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Original research paper

Synthesis, antiproliferative and antiplasmodial evaluation of new chloroquine and mefloquine-based harmiquins

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ABSTRACT

Here we present the synthesis and evaluation of the biological activity of new hybrid compounds, ureido-type (UT) harmiquins, based on chloroquine (CQ) or mefloquine (MQ) scaffolds and β-carboline alkaloid harmine against cancer cell lines and *Plasmodium falciparum*. The hybrids were prepared from the corresponding amines by 1,1′-carbonyldiimidazole (CDI)-mediated synthesis. *In vitro* evaluation of the biological activity of the title compounds revealed two hit compounds. Testing of the antiproliferative activity of the new UT harmiquins, and previously prepared triazole- (TT) and amide-type (AT) CQ-based harmiquins, against a panel of human cell lines, revealed TT harmiquine **16** as the most promising compound, as it showed pronounced and selective activity against the tumor cell line HepG2 (IC_{50} = 5.48 ± 3.35 μmol L^{-1}). Screening of the antiplasmodial activities of UT harmiquins against erythrocytic stages of the *Plasmodium* life cycle identified CQ-based UT harmiquine **12** as a novel antiplasmodial hit because it displayed low IC_{50} values in the submicromolar range against CQ-sensitive and resistant strains (IC_{50} 0.06 ± 0.01, and 0.19 ± 0.02 μmol L^{-1}, respectively), and exhibited high selectivity against *Plasmodium*, compared to mammalian cells (SI = 92).

Keywords: chloroquine, mefloquine, β-carboline, harmine, antiplasmodial activity, antiproliferative activity

Cancer and malaria are pathophysiologically and etiologically completely different diseases. However, according to the World Health Organization, both diseases are global health threats, have high incidence rates, and are leading causes of mortality worldwide, especially in low and middle-income countries with reduced access to quality healthcare. In 2021, there were 247 million malaria cases and 619,000 deaths, representing an increase in mortality of approximately 10 % compared with 2019, attributed to the disruption of services due to COVID-19 (1). Despite the significant progress that has been made in the field of cancer therapies, cancer continues to have a major impact around the world and

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was the cause of nearly 10 million deaths worldwide in 2020 (2). The problem of drug resistance remains the most important limiting factor in curing cancer patients and has similarities to drug resistance in the field of malaria control, as therapies for both diseases are challenged by highly proliferating aggressors that can adapt and overcome the effects of drugs (3). As a consequence, the treatments of both diseases are based on combination therapies to avoid early drug failure.

Interestingly, several anticancer drugs show antiplasmodial activities and are used as valuable lead compounds for the discovery of new antimalarial agents (4). Conversely, some antimalarial drugs have been shown to have anticancer effects and have been implicated in the therapy of various cancers (5). For example, the known antimalarial drugs chloroquine (CQ) and mefloquine (MQ), 4-substituted quinolines, exhibit anticancer properties through inhibition of autophagy, affection of the Toll-like receptor 9/nuclear factor kappa B, CXCL12/CXCR4, and the p53 signaling pathways, tumor vasculature and immune system, augmentation of TRAIL-induced apoptosis and induction of G2/M phase arrest in human cancer cells (6, 7).

Harmine, a naturally occurring alkaloid of the β-carboline type, is one of the components of the extract of *Peganum harmala*, which is used in traditional Chinese medicine for the treatment of cancer and malaria (8). Numerous studies show that harmine significantly influences the cell cycle of various cancer cell lines by induction of G2/M cell-cycle arrest, intercalation into DNA and induction of DNA fragmentation, upregulation of p53 and p21 expression, and downregulation of cyclin B1, p-cdc2, cdc2, cdc25, and p-cdc25 expression necessary for G2/M transition (9, 10). On the other hand, harmine was identified as a selective inhibitor of *P. falciparum* heat shock protein 90 (*Pf*Hsp90), and its antimalarial activity was corroborated *in vitro* and *in vivo* (11).

Such findings resulted in extensive research on the design and synthesis of β-carboline-based antitumour and antimalarial agents. Most of the modifications could be divided into several groups: 1) substitutions at positions 1, 2, 3, 6, and 9 of the β-carboline core, resulting in mono, di- and trisubstituted β-carbolines, 2) dimerization of β-carbolines employing various spacers resulting in bivalent β-carbolines, 3) hybridization of β-carbolines with other biologically active scaffolds and 4) preparation of β-carboline-based metal complexes (ruthenium(II), rhenium(I), copper(II), nickel(II), iridium (II)) (12–18). Different biological targets and mechanisms of anticancer and antimalarial action were explored, for example, DNA intercalation or groove binding, enzyme inhibition (topoisomerase, kinases, such as DYRK1A, haspin kinase and cyclin-dependent kinases, histone deacetylase, *Pf* phosphodiesterase, *Pf*ATP4), *Pf*Hsp90 and others (14, 15, 17, 18).

As already stated above, standard-of-care for both cancer and malaria includes drug combinations, although drug-drug interactions and lower treatment adherence could pose a problem for successful therapy. One of the approaches that may overcome pitfalls of drug combinations is the molecular hybridization concept, a rational strategy in the drug design where two or more biologically active molecules or their pharmacophores are combined to form a new hybrid compound (19). We have applied the same concept for the design and synthesis of a library of CQ-based triazole- (TT) and amide-type (AT) harmiquins, which showed excellent antiplasmodial properties (20).

To broaden our knowledge about the influence of the 4-aminoquinoline and the linker on the harmiquins biological activity, we synthesized novel hybrid compounds, comprising β-carboline and CQ, or MQ-based 4-aminoquinoline motifs linked by a urea, *i.e.* ureido-type

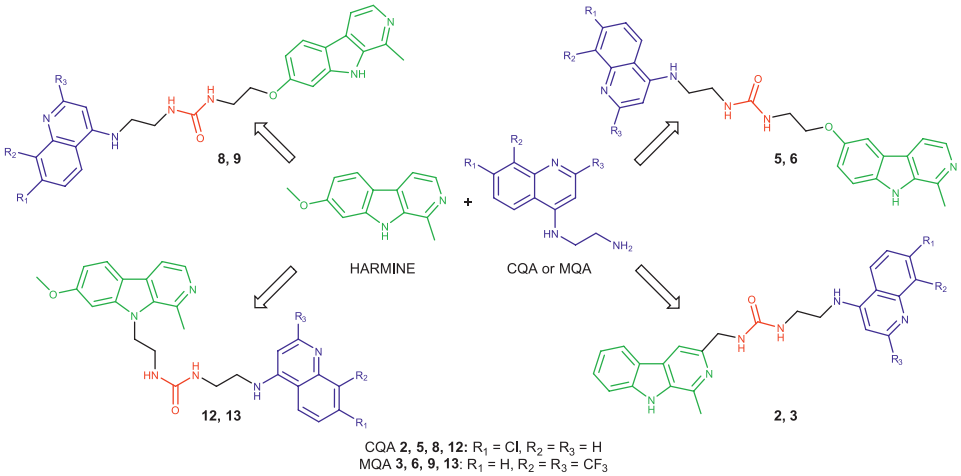


Fig. 1. Structures of novel UT harmiquins.

(UT) harmiquins (Fig. 1). As both constituents of hybrid drugs exert anticancer and antiplasmodial properties, we also conducted a screening of their biological activities against a panel of human cell lines and erythrocytic stages of the *P. falciparum* life cycle.

EXPERIMENTAL

General

Melting points were determined on a Stuart Melting Point (SMP3) apparatus (Barloworld Scientific, UK) in open capillaries with uncorrected values. Microwave-assisted reactions were performed in a microwave reactor CEM Discover (CEM, USA) in a glass reaction vessel. FTIR-ATR spectra were recorded using a Fourier-Transform Infrared Attenuated Total Reflection Spectrum Two spectrophotometer (PerkinElmer, USA) in the range from 450 to 4000 cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD operating at 400 or 600 MHz for the ^1H and 100, 101, or 151 MHz for the ^{13}C nuclei (Bruker, USA). Samples were measured in DMSO-d_6 solutions at 20 °C in 5 mm NMR tubes. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as a reference in the ^1H and DMSO residual peak as a reference in the ^{13}C spectra (39.52 ppm). Coupling constants (*J*) are reported in hertz (Hz). Mass spectra were recorded on Agilent 1200 Series HPLC coupled with Agilent 6410 Triple Quad (Agilent Technologies, USA). Mass determination was performed using electron spray ionization (ESI) in positive mode. The purities of all final compounds were determined on an Agilent Ultivo LC/TQ system, and all were > 95 %. A Phenomenex Gemini C18 column (4.6 × 150 mm, 5 μm) was used as a stationary phase. The column temperature was 25 °C, with a sample injection volume of 10 μL and a flow rate of 0.8 mL/min. The mobile phase consisted of ultrapure water with 0.1 % formic acid (LC/MS grade, Sigma-Aldrich) (eluent A) and MeOH (LC/MS grade, Carlo Erba) with 0.1 % formic acid (eluent B). The gradient was 0–10 min 10–100 % B; 10–15 min 100 % B; 15–16 min 100–10 % B. The absorption was measured using a diode array detector at 230, 254, 300, 330, and 366 nm. All compounds were routinely checked by TLC with

Merck silica gel 60F-254 glass plates (Merck, Germany) using cyclohexane/ethyl acetate/methanol 3:1:0.5 or 1:1:0.5 and dichloromethane/methanol 7.5:2.5, 8:1 or 1:1 as solvent systems. Spots were visualized by short- (λ = 254 nm) and long-wave UV light (λ = 366 nm), and iodine vapor. Column chromatography was performed on silica gel 0.063–0.200 mm (Sigma-Aldrich, USA) with the same eluents used for TLC.

