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

Journal article, Published version<br>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
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Download date / Datum preuzimanja: 2024-04-16


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DIGITALNI AKADEMSKI ARHIVI I REPOZITORIII

# Antiproliferative evaluation of various aminoquinoline derivatives 

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Accepted June 24, 2019
Published online July 31, 2019

Four classes of aminoquinoline derivatives were prepared: primaquine ureas 1a-f, primaquine bis-ureas $\mathbf{2 a - f}$, chloroquine fumardiamides $\mathbf{3 a}-\mathbf{f}$ 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 $I C_{50}$ values in low micromolar range against all tested cancer cell lines.

Keywords: primaquine, chloroquine, mefloquine, fumardiamide, 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-

[^0]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 $\mathbf{2 a - f}$, chloroquine fumardiamides $\mathbf{3 a - f}$ and mefloquine fumardiamides $\mathbf{4 a - f}$.

## 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-[(1S,3R)-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-(hydroxy-methyl)cyclobutyl]carbamoyl\}amino)-1-\{4-[(6-methoxyquinolin-8-yl)aminolpentyl\}urea (2b), 3-(\{[(1S,3R)-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-hydroxy-phenyl)ethyl]carbamoyl\}amino)-1-\{4-[(6-methoxyquinolin-8-yl)amino]pentyl\}urea (2f),
 (2E)- $N^{\prime}-\{4-[(7-$ chloroquinolin-4-yl)amino]butyl\}-N-(4-fluorophenyl)but-2-enediamide (3b), (2E)-N-(3-chlorophenyl)- $N^{\prime}-\{4-[(7-$ chloroquinolin-4-yl)amino]butyl\}but-2-enediamide (3c), (2E)-N-(4-chlorophenyl)- $N^{\prime}$-\{4-[(7-chloroquinolin-4-yl)amino]butyl\}but-2-enediamide (3d),
(2E)- $N^{\prime}-\{4-[(7-$ chloroquinolin-4-yl)amino]butyl\}-N-[3-(trifluoromethyl)phenyl]but-2enediamide (3e) and (2E)- $N^{\prime}-\{4-[(7-c h l o r o q u i n o l i n-4-y l) a m i n o] b u t y l\}-N-[4-(t r i f l u o r o m e t h y l) ~$ phenyl]but-2-enediamide (3f), (2E)- $N^{\prime}$-(4-\{[2,8-bis(trifluoromethyl)quinolin-4-yl]amino\} butyl)- $N$-(3-fluorophenyl)but-2-enediamide (4a), (2E)- $N^{\prime}$-(4-\{[2,8-bis(trifluoromethyl) quinolin-4-yl]amino\}butyl)- $N$-(4-fluorophenyl)but-2-enediamide (4b), (2E)-N'-(4-\{[2,8-bis(trifluoromethyl)quinolin-4-yl]aminołbutyl)- N -(3-chlorophenyl)but-2-enediamide (4c), (2E)- $N^{\prime}$-(4-\{[2,8-bis(trifluoromethyl)quinolin-4-yl]amino\}butyl)- $N$-(4-chlorophenyl)but-2enediamide ( 4 d ), ( $2 E$ )- $N^{\prime}$-(4-\{[2,8-bis(trifluoromethyl)quinolin-4-yl]amino\}butyl)- $N$-[3-(trifluoromethyl)phenyl]but-2-enediamide (4e), (2E)- $N^{\prime}$-(4-\{[2,8-bis(trifluoromethyl) quinolin-4-yl]aminołbutyl)- $N$-[4-(trifluoromethyl)phenyl]but-2-enediamide (4f). $R_{\mathrm{f}}$ values and IR, ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ 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 $\mathrm{mmol} \mathrm{L}^{-1}$ glutamine, $100 \mathrm{U} \mathrm{mL}^{-1}$ penicillin and $100 \mu \mathrm{~g} \mathrm{~mL}{ }^{-1}$ streptomycin in a humidified atmosphere with $5 \% \mathrm{CO}_{2}$ at $37^{\circ} \mathrm{C}$.

Proliferation assay. - The panel cell lines were inoculated in parallel onto a series of standard 96-well microtiter plates on day 0 , at $1 \times 10^{4}$ to $3 \times 10^{4}$ 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 $10^{-4} \mathrm{M}$ ) 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 $0.5 \mu \mathrm{~g} \mu \mathrm{~L}^{-1}$. After four hours of incubation, the precipitates were dissolved in DMSO $(160 \mu \mathrm{~L})$. The absorbance ( $O D$, optical density) was measured on a microplate reader at 570 nm . The absorbance is directly proportional to the cell viability. The percentage of growth $(P G)$ of the cell lines was calculated according to one or the other of the following two expressions:

$$
\begin{aligned}
& \text { If }\left(\text { mean } O D_{\text {test }}-\text { mean } O D_{\text {tzero }}\right) \geq 0 \text { then } \\
& P G=100 \times\left(\text { mean } O D_{\text {test }}-\text { mean } O D_{\text {tzero }}\right) /\left(\text { mean } O D_{\text {ctrl }}-\text { mean } O D_{\text {tzero }}\right) ; \\
& \text { If }\left(\text { mean } O D_{\text {test }}-\text { mean } O D_{\text {tero }}\right)<0 \text { then } \\
& P G=100 \times\left(\text { mean } O D_{\text {test }}-\text { mean } O D_{\text {tzero }}\right) / O D_{\text {tzero }} ;
\end{aligned}
$$

