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Recycling antimalarial leads for cancer: Antiproliferative properties of *N*-cinnamoyl chloroquine analogues

Bianca C. Pérez [a,†], Iva Fernandes [a,†], Nuno Mateus [a], Cátia Teixeira [a,b], Paula Gomes [a,*]

[a] Centro de Investigação em Química da Universidade do Porto, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, P-4169-007 Porto, Portugal
[b] CICECO, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

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ABSTRACT

Cinnamic acids and quinolines are known as useful scaffolds in the discovery of antitumor agents. Therefore, N-cinnamoylated analogues of chloroquine, recently reported as potent dual-action antimalarials, were evaluated against three different cancer cell lines: MKN-28, Caco-2, and MCF-7. All compounds display anti-proliferative activity in the micromolar range against the three cell lines tested, and most of them were more active than their parent drug, chloroquine, against all cell lines tested. Hence, *N*-cinnamoyl-chloroquine analogues are a good start towards development of affordable antitumor leads.

Cancer remains a life threatening disease worldwide despite of available conventional treatments such as surgery, radiotherapy and/or chemotherapy. The still considerable limitations of cancer chemotherapy are mainly associated with low efficacy, high toxicity, and emergence of drug resistance, and not less important their high cost.[1] Hence, there is a wide range of scientific approaches to find better chemotherapeutic agents, including those based on recycling or repositioning of well-known drugs used to treat diseases other than cancer.[2] In this connection, both quinolines and cinnamic acids, which are found in different natural resources and widely used for diverse medicinal purposes,[3,4] have demonstrated to constitute scaffolds of great interest for the development of new antitumor agents.[5,6] In one hand, quinoline synthetic versatility promotes the development of large diversity of quinoline analogues. On the other, the 3-phenyl acrylic acid moiety offers three main reactive sites: substitutions at the phenyl ring, additions at the α,β-unsaturated carbonyl moiety, and the carboxylic acid functionality reactions.[5]

The specific mechanisms of antitumor action of either quinoline-based or cinnamic acid-based compounds remain unclear. However, quinoline derivatives and quinoline alone can inhibit tyrosine kinase, DNA repair, tubulin polymerization, and proteasome, and these are just few of the mechanisms of action which this type of compounds can exert.[6] For instance, chloroquine (CQ), **1** in Figure 1, which is known to inhibit proliferation of breast cancer cells,[7] can cause defects in DNA synthesis and repair, and also induce cell apoptosis and necrosis.[2,8] In the case of cinnamic acid, it has been reported that one of its modes of action seems to be inhibition of protein isoprenylation, which blocks mitogenic signal transduction.[4] Still, cinnamic acid analogues have also shown to be antiangiogenic, antileukemic as well as inhibitors of transglutaminase, aminopeptidase N, DNA synthesis, and of a specific tyrosine kinase.[5,9] The introduction of a cinnamic acid moiety has also demonstrated to boost the antitumor activity of parent antitumor compounds; for instance, distamycin A, **2** in Figure 1, (IC_{50} = 7.2 ng/mL) which includes a cinnamoyl functionality, showed antileukemic activity superior to that presented by tallimustine, **3** in Figure 1 (IC_{50} = 50.3 ng/mL).[10,11]

In view of the above, the use of cinnamic acid or quinoline scaffolds may be beneficial for the development of new antitumor agents. However, to our knowledge, up today there is no report in the literature that evaluates the antiproliferative properties of structures were both the quinoline and cinnamoyl moieties are linked together. Based on this, and following the drug recycling, or repurposing, concept,[12] we herein report the in vitro antiproliferative properties of chloroquine analogues **4** (Fig. 2), recently reported by us as potent dual-action antimalarials.[13,14] Other

* Corresponding author. Tel./fax: +351 220402563.
E-mail address: pgomes@fc.up.pt (P. Gomes).
† These authors have contributed equally to this work.

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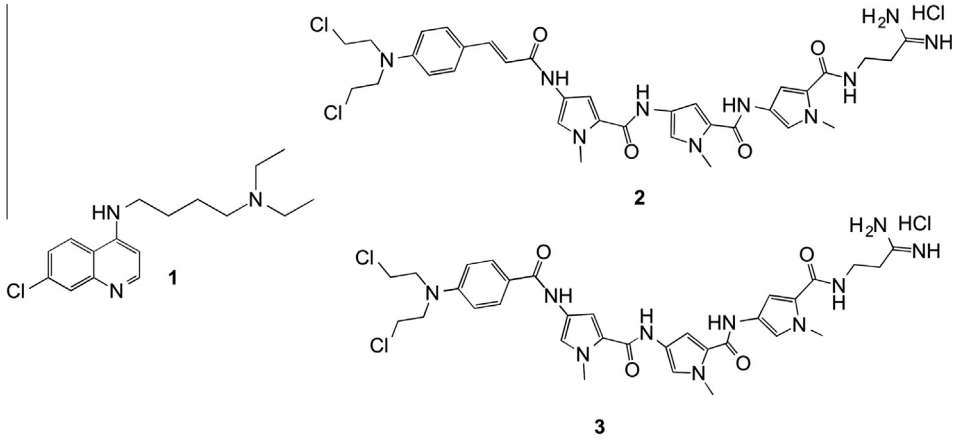


Figure 1. Reported examples of quinoline- and cinnamic acid-based compounds with antitumor properties: chloroquine (**1**), distamicyn A (**2**) and tallismustine (**3**).

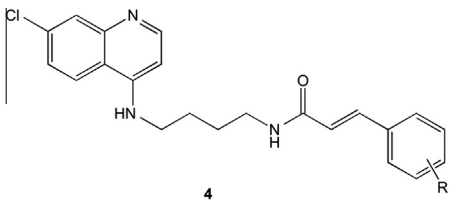


Figure 2. Chloroquine analogues previously reported as dual-action antimalarials.[14]

related structures (**5**–**10**), as well as the parent compound, CQ, were included in the study for a better understanding of structure–activity relationships (SAR).

Compounds **4**–**10** were prepared following a straightforward low-cost synthetic pathway, previously reported, and the analytical and structural data were in agreement with formerly published data.[13,14] Compounds' antiproliferative properties against MKN-28 (gastric cancer), Caco-2 (colorectal adenocarcinoma), MCF-7 (breast cancer) and HFF-1 (human foreskin fibroblasts) cell lines were determined according to the National Cancer Institute (NCI, USA) procedure, which uses the protein-binding dye sulforhodamine B.[15]

As shown by data on Table 1, all compounds resulted active in the micromolar range against the three cancer cell lines used. Furthermore, with the only exceptions of compounds **5**, **7** and **8**, the test compounds showed selectivity for MKN-28, Caco-2, and MCF-7 tumor cells over normal HFF-1 cells. Relevantly, data obtained clearly shows that the CQ's heterocyclic core, 4-amino-7-chloroquinoline, contributes to the enhancement of anti-tumor activity since (i) its replacement by the 4-aminopyridine one (**9** vs **4c**) led to a threefold or higher loss in anti-proliferative activity against the three cancer cell lines tested; (ii) its replacement by the morpholine core (**10** vs **4b**) led to an above threefold (MKN-28) activity loss. Also, all CQ analogues were more active than CQ itself against the three cancer cell lines tested, except for **5** on Caco-2 cells.

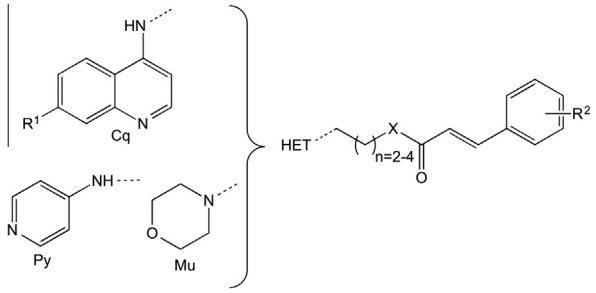
Additional SAR could be devised by close inspection of in vitro data of CQ analogues (**4a**–**g**, **5**–**8**):

- increasing the length of the alkyl chain leads to an increase of anti-proliferative activity against Caco-2 and MKN-28 cells, but has the opposite effect against MFC-7 cells, as shown by comparison of compounds **4b** vs **6**, and **5** vs **4e**; also, data from this former couple of compounds suggest that decreasing chain length is unfavorable for selectivity;
- results against the three tumor cell lines used also suggest that *ortho*- and *para*-substituted cinnamoyl groups are preferred over *meta*-substituted ones, by comparison of **4f** vs **4g** and **4d** vs **4e**;
- removal of the chlorine substituent at the quinoline's C-7 (**7** vs **4b**), though leading to an increase of anti-proliferative activity against all tumor cell lines, had the same effect also against normal HFF-1 cells, that is, was detrimental for compound selectivity;
- replacement of the amide bond in **4c** by an ester functionality, as in **8**, had a similar effect to that of chlorine removal, since **8** presented higher anti-proliferative activity than **4c** against the four cell lines tested, that is, activity was increased at the expense of selectivity loss;
- no obvious correlation could be devised between the stereoelectronic properties of the cinnamoyl substituents (R^2) and anti-proliferative activity.

Finally, we investigated whether the activity displayed by the CQ analogues could be due to degradation products or metabolites formed in the course of the experiments. To this end, the stability of selected test compounds (**4b**, **6**, **7**, **9** and **10**) in cell culture media was evaluated through analysis by liquid chromatography hyphenated with electrospray ionization/ion trap mass spectrometry (LC/ESI-IT MS). All the compounds were confirmed to be stable in those media, as only one MS peak corresponding to the *quasi*-molecular (MH^+) ion of each compound was observed in all cases (data not shown).

The mechanism(s) of action (MOA) through which these CQ analogues **4** exert their anti-proliferative activity is to be elucidated. Due to the planarity of their aromatic moieties, and resemblance to CQ, these compounds might be able to inhibit DNA replication.[16] On the other hand, though with a different global structure, these CQ analogues share the 4-aminoquinoline and α,β-unsaturated carbonyl moieties with HKI-272 (**11**, Fig. 3), a potent inhibitor (IC_{50} = 59 nM) of the human epidermal growth factor receptor-2 (HER-2). HER-2 is over-expressed in all three cancer cell lines used in the present study.[17–19] According to the literature, HKI-272 most likely acts by irreversibly binding to a cysteine in the ATP-binding pocket of HER-2,[20] a behavior that might also be displayed by our compounds, as their Michael acceptor moiety could engage in the S-alkylation of the critical Cys residue in HER-2. Still, this is a highly hypothetical example of what could be the MOA for our compounds, amongst the wide range of inhibition processes in which both the quinoline and the Michael acceptor moieties could participate, leading to impairment of tumor cell growth.[5,6,21] It could be also argued that, since replacement of the

Table 1
In vitro data of test compounds for antiproliferative activity against MKN-28, Caco-2, and MCF-7