All chemicals and solvents were of analytical grade and purchased from commercial sources. Anhydrous solvents were dried and redistilled prior to use. N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine (CQA), N^1-(2,8-*bis*(trifluoromethyl)quinolin-4-yl)ethane-1,2-diamine (MQA), harmine-based amines **1**, **4**, **7**, and **10**, and harmine benzotriazolide **11** were synthesized according to our previously described procedures (20–25).

Syntheses

N^1-*(7-chloroquinolin-4-yl)ethane-1,2-diamine (CQA)*

CQA was prepared from 4,7-dichloroquinoline and ethylenediamine according to the previously published procedure (20).

N^1-*(2,8-bis(trifluoromethyl)quinolin-4-yl)ethane-1,2-diamine (MQA)*

A mixture of 4-chloro-2,8-*bis*(trifluoromethyl)quinoline (0.400 g, 1.335 mmol) and ethylenediamine (0.895 mL, 13.351 mmol) was stirred under microwave irradiation (150 W) at 95 °C. After 60 min, the reaction mixture was diluted with dichloromethane (70 mL), extracted with 5 % NaOH (3 × 50 mL), and washed with water (2 × 50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. After trituration with diethyl ether, a pale pink solid MQA was obtained (0.309 g, 72 %) (22).

*(1-Methyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methanamine* (***1***)

Harmine-based amine **1** was prepared from tryptophane methyl ester according to the previously published procedure (23).

*1-(2-((7-Chloroquinolin-4-yl)amino)ethyl)-3-((1-methyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methyl)urea (**2**)*

A solution of amine **1** (0.036 g, 0.170 mmol) in dry DMF (1 mL) was added dropwise to a solution of CDI (0.036 g, 0.220 mmol) in dry DMF (1 mL). The reaction mixture was stirred at 0 °C. After 2 h, CQA (0.034 g, 0.153 mmol) was added, and the mixture was stirred at r.t. for 3 days. The reaction was quenched with water, and the resulting precipitate was filtered off and recrystallized from ethanol to give a pale purple solid **2** (0.023 g, 33 %).

*1-(2-((2,8-bis(Trifluoromethyl)quinolin-4-yl)amino)ethyl)-3-((1-methyl-9*H*-pyrido[3,4-*b*]indol-3-yl)methyl)urea (**3**)*

A solution of amine **1** (0.019 g, 0.089 mmol) in dry DMF (1 mL) was added dropwise to a solution of CDI (0.017 g, 0.107 mmol) in dry DMF (1 mL). The reaction mixture was stirred at 0 °C. After 4.5 h, MQA (0.035 g, 0.107 mmol) was added, and the mixture was stirred at r.t. for 2 days. The reaction was quenched with water and the resulting precipitate was filtered off. After purification by column chromatography (cyclohexane/ethyl acetate/methanol 1:1:0.5), and precipitation from ethanol/water, a white solid **3** (0.014 g, 28 %) was obtained.

*2-((1-Methyl-9*H*-pyrido[3,4-*b*]indol-6-yl)oxy)ethan-1-amine (**4**)*

Harmine-based amine **4** was prepared from 5-methoxytryptamine according to the previously published procedure (23).

*1-(2-((7-Chloroquinolin-4-yl)amino)ethyl)-3-(2-((1-methyl-9*H*-pyrido[3,4-*b*]indol-6-yl)oxy)ethyl) urea (**5**)*

A solution of amine **4** (0.042 g, 0.172 mmol) in dry DMF (1 mL) was added dropwise to a solution of CDI (0.033 g, 0.204 mmol) in dry DMF (1 mL). The reaction mixture was stirred at 0 °C. After 2 h, CQA (0.032 g, 0.143 mmol) was added, and the mixture was stirred at r.t. for 3 days. The reaction was quenched with water and the resulting precipitate was filtered off. After consecutive trituration with methanol, DMF, water, and diethyl ether, a pale yellow solid **5** (0.029 g, 41 %) was obtained.

*1-(2-((2,8-bis(Trifluoromethyl)quinolin-4-yl)amino)ethyl)-3-(2-((1-methyl-9*H*-pyrido[3,4-*b*]indol--6-yl)oxy)ethyl)urea (**6**)*

A solution of amine **4** (0.029 g, 0.119 mmol) in dry DMF (1 mL) was added dropwise to a solution of CDI (0.023 g, 0.142 mmol) in dry DMF (1 mL). The reaction mixture was stirred at 0 °C. After 2.5 h, MQA (0.046 g, 0.142 mmol) was added, and the mixture was stirred at r.t. for 2 days. The reaction was quenched with water and the resulting precipitate was filtered off. The crude product was purified by column chromatography with dichloromethane/methanol 7.5:2.5 as a mobile phase and triturated with diethyl ether to give a pale yellow solid **6** (0.049 g, 70 %).

*2-((1-Methyl-9*H*-pyrido[3,4-*b*]indol-7-yl)oxy)ethan-1-amine (**7**)*

Harmine-based amine **7** was prepared from harmole according to the previously published procedure (24).

*1-(2-((7-Chloroquinolin-4-yl)amino)ethyl)-3-(2-((1-methyl-9*H*-pyrido[3,4-*b*]indol-7-yl)oxy)ethyl) urea (**8**)*

A solution of amine **7** (0.032 g, 0.133 mmol) in dry DMF (1 mL) was added dropwise to a solution of CDI (0.026 g, 0.159 mmol) in dry DMF (1 mL). The reaction mixture was stirred at 0 °C. After 1 h, CQA (0.035 g, 0.160 mmol) was added, and the mixture was stirred at r.t. overnight. The resulting precipitate was filtered off and washed with water, ethyl acetate, and ethanol to give a white solid **8** (0.028 g, 43 %).

*1-(2-((2,8-bis(Trifluoromethyl)quinolin-4-yl)amino)ethyl)-3-(2-((1-methyl-9*H*-pyrido[3,4-*b*]indol-7-yl)oxy)ethyl)urea (**9**)*

A solution of amine **7** (0.029 g, 0.119 mmol) in dry DMF (1 mL) was added dropwise to a solution of CDI (0.023 g, 0.142 mmol) in dry DMF (1 mL). The reaction mixture was stirred at 0 °C. After 1 h, MQA (0.046 g, 0.142 mmol) was added, and the mixture was stirred at r.t. overnight. The reaction was quenched with water and the resulting precipitate was filtered off. The crude product was dissolved in water, pH adjusted with 5 % NaOH to 11, and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. After trituration with diethyl ether, a white solid **9** (0.044 g, 63 %) was collected.

*2-(7-Methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indol-9-yl)ethan-1-amine (**10**)*

Harmine-based amine **10** was prepared from harmine according to the previously published procedure (24).

*N-(2-(7-Methoxy-1-methyl-9*H*-pyrido[3,4-b]indol-9-yl)ethyl)-1*H*-benzo[*d*][1,2,3]triazole-1-carboxamide (**11**)*

Harmine benzotriazolide **11** was prepared from amine **10** and 1-benzotriazole carboxylic acid chloride according to the previously published procedure (25).

*1-(2-((7-Chloroquinolin-4-yl)amino)ethyl)-3-(2-(7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indol-9-yl) ethyl)urea (**12**)*

A mixture of amine **10** (0.064 g, 0.290 mmol), harmine benzotriazolide **11** (0.058 g, 0.145 mmol), and TEA (0.040 mL, 0.290 mmol) in dichloromethane (2 mL) was stirred under microwave irradiation (150 W) at 65 °C for 30 min. Upon completion, the precipitate was filtered off. After consecutive trituration of the residue with diethyl ether and recrystallization from ethanol, a white solid **12** (0.039g, 53 %) was obtained.

*1-(2-((2,8-bis(Trifluoromethyl)quinolin-4-yl)amino)ethyl)-3-(2-(7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indol-9-yl)ethyl)urea (**13**)*

A solution of amine **10** (0.029 g, 0.119 mmol) in dry DMF (1 mL) was added dropwise to a solution of CDI (0.033 g, 0.204 mmol) in dry DMF (1 mL). The reaction mixture was stirred at 0 °C. After 1.5 h, MQA (0.046 g, 0.142 mmol) was added, and the mixture was stirred at r.t. overnight. The reaction was quenched with water and the resulting precipitate was filtered off. The crude product was purified by column chromatography with dichloromethane/methanol 8:2 as a mobile phase and triturated with diethyl ether to give a white solid (0.042 g, 58 %).

