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# Quantitative structure retention/activity relationships of biologically relevant 4-amino-7-chloroquinoline based compounds

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ABSTRACT

The chromatographic behaviour of series of 4-amino-7-chloroquinoline (4,7-ACQ) based compounds was studied by reversed-phase thin-layer chromatography (RPTLC) with binary mobile phases containing water and the organic modifiers, DMSO or acetone. The lipophilicity of the studied compounds was determined by extrapolation of retention parameters $R_M$ to pure water content in mobile phase. In order to obtain some basic insight into the chromatographic behaviour and structural features of investigated compounds, PCA was performed on both chromatographic data ($R_M$ values) and calculated 2D and 3D structural descriptors. Both QSRR and QSAR models were built by means of the partial least squares (PLS) statistical method. It was found that descriptors which encode hydrophobic (dispersive) interactions have positive influence on retention, while influence of descriptors encoding polar interactions was negative. According to the obtained PLS model for inhibition of botulinum neurotoxin serotype A light chain, hydrophobic interactions influence positively on the mechanism of action of the investigated 4,7-ACQ, while polar interactions are less favoured. Contrary, the results of PLS modelling of activity against *Plasmodium falciparum* strains (W2, D6 and TM91C235) indicate that higher polarity of 4,7-ACQ contribute to their higher antimalarial activity.



## 1. Introduction

Lipophilicity is an important physico-chemical parameter which influences the partition of a substance in biological media and, hence, is essential for its biological activity, describing both pