

Antiproliferative evaluation of various aminoquinoline derivatives

Zorc, Branka; Rajić, Zrinka; Perković, Ivana

Source / Izvornik: **Acta Pharmaceutica, 2019, 69, 661 - 672**

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

<https://doi.org/10.2478/acph-2020-0048>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:163:918336>

Rights / Prava: [In copyright](#) / [Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2024-12-23**



Repository / Repozitorij:

[Repository of Faculty of Pharmacy and Biochemistry University of Zagreb](#)



Antiproliferative evaluation of various aminoquinoline derivatives

BRANKA ZORC*
ZRINKA RAJIĆ
IVANA PERKOVIĆ

University of Zagreb Faculty
of Pharmacy and Biochemistry
Department of Medicinal Chemistry
10 000 Zagreb, Croatia

Accepted June 24, 2019
Published online July 31, 2019

Four classes of aminoquinoline derivatives were prepared: primaquine ureas **1a–f**, primaquine bis-ureas **2a–f**, chloroquine fumardiamides **3a–f** and mefloquine fumardiamides **4a–f**. Their antiproliferative activities against breast adenocarcinoma (MCF-7), lung carcinoma (H460) and colon carcinoma (HCT 116 and SW620) cell lines were evaluated *in vitro*, using MTT cell proliferation assay. The results revealed a low activity of primaquine urea and bis-urea derivatives and high activity of all fumardiamides, with IC_{50} values in low micromolar range against all tested cancer cell lines.

Keywords: primaquine, chloroquine, mefloquine, fumar-diamide, antiproliferative activity

Finding novel therapeutic indications for already approved drugs (drug repurposing), is one of the possible strategies in the search of novel medicines (1, 2). Repurposing of antimalarial drugs as anticancer agents is very promising since different classes of antimalarials change the sensitivity of resistant tumour cell lines, inhibit the development of drug resistance, or show synergistic effects with clinically approved anticancer drugs (3–16). Anticancer effects of 14 registered antimalarial drugs have been reported and many of them (hydroxychloroquine, chloroquine, quinacrine, artemisinin, artemether, artesunate, quinine, atovaquone, doxycycline) were evaluated or are currently under evaluation in approximately a hundred and fifty clinical anticancer trials, mainly in the combination with conventional anticancer drugs (4, 17).

Primaquine (PQ), chloroquine (CQ) and mefloquine (MQ) are 8- or 4-aminoquinoline antimalarial drugs, recognized by the World Health Organization as essential medicines (18). Numerous modifications of their structures, both at the quinoline heterocycle and at the side chain, were performed in order to avoid drug resistance, to obtain the antimalarial agents with reduced toxicity and/or increased activity or to get biologically active compounds outside the antimalarial field (19–22).

During the last ten years, derivatization of the antimalarial drugs was the main focus of our research group as well. We have prepared a number of novel CQ-based (23, 24), MQ-

* Correspondence; e-mail: bzorc@pharma.hr

based derivatives (25) and approximately 150 novel PQ-derivatives, and evaluated their antiplasmodial, anticancer, antioxidative and/or antimicrobial activities (26–37). Among others, we have prepared PQ-urea (**1**) and bis-urea (**2**) derivatives (38) and hybrid molecules **3**, composed of CQ-pharmacophore, fumaric acid and halogenaniline fragments, and analogues compounds **4** bearing the MQ-scaffold (Fig. 1) (25). Their antiplasmodial and/or antimycobacterial activity was also reported in combination with synthesis. To view their full biological profile, we additionally evaluated their antiproliferative activity and report the results in this paper.

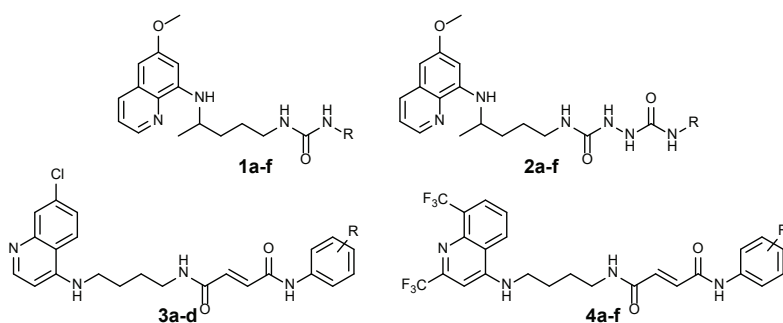


Fig. 1. Structure of primaquine-ureas **1a–f**, primaquine-bis-ureas **2a–f**, chloroquine fumardiamides **3a–f** and mefloquine fumardiamides **4a–f**.