In vitro *drug sensitivity assay against erythrocytic stages of* P. falciparum

Antiplasmodial activity of new harmiquins was evaluated against two strains of *P. falciparum* (3D7 – CQ-sensitive, provided by BEI resources, MRA-102 and Dd2 – multidrug-resistant, provided by BEI resources, MRA-159), as previously described, using the histidine-rich protein 2 (HRP2) assay (26, 27). Briefly, 96-well plates were pre-coated with the tested compounds in a three-fold dilution before ring-stage parasites were added to the complete culture medium at a hematocrit of 1.5 % and parasitaemia of 0.05 %. After three days of incubation at 37 °C, 5 % CO_2, and 5 % oxygen, plates were frozen until analyzed by HRP2-ELISA. All compounds were evaluated in duplicate in at least two independent experiments. The IC_{50} was determined by nonlinear regression analysis of log concentration-response curves using the drc-package v0.9.0 of R v2.6.1 (28).

Cytotoxicity assay in human cell lines

The experiments were carried out on five human cell lines purchased from American Type Culture Collection (ATCC): HepG2 (hepatocellular carcinoma; ATCC® HB-8065™), SW620 (colorectal adenocarcinoma; ATCC® CCL-227™), HCT116 (colorectal carcinoma; ATCC® CCL-247™), MCF-7 (breast adenocarcinoma; ATCC® HTB-22™), and Hek293T (embryonic kidney cells; ATCC® CRL-3216™). All cell lines were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) (Capricorn Scientific, USA),

supplemented with 10 % fetal bovine serum (FBS) (Capricorn Scientific, USA), 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (Capricorn Scientific, USA) in a humidified atmosphere with 5 % CO_2 at 37 °C. Cells were seeded in 96-well plates (Corning, USA) at 5,000–7,000 cells per well (depending on the cell doubling time of a specific cell line) in 0.1 mL media and cultured for 24 h. The next day, the medium was aspirated, and cells were treated for 72 h. Only the compounds that led to more than a 50 % reduction in mitochondrial metabolic activity at a concentration of 50 μmol L^{-1} were selected for further analysis. The following concentrations of selected compounds were used: 25, 10, 5, and 1 μmol L^{-1}. Working dilutions were freshly prepared on the day of the testing. A fresh growth medium was added to untreated control cells, which were defined as 100 % viable. DMSO (0.13 %) in DMEM was considered a negative control. 5-Fluorouracil (5-FU) and harmine were used as positive controls. At the end of treatment, media was removed, and cells were incubated for 1 h with 0.5 mg mL^{-1} MTT (Abcam, USA) dissolved in serum-deprived DMEM. The MTT-containing media was then removed, and 0.1 mL isopropanol was added per well to lyse cells and dissolve formazan. The optical density was measured at 570 nm using a microplate reader (VICTOR3, PerkinElmer). Each test point was performed in triplicate. The absorbance was directly proportional to cell viability. The IC_{50} values were calculated by using linear regression on the sigmoidal dose-response plots and are expressed as mean ± SD.

RESULTS AND DISCUSSION

Chemistry

In this work, we report the synthesis of new hybrid compounds, UT harmiquins, that differ in the type of the incorporated 4-aminoquinoline. CQ-based UT harmiquins **2**, **5**, **8,** and **12** contain 7-chloroquinoline, whereas MQ-based UT harmiquins **3**, **6**, **9**, and **13** employ 2,8-trifluoromethylquinoline in their structures.

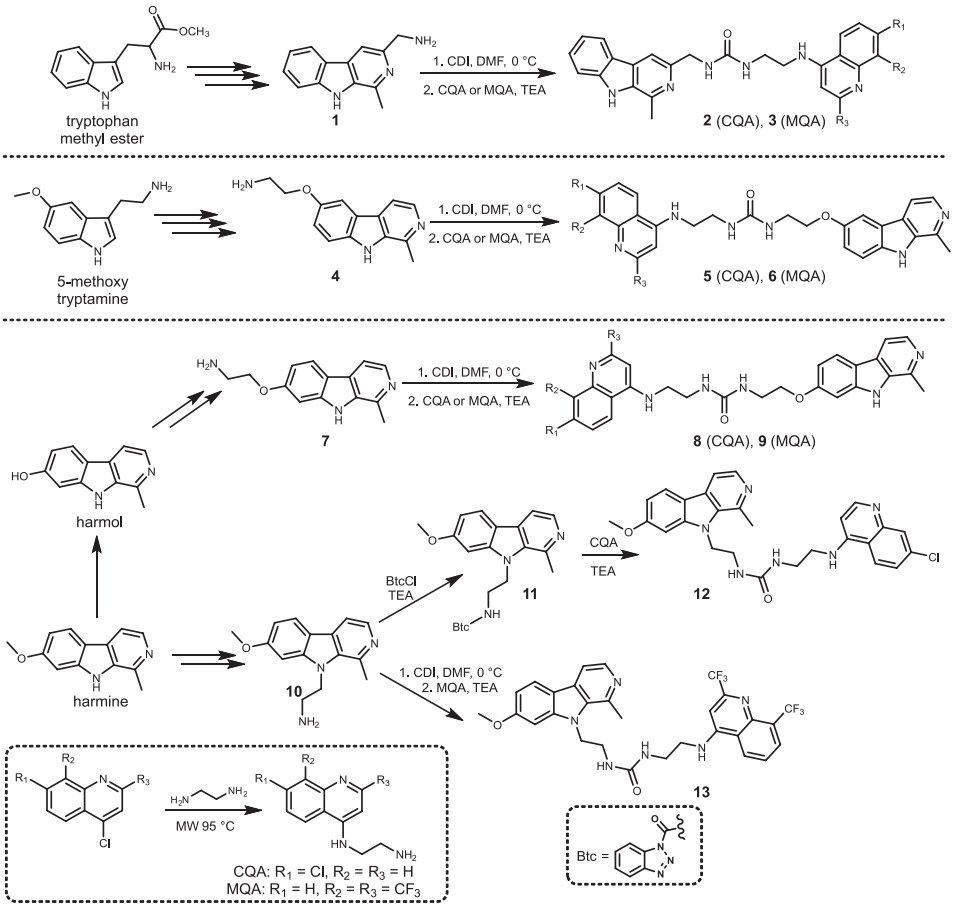
We have decided to explore the utility of the urea linker due to the important role of ureas in medicinal chemistry. Urea can form multiple stable hydrogen bonds as both hydrogen bond donor and acceptor, which leads to the stabilization of drug-target interactions and an increase in drug activity and selectivity (29). As a result, urea moiety has been incorporated in registered anticancer (sorafenib, lenvatinib), antiviral (boceprevir, ritonavir), antipsychotic (ceriprazine), and antiparkinson (lisuride) drugs, as well as in numerous biological active agents (anti-HIV, anticancer, antibacterial, antidiabetic agents) (30).

After careful consideration, we decided to prepare UT harmiquins by the 1,1′-carbonyldiimidazole (CDI)-mediated synthesis, as synthetic routes to the starting compounds, namely 4-aminoquinoline-based amines CQA and MQA, and β-carboline-based amines **1**, **4**, **7**, **10** were already established by us (20, 21, 23, 24). Shortly, CQA and MQA were obtained from commercially available 4,7-dichloroquinoline/4-chloro-2,8-bis-(trifluoromethyl)-quinoline and ethane-1,2-diamine, under microwave irradiation, in excellent yields. On the contrary, the synthesis of amines **1**, **4**, **7,** and **10** involved several steps. The β-carbolines required for the synthesis of amines **1** and **4** were prepared by Pictet-Spengler condensation of tryptophane methyl ester/5-methoxytryptamine and acetaldehyde dimethyl acetal under acidic conditions to give tetrahydro-β-carbolines, followed by oxidation. Further reactions included: 1) reduction of ester in the position C-3 to yield the corresponding

alcohol, its conversion to azide using ADMP/DBU and catalytic hydrogenation (amine **1**); 2) acid-mediated hydrolysis of ethers in positions O-6 and O-7 to obtain corresponding phenols and 3) *O*-alkylation of phenols or *N*-alkylation of secondary amine at N-9 with 2-(Boc-amino)ethyl bromide/Cs_2CO_3, followed by the removal of the Boc protecting group under acidic conditions (amines **4**, **7**, and **10**).

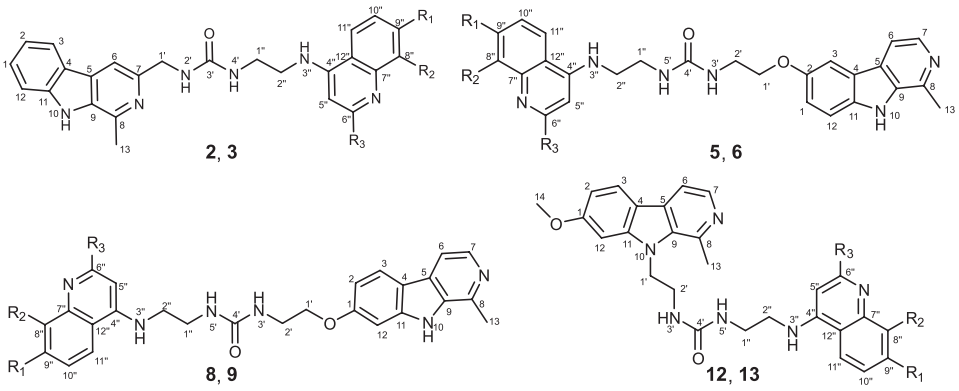
In general, the preparation of UT harmiquins started by the *in situ* formation of *N*-substituted carbonylimidazolide from β-carboline-based amine and CDI in DMF, which subsequently reacted with either CQA or MQA. Interestingly, amine **10** and CQA failed to react under those reaction conditions. Instead, amine **10** was converted with 1-benzotriazolecarboxylic acid chloride (BtcCl) to the corresponding benzotriazolide, followed by the nucleophilic attack of the primary amino group of CQA to obtain urea **12**.

The synthetic pathway towards new CQ- and MQ-based UT harmiquins is depicted in Scheme 1.



Scheme 1

*Table I. Analytical data for UT harmiquins **2**, **3**, **5**, **6**, **8**, **9**, **12** and **13***



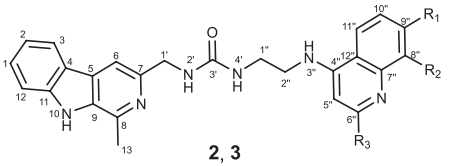
Compd.	R_1	R_2	R_3	Yield (%)	Molecular formula	M_r (exact mass)	MS (*m*/*z*)	t_t (°C)	HPLC purity (%)
2	Cl	H	H	33	$C_{25}H_{23}ClN_6O$	458.95 (458.16)	459.1 $(M+1)^+$	> 240	< 95
3	H	CF_3	CF_3	28	$C_{27}H_{22}F_6N_6O$	560.50 (560.18)	561.1 $(M+1)^+$	137.0–139.5	98.5
5	Cl	H	H	41	$C_{26}H_{25}ClN_6O_2$	488.98 (488.17)	489.1 $(M+1)^+$	259.5 (decomp.)	97
6	H	CF_3	CF_3	70	$C_{28}H_{24}F_6N_6O_2$	590.53 (590.19)	591.1 $(M+1)^+$	254.5 (decomp.)	99.7
8	Cl	H	H	43	$C_{26}H_{25}ClN_6O_2$	488.98 (488.17)	489.1 $(M+1)^+$	257.5 (decomp.)	99
9	H	CF_3	CF_3	63	$C_{28}H_{24}F_6N_6O_2$	590.53 (590.1865)	591.1968 $(M+1)^+$	196.0 (decomp.)	98
12	Cl	H	H	42	$C_{27}H_{27}ClN_6O_2$	503.0 (502.19)	503.1 $(M+1)^+$	216.0 (decomp.)	99
13	H	CF_3	CF_3	58	$C_{29}H_{26}F_6N_6O_2$	604.56 (604.20)	605.1 $(M+1)^+$	236.4 (decomp.)	99.7

Structures of new UT harmiquins were confirmed by MS, IR, ^{1}H, and ^{13}C NMR spectra. All compounds, except inactive hybrid **2**, showed a high degree of purity, > 98 % (assessed by HPLC analysis). Analytical and spectral data are given in Tables I and II.

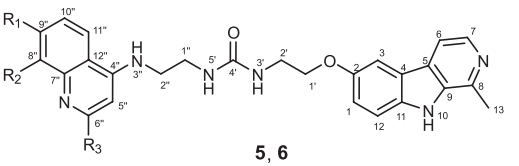
Antiproliferative activity

Screening of the antiproliferative activity of UT harmiquins was performed against four human tumor cell lines: MCF-7, HepG2, SW 620, and HCT 116, and one non-tumor cell line, Hek293T (Table III), using the method described previously (31). The anticancer drug

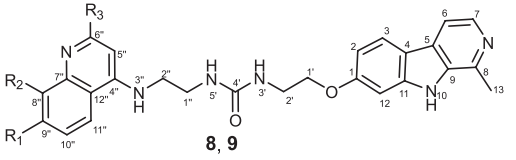
Table II. IR, ^{1}H and ^{13}C NMR spectroscopic data for UT harmiquins ***2****,* ***3****,* ***5****,* ***6****,* ***8****,* ***9****,* ***12*** *and* ***13***



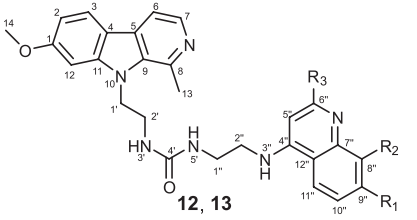
2, **3**



5, **6**



8, **9**



12, **13**

Compd.	R_1	R_2	R_3	IR (υ/ cm^{-1})	^{1}H NMR (600 MHz, DMSO-d_6, δ ppm)	^{13}C NMR (151 MHz, DMSO-d_6, δ ppm)
2	Cl	H	H	3370, 1739, 1614, 1585, 1536, 1487, 1452, 1437, 1424, 1361, 1336, 1269, 1249, 1200, 1144, 1081, 928, 907, 882, 856, 821, 764, 735, 669, 632, 602, 582, 556, 510, 472	11.46 (s, 1H, 10), 8.37 (d, *J* = 5.2 Hz, 1H, 6''), 8.14 (d, *J* = 9.0 Hz, 1H, 11''), 8.00 (d, *J* = 7.9 Hz, 1H, 3), 7.78 (s, 1H, 6), 7.76 (s, 1H, 8''), 7.58–7.52 (m, 2H, 12, 3''), 7.50 (t, *J* = 7.7 Hz, 1H, 1), 7.39 (d, *J* = 8.9 Hz, 1H, 10''), 7.16 (t, *J* = 7.5 Hz, 1H, 2), 6.69 (t, *J* = 6 Hz, 1H, 2'), 6.53 (d, *J* = 5.4 Hz, 1H, 5''), 6.44 (s, 1H, 4'), 4.44 (d, *J* = 5.9 Hz, 2H, 1'), 3.45–3.35 (m, 4H, 1'', 2''), 2.73 (s, 3H, 13)	158.96 (3'), 151.95 (6''), 150.12 (4''), 149.00 (7''), 147.31 (7), 141.13 (8), 140.71 (11), 133.43 (9), 133.35 (9''), 127.75 (10''), 127.69 (4), 127.51 (3), 124.12 (8''), 123.70 (11''), 121.45 (1), 120.96 (5), 119.05 (2), 117.34 (12''), 111.89 (12), 109.51 (6), 98.56 (5''), 45.30 (1'), 43.99 (2''), 38.06 (1''), 20.32 (2)
3	H	$^{14''}CF_3$	$^{13''}CF_3$	3323, 2971, 2945, 1739, 1625, 1597, 1579, 1550, 1497, 1471, 1440, 1333, 1313, 1290, 1249, 1218, 1193, 1177, 1061, 968, 905, 877, 837, 821, 760, 744, 727, 667, 634, 620, 585, 521, 517, 473	11.47 (s, 1H, 10), 8.47 (d, *J* = 8.6 Hz, 1H, 9''), 8.27 (s, 1H, 3''), 8.08 (d, *J* = 7.4 Hz, 1H, 11''), 7.96 (d, *J* = 7.8 Hz, 1H, 3), 7.78 (s, 1H, 6), 7.65–7.40 (m, 3H, 1, 12, 10''), 7.13 (t, *J* = 7.4 Hz, 1H, 2), 6.97 (s, 1H, 5''), 6.72 (t, *J* = 5.8 Hz, 1H, 2'), 6.52 (s, 1H, 4'), 4.46 (d, *J* = 5.8 Hz, 2H, 1'), 3.44 (dt, *J* = 14.3, 5.9 Hz, 4H, 1'', 2''), 2.73 (s, 3H, 13)	159.12 (3'), 152.43 (4''), 148.27–147.62 (q, J_2 = 33.0 Hz, 6''), 147.11 (7), 143.74 (7''), 141.03 (8), 140.75 (11), 133.40 (9), 128.89-128.79 (q, J_3 = 5 Hz, 9''), 127.82 (3), 126.70–121.28 (q, J_1 = 273 Hz, 13''), 126.61–126.04 (q, J_2 = 29 Hz, 8''), 124.47 (10''), 126.53 (11''), 126.42 (4), 124.41–118.93 (q, J_1 = 276 Hz, 14''), 121.37 (1), 120.88 (5), 119.25 (12''), 119.02 (2), 111.88 (12), 109.45 (6), 94.16 (5''), 45.18 (1'), 44.16 (2''), 37.97 (1''), 20.20 (13)

					11.35 (s, 1H, 10), 8.39 (d, J = 5.4 Hz, 1H, 6′), 8.18 (d, J = 8.9 Hz, 1H, 11″), 8.15 (d, J = 5.3 Hz, 1H, 7),		
				3322, 1615, 1574, 1539, 1500, 1448, 1409, 1370, 1335, 1290, 1250, 1197, 1173, 1143, 1069, 1048, 979, 947, 905, 871, 805, 760, 600, 580, 546, 500	7.88 (d, J = 5.4 Hz, 1H, 6), 7.78 (s, 1H, 8″), 7.75 (s, 1H, 3), 7.52 (t, J = 5.1 Hz, 1H, 3″), 7.48 (d, J = 8.8 Hz, 1H, 12), 7.44 (d, J = 9.0 Hz, 1H, 10″), 7.16 (d, J = 9.2 Hz, 1H, 1), 6.54 (d, J = 5.5 Hz, 1H, 5″), 6.40 (t, J = 5.7 Hz, 1H, 3′), 6.35 (t, J = 5.9 Hz, 1H, 5′), 4.07 (t, J = 5.7 Hz, 2H, 1′), 3.47 (q, J = 5.8 Hz, 2H, 2′), 3.36–3.33 (m, J = 8.8 Hz, 4H, 1″, 2″), 2.74 (s, 4H, 13)	159.30 (4′), 152.83 (2), 152.45 (6″), 150.59 (4″), 149.51 (7″), 142.65 (8), 137.40 (7), 135.75, 135.54 (9, 11), 133.84 (9″), 128.04 (10″), 127.16 (4), 124.61 (8″), 124.24 (11″), 121.88 (5), 118.62 (1), 117.85 (12″), 113.17, 113.14 (6, 12), 105.06 (3), 99.08 (5″), 68.42 (1′), 44.25 (2″), 39.64 (1″), 38.47 (2′), 20.88 (13)
5	Cl	H	H				
					11.43 (s, 1H, 10), 8.59 (dd, J = 8.7, 1.3 Hz, 1H, 9″), 8.31 (t, J = 5.2 Hz, 1H, 3″), 8.15 (d, J = 5.3 Hz, 1H, 7), 8.12 (dd, J = 7.3, 1.2 Hz, 1H, 11″), 7.87 (d, J = 5.3 Hz, 1H, 6), 7.75 (d, J = 2.5 Hz, 1H, 3), 7.66 (dd, J = 8.5, 7.3 Hz, 1H, 10″), 7.49 (d, J = 8.9 Hz, 1H, 12), 7.16 (dd, J = 8.8, 2.5 Hz, 1H, 1), 6.98 (s, 1H, 5″), 6.47–6.43 (m, 2H, 3′, 5′), 4.06 (t, J = 5.7 Hz, 2H, 1′), 3.49–3.43 (m, 3H, 2′, 2″), 3.39–3.37 (m, J = 5.8 Hz, 2H, 1″), 2.74 (s, 3H, 13)	158.92 (4′), 152.51 (4″), 152.35 (2), 148.30–147.64 (q, J_2 = 33.0 Hz, 6″), 143.81 (7″), 142.14 (8), 136.74 (7), 135.34 (11), 135.05 (9), 128.99–128.89 (q, J_3 = 5 Hz, 9″), 126.80 (11″), 126.74 (4), 126.74–121.30 (q, J_1 = 272.0 Hz, 13″), 126.63–126.06 (q, J_2 = 28.7 Hz, 8″), 124.53 (10″), 124.42–118.94 (q, J_1 = 276.0 Hz, 14″), 121.36 (5), 119.33 (12″), 118.22 (1), 112.72, 112.68 (6, 12), 104.57 (3), 94.25–94.20 (q, J_3 = 2 Hz, 5″), 67.89 (1′), 43.79 (2″), 39.66 (1″), 37.97 (2′), 20.34 (13)
6	H	$^{14''}CF_3$	$^{13''}CF_3$	3354, 1602, 1579, 1547, 1505, 1461, 1441, 1317, 1294, 1210, 1158, 1128, 1072, 972, 843, 820, 808, 765, 731, 622, 520			
					11.40 (s, 1H, 10), 8.39 (d, J = 5.4 Hz, 1H, 6′), 8.19 (d, J = 8.9 Hz, 1H, 11″), 8.15 (d, J = 5.3 Hz, 1H, 7), 8.03 (d, J = 8.5 Hz, 1H, 3), 7.80–7.78 (m, 2H, 6, 8″), 7.50 (t, J = 4.9 Hz, 1H, 3″), 7.44 (dd, J = 9.0, 2.0 Hz, 1H, 10″), 7.02 (d, J = 3 Hz, 1H, 12), 6.84 (dd, J = 8.0, 2.0 Hz, 1H, 2), 6.55 (d, J = 5.4 Hz, 1H, 5″), 6.39 (t, J = 5.5 Hz, 1H, 5′), 6.32 (t, J = 5.8 Hz, 1H, 3′), 4.09 (t, J = 5.4 Hz, 2H, 1′), 3.48 (q, J = 5.6 Hz, 2H, 2′), 3.38–3.31 (m, 4H, 1″, 2″), 2.72 (s, 3H, 13)	159.18 (4′), 158.75 (1), 151.97 (6″), 150.12 (4″), 149.03 (7″), 141.85 (8), 141.29 (11), 137.75 (7), 134.56 (9), 133.36 (9″), 127.56 (10″), 127.16 (4), 124.13 (8″), 123.79 (11″), 122.61 (3), 117.38 (12″), 114.97 (5), 111.91 (6), 109.25 (2), 98.62 (5″), 95.36 (12), 67.49 (1′), 43.68 (2″), 39.03 (1″), 38.00 (2), 20.35 (13)
8	Cl	H	H	3336, 3074, 2942, 2866, 1612, 1578, 1568, 1543, 1511, 1487, 1473, 1450, 1433, 1401, 1370, 1336, 1322, 1296, 1279, 1258, 1235, 1183, 1172, 1128, 1101, 1079, 1058, 972, 924, 902, 871, 845, 812, 798, 792, 766, 741, 633, 584, 568, 557, 516, 496			

				11.40 (s, 1H, 10), 8.55 (d, J = 8.4 Hz, 1H, 9''), 8.23 (t, J = 5.2 Hz, 1H, 3''), 8.15 (d, J = 5.3 Hz, 1H, 7), 8.13 (d, J = 7.3 Hz, 1H, 11''), 8.03 (d, J = 8.6 Hz, 1H, 3), 7.80 (d, J = 5.3 Hz, 1H, 6), 7.69–7.64 (m, 1H, 10''), 7.01 (d, J = 2.2 Hz, 1H, 12), 6.98 (s, 1H, 5''), 6.83 (dd, J = 8.6, 2.2 Hz, 1H, 2), 6.40 (t, J = 5.7 Hz, 1H, 5'), 6.36 (t, J = 5.9 Hz, 1H, 3'), 4.08 (t, J = 5.6 Hz, 2H, 1'), 3.48 (q, J = 5.6 Hz, 2H, 2'), 3.45 (q, J = 5.9 Hz, 2H, 2''), 3.37 (dt, J = 12.4, 6.0 Hz, 2H, 1''), 2.72 (s, 3H, 13)	159.11 (1), 158.73 (4'), 152.40 (4''), 148.20–147.55 (q, J_2 = 33 Hz, 6''), 143.71 (7''), 141.80 (8), 141.14 (11), 137.54 (7), 134.45 (9), 128.91–128.81 (q, J_3 = 5 Hz, 9''), 127.13 (4), 126.58 (11''), 126.63–121.19 (q, J_1 = 273 Hz, 13''), 126.63–126.00 (q, J_2 = 36 Hz, 8''), 124.47 (10''), 124.31–118.83 (q, J_1 = 278 Hz, 14''), 122.53 (3), 119.22 (12''), 114.86 (5), 111.84 (6), 109.19 (2), 95.25 (12), 94.19–94.14 (q, J_3 = 2 Hz, 5''), 67.37 (1'), 43.64 (2''), 39.01 (1''), 37.91 (2'), 20.19 (13)
9	H	$^{14''}CF_3$	$^{13''}CF_3$	3376, 3323, 1633, 1599, 1578, 1544, 1474, 1443, 1399, 1319, 1291, 1235, 1180, 1124, 1064, 971, 931, 821, 802, 771, 729, 567		
				8.41 (d, J = 5.4 Hz, 1H, 6''), 8.18 (d, J = 9.0 Hz, 1H, 11''), 8.16 (d, J = 5.2 Hz, 1H, 7), 8.07 (d, J = 8.6 Hz, 1H, 3), 7.86 (d, J = 5.2 Hz, 1H, 6), 7.79 (s, 1H, 8''), 7.50 (t, J = 4.9 Hz, 1H, 3''), 7.42 (d, J = 8.9 Hz, 1H, 10''), 7.30 (s, 1H, 12), 6.87 (d, J = 8.7 Hz, 1H, 2), 6.53 (d, J = 5.4 Hz, 1H, 5''), 6.37–6.33 (m, 2H, 3', 5'), 4.57 (t, J = 6.9 Hz, 2H, 1'), 3.91 (s, 3H, 14), 3.44 (h, J = 6.7 Hz, 4H, 2', 2''), 3.25 (q, J = 6.1 Hz, 2H, 1''), 2.97 (s, 3H, 13)	160.49 (1), 158.92 (4'), 151.94 (6''), 150.11 (4''), 149.00 (7'), 142.92 (8), 140.70 (11), 137.71 (7), 134.72 (9), 133.37 (9''), 128.32 (4), 127.53 (10''), 124.11 (8''), 123.76 (11''), 122.31 (3), 117.38 (12''), 114.28 (5), 112.21 (6), 109.11 (2), 98.60 (5''), 93.81 (12), 55.46 (14), 44.37 (1'), 43.66 (2''), 39.62 (1''), 37.99 (2'), 23.11 (13)
12	Cl	H	H	3296, 1622, 1568, 1500, 1449, 1409, 1367, 1345, 1280, 1251, 1200, 1173, 1140, 1080, 1048, 1025, 978, 948, 906, 870, 805, 764, 599, 545, 496		
				8.54 (d, J = 8.5 Hz, 1H, 9''), 8.22 (t, J = 5.1 Hz, 1H, 3''), 8.15 (d, J = 5.1 Hz, 1H, 7), 8.14 (s, 1H, 11''), 8.06 (d, J = 8.6 Hz, 1H, 3), 7.85 (d, J = 5.2 Hz, 1H, 6), 7.65 (t, J = 7.9 Hz, 1H, 10''), 7.27 (d, J = 2.2 Hz, 1H, 12), 6.98 (s, 1H, 5''), 6.86 (dd, J = 8.6, 2.1 Hz, 1H, 2), 6.39 (dt, J = 12.3, 5.9 Hz, 2H, 3', 5'), 4.55 (t, J = 7.1 Hz, 2H, 1'), 3.89 (s, 3H, 14), 3.44–3.36 (m, 6H, 2', 1'', 2''), 2.97 (s, 3H, 13)	159.92 (1), 158.36 (4'), 151.87 (4''), 147.71–147.05 (q, J_2 = 33.0 Hz, 6''), 143.22 (7''), 142.33 (8), 140.05 (11), 137.00 (7), 134.08 (9), 128.43–128.33 (q, J_3 = 6 Hz, 9''), 127.78 (4), 126.07 (11''), 126.13–120.70 (q, J_1 = 273 Hz, 13''), 125.88–125.50 (q, J_2 = 28 Hz, 8''), 123.91 (10''), 123.83–118.35 (q, J_1 = 273 Hz, 14''), 121.71 (3), 118.73 (12''), 113.66 (5), 111.61 (6), 108.53 (2), 93.67–93.62 (q, J_3 = 2 Hz, 5''), 93.17 (12), 54.84 (14), 43.72 (1'), 43.10 (2''), 39.20 (1''), 37.39 (2'), 22.40 (13)
13	H	$^{14''}CF_3$	$^{13''}CF_3$	3332, 1624, 1601, 1579, 1556, 1500, 1440, 1409, 1317, 1292, 1253, 1202, 1175, 1118, 971, 946, 816, 762, 727, 620		

