**Introduction**

Malaria, caused by parasites of the genus *Plasmodium*, is one of the most important life-threatening infectious diseases in humans. According to World Health Organization, 241 million malaria cases and 627 000 deaths were reported in 2020, mainly in tropical and subtropical regions. Over the past two decades, malaria cases and deaths have declined worldwide, but progress is now stagnating due to the widespread emergence of multidrug-resistant strains. Several strategies are currently being used in parallel to combat this disease, including vector control methods, vaccine development, and novel drugs. RTS,S/AS01 is the leading malaria vaccine, but has shown only moderate efficacy in preventing clinical *P. falciparum* malaria. In addition, first-line treatments, including artemisinin-based combination therapies (ACTs), are also at risk due to the emergence of resistant *P. falciparum* strains in Southeast Asia and Africa, so there is an urgent need to discover and develop new drugs with different mechanisms of action. One attractive approach commonly used in drug discovery is molecular hybridization. A hybrid combining two or more bioactive molecules offers the potential to increase the efficacy of each bioactive molecule and overcome drug resistance. In this work, harmine/β-carboline and cinnamic acid derivatives (CADs), were covalently linked, resulting in harmicines. Harmine is a naturally occurring β-carboline alkaloid and a potent and selective inhibitor of *P. falciparum* Hsp90. Cinnamic acid and its derivatives are also natural products whose numerous biological activities, including antimalarial activity, have been extensively reported. In this work, the synthesis, characterization and biological evaluation of harmicines were reported. In addition, detailed QSAR study was performed, leading to a predictive model that enabled the design and synthesis of new harmicines**.**

**Materials and methods**

The preparation of the amide-type harmicines (AT) was straightforward and involved: 1) synthesis of the primary β-carboline amines **6**, **12**, **18**, **21**, and **24** and 2) coupling reactions with cinnamic acid and various CADs. The β-carboline amines **6**, **12**, and **18** in positions C-1, C-3, and O-6, respectively, were prepared *via* a multistep reaction pathway. Pictet-Spengler condensation of 1) tryptamine with 2,2-dimethoxyacetaldehyde, 2) tryptophan methyl ester with acetaldehyde dimethyl acetal, 3) 5-methoxytryptamine with acetaldehyde dimethyl acetal and subsequent oxidation of the tetrahydro-β-carboline intermediates resulted in the preparation of the corresponding β-carbolines **2**, **9** and **15**, respectively. The acetal group of β-carboline **2** was hydrolyzed in a CH3COOH/H2O mixture, and the obtained aldehyde **3** was reduced with LiAlH4 to give alcohol **4**. The ester groups of β-carbolines **9a** and **9b** were reduced under similar reaction conditions, yielding alcohol **10**. Alcohols **4** and **10** reacted with 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (ADMP) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give azides **5** and **11**, respectively. Reduction of azides with H2/Pd/C gave rise to amines **6** and **12**. The ether group of β-carboline **15** was hydrolyzed in a CH3COOH/HBr mixture. Subsequently, the obtained phenol **16** was alkylated with BocNH(CH2)2Br in the presence of Cs2CO3 and tetrabutylammonium hydrogen sulfate (TBAHS) to give the Boc-protected amine **17**. The latter was converted to amine **18** after the removal of the Boc protecting group in HCl. The synthesis of amines in the positions O-7 and N-9 was carried out by the alkylation of harmole (obtained by hydrolysis of harmine in a CH3COOH/HBr mixture) or harmine with BocNH(CH2)2Br in the presence of Cs2CO3. The resulting Boc-protected amines **20** and **23** were converted to amines **21** and **24** as described previously.

For the preparation of carbamate- and ureido-type harmicines, CADs were converted into corresponding alcohols and amines. First, CADs were converted to acyl chlorides **26b-k** using SOCl2 and DMF in toluene, which were immediately reduced with NaBH4, either in a mixture of THF and MeOH, giving alcohols **27b-d**, or in a mixture of diethyl ether and MeOH, yielding alcohols **27e-k**. Cinnamyl alcohols **27b**,**c** were then converted to azides **29b**,**c** with ADMP in the presence of DBU and reduced to amines **30b**,**c** with LiAlH4. Reaction of BtcCl and β-carboline amine **24** in the presence of TEA in DCM gave harmine benzotriazolide (**31**).