EXPERIMENTAL

Chemistry

The following compounds were prepared: 3-[1-(hydroxymethyl)cyclopropyl]-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**1a**), 3-[1-(hydroxymethyl)cyclobutyl]-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**1b**), 3-[(1*S*,3*R*)-3-hydroxycyclopentyl]-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**1c**), 3-(4-fluoro-1-hydroxybutan-2-yl)-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**1d**), 1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]-3-(3,3,3-trifluoro-2-hydroxypropyl)urea (**1e**), 3-[2-(4-hydroxyphenyl)ethyl]-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**1f**), 3-([1-(hydroxymethyl)cyclopropyl]carbamoyl)amino)-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**2a**), 3-([1-(hydroxymethyl)cyclobutyl]carbamoyl)amino)-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**2b**), 3-([(1*S*,3*R*)-3-hydroxycyclopentyl]carbamoyl)amino)-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**2c**), 3-[(4-fluoro-1-hydroxybutan-2-yl)carbamoyl]amino)-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**2d**), 1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]-3-[(3,3,3-trifluoro-2-hydroxypropyl)carbamoyl]amino)urea (**2e**), 3-([(2-(4-hydroxyphenyl)ethyl)carbamoyl]amino)-1-[4-[(6-methoxyquinolin-8-yl)amino]pentyl]urea (**2f**), (2*E*)-*N'*-{4-[(7-chloroquinolin-4-yl)amino]butyl}-*N*-(3-fluorophenyl)but-2-enediamide (**3a**), (2*E*)-*N'*-{4-[(7-chloroquinolin-4-yl)amino]butyl}-*N*-(4-fluorophenyl)but-2-enediamide (**3b**), (2*E*)-*N*-(3-chlorophenyl)-*N'*-{4-[(7-chloroquinolin-4-yl)amino]butyl}but-2-enediamide (**3c**), (2*E*)-*N*-(4-chlorophenyl)-*N'*-{4-[(7-chloroquinolin-4-yl)amino]butyl}but-2-enediamide (**3d**),

(2*E*)-*N'*-[4-[(7-chloroquinolin-4-yl)amino]butyl]-*N*-[3-(trifluoromethyl)phenyl]but-2-enediamide (**3e**) and (2*E*)-*N'*-[4-[(7-chloroquinolin-4-yl)amino]butyl]-*N*-[4-(trifluoromethyl)phenyl]but-2-enediamide (**3f**), (2*E*)-*N'*-[4-[[2,8-bis(trifluoromethyl)quinolin-4-yl]amino]butyl]-*N*-(3-fluorophenyl)but-2-enediamide (**4a**), (2*E*)-*N'*-[4-[[2,8-bis(trifluoromethyl)quinolin-4-yl]amino]butyl]-*N*-(4-fluorophenyl)but-2-enediamide (**4b**), (2*E*)-*N'*-[4-[[2,8-bis(trifluoromethyl)quinolin-4-yl]amino]butyl]-*N*-(3-chlorophenyl)but-2-enediamide (**4c**), (2*E*)-*N'*-[4-[[2,8-bis(trifluoromethyl)quinolin-4-yl]amino]butyl]-*N*-(4-chlorophenyl)but-2-enediamide (**4d**), (2*E*)-*N'*-[4-[[2,8-bis(trifluoromethyl)quinolin-4-yl]amino]butyl]-*N*-[3-(trifluoromethyl)phenyl]but-2-enediamide (**4e**), (2*E*)-*N'*-[4-[[2,8-bis(trifluoromethyl)quinolin-4-yl]amino]butyl]-*N*-[3-(trifluoromethyl)phenyl]but-2-enediamide (**4f**). R_f values and IR, ^1H and ^{13}C NMR spectra of all compounds were in accord with the previously published data (25, 38).

Biology

Antiproliferative evaluation

Cell lines. – The antiproliferative evaluation was carried out on four human cancer cell lines: MCF-7 (breast adenocarcinoma), H460 (lung carcinoma), HCT 116 and SW620 (colon carcinoma), following the previously published procedure (29).

Cell culturing. – The cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM), supplemented with 10 % foetal bovine serum (FBS), 2 mmol L⁻¹ glutamine, 100 U mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin in a humidified atmosphere with 5 % CO₂ at 37 °C.

Proliferation assay. – The panel cell lines were inoculated in parallel onto a series of standard 96-well microtiter plates on day 0, at 1 × 10⁴ to 3 × 10⁴ cells per mL, depending on the doubling time of the specific cell line. Test compounds were then added in five 10-fold dilutions (10⁻⁸ to 10⁻⁴ M) and incubated for the next 72 hours. Working dilutions were freshly prepared on the day of the testing. After 72 hours of incubation, the cell growth rate was evaluated by performing the MTT cell proliferation assay, which detects dehydrogenase activity in viable cells. The MTT assay is a colorimetric assay, which measures the reduction of the tetrazolium component (MTT) into the insoluble formazan product by the mitochondria of viable cells. For this purpose, the substance treated medium was discarded and MTT was added to each well at a concentration of 0.5 µg µL⁻¹. After four hours of incubation, the precipitates were dissolved in DMSO (160 µL). The absorbance (*OD*, optical density) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the cell viability. The percentage of growth (*PG*) of the cell lines was calculated according to one or the other of the following two expressions:

If (mean OD_{test} – mean OD_{zero}) ≥ 0 then

$$PG = 100 \times (\text{mean } OD_{\text{test}} - \text{mean } OD_{\text{zero}}) / (\text{mean } OD_{\text{ctrl}} - \text{mean } OD_{\text{zero}});$$

If (mean OD_{test} – mean OD_{zero}) < 0 then

$$PG = 100 \times (\text{mean } OD_{\text{test}} - \text{mean } OD_{\text{zero}}) / OD_{\text{zero}};$$

where mean OD_{zero} is the average of optical density measurements before exposure of cells to the test compound, mean OD_{test} is the average of optical density measurements after the desired period of time and mean OD_{ctrl} is the average of optical density measurements

after the desired period of time with no exposure of cells to the test compound. Each test point was performed in quadruplicate in three individual experiments. The results were expressed as IC_{50} , a concentration necessary for 50 % of inhibition.

IC₅₀ calculations. – The concentration that causes 50 % growth inhibition (IC_{50}) for each compound was calculated from dose-response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the respective reference value (e.g. 50 for IC_{50}). Therefore, a real value for any of the response parameters was obtained only if at least one of the tested drug concentrations fell above, and likewise at least one fell below the respective reference value. If however, for a given cell line all of the tested concentrations produced PG s exceeding the respective reference level of the effect (e.g. PG value of 50), then the highest tested concentration was assigned as the default value, preceded by the sign >.

Interaction with glutathione (GSH)

CQ-fumardiamide **3c** (1.25 μ M) was incubated with GSH (125 μ M) in ammonium formate buffer (pH = 7.4) containing 10 % acetonitrile at 37 °C for four days (39). The progress of the reactions was monitored with the percent of remaining fumardiamide determined by mass spectroscopy using an internal standard (chloroquine). Aliquots of the reaction mixture (taken after 0, 4.5, 24, 48, 72 and 96 h) were analysed with Synapt G2-Si ESI-QTOF-MS system (Waters, Milford, USA). The aliquots were diluted 10 times with acetonitrile and sprayed at a flow rate of 50 μ L min^{-1} using the fluidics system of the instrument. MS conditions were set as follows: positive ion mode, capillary voltage 3 kV, sampling cone voltage 10 V, source temperature 120 °C, desolvation temperature 350 °C, desolvation gas flow 800 L h^{-1} . Mass spectra were recorded from 100–1000 m/z at a frequency of 1 Hz. Data were acquired and analysed with Waters MassLynx v4.1 software. The analogue experiment was performed with MQ-fumardiamide **4a**.

RESULTS AND DISCUSSION

Chemistry

Four classes of aminoquinoline derivatives were prepared: PQ-ureas **1a–f**, PQ-bis-ureas **2a–f**, CQ-fumardiamides **3a–f** and MQ-fumardiamides **4a–f**. Their general structures are given in Fig. 1 and chemical structures of each particular compound in Tables I–IV.

All tested compounds were prepared according to our previously published methods. The procedure leading to PQ-derivatives **1a–f** consisted of: a) synthesis of PQ-benzotriazolide from PQ base and 1-benzotriazole carboxylic acid chloride (26), b) reaction of PQ-benzotriazolide and the corresponding amine (1-aminocyclopropyl)methanol, (1-aminocyclobutyl)methanol, (1*R*,3*S*)-3-aminocyclopentanol, 2-amino-4-fluorobutan-1-ol, 3-amino-1,1,1-trifluoropropan-2-ol or 4-(2-aminoethyl)phenol (38). The starting PQ-benzotriazolide was prepared by the acylation of PQ with 1-benzotriazole carboxylic acid chloride (26).