5-FU and harmine were used as positive controls. In addition, we decided to evaluate the antiproliferative activity of the previously established library of AT and TT harmiquins (21), to determine the structural features of CQ-based harmiquins relevant to antiproliferative activity (Table III, compounds **14**–**28**). Pre-screening against tumor cell lines was performed using 50 μmol L^{-1} of the compound tested. Only compounds that resulted in more than a 50 % reduction in mitochondrial metabolic activity at 50 μmol L^{-1} concentration were selected for further analysis. Evaluation of antiproliferative activity against Hek293T was performed only if the hybrid compound had an IC_{50} value of less than 50 μmol L^{-1} against any tumor cell line.

First, we turned our attention to UT (**2**, **5**, **8**, **12**), TT (**14**-**21**), and AT (**22**–**28**) CQ-based harmiquins and carefully analysed the influence of the linker between two structural motifs and of the position of the β-carboline substitution. We have found that, in general, AT harmiquins exert the strongest and nonselective activity against all cell lines tested, followed by TT harmiquins, whereas only one UT harmiquine, compound **12**, showed pronounced antiproliferative activity. Significant differences in the activity of the compounds prepared at different β-carboline positions were also found. TT harmiquine **14**, prepared at C-1, showed selective activity against HCT 116 only, whereas the corresponding AT harmiquine **22** exerted more pronounced activity against all cell lines tested. There was some degree of selectivity against tumor cell lines, albeit a small one. At C-3, both UT (**2**) and TT (**15**) harmiquins were inactive, whereas AT harmiquine **23** had pronounced, but non-selective activity. At O-6, UT (**5**) and AT (**24**) harmiquins were inactive, but TT harmiquine **16** showed a single-digit IC_{50} value against HepG2. Fortunately, it was inactive against non-tumor cells. UT harmiquine (**8**), prepared at O-7, was inactive, whereas TT (**18**, **19**) and AT (**25**) harmiquins showed significant non-selective activity. Surprisingly, the most active compound prepared at the N-9 position of the β-carboline was UT harmiquine **12**, followed by TT harmiquins **20** and **21**, and in the third place AT harmiquins **27** and **28**.

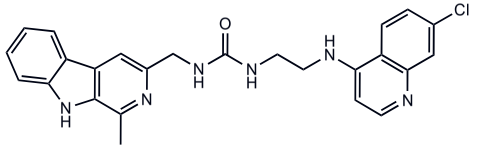
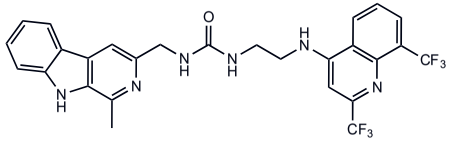
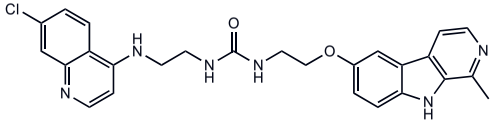
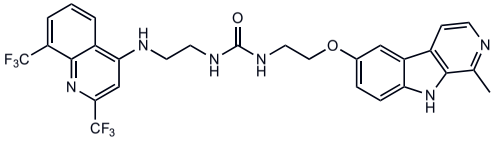
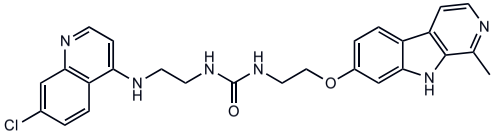
Altogether, the most active compounds among CQ derivatives were UT harmiquine **12**, and TT harmiquins **18** and **19**, which showed IC_{50} values in the one-digit micromolar range against all cell lines tested, while TT harmiquine **16** was the only selective compound against MCF-7 cell line, in comparison to Hek293T (selectivity index, calculated as the fractional ratio between the IC_{50} values for Hek293T and MCF-7, was above 9).

MQ-based UT harmiquins **3**, **6**, **9**, and **13** showed predominantly potent and non-selective activity against all cell lines (only compound **9** exerted low selectivity against tumor cell lines). In particular, the antiproliferative activity of hybrids prepared at positions C-3, O-6, and O-7 of the β-carboline ring was more pronounced than the activity of their CQ-based counterparts **2**, **5**, **8**. The activity of the two N-9 substituted UT harmiquins, **12** and **13**, was similar.

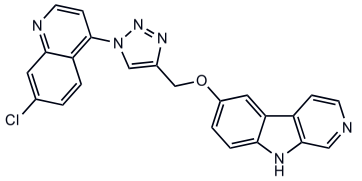
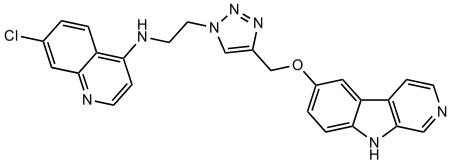
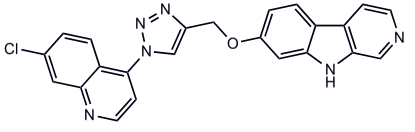
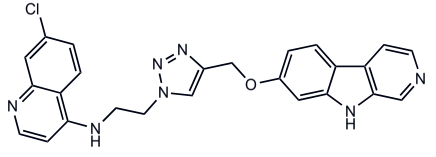
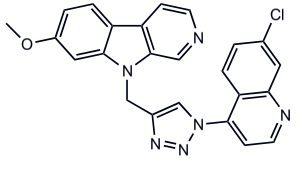
Antiplasmodial activity

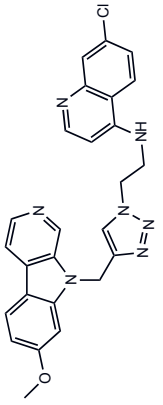
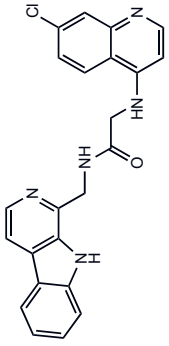
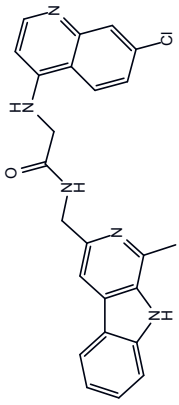
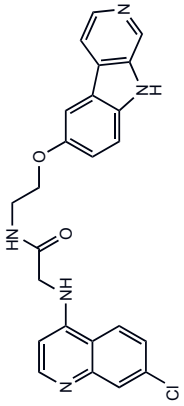
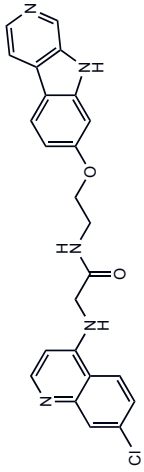
Antiplasmodial activity of UT harmiquins against the erythrocytic stage of the *Plasmodium* life cycle was evaluated *in vitro*, against two strains of *P. falciparum*, CQ-sensitive (*Pf*3D7) and CQ-resistant (*Pf*Dd2), according to the method described previously (Table IV) (26–28). Harmine and CQ were used as positive controls. The selectivity of UT harmiquins against *P. falciparum* was assessed by calculating the selectivity index (SI), as a fractional ratio between the IC_{50} values for Hek293T and *P. falciparum* 3D7 strain.