All chemicals were obtained from commercial sources. The progress of the chemical reactions was monitored by thin-layer chromatography (TLC). The synthesized products were purified by extraction, column chromatography, trituration with diethyl ether/petroleum ether and fully characterized by 1H and 13C NMR, IR and MS.

Harmicines’ antimalarial activities against the erythrocytic and hepatic stages of the *Plasmodium* life cycle and cytotoxicity against the hepatocellular carcinoma cell line (HepG2) were investigated. Antimalarial activity against the erythrocytic stage was evaluated *in vitro* against two *P. falciparum* laboratory strains: 3D7 (CQ-sensitive) and Dd2 (CQ-resistant), using the histidine-rich protein-2 assay (HRP-2), with CQ and harmine as positive controls. Antimalarial activity against hepatic stages of the *Plasmodium* life cycle was evaluated *in vitro* against hepatocellular carcinoma cells (Huh7) infected with *P. berghei* using a bioluminescence assay, with primaquine (PQ) and harmine as positive controls. AT harmicines were initially tested at two concentrations, 1 and 10 µM. Cytotoxicity against HepG2 was evaluated using the neutral red assay. For safety evaluation, a selectivity index (SI) was calculated for each compound tested as a fraction of the ratio between the *IC*50 values for HepG2 and the *Pf*3D7 strain.

In addition, a QSAR study was performed to correlate the biological activity with structural and physicochemical properties of AT harmicines, *i.e.* the molecular descriptors using multiple linear regression (MLR) technique. The molecular descriptors for 40 synthesized AT harmicines were generated using the online platform Chemicalize.com, the web tool Swissadme.ch and the software programs MarvinSketch, ACD/ChemSketch, Mnova and ChemDraw Professional. A total set of molecular descriptors were statistically processed in R 4.0.3 using RStudio 1.3.1093 and the libraries "tidyverse," "ggplot2," "readxl," and "caret." First, the initial set of molecular descriptors was reduced by removing descriptors with "0" and constant values for each compound. Then, the correlation matrix was calculated and descriptors with correlation coefficient > 0.95 and < -0.95 were removed. A reduced set of 67 molecular descriptors was subjected to a stepwise forward regression procedure to determine which combination of descriptors was most strongly correlated with the logarithm of the *IC*50 value against the *Pf*3D7 strain. The predictive model was constructed by selecting two descriptors, isoelectric point (pI) and Abraham descriptor E (Abr E) based on the calculated statistical parameters (R2, coefficient of determination; Ra2, adjusted coefficient of determination; p value; F value; MAE, mean absolute error; and RMSE, root mean square error). Internal validation of the constructed model was performed with 10-fold cross-validation. Subsequently, the constructed model was used to calculate the predictive log *IC*50 values of 356 newly designed harmicines.

**Results and discussion**

AT harmicines **7**, **13**, **19**, **22**, and **25** were prepared by coupling reactions of cinnamic acid/CADs and β-carboline-based amines **6**, **12**, **18**, **21**, and **24**, respectively, using HATU and DIEA in DCM. The target hybrids were obtained in good to excellent yields. CT harmicines were prepared in the position N-9 by reacting β-carboline-based amine **24**, CDI, and cinnamyl alcohols **27a-k** using two different approaches. Preparation of CT harmicines **28a-d** started with a reaction of amine **24** and CDI in DMF, followed by the *in situ* reaction of the obtained precursor with cinnamyl alcohols **27a-d**. On the other hand, in the synthetic pathway leading to CT harmicines **28e-k**, in the first step cinnamyl alcohols **27e-k** reacted with CDI. After isolation of the obtained precursor, reaction with amine **24** gave rise to CT harmicines in low to moderate yields. Reaction of cinnamyl amines **30b**,**c** and harmine benzotriazolide **31** gave UT harmicines **32b**,**c** in moderate yield.