Synthesis of PQ-derivatives **2a–f** was more complex. Synthesis of bis-ureas **2a–f** included the preparation of PQ-benzotriazolide, *N*-(4-((6-methoxyquinolin-8-yl)amino)-

Table I. PQ-ureas **1a-f**: growth inhibition of tumour cell lines in vitro

Cmpd.	Structure	IC_{50} ($\mu\text{mol L}^{-1}$) ^a			
		MCF-7	HCT 116	H460	SW620
1a		41 ± 13	≥ 100	≥ 100	≥ 100
1b		21 ± 5	35 ± 5	48 ± 8	52 ± 1
1c		42 ± 19	≥ 100	≥ 100	≥ 100
1d		21 ± 9	≥ 100	≥ 100	≥ 100
1e		39 ± 19	79 ± 4	≥ 100	≥ 100
1f		18 ± 1	25 ± 6	21 ± 2	32 ± 2
PQ ^b		9 ± 4	14 ± 5	20 ± 11	20 ± 6

^a IC_{50} – a concentration that causes 50 % growth inhibition; ^b PQ – primaquine

pentyl)hydrazinecarboxamide and its benzotriazolide (**29**). The final step was, again, aminolysis with the corresponding amino alcohols under microwave irradiation (**38**).

Synthesis of fumardiamides **3** and **4** proceeded *via* multi-step reactions, in which two amide bonds were formed (**25**). The amide bond between mono-ethyl fumarate and *N*¹-(7-chloroquinolin-4-yl)butane-1,4-diamine (CQ-pharmacophore) or *N*¹-(2,8-bis(trifluoromethyl)quinolin-4-yl)butane-1,4-diamine (MQ-pharmacophore) was achieved using standard coupling conditions (HATU/DIEA). The obtained amidoesters were further hydrolyzed to afford intermediates with free carboxylic groups, which then reacted with the selected halogenanilines in the presence of HATU/DIEA and formed products **3** and **4**, respectively.

Table II. PQ-bis-ureas **2a-f**: growth inhibition of tumour cell lines in vitro

Cmpd.	Structure	IC_{50} ($\mu\text{mol L}^{-1}$) ^a			
		MCF-7	HCT 116	H460	SW620
2a		40 ± 5	≥ 100	≥ 100	≥ 100
2b		40 ± 25	≥ 100	≥ 100	≥ 100
2c		56 ± 21	≥ 100	≥ 100	≥ 100
2d		≥ 100	≥ 100	≥ 100	≥ 100
2e		43 ± 24	≥ 100	≥ 100	≥ 100
2f		24 ± 13	44 ± 7	46 ± 5	45 ± 19
PQ ^b		9 ± 4	14 ± 5	20 ± 11	20 ± 6
Dox ^c		0.01 ± 0.001	0.01 ± 0.006	0.003 ± 0.002	–

^a IC_{50} – a concentration that causes 50 % growth inhibition; ^b PQ – primaquine; ^c Dox – doxorubicin

Antiproliferative activity

Antiproliferative evaluation was based on the MTT assay. Standard anticancer drug doxorubicin (Dox) was used as positive control. All PQ-ureas **1a–f** showed moderate activity against MCF-7 cells, but lower than the parent compound (Table I). Ureas derived from various amino alcohols **1a–e** were practically inactive against the other three cell

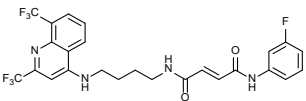
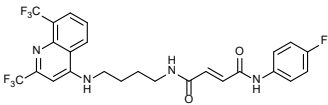
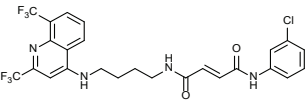
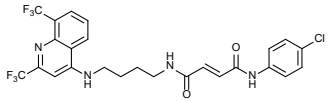
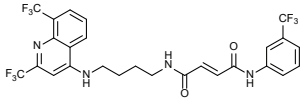
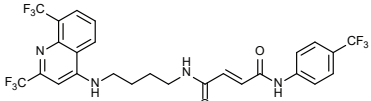
Table III. CQ-fumardiamides **3a-f**: antiproliferative evaluation against embryonic kidney Hek293 cells and selected cancer cell lines in vitro

Cmpd.	Structure	IC_{50} ($\mu\text{mol L}^{-1}$) ^a			
		Hek293	MCF-7	HCT 116	H460
3a		22.3 ± 6.6	1 ± 0.1	1 ± 0.3	2 ± 0.2
3b		30.9 ± 4.7	2 ± 0.2	2 ± 0.2	5 ± 2
3c		96 ± 41.0	1 ± 0.1	2 ± 0.1	2 ± 0.2
3d		7.0 ± 2.9	1 ± 0.1	2 ± 0.2	2 ± 0.2
3e		41.3 ± 18.4	14 ± 2	14 ± 2	16 ± 1
3f		10.9 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	1 ± 0.1
CQ ^b		–	3 ± 1	2 ± 1	2 ± 1
Dox ^c		–	0.01 ± 0.001	0.01 ± 0.006	0.003 ± 0.002

^a IC_{50} – a concentration that causes 50 % growth inhibition; ^b CQ – chloroquine; ^c Dox – doxorubicin

lines, whereas compound **1f** prepared from 4-(2-aminoethyl)phenol showed moderate activity. Very similar results were obtained for PQ-bis-ureas **2a–f** (Table II). Previously, we have prepared analogues PQ-urea and bis-urea compounds derived from various aromatic amines, which exerted much higher antiproliferative activity (26, 30, 36). Obviously, the replacement of the aromatic amines with amino alcohols was not beneficial for the activity. However, activity against MCF-7 cell line still remained. Such observation is not surprising

Table IV. IC_{50} values of MQ-derivatives **4a-f** against selected cancer cell lines in vitro

Cmpd.	Structure	IC_{50} ($\mu\text{mol L}^{-1}$) ^a		
		MCF-7	HCT 116	H460
4a		1 ± 0.2	2 ± 1	3 ± 2
4b		2 ± 0.2	5 ± 0.2	12 ± 2
4c		2 ± 0.3	2 ± 0.3	2 ± 0.1
4d		0.4 ± 0.1	2 ± 0.1	21 ± 14
4e		2 ± 0.3	2 ± 1	3 ± 1
4f		0.3 ± 0.2	1 ± 1	23 ± 9
MQ ^b		1.3 ± 0.2	1.0 ± 0.1	1.5 ± 0.19
Dox ^c		0.01 ± 0.001	0.01 ± 0.006	0.003 ± 0.002

^a IC_{50} – a concentration that causes 50 % growth inhibition; ^b MQ – mefloquine; ^c Dox – doxorubicin

since the sensitivity of MCF-7 cell line to primaquine and other antimalarial drugs has been observed by our research group and others (40–42). On the other hand, almost all CQ-fumardiamides **3a-f** exerted antiproliferative effects in single-digit micromolar concentrations against all tested cancer cell lines and moderate activity against human embryonic kidney Hek293 (selectivity index ranging from 2.9 to 96, depending on the cell line). CQ-fumardiamide derived from *p*-CF₃-aniline (compound **3f**) was the most active compound in the series, with $IC_{50} = 0.4 \pm 0.1 \mu\text{mol L}^{-1}$ against MCF-7 and $0.3 \pm 0.1 \mu\text{mol L}^{-1}$ against HCT 116 cells. The analogous MQ-derivatives showed practically the same antiproliferative effects. *p*-Chloro (**4d**) and *p*-CF₃-derivative (**4f**) inhibited proliferation of MCF-7 cells in low micromolar concentrations, with $IC_{50} = 0.4 \pm 0.1$ and $0.3 \pm 0.1 \mu\text{mol L}^{-1}$, respectively.

Interactions with glutathione (GSH)

The interaction of two fumarmides, **3c** and **4a**, with GSH in buffer solution (pH = 7.4) containing 10 % acetonitrile at 37 °C was followed for four days. The rate of fumardiamides-GSH consumption was slow and incomplete (4 and 5 %, respectively).

CONCLUSIONS

Antiproliferative screening *in vitro* revealed low to moderate activity of PQ-ureas (**1**) and bis-ureas (**2**). On the other hand, the antiproliferative activity of CQ- and MQ-fumardiamides (**3** and **4**) was high. Almost all fumardiamides exerted antiproliferative effects in single-digit micromolar concentrations against all tested cancer cell lines. They represent interesting lead compounds that might be useful in the design of new anticancer agents.

Acknowledgments. – The study was supported by the Croatian Science Foundation (research project IP-2014-09-1501). We thank Katja Ester, Lidija Uzelac and Marijeta Kralj for antiproliferative screening and Toma Keser for the experiments with glutathione.

Abbreviations, acronyms, symbols. – CQ, chloroquine; DIEA, *N,N*-diisopropylethylamine; DMEM, Dulbecco's modified Eagle's medium; GSH, glutathione; H460, lung carcinoma cell line; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium; 3-oxide hexafluorophosphate; HCT 116, colorectal carcinoma cell line; Hek293, human embryonic kidney cell line; FBS, foetal bovine serum; IC_{50} , concentration that causes 50 % growth inhibition; MQ, mefloquine; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OD, optical density; PG, percentage of growth; PQ, primaquine; MCF-7, breast adenocarcinoma cell line; SW620, colon carcinoma cell line.

REFERENCES

1. T. I. Oprea and J. Mestres, Drug repurposing: Far beyond new targets for old drugs, *AAPS J.* **14** (2012) 759–763.
2. T. T. Ashburn and K. B. Thor, Drug repositioning: identifying and developing new uses for existing drugs, *Nat. Rev. Drug Discov.* **3** (2004) 673–683; <https://doi.org/10.1038/nrd1468>
3. V. R. Solomon and H. Lee, Chloroquine and its analogs: A new promise of an old drug for effective and safe cancer therapies, *Eur. J. Pharmacol.* **625** (2009) 220–233; <https://doi.org/10.1016/j.ejphar.2009.06.063>
4. R. Duffy, C. Wade and R. Chang, Discovery of anticancer drugs from antimalarial natural products: a MEDLINE literature review, *Drug Discov. Today* **17** (2012) 942–953; <https://doi.org/10.1016/j.drudis.2012.03.013>
5. T. Kimura, Y. Takabatake, A. Takahashi and Y. Isaka, Chloroquine in cancer therapy: A double-edged sword of autophagy, *Cancer Res.* **73** (2013) 3–7; <https://doi.org/10.1158/0008-5472>
6. R. H. van Huijsduijnen, R. Kiplin Guy, K. Chibale, R. K. Haynes, I. Peitz, G. Kelter, M. A. Phillips, J. L. Vennerstrom, Y. Yuthavong and T. N. C. Wells, Anticancer properties of distinct antimalarial drug classes, *PLoS One* **8** (2013) e82962.
7. A. K. Abdel-Aziz, S. Shouman, E. El-Demerdash, M. Elgendy and A. B. Abdel-Naim, Chloroquine as a promising adjuvant chemotherapy together with sunitinib, *Sci. Proc.* **1** (2014) Article ID e384; <https://doi.org/10.14800/sp.384>
8. F. Liu, Y. Shang and S.-Z. Chen, Chloroquine potentiates the anti-cancer effect of lidamycin on non-small cell lung cancer cells *in vitro*, *Acta Pharmacol. Sin.* **35** (2014) 645–652; <https://doi.org/10.1038/aps.2014.3>

9. A. R. Choi, J. H. Kim, Y. H. Woo, H. S. Kim and S. Yoon, Anti-malarial drugs primaquine and chloroquine have different sensitization effects with anti-mitotic drugs in resistant cancer cells, *Anticancer Res.* **36** (2016) 1641–1648.
10. A. Ganguli, D. Choudhury, S. Datta, S. Bhattacharya and G. Chakrabarti, Inhibition of autophagy by chloroquine potentiates synergistically anti-cancer property of artemisinin by promoting ROS dependent apoptosis, *Biochimie* **107** (2014) 338–349; <https://doi.org/10.1016/j.biochi.2014.10.001>
11. C. Verbaanderd, H. Maes, M. B. Schaaf, V. P. Sukhatme, P. Pantziarka, V. Sukhatme, P. Agostinis and G. Bouche, Repurposing drugs in oncology (ReDO) – chloroquine and hydroxychloroquine as anti-cancer agents, *eCancer* **11** (2017) Article ID 781; <https://doi.org/10.3332/ecancer.2017.781>
12. F. Wang, J. Tang, P. Li, S. Si, H. Yu, X. Yang, J. Tao, Q. Lv, M. Gu, H. Yang and Z. Wang, Chloroquine enhances the radiosensitivity of bladder cancer cells by inhibiting autophagy and activating apoptosis, *Cell. Physiol. Biochem.* **45** (2018) 54–66; <https://doi.org/10.1159/000486222>
13. L. Liu, C. Han, H. Yu, W. Zhu, H. Cui, L. Zheng, C. Zhang and L. Yue, Chloroquine inhibits cell growth in human A549 lung cancer cells by blocking autophagy and inducing mitochondrial-mediated apoptosis, *Oncol. Rep.* **39** (2018) 2807–2816.