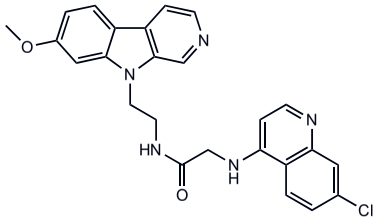
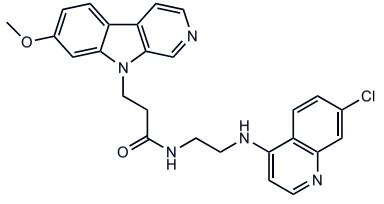
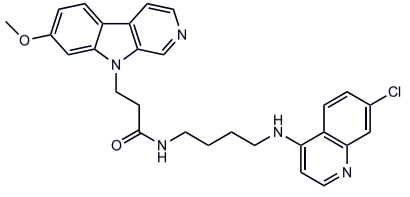
Table III. In vitro *antiproliferative activity of UT harmiquins against human cell lines (MCF-7, HepG2, SW 620, HCT 116, Hek293T)*

Compd.	Structure	IC_{50}[a] (μmol L^{-1})				
		MCF-7	HepG2	SW 620	HCT 116	Hek293T
2		> 50 [b]	> 50	> 50	> 50	n.d.
3		12.04 ± 0.11[c]	14.71 ± 2.3	15.21 ± 0.61	12.62 ± 1.65	15.72 ± 2.09
5		> 50	> 50	> 50	> 50	n.d.
6		4.15 ± 0.2	3.18 ± 0.61	3.07 ± 0.19	4.06 ± 1.39	3.13 ± 0.03
8		> 50	> 50	> 50	38.78 ± 4.48	n.d.

9		4.61 ± 1.81	8.15 ± 0.83	16.51 ± 1.44	6.58 ± 0.04	16.76 ± 0.23
12		3.24 ± 0.02	4.44 ± 1.35	3.38 ± 0.22	3.08 ± 0.01	5.49 ± 0.30
13		4.08 ± 0.69	6.53 ± 1.61	7.75 ± 0.16	7.32 ± 0.44	7.75 ± 0.10
14		> 50	> 25	> 50	12.66 ± 0.98	> 50
15		> 50	> 100	> 50	> 50	> 50

16		21.17 ± 0.39	5.48 ± 3.35	> 50	12.89 ± 0.7	> 50
17		12.38 ± 0.49	15.03 ± 1.46	7.08 ± 0.34	4.56 ± 0.24	16.23 ± 0.61
18		1.58 ± 0.14	6.40 ± 4.16	8.20 ± 0.26	2.01 ± 0.05	5.66 ± 0.13
19		1.88 ± 0.33	6.27 ± 1.90	3.43 ± 0.15	2.81 ± 0.58	4.59 ± 0.42
20		13.05 ± 0.11	19.72 ± 8.34	> 50	7.73 ± 0.36	10.59 ± 0.68

21		13.03 ± 1.33	13.99 ± 1.40	18.12 ± 0.32	12.84 ± 0.98	26.09 ± 0.39
22		7.08 ± 0.24	7.85 ± 1.49	11.21 ± 1.41	7.42 ± 0.35	22.69 ± 0.51
23		6.18 ± 0.56	18.62 ± 7.34	10.45 ± 0.76	6.00 ± 0.53	6.18 ± 0.5
24		> 50	> 20	> 50	> 50	> 50
25		10.83 ± 0.77	15.93 ± 0.84	9.37 ± 0.64	7.33 ± 0.36	12.26 ± 1.70

26		7.74 ± 0.19	8.94 ± 1.04	3.31 ± 0.46	5.06 ± 0.21	11.76 ± 1.1
27		6.79 ± 0.11	12.84 ± 2.38	6.31 ± 0.17	6.04 ± 0.26	7.36 ± 0.69
28		> 50	> 25	> 50	7.23 ± 0.29	11.14 ± 1.24
HAR	-	13.50 ± 1.10	18.70 ± 0.80	7.32 ± 2.69	7.64 ± 1.70	20.98 ± 3.04
5-FU	-	23.90 ± 5.70	5.50 ± 0.60	9.01 ± 0.3	14.43 ± 1.92	17.57 ± 1.94

HAR, harmine; 5-FU, 5-fluorouracil; n.d., not determined; [a] IC_{50}, the concentration that causes 50 % growth inhibition; [b] the exact IC_{50} could not be obtained, as activity could only be detected at the highest tested concentration; [c] results represent mean ± SD, $n \geq 2$.

Table IV. In vitro *antiplasmodial activity of UT harmiquins against the erythrocytic stage of* P. falciparum *(Pf3D7 and PfDd2 strains)*

Compd.	IC_{50} (mmol L^{-1})[a]		SI [b]
	*Pf*3D7	*Pf*Dd2	
2	0.21 ± 0.003 [c]	13.11 ± 5.51	n.d.
3	2.98 ± 0.09	8.01 ± 0.96	5.3
5	0.89 ± 0.27	1.64 ± 0.22	n.d.
6	0.43 ± 0.06	3.94 ± 1.51	7.28
8	0.46 ± 0.11	0.32 ± 0.01	n.d.
9	0.86 ± 0.22	> 5.5 [d]	19.5
12	0.06 ± 0.01	0.19 ± 0.02	91.5
13	0.75 ± 0.002	0.87 ± 0.005	10.3
CQ	0.01 ± 0.002	0.34 ± 0.08	–
HAR	8.25 ± 2.83	> 27.7	–

CQ, chloroquine; HAR, harmine; n.d., not determined; [a] IC_{50}, the concentration of the tested compound necessary for 50 % growth inhibition; [b] SI, selectivity index, the ratio between IC_{50} (HepHek293T) and IC_{50} (*Pf*3D7); [c] results represent mean ± SD, $n \geq 2$; [d] the exact IC_{50} could not be obtained, as activity could only be detected at the highest tested concentration.

In general, UT harmiquins showed antiplasmodial activity, against both strains tested, significantly higher than that of the parent compound harmine, in the submicromolar and low micromolar range. CQ-based UT harmiquins were more active than their MQ-based counterparts, apart from the hybrids prepared in the O-6 position. A comparison of harmiquins' activities against both strains revealed stronger activity against *Pf*3D7, but unfortunately, these were lower than that of the reference drug CQ. Surprisingly, CQ-based hybrid **8**, prepared in the O-7 position, showed 30 % higher activity against *Pf*Dd2 than against *Pf*3D7, and similar activity to CQ.

The most active hybrid was CQ-based UT harmiquine **12**, prepared in the N-9 position (IC_{50} (*Pf*3D7 = 0.06 ± 0.01 μmol L^{-1}). Moreover, its activity against *Pf*Dd2 was 1.8 times stronger than the activity of CQ itself. Since it was the most active compound against *P. falciparum*, compound **12** had the most favourable SI, *i.e.* it was the most selective compared to mammalian cells (SI = 91).

CONCLUSIONS

Both malaria and cancer remain global health problems, due to the development of resistance to the currently available drugs. Therefore, the exploration of new agents against both diseases is of utmost importance. In this study, we employed molecular hybridization to synthesize new hybrid compounds, UT harmiquins, composed of CQ or MQ-based

4-aminoquinoline motifs and β-carboline alkaloid harmine. The hybrids were prepared from the corresponding 4-aminoquinoline amines and β-carboline amines in four positions of the β-carboline core, C-3, O-6, O-7, and N-9, by the CDI-mediated synthesis. We further studied their antiproliferative activities *in vitro*, along with their CQ-based TT and AT counterparts. The results showed that the 4-aminoquinoline moiety, the linker, and the position of the substitution at the β-carboline core influenced the activity. Testing of UT harmiquins antiplasmodial activity revealed CQ-based UT harmiquins as more effective compounds than their MQ-based analogues. A careful analysis of all obtained results revealed two valuable hits, namely TT harmiquine **16** as an anticancer and UT harmiquine **12** as an antiplasmodial hit. In our further research, we will focus our efforts on the enrichment of the compound library to determine the structure-activity relationship and develop new, more potent, and selective compounds.

Supplementary Material is available upon request.

Abbreviations, acronyms, symbols. – AT – amide-type, ATR, attenuated total reflection, Boc – *tert*-butyloxycarbonyl, BtH – benzotriazole, CDI – 1,1′-carbonyldiimidazole, CQ – chloroquine, CQA – chloroquine-based amine, DMF – *N*,*N*-dimethylformamide, ESI – electrospray ionization, 5-FU – 5-fluorouracil, HAR – harmine, HCT 116 – human colorectal carcinoma cell line, Hek293 – human embryonic kidney cell line, HEPES – 2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanesulfonic acid – HepG2 – human liver hepatocellular carcinoma cell line, hpi – hours post infection, HRP2 – histidine-rich protein 2, IC_{50} – the concentration of the tested compound necessary for 50 % growth inhibition, MCF-7 – human breast adenocarcinoma cell line, MQ – mefloquine, MQA – mefloquine-based amine, *Pf*3D7 – chloroquine-sensitive strain of *P. falciparum*, *Pf*Dd2 – chloroquine-resistant strain of *P. falciparum*, *Pf*Hsp90 – *P. falciparum* heat shock protein 90, SW 620 – human colorectal adenocarcinoma cell line, TEA – triethylamine, TMS – tetramethylsilane, TT – triazole-type, TWC – total wavelength chromatogram, UT – ureido-type.