Compound	HET	*n*	X	R^2	GI_{50}[a] (MKN-28)	GI_{50} (Caco-2)	GI_{50} (MCF-7)	GI_{50} (HFF-1)
4a	Cq (R^1 = Cl)	3	NH	H	16.21 ± 0.50	24.14 ± 0.93	14.85 ± 0.90	>100
4b		3	NH	*p*-iPr	22.31 ± 0.37	11.90 ± 0.96	8.09 ± 0.19	>100
4c		3	NH	*p*-OMe	16.90 ± 0.46	30.67 ± 0.63	25.23 ± 0.54	>100
4d		3	NH	*m*-F	15.27 ± 0.73	25.38 ± 0.19	14.85 ± 1.19	>100
4e		3	NH	*p*-F	11.76 ± 0.26	15.03 ± 0.35	12.52 ± 1.94	>100
4f		3	NH	*o*-NO_2	15.14 ± 0.16	11.94 ± 0.69	13.30 ± 0.39	>100
4g		3		*m*-NO_2	16.16 ± 0.15	19.32 ± 0.13	59.28 ± 0.56	>100
5		2	NH	*p*-F	34.68 ± 0.85	44.11 ± 0.43	5.24 ± 0.46	44.84 ± 1.95
6		4	NH	*p*-iPr	14.04 ± 0.80	9.16 ± 0.33	20.14 ± 0.68	>100
7	Cq (R^1 = H)	3	NH	*p*-iPr	5.55 ± 0.20	6.75 ± 0.26	5.24 ± 0.46	39.05 ± 0.60
8	Cq (R^1 = Cl)	3	O	*p*-OMe	6.60 ± 0.12	17.27 ± 0.64	5.08 ± 0.14	75.21 ± 6.18
9	Py	3	NH	*p*-OMe	84.52 ± 1.16	>100	77.06 ± 1.79	>100
10	Mu	3	NH	*p*-iPr	72.48 ± 2.89	29.61 ± 3.52	32.69 ± 2.58	>100
CQ					44.44 ± 4.67	38.67 ± 2.64	64.35 ± 2.97	>100
Doxorubicin					0.24 ± 0.03	0.32 ± 0.02	0.31 ± 0.05	>400

[a] GI_{50}: compound concentration (in μM) causing an inhibition by 50% of cell growth.

quinoline ring (in **4b**) by a morpholine ring (in **10**) does not dramatically decrease activity, the antiproliferative activity of these compounds could be more closely associated to the *N*-alkylcinnamoyl building block; that might bring to the scene MOA related to cinnamic acid derivatives, as inhibition of (i) phenyltransferases, (ii) protein isoprenylation inhibition, or (iii) DNA synthesis in growing cells, amongst many other putative MOA of cinnamoylated compounds. Therefore, any discussion regarding the possible MOA of these compounds is at this stage highly speculative and ongoing investigations, in order to identify a putative target for these compounds, will hopefully shed some light on this aspect.

In summary, chloroquine analogues **4** have been found to exhibit micromolar activity against three different cancer cell lines: MKN-28, Caco-2, and MCF-7. Their MOA is yet to be elucidated, the relevance of the quinoline core for the activity was clearly demonstrated, since substitution of the quinoline by a pyridine ring significantly decreased the activity of the compounds. Moreover, the cinnamoyl moieties seem to contribute to antiproliferative activity of the N-cinnamoylated chloroquinoline analogues as all compounds **4** displayed activity comparable or higher than chloroquine alone. Compounds **4** represent a promising start towards the development of novel affordable leads against malignant tumor cells, which is a key aspect given that low and middle-income countries account for about 70% of all cancer deaths.[22] Further studies are envisaged to inquire the possible mechanism(s), such as DNA intercalation, underlying antiproliferative properties displayed by these N-cinnamoylated chloroquine analogues, and either the reported compounds act prompting necrosis or apoptosis. Finally, these data reinforce the considerable therapeutic potential that may be offered by repurposing antimalarials such as chloroquine and its derivatives against cancer, a huge global health burden which is projected to increase in the years to come.

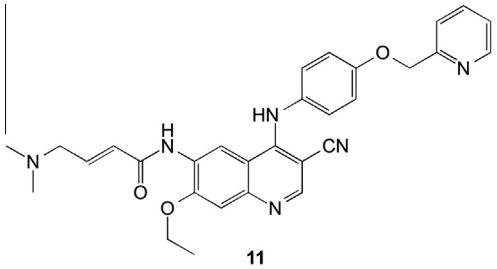


Figure 3. Structure of the HER-2 inhibitor HKI-272 (**11**).

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Supplementary data

Supplementary data associated with (details regarding synthetic procedures for compounds **4**–**10**, procedures for Sulforhodamine B assay and procedures for LC–MS analysis) this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.10.025.

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