14. A. Kamal A. Aziz, S. Shouman, E. El-Demerdash, M. Elgendy and A. B. Abdel-Naim, Chloroquine synergizes sunitinib cytotoxicity via modulating autophagic, apoptotic and angiogenic machineries, *Chem. Biol. Interact.* **217** (2014) 28–40; <https://doi.org/10.1016/j.cbi.2014.04.007>
15. G. W. Soo, J. H. Law, E. Kan, S. Y. Tan, W. Y. Lim, G. Chay, N. I. Bukhari and I. Segarra, Differential effects of ketoconazole and primaquine on the pharmacokinetics and tissue distribution of imatinib in mice, *Anticancer Drugs* **21** (2010) 695–703.
16. Y. K. Wong, C. Xu, K. A. Kalesh, Y. He, Q. Lin, W. S. F. Wong, H. M. Shen and J. Wang, Artemisinin as an anticancer drug: Recent advances in target profiling and mechanisms of action, *Med. Res. Rev.* **37** (2017) 1492–1517.
17. <https://clinicaltrials.gov/ct2/home> (last access May 26, 2019)
18. https://en.wikipedia.org/wiki/WHO_Model_List_of_Essential_Medicines (last access May 27, 2019)
19. N. Vale, R. Moreira and P. Gomes, Primaquine revisited six decades after its discovery, *Eur. J. Med. Chem.* **44** (2009) 937–953; <https://doi.org/10.1016/j.ejmech.2008.08.011>
20. P. M. Njaria, J. Okombo, N. M. Njuguna and K. Chibale, Chloroquine-containing compounds: a patent review (2010 – 2014), *Expert Opin. Therap. Patents* **25** (2015) 1003–1024; <https://doi.org/10.1517/13543776.2015.1050791>
21. S-J. Yeo, D-X. Liu, H-S. Kim and H. Park, Anti-malarial effect of novel chloroquine derivatives as agents for the treatment of malaria, *Malaria J.* **16** (2017) 80; <https://doi.org/10.1186/s12936-017-1725-z>
22. M. A. Avery, D. J. Weldon and K. M. Muraleedharan, *Advances in the Discovery of New Antimalarials*, in *Comprehensive Medicinal Chemistry II* (Eds. J. B. Taylor and D. J. Triggle), Vol. 7, Elsevier Ltd., 2007, pp. 765–814; <https://doi.org/10.1016/B0-08-045044-X/00227-3>
23. K. Pavić, Z. Rajić, Z. Mlinarić, L. Uzelac, M. Kralj and B. Zorc, Chloroquine urea derivatives: synthesis and antitumor activity *in vitro*, *Acta Pharm.* **68** (2018) 471–483.
24. M. Beus, L. Persoons, D. Schols, L. Uzelac, M. Kralj, Z. Rajić and B. Zorc, Cytotoxicity studies of primaquine and chloroquine fumardiamides, 6th Croatian Congress on Pharmacy with International Participation, Book of Abstract, PO-16, Dubrovnik, April 4–6, 2019
25. M. Beus, D. Fontinha, J. Held, Z. Rajić, M. Prudêncio and B. Zorc, Synthesis and antiplasmodial evaluation of novel mefloquine-based fumardiamides, *Acta Pharm.* **69** (2019) 233–248; <https://doi.org/10.2478/acph-2019-0019>
26. G. Džimbeg, B. Zorc, M. Kralj, K. Ester, K. Pavelić, J. Balzarini, E. De Clercq and M. Mintas, The novel primaquine derivatives of *N*-alkyl, cycloalkyl or aryl urea: synthesis, cytostatic and antiviral activity evaluations, *Eur. J. Med. Chem.* **43** (2008) 1180–1187; <https://doi.org/10.1016/j.ejmech.2007.09.001>

