; H, 4.49; N, 13.12. Found: C, 70.28; H, 4.59; N, 13.21.

*4.1.3.4. 3-[4-(7-Chloroquinolin-4-ylamino)phenyl]-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***7d***. Yellow solid; 67% yield; mp: 208–211 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3478 (NH), 3025 (=C–H), 1676 (C=O), 1618 and 1593 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 3.18 (dd, $J$ = 18.0, 4.7 Hz, 1H, H-4′a), 3.72 (s, 3H, $OCH_3$), 3.88 (dd, $J$ = 18.0, 11.7 Hz, 1H, H-4′b), 5.48 (dd, $J$ = 11.7, 4.7 Hz, 1H, H-5′), 6.89 (d, $J$ = 8.5 Hz, 2H, Ho′), 7.12 (d, $J$ = 5.5 Hz, 1H, H-3), 7.16 (d, $J$ = 8.5 Hz, 2H, H*m*′), 7.44 (d, $J$ = 8.5 Hz, 2H, Ho), 7.61 (dd, $J$ = 9.0, 2.3 Hz, 1H, H-6), 7.81 (d, $J$ = 8.5 Hz, 2H, H*m*), 7.93 (d, $J$ = 2.3 Hz, 1H, H-8), 8.13 (s, 1H, NH), 8.42 (d, $J$ = 9.0 Hz, 1H, H-5), 8.54 (d, $J$ = 5.5 Hz, 1H, H-2), 8.86 (s, 1H, CHO). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ ppm 42.3, 55.1, 58.0, 103.4, 114.1, 118.7, 121.1, 124.7, 125.4, 125.6, 127.0, 127.2, 128.0, 133.4, 134.4, 142.6, 147.2, 148.9, 151.4, 155.7, 158.6, 159.4. MS (70 eV) *m*/*z* (%): 456/458 [$M^+$] (100/36), 427 (27), 429 (10), 321 (20), 295 (33), 297 (10), 280 (38), 282 (12). Anal. Calcd. For $C_{26}H_{21}ClN_4O_2$: C, 68.34; H, 4.63; N, 12.26. Found: C, 68.28; H, 4.53; N, 12.19.

*4.1.3.5. 3-[4-(7-Chloroquinolin-4-ylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***7e***. Yellow solid; 63% yield; mp: 203–205 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3484 (NH), 3061 (=C–H), 1670 (C=O), 1622 and 1594 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 3.28 (dd, $J$ = 18.1, 5.3 Hz, 1H, H-4′a), 3.64 (s, 3H, $OCH_3$), 3.75 (s, 6H, $OCH_3$), 3.94 (dd, $J$ = 18.1, 11.8 Hz, 1H, H-4′b), 5.52 (dd, $J$ = 11.8, 5.3 Hz, 1H, H-5′), 6.54 (s, 2H, Ho′), 6.99 (d, $J$ = 7.0 Hz, 1H, H-3), 7.58 (d, $J$ = 8.5 Hz, 2H, Ho), 7.91 (dd, $J$ = 9.2, 2.0 Hz, 1H, H-6), 7.96 (d, $J$ = 8.5 Hz, 2H, H*m*), 8.02 (d, $J$ = 2.0 Hz, 1H, H-8), 8.59 (d, $J$ = 7.0 Hz, 1H, H-2), 8.70 (d, $J$ = 9.2 Hz, 1H, H-5), 8.95 (s, 1H, CHO), Not observed (br s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ ppm 42.5, 55.9, 58.9, 59.9, 100.9, 102.8, 116.3, 119.9, 124.9, 125.6, 125.7, 127.6, 128.3, 129.5, 136.8, 136.9, 138.5, 139.0, 139.5, 144.4, 153.1, 154.1, 159.9. MS (70 eV) *m*/*z* (%): 516/518 [$M^+$] (100/35), 487 (13), 321 (14), 280 (19). Anal. Calcd. For $C_{28}H_{25}ClN_4O_4$: C, 65.05; H, 4.87; N, 12.84. Found: C, 65.16; H, 4.80; N, 12.77.