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Conflicts of interest. – The authors declare no conflict of interest.

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REFERENCES

1. World Health Organization, *World malaria report*, December 2022; https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022; last access date March 30, 2023.
2. World Health Organization, *Cancer*, February 2022; https://www.who.int/news-room/fact-sheets/detail/cancer; last access date March 30, 2023.
3. N. Vasan, J. Baselga and D. M. Hyman, A view on drug resistance in cancer, *Nature* **575**(7782) (2019) 299–309; https://doi.org/10.1038/s41586-019-1730-1
4. Y. Le Govic, S. Houzé and N. Papon, Repurposing anticancer drugs to tackle malaria, *ChemMedChem* **16**(14) (2021) 2192–2194; https://doi.org/10.1002/cmdc.202100176

5. T. Ellis, E. Eze and B. T. Raimi-Abraham, Malaria and cancer: a critical review on the established associations and new perspectives, *Infect. Agent Cancer* **16**(33) (2021) (14 pages); https://doi.org/10.1186/s13027-021-00370-7
6. 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, *Ecancermedicalscience* **23**(11) (2017) Article ID 781 (35 pages); https://doi.org/10.3332/ecancer.2017.781
7. H. Monma, Y. Iida, T. Moritani, T. Okimoto, R. Tanino, Y. Tajima and H. Mamoru, Chloroquine augments TRAIL-induced apoptosis and induces G2/M phase arrest in human pancreatic cancer cells, *PLoS One* **13**(3) (2018) e0193990 (16 pages); https://doi.org/10.1371/journal.pone.0193990
8. R. Cao, W. Fan, L. Guo, Q. Ma, G. Zhang, J. Li, X. Chen, Z. Ren and L. Qiu, Synthesis and structure--activity relationships of harmine derivatives as potential antitumor agents, *Eur. J. Med. Chem.* **60** (2013) 135–143; https://doi.org/10.1016/j.ejmech.2012.11.045
9. G. D. Kim, Harmine hydrochloride triggers G2/M cell cycle arrest and apoptosis in HCT116 cells through ERK and PI3K/AKT/mTOR signaling pathways, *Prev. Nutr. Food Sci.* **26**(4) (2021) 445–452; https://doi.org/10.3746/pnf.2021.26.4.445
10. N. S. R. S. Mota, M. R. Kviecinski, K. B. Felipe, V. M. A. S. Grinevicius, T. Siminski, G. M. Almeida, R. C. Zeferino, C. T. Pich, D. W. Filho and R. C. Pedrosa, β-carboline alkaloid harmine induces DNA damage and triggers apoptosis by a mitochondrial pathway: study *in silico*, *in vitro* and *in vivo*, *Int. J. Funct. Nutr.* **1**(1) (2023) (12 pages); https://doi.org/10.3892/ijfn.2020.1
11. D. Shahinas, G. MacMullin, C. Benedict, I. Crandall and D. R. Pillai, Harmine is a potent antimalarial targeting Hsp90 and synergizes with chloroquine and artemisinin, *Antimicrob. Agents Chemother.* **56**(8) (2012) 4207–4213; https://doi.org/10.1128/AAC.00328-12
12. S. Kumar, A. Singh, K. Kumar and V. Kumar, Recent insights into synthetic β-carbolines with anti-cancer activities, *Eur. J. Med. Chem.* **142** (2017) 48–73; https://doi.org/10.1016/j.ejmech.2017.05.059
13. D. Jiangkun, D. Wenjia, S. Uwe and W. Junru, β-Carboline alkaloid monomers and dimers: occurrence, structural diversity, and biological activities, *Eur. J. Med. Chem.* **157** (2018) 622–656; https://doi.org/10.1016/j.ejmech.2018.08.027
14. S. Aaghaz, K. Sharma, R. Jain and A. Kamal, β-Carbolines as potential anticancer agents, *Eur. J. Med. Chem.* **216** (2021) Article ID 113321 (25 pages); https://doi.org/10.1016/j.ejmech.2021.113321
15. B. Luo and X. Song, A comprehensive overview of β-carbolines and its derivatives as anticancer agents, *Eur. J. Med. Chem.* **224** (2021) Article ID 113688 (42 pages); https://doi.org/10.1016/j.ejmech.2021.113688
16. J. P. Soni, Y. Yeole and N. Shankaraiah, β-Carboline-based molecular hybrids as anticancer agents: a brief sketch, *RSC Med. Chem.* **12**(5) (2021) 730–750; https://doi.org/10.1039/d0md00422g
17. A. Kamboj, B. Sihag, D. S. Brar, A. Kaur and D. B. Salunke, Structure activity relationship in β-carboline derived anti-malarial agents, *Eur. J. Med. Chem.* **221** (2021) Article ID 113536 (15 pages); https://doi.org/10.1016/j.ejmech.2021.113536
18. P. Kushwaha, V. Kumar and B. Saha, Current development of β-carboline derived potential antimalarial scaffolds, *Eur. J. Med. Chem.* **252** (2023) Article ID 115247 (15 pages); https://doi.org/10.1016/j.ejmech.2023.115247
19. Q. Zhao and G. Huang, *Anticancer Hybrids,* in *Design of Hybrid Molecules for Drug Development* (Ed. M. Decker), 1st ed., Elsevier, Amsterdam 2017, pp. 193–218.
20. G. Poje, L. Pessanha de Carvalho, J. Held, D. Moita, M. Prudêncio, I. Perković, T. Tandarić, R. Vianello and Z. Rajić, Design and synthesis of harmiquins, harmine and chloroquine hybrids as potent antiplasmodial agents, *Eur. J. Med. Chem.* **238** (2022) Article ID 114408 (15 pages); https://doi.org/10.1016/j.ejmech.2022.114408

21. 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**(2) (2019) 233–248; https://doi.org/10.2478/acph-2019-0019
22. G. Barbosa-Lima, A. M. Moraes, A. S. Araújo, E. T. da Silva, C. S. de Freitas, Y. R. Vieira, A. Marttorelli, J. C. Neto, P. T. Bozza, M. V. N. de Souza and T. M. L. Souza, 2,8-*Bis*(trifluoromethyl)quinoline analogs show improved anti-Zika virus activity, compared to mefloquine, *Eur. J. Med. Chem.* **127** (2017) 334–340; https://doi.org/10.1016/j.ejmech.2016.12.058
23. M. Marinović, G. Poje, I. Perković, D. Fontinha, M. Prudêncio, J. Held, L. Pessanha de Carvalho, T. Tandarić, R. Vianello and Z. Rajić, Further investigation of harmicines as novel antiplasmodial agents: synthesis, structure-activity relationship and insight into the mechanism of action, *Eur. J. Med. Chem.* **224** (2021) Article ID 113687 (20 pages); https://doi.org/10.1016/j.ejmech.2021.113687
24. M. Marinović, I. Perković, D. Fontinha, M. Prudêncio, J. Held, L. Pessanha de Carvalho, T. Tandarić, R. Vianello, B. Zorc and Z. Rajić, Novel harmicines with improved potency against *Plasmodium*, *Molecules* **25**(19) (2020) Article ID 4376 (19 pages); https://doi.org/10.3390/molecules25194376
25. M. Marinović, *Amide-type Harmicines as Potential Antimalarial Agents*, doctoral dissertation, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb 2023, pp 104.
26. J. Held, T. Gebru, M. Kalesse, R. Jansen, K. Gerth, R. Müller and B. Mordmüller, Antimalarial activity of the myxobacterial macrolide chlorotonil A, *Antimicrob. Agents Chemother.* **58**(11) (2023) 6378–6384; https://doi.org/10.1128/AAC.03326-14
27. H. Noedl, J. Bronnert, K. Yingyuen, B. Attlmayr, H. Kollaritsch and M. Fukuda, Simple histidine-rich protein 2 double-site sandwich enzyme-linked immunosorbent assay for use in malaria drug sensitivity testing, *Antimicrob. Agents Chemother.* **49**(8) (2005) 3575–3577; https://doi.org/10.1128/AAC.49.8.3575-3577.2005
28. R Core Team, *R: A language and environment for statistical computing version 4.1.3*. R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/; last access December 10, 2022.
29. T. J. Maher and D. A. Johson, *Receptors and Drug Action*, in *Foye's Principles of Medicinal Chemistry* (Ed. T. L. Lemke, D. A. Williams, S. W. Zito, Ph. D. Roche, F. Victoria), 6th ed., Lippincott, Williams and Wilkins, Philadelphia, 2007.
30. A. K. Ghosh and M. Brindisi, Urea derivatives in modern drug discovery and medicinal chemistry, *J. Med. Chem.* **63**(6) (2020) 2751–2788; https://doi.org/10.1021/acs.jmedchem.9b01541
31. K. Pavić, M. Beus, G. Poje, L. Uzelac, M. Kralj and Z. Rajić, Synthesis and biological evaluation of harmirins, novel harmine-coumarin hybrids as potential anticancer agents, *Molecules* **26**(21) (2021) Article ID 6490 (19 pages); https://doi.org/10.3390/molecules26216490