27. M. Šimunović, I. Perković, B. Zorc, K. Ester, M. Kralj, D. Hadjipavlou-Litina and E. Pontiki, Urea and carbamate derivatives of primaquine: synthesis, cytostatic and antioxidant activities, *Bioorg. Med. Chem.* **17** (2009) 5605–5613; <https://doi.org/10.1016/j.bmc.2009.06.030>
28. I. Perković, S. Tršinar, J. Žanetić, M. Kralj, I. Martin-Kleiner, J. Balzarini, D. Hadjipavlou-Litina and A. M. Katsori, Novel 1-acyl-4-substituted semicarbazide derivatives of primaquine – synthesis, cytostatic, antiviral and antioxidative studies, *J. Enzyme Inhib. Med. Chem.* **28** (2013) 601–610; <https://doi.org/10.3109/14756366.2012.663366>
29. K. Pavić, I. Perković, M. Cindrić, M. Pranjčić, I. Martin-Kleiner, M. Kralj, D. Schols, D. Hadjipavlou-Litina, A.-M. Katsori and B. Zorc, Novel semicarbazides and ureas of primaquine with bulky aryl or hydroxyalkyl substituents: Synthesis, cytostatic and antioxidative activity, *Eur. J. Med. Chem.* **86** (2014) 502–514; <https://doi.org/10.1016/j.ejmech.2014.09.013>
30. I. Perković, M. Antunović, I. Marijanović, K. Pavić, K. Ester, M. Kralj, J. Vlainić, I. Kosalec, D. Schols, D. Hadjipavlou-Litina, E. Pontiki and B. Zorc, Novel urea and bis-urea primaquine derivatives with hydroxyphenyl and halogenphenyl substituents: synthesis and biological evaluation, *Eur. J. Med. Chem.* **124** (2016) 622–636; <https://doi.org/10.1016/j.ejmech.2016.08.021>
31. K. Pavić, I. Perković, P. Gilja, F. Kozlina, K. Ester, M. Kralj, D. Schols, D. Hadjipavlou-Litina, E. Pontiki and B. Zorc, Design, synthesis and biological evaluation of novel primaquine-cinnamic acid conjugates of amide and acylsemicarbazide type, *Molecules* **21** (2016) 1629–1653; <https://doi.org/10.3390/molecules21121629>
32. K. Pavić, I. Perković, Š. Pospíšilová, M. Machado, D. Fontinha, M. Prudêncio, J. Jampilek, A. Coffey, L. Endersen, H. Rimac and B. Zorc, Primaquine hybrids as promising antimycobacterial and antimalarial agents, *Eur. J. Med. Chem.* **143** (2018) 769–779; <https://doi.org/10.1016/j.ejmech.2017.11.083>
33. J. Vlainić, I. Kosalec, K. Pavić, D. Hadjipavlou-Litina, E. Pontiki and B. Zorc, Insights into biological activity of ureidoamides with primaquine and amino acid moieties, *J. Enzyme Inhib. Med. Chem.* **33** (2018) 376–382; <https://doi.org/10.1080/14756366.2017.1423067>
34. J. Levatić, K. Pavić, I. Perković, L. Uzelac, K. Ester, M. Kralj, M. Kaiser, M. Rottmann, F. Supek and B. Zorc, Machine learning prioritizes synthesis of primaquine ureidoamides with high antimalarial activity and attenuated cytotoxicity, *Eur. J. Med. Chem.* **146** (2018) 651–667; <https://doi.org/10.1016/j.ejmech.2018.01.062>
35. M. Beus, Z. Rajić, D. Maysinger, Z. Mlinarić, M. Antunović, I. Marijanović, D. Fontinha, M. Prudêncio, J. Held, S. Olgen and B. Zorc, SAHAquines, novel hybrids based on SAHA and primaquine motifs, as potential anticancer and antiplasmodial agents, *ChemistryOpen* **7** (2018) 624–638; <https://doi.org/10.1002/open.201800117>
36. Z. Rajić, M. Beus, H. Michnova, J. Vlainić, L. Persoons, I. Kosalec, J. Jampilek, D. Schols, T. Keser and B. Zorc, Asymmetric primaquine and halogenaniline fumardiamides as novel biologically active Michael acceptors, *Molecules* **23** (2018) 1724; <https://doi.org/10.3390/molecules23071724>
37. I. Zhang, M. Beus, U. Stochaj, P. U. Le, B. Zorc, Z. Rajić, K. Petrecca and D. Maysinger, Inhibition of glioblastoma cell proliferation, invasion, and mechanism of action of a novel hydroxamic acid hybrid molecule, *Cell Death Discov.* **5** (2019) 41; <https://doi.org/10.1038/s41420-018-0103-0>
38. K. Pavić, Z. Rajić, H. Michnová, J. Jampilek, I. Perković and B. Zorc, Second generation of primaquine ureas and bis-ureas as potential antimycobacterial agents, *Mol. Diver.* (2018); <https://doi.org/10.1007/s11030-018-9899-z>
39. M. E. Flanagan, J. A. Abramite, D. P. Anderson, A. Aulabaugh, U. P. Dahal, A. M. Gilbert, C. Li, J. Montgomery, S. R. Oppenheimer, T. Ryder, B. P. Schuff, D. P. Uccello, G. S. Walker, Y. Wu, M. F. Brown, J. M. Chen, M. M. Hayward, M. C. Noe, R. S. Obach, L. Philippe, V. Shanmugasundaram, M. J. Shapiro, J. Starr, J. Stroh and Y. Che, Chemical and computational methods for the characterization of covalent reactive groups for the prospective design of irreversible inhibitors, *J. Med. Chem.* **57** (2014) 10072–10079; <https://doi.org/10.1021/jm501412a>

40. I. Fernandes, N. Vale, V. de Freitas, R. Moreira, N. Mateus and P. Gomes, Anti-tumoral activity of imidazoquines, a new class of antimalarials derived from primaquine, *Bioorg. Med. Chem. Lett.* **19** (2009) 6914–6917; <https://doi.org/10.1016/j.bmcl.2009.10.081>
41. T. Rossi, A. Coppi, E. Bruni, A. Ruberto, S. Santachiara and G. A. Baggio, Effects of anti-malarial drugs on MCF-7 and Vero cell replication, *Anticancer Res.* **27** (2007) 2555–2559.
42. A. R. Martirosyan, R. Rahim-Bata, A. B. Freeman, C. D. Clarke, R. L. Howard and J. S. Strobl, Differentiation-inducing quinolines as experimental breast cancer agents in the MCF-7 human breast cancer cell model, *Biochem. Pharmacol.* **68** (2004) 1729–1738.