*4.1.3.6. 3-[4-(7-Chloroquinolin-4-ylamino)phenyl]-5-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***7f***. Yellow solid; 70% yield; mp: 208–211 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3481 (NH), 3059 (=C–H), 1679 (C=O), 1618 and 1593 (C=N and C=C). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 2.27 (s, 3H, $CH_3$), 3.17 (dd, $J$ = 18.0, 4.6 Hz, 1H, H-4′a), 3.89 (dd, $J$ = 18.0, 11.7 Hz, 1H, H-4′b), 5.48 (dd, $J$ = 11.7, 4.6 Hz, 1H, H-5′), 7.09–7.17 (m, 5H, Ho′, Hm′, H-3), 7.43 (d, $J$ = 8.5 Hz, 2H, Ho), 7.61 (dd, $J$ = 9.0, 2.1 Hz, 1H, H-6), 7.80 (d, $J$ = 8.5 Hz, 2H, Hm), 7.93 (d, $J$ = 2.1 Hz, 1H, H-8), 8.13 (s, 1H, NH), 8.41 (d, $J$ = 9.0 Hz, 1H, H-5), 8.54 (d, $J$ = 5.3 Hz, 1H, H-2), 8.87 (s, 1H, CHO). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ ppm 20.6, 42.3, 58.2, 103.4, 118.7, 121.0, 124.7, 125.4, 125.5, 125.6, 127.2, 128.0, 129.2, 134.3, 136.7, 138.5, 142.6, 147.1, 151.5, 155.7, 159.0, 159.4. MS (70 eV) $m/z$ (%): 440/442 [$M^+$] (100/35), 411 (31), 413 (12), 321 (32), 323 (11), 280 (35), 282 (12). Anal. Calcd. For $C_{26}H_{21}ClN_4O$: C, 70.82; H, 4.80; N, 12.71. Found: C, 70.76; H, 4.89; N, 12.73.

*4.1.4. General procedure for the synthesis of 1-(3-[3-(7-chloroquinolin-4yl)amino) phenyl]-5-substitutedphenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***8a–f***

A mixture of 3-(7-chloroquinolin-4-yl)aminochalcone **5** (0.32 mmol), boron trifluoride diethyl etherate (2 drops) and hydrazine hydrate (0.42 mmol) was stirred at room temperature for 4 h until complete consumption of the chalcone **5** (TLC control). Then, formic acid (2.0 mL) was added to the reaction mixture and the stirring, at room temperature, was continued for 1 h further. Once the reaction finished, ethanol (3.0 mL) was added and the solid formed was removed by filtration and discarded. Then the resulting filtrate was concentrate to dryness under reduced pressure and the crude obtained was purified by column chromatography on silica gel by using a mixture $CHCl_3$:MeOH (30:1) as eluent.

*4.1.4.1. 5-(4-Bromophenyl)-3-[3-(7-chloroquinolin-4-ylamino) phenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***8a***. Yellow solid; 75% yield; mp: 154–155 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3481 (NH), 3061 (=C–H), 1670 (C=O) and 1569 (C=N and C=C). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 3.19 (dd, $J$ = 17.7, 4.6 Hz, 1H, H-4′a), 3.83 (dd, $J$ = 17.7, 11.7 Hz, 1H, H-4′b), 5.52 (dd, $J$ = 11.7, 4.6 Hz, 1H, H-5′), 7.00 (d, $J$ = 4.9 Hz, 1H, H-3), 7.15 (d, $J$ = 7.8 Hz, 2H, Ho″), 7.38–7.58 (m, 7H, H-6, Hm″, Ho, Hm, Hp, NH), 7.75 (s, 1H, Ho′), 8.06 (s, 1H, H-8), 8.59 (d, $J$ = 4.9 Hz, 1H, H-2), 7.92 (d, $J$ = 9.0 Hz, 1H, H-5), 8.95 (s, 1H, CHO). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ ppm 42.4, 58.7, 100.1, 120.1, 121.3, 122.1, 123.0, 124.5, 126.5, 127.5, 128.7, 129.5, 130.3, 132.3, 132.5, 136.7, 137.2, 139.4, 140.3, 146.2, 151.5, 154.8, 160.1. MS (70 eV) $m/z$ (%): 504/506 [$M^+$] (76/100), 475 (18), 477 (19), 447 (27), 449 (35), 321 (33), 280 (25). Anal. Calcd. For $C_{25}H_{18}BrClN_4O$: C, 59.37; H, 3.59; N, 11.08. Found: C, 59.43; H, 3.64; N, 10.15.