where mean $O D_{\text {tzero }}$ is the average of optical density measurements before exposure of cells to the test compound, mean $O D_{\text {test }}$ is the average of optical density measurements after the desired period of time and mean $O D_{\text {ctrl }}$ 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 $I C_{50}$, a concentration necessary for $50 \%$ of inhibition.
$\mathrm{IC}_{50}$ calculations. - The concentration that causes $50 \%$ growth inhibition $\left(\mathrm{IC}_{50}\right)$ for each compound was calculated from dose-response curves using linear regression analysis by fitting the test concentrations that give $P G$ values above and below the respective reference value (e.g. 50 for $I C_{50}$ ). 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 PGs exceeding the respective reference level of the effect (e.g. $P G$ value of 50 ), then the highest tested concentration was assigned as the default value, preceded by the sign >.

## Interaction with glutathione (GSH)

CQ-fumardiamide $3 \mathrm{c}(1.25 \mu \mathrm{M})$ was incubated with GSH ( $125 \mu \mathrm{M}$ ) in ammonium formate buffer ( $\mathrm{pH}=7.4$ ) containing $10 \%$ acetonitrile at $37^{\circ} \mathrm{C}$ 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 $50 \mu \mathrm{~L} \mathrm{~min}{ }^{-1}$ 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 $120^{\circ} \mathrm{C}$, desolvation temperature $350^{\circ} \mathrm{C}$, desolvation gas flow $800 \mathrm{~L} \mathrm{~h}^{-1}$. Mass spectra were recorded from 100-1000 $\mathrm{m} / \mathrm{z}$ 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 $\mathbf{2 a - f}$, CQ-fumardiamides $\mathbf{3 a} \mathbf{- f}$ and MQ-fumardiamides $\mathbf{4 a} \mathbf{- f}$. 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, (1R,3S)-3-aminocyclopentanol, 2-amino-4-fluorobutan-1-ol, 3-amino-1,1,1-tri-fluoropropan-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.
${ }^{\mathrm{a}} I C_{50}$ - a concentration that causes $50 \%$ growth inhibition; ${ }^{\mathrm{b}} \mathrm{PQ}$ - 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 $\mathbf{3}$ and $\mathbf{4}$ 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(trifluoro-methyl)quinolin-4-yl)butane-1,4-diamine (MQ-pharmacophore) was achieved using standard coupling conditions (HATU/DIEA). The obtained amidoesters were further hydrolized 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.
${ }^{\mathrm{a}} I C_{50}$ - a concentration that causes $50 \%$ growth inhibition; ${ }^{\mathrm{b}} \mathrm{PQ}$ - primaquine; ${ }^{\mathrm{c}}$ 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
(
${ }^{\mathrm{a}} I C_{50}$ - a concentration that causes $50 \%$ growth inhibition; ${ }^{\mathrm{b}} \mathrm{CQ}$ - chloroquine; ${ }^{\mathrm{c}}$ 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. $\mathrm{IC}_{50}$ values of $M Q$-derivatives $4 a$-f against selected cancer cell lines in vitro
Cmpd.
${ }^{\mathrm{a}} I C_{50}$ - a concentration that causes $50 \%$ growth inhibition; ${ }^{\mathrm{b}} \mathrm{MQ}$ - mefloquine; ${ }^{\mathrm{c}}$ 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 $p-\mathrm{CF}_{3}$-aniline (compound 3 f ) was the most active compound in the series, with $I C_{50}=0.4 \pm 0.1 \mu \mathrm{~mol} \mathrm{~L}{ }^{-1}$ against MCF-7 and $0.3 \pm 0.1 \mu \mathrm{~mol} \mathrm{~L}^{-1}$ against HCT 116 cells. The analogous MQ-derivatives showed practically the same antiproliferative effects. $p$-Chloro (4d) and $p-\mathrm{CF}_{3}$-derivative (4f) inhibited proliferation of MCF-7 cells in low micromolar concentrations, with $I C_{50}=0.4 \pm 0.1$ and $0.3 \pm 0.1 \mu \mathrm{~mol} \mathrm{~L}^{-1}$, respectively.

## Interactions with glutathione (GSH)

The interaction of two fumarmides, $\mathbf{3 c}$ and $\mathbf{4 a}$, with GSH in buffer solution ( $\mathrm{pH}=7.4$ ) containing $10 \%$ acetonitrile at $37^{\circ} \mathrm{C}$ was followed for four days. The rate of fumardiamidesGSH 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 MQfumardiamides ( 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]-1H-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; $I C_{50}$ concentration that causes $50 \%$ growth inhibition; MQ, mefloquine; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; $O D$, optical density; $P G$, percentage of growth; PQ , primaquine; MCF-7, breast adenocarcinoma cell line; SW620, colon carcinoma cell line.

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