The results of the *in vitro* screening of harmicines' antiplasmodial activity against the erythrocytic stage of two *P. falciparum* strains (*Pf*3D7 and *Pf*Dd2) showed that all compounds exert higher activity than the parent compound harmine (*IC*50 values in low micromolar and submicromolar concentrations), with the exception of AT harmicines (**7a-h**) in the position C-1, which were inactive at the highest concentration tested. In general, harmicines were more active against *Pf*3D7 strain. The most active compounds among all harmicines were AT harmicines in the position N-9 (**25b-e**,**i**,**j**), which showed at least two orders of magnitude stronger activities (*IC*50 = 0.04 – 0.09 µM) against the *Pf*3D7 strain, than the parent compound harmine. AT harmicines in the position C-3 were the least active against both strains, while O-6 and O-7 AT harmicines showed good to moderate activities, except α-CH3-substituted AT harmicine in the position O-7 (**22h**), which showed the weakest activity among all harmicines. In the series of CT harmicines, *p*-NO2-substituted harmicine **28k** showed the strongest activities against both strains (*IC*50 (*Pf*3D7) = 0.36 ± 0.001 µM and *IC*50 (*Pf*Dd2) = 0.33 ± 0.04 µM). Among UT harmicines, *m*-CF3-substituted harmicine **32c** exhibited two-fold stronger activity against *Pf*3D7 (*IC*50 = 0.22 ± 0.04 µM) than the *p*-propoxy-substituted harmicine **32b** (*IC*50 = 0.42 ± 0.0004 µM).

On the other hand, the results of harmicines’ antiplasmodial activity screening against hepatic stages of *P. berghei* were modest. As expected, all AT harmicines, except **7a** and **7b**, were more active at 10 µM. The marked cytotoxicity to Huh7 cells observed with most of the harmicines precluded the determination of the corresponding *IC*50 values. Only *m*-CF3-substituted AT harmicine in the position N-9 (**25i**) was selected for *IC*50 determination. The *IC*50 value obtained was 4.3-fold lower than the *IC*50 value of the reference drug primaquine (1.94 ± 0.68 µM versus 8.4 ± 3.4 µM).

The results of the cytotoxicity assay showed that AT harmicines in the position O-6 **19e-g** and CT harmicines **28a**,**c** were not cytotoxic at all in the highest concentration tested. The least cytotoxic compound with *IC*50 = 350.31 ± 13.02 µM was unsubstituted AT harmicine in the position N-9 (**25a**). The most selective compound was AT harmicine **25i** (SI = 1105), followed by **25b** (*m*-F-substituted AT harmicine, SI = 773). Notably, the latter harmicines were among the most active compounds.

The QSAR model for prediction of antiplasmodial activity (log *IC*50 against the *Pf*3D7 strain) was constructed as follows: the initial set of molecular descriptors was reduced and after applying forward stepwise regression method followed by selecting two descriptors (pI and Abr E), the linear model was obtained. The quality of the constructed QSAR model was evaluated based on various statistical parameters (R2 = 0.89, Ra2 = 0.88, MAEMLR = 0.248, RMSEMLR = 0.310, F-test = 148.4). The coefficient of determination and the adjusted coefficient of determination indicate the goodness of fit, and both values were greater than the required criteria (R2 > 0.6). The constructed QSAR model was also validated by 10-fold cross-validation and by calculating statistical parameters: Q2 = 0.85, MAECV = 0.282, RMSECV = 0.355. The value of Q2 was greater than the required value of 0.5, indicating high predictive power of the model obtained. For confirmation, a series of 356 novel harmicines was designed, and their log *IC*50 values were calculated. Further, we created and synthesized a focused library of novel AT (**25k-p**), CT (**28a-k**), and UT (**32b**,**c**) harmicines, and evaluated their antiplasmodial activities against the erythrocytic stage of *P. falciparum*.

**Conclusion**

In this thesis we represent design, synthesis, *in vitro* evaluation of antiplasmodial activity as well as cytotoxicity and QSAR study of novel harmicines. By applying molecular hybridization approach, *i.e.* conjugation of harmine with various CADs, we have successfully prepared hybrid compounds harmicines. The most of harmicines exhibited pronounced antiplasmodial activities against the erythrocytic stage of *P. falciparum*, which was higher than that of the parent compound harmine. In addition, structures and biological activities of 40 novel AT harmicines served as a basis for the creation of QSAR model, followed by the prediction, selection, and synthesis of novel AT, CT, and UT harmicines. The most active and selective harmicines were AT harmicines prepared in the position N-9 of the b-carboline ring. Thus, those harmicines could be considered as lead compounds for further development of novel antimalarial agents.