*4.1.4.2. 5-(4-Chlorophenyl)-3-[3-(7-chloroquinolin-4-ylamino) phenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***8b***. Yellow solid; 71% yield; mp: 139–141 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3484 (NH), 3061 (=C–H), 1669 (C=O) and 1566 (C=N and C=C). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 3.26 (dd, $J$ = 18.2, 5.1 Hz, 1H, H-4′a), 3.96 (dd, $J$ = 18.2, 11.9 Hz, 1H, H-4′b), 5.59 (dd, $J$ = 11.9, 5.1 Hz, 1H, H-5′), 6.93 (d, $J$ = 6.8 Hz, 1H, H-3), 7.29 (d, $J$ = 8.4 Hz, 2H, Ho″), 7.42 (d, $J$ = 8.4 Hz, 2H, Hm″), 7.57–7.63 (m, 1H, Ho), 7.68 (t, $J$ = 7.8 Hz, 1H, Hm), 7.79 (d, $J$ = 7.8 Hz, 1H, Hp), 7.86–7.92 (m, 2H, H-6, Ho′), 8.10–8.18 (br s, 1H, NH), 8.13–8.17 (m, 1H, H-8), 8.56 (d, $J$ = 6.8 Hz, 1H, H-2), 8.67 (d, $J$ = 9.0 Hz, 1H, H-5), 8.90 (s, 1H, CHO). $^{13}$C NMR (100 MHz, CDCl3) $\delta$ ppm 42.1, 58.1, 100.8, 116.3, 120.4, 122.8, 125.5, 125.6, 126.8, 127.4, 127.8, 128.7, 130.6, 132.1, 132.5, 137.9, 138.1, 140.1, 144.9, 154.0, 155.5, 159.0, 159.8. MS (70 eV) $m/z$ (%): 460/462 [$M^+$] (100/66), 431 (19), 403 (42), 405 (28), 321 (33), 280 (24). Anal. Calcd. For $C_{25}H_{18}Cl_2N_4O$: C, 65.09; H, 3.93; N, 12.14. Found: C, 65.02; H, 3.90; N, 12.19.

*4.1.4.3. 3-[3-(7-Chloroquinolin-4-ylamino)phenyl]-5-phenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***8c***. Yellow solid; 61% yield; mp: 134–135 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3356 (NH), 3048 (=C–H), 1670 (C=O) and 1570 (C=N and C=C). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 3.10 (dd, $J$ = 17.8, 4.9 Hz, 1H, H-4′a), 3.68 (dd, $J$ = 17.8, 11.7 Hz, 1H, H-4′b), 5.43 (dd, $J$ = 11.7, 4.9 Hz, 1H, H-5′), 6.90 (d, $J$ = 5.5 Hz, 1H, H-3), 7.11–7.24 (m, 5H, H-6, Ho, Hm, Hp, Hp″), 7.28–7.39 (m, 4H, Ho″, Hm″), 7.63 (s, 1H, Ho′), 7.88 (d, $J$ = 9.0 Hz, 1H, H-5), 7.92 (d, $J$ = 2.0 Hz, 1H, H-8), 8.46 (d, $J$ = 5.5 Hz, 1H, H-2), 8.90 (s, 1H, CHO), Not observed (br s, 1H, NH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ ppm 42.6, 59.3, 102.8, 118.5, 120.1, 122.0, 122.8, 124.3, 125.6, 126.2, 128.1, 128.7, 129.1, 130.1, 132.5, 135.5, 140.3, 140.6, 147.5, 149.7, 151.8, 155.4, 160.3. MS (70 eV) $m/z$ (%): 426/428 [$M^+$] (100/35), 369 (44), 371 (15), 321 (34), 323 (11), 280 (17). Anal. Calcd. For $C_{25}H_{19}ClN_4O$: C, 70.34; H, 4.49; N, 13.12. Found: C, 70.25; H, 4.54; N, 13.19.

*4.1.4.4. 3-[3-(7-Chloroquinolin-4-ylamino) phenyl]-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***8d***. Yellow solid; 47% yield; mp: 222–224 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3340 (NH), 3058 (=C–H), 1666 (C=O), 1609 and 1567 (C=N and C=C). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 3.22 (dd, $J$ = 17.8, 4.7 Hz, 1H, H-4′a), 3.78 (m, 4H, $OCH_3$, H-4′b), 5.51 (dd, $J$ = 11.5, 4.7 Hz, 1H, H-5′), 6.86 (d, $J$ = 8.3 Hz, 2H, Ho″), 6.90–6.97 (m, 1H, H-3), 7.19 (d, $J$ = 8.3 Hz, 2H, Hm″), 7.28 (s, 1H, H-6), 7.37–7.54 (m, 4H, Ho, Hm, Hp, NH), 7.77 (s, 1H, Ho′), 7.97–8.05 (m, 2H, H-5, H-8), 8.43–8.50 (m, 1H, H-2), 8.96 (s, 1H, CHO). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ ppm 42.8, 59.5, 60.8, 101.2, 117.2, 121.2, 123.8, 124.1, 124.4, 125.2, 127.0, 130.4, 132.7, 136.0, 137.6, 137.7, 139.5, 144.7, 147.1, 150.9, 153.8, 155.1, 160.3, 168.6. MS (70 eV) $m/z$ (%): 456/458 [$M^+$] (100/35), 427 (43), 429 (15), 321 (16), 280 (64), 282 (20). Anal. Calcd. For $C_{26}H_{21}ClN_4O_2$: C, 68.34; H, 4.63; N, 12.26. Found: C, 68.27; H, 4.57; N, 12.29.

*4.1.4.5. 3-[3-(7-Chloroquinolin-4-ylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***8e***. Yellow solid; 53% yield; mp: 127–129 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3262 (NH), 3065 (=C–H), 1674 (C=O) and 1593 (C=N and C=C). $^1$H NMR (400 MHz $CDCl_3$) $\delta$ ppm 3.21 (dd, $J$ = 17.8, 4.7 Hz, 1H, H-4′a), 3.67–3.94 (m, 10H, ($OCH_3$ × 3), H-4′b), 5.49 (dd, $J$ = 11.4, 4.7 Hz, 1H, H-5′), 6.44 (s, 2H, Ho″), 6.83 (d, $J$ = 4.8 Hz, 1H, H-3), 7.32–7.61 (m, 4H, H-6, Ho, Hm, Hp), 7.81 (s, 1H, Ho′), 7.93 (s, 1H, H-8), 8.18 (d, $J$ = 8.5 Hz, 1H, H-5), 8.29 (d, $J$ = 4.8 Hz, 1H, H-2), 8.99 (s, 1H, CHO), Not observed (br s, 1H, NH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ ppm 42.8, 56.2, 59.5, 60.8, 101.4, 102.5, 117.2, 121.2, 123.6, 124.3, 125.2, 126.9, 130.4, 132.7, 136.0, 137.6, 137.7, 139.3, 144.6, 147.0, 150.9, 153.8, 155.1, 160.3, 168.6. MS (70 eV) $m/z$ (%): 516/518 [$M^+$] (64/23), 501 (100), 487 (18), 321 (11), 280 (65), 282 (20). Anal. Calcd. For $C_{28}H_{25}ClN_4O_4$: C, 65.05; H, 4.87; N, 12.84. Found: C, 65.13; H, 4.92; N, 12.94.

*4.1.4.6. 3-[3-(7-Chloroquinolin-4-ylamino)phenyl]-5-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde* ***8f***. Yellow solid; 60% yield; mp: 261–263 °C. FTIR (KBr) $\upsilon$ ($cm^{-1}$): 3288 (NH), 3060 (=C–H), 1662 (C=O), 1608 and 1567 (C=N and C=C). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 2.31 (s, 3H, $CH_3$), 3.21 (dd, $J$ = 17.8, 4.9 Hz, 1H, H-4′a), 3.79 (dd, $J$ = 17.8, 11.8 Hz, 1H, H-4′b), 5.52 (dd, $J$ = 11.8, 4.9 Hz, 1H, H-5′), 6.85 (d, $J$ = 5.0 Hz, 1H, H-3), 7.09–7.55 (m, 9H, H-6, Ho, Hm, Hp, Ho″, Hm″, NH), 7.80 (s, 1H, Ho′), 7.95 (s, 1H, H-8), 8.14 (d, $J$ = 8.8 Hz, 1H, H-5), 8.32 (d, $J$ = 5.0 Hz, 1H, H-2), 8.96 (s, 1H, CHO). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ ppm 21.1, 42.6, 59.2, 101.2, 117.1, 121.2, 123.7, 124.0, 124.2, 125.2, 125.6, 127.0, 129.7, 130.4, 132.9, 137.4, 137.8, 137.9, 139.2, 144.1, 146.6, 151.2, 154.9, 160.2. MS (70 eV) $m/z$ (%): 440/442 [$M^+$] (100/35), 411 (16), 321 (16), 280 (17). Anal. Calcd. For $C_{26}H_{21}ClN_4O$: C, 70.82; H, 4.80; N, 12.71. Found: C, 70.72; H, 4.82; N, 12.81.

### 4.2. Biological evaluation

#### 4.2.1. Anticancer activity

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% $CO_2$, 95% air and 100% relative humidity for 24 h prior to addition of tested compounds. After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of sample addition ($T_z$). The samples were solubilized in dimethyl sulfoxide (DMSO) at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of compounds addition, an aliquot of frozen concentrate was thawed and diluted to twice, the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different sample dilutions were added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final sample concentrations [44]. After the tested compounds were added, the plates were incubated for an additional 48 h at 37 °C, 5% $CO_2$, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 μL of cold 50% (*w*/*v*) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (*w*/*v*) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero ($T_z$), control growth in the absence of drug ($C$), and test growth in the presence of drug at the five concentration levels ($T_i$)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as: $[(T_i - T_Z)/(C - T_Z)] \times 100$ for concentrations for which $T_i > T_z$, and $[(T_i - T_Z)/T_Z] \times 100$ for concentrations for which $T_i < T_z$. Three dose response parameters were calculated for each compound. Growth inhibition of 50% ($GI_{50}$) was calculated from $[(T_i - T_Z)/(C - T_Z)] \times 100 = 50$, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from $T_i = T_z$. The $LC_{50}$ (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(T_i - T_Z)/T_Z] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested [44–47].

#### 4.2.2. Antiplasmodial activity

The antiplasmodial activity was evaluated *in vitro* on asynchronic cultures of *P. falciparum* (NF54 strain), maintained in standard culture conditions [48]. The effect of the evaluated compounds over the growing of the parasites was evaluated according to the fluorometric method by quantifying the parasite DNA by staining with ethidium bromide (EtBr). The fluorescence intensity is directly proportional to the amount of DNA and this, in turn, is proportional to the amount of viable parasites. The amount of ADN detected is directly related with the concentration of the evaluated compound and in turn with the inhibition of the replication process of the parasite [49–51]. Briefly, the *P. falciparum* cultures with total forms were adjusted to a parasitemia (1.5%–2%) and hematocrit (5%) in 10% RPMI medium of human serum (total medium). Then, in each well of a 24-wells plate it was simultaneously placed 500 μL of a suspension of the parasites and 500 μL of dilutions of each compound to be evaluated (Dilutions were prepared from a concentrate mother solution of 100 μg/mL). Concentration of the positive chloroquine (CQ) control was 22.2 μg/mL. A well with the suspension of the parasites free of any compound was used for both growing and viability control. The plates were incubated during 48 h at 37 °C in $N_2$ (90%), $CO_2$ (5%) and $O_2$ (5%) atmosphere. After the incubation finished, extraction and purification of DNA was carried out by using a lysis solution containing proteinase K.

In this sense, the anti-*Plasmodium* activity of each evaluated compound was evidenced by the reduction of the fluorescence (Flu). Indeed, the viability percentage was calculated through the following formula:

$$\text{Viability (\%)} = \frac{\text{(Flu) of parasites with compound} - \text{(Flu) of the medium}}{\text{(Flu) of parasites without compound} - \text{(Flu) of the medium}} * 100$$

Then, the inhibition growing percentage was calculated according to the following formula:

$$\text{Inhibition (\%)} = 100 - \text{Viability (\%)}$$

All determinations were carried out by triplicate. CQ was used as control of activity for each case.

#### 4.2.3. Hemolytic activity

The ability to induce hemolysis was evaluated specifically to compounds which showed antiplasmodial activity following the method of cytotoxicity by spectrophotometry on 96-wells plates. Red blood cells (hematocrit 5%) were placed into the wells along with the RPMI medium 1640 and subsequently were exposed against three different concentrations of compounds (20, 40 and 80 μg/mL). Detection of free hemoglobin, after 48 h of incubation at 37 °C, should be an evidence of the capability of the compound to induce hemolysis. The measurement of the concentration of free hemoglobin was performed spectrophotometrically at 542 nm.

#### 4.2.4. Determination of the $IC_{50}$

The inhibitory concentration ($IC_{50}$) corresponding to the concentration of the compound which produces a 50% reduction of

growing of the parasite was determined by mean of the statistical program Probit. It is a parametric method of linear regression which permits the analysis of the dose–response relationship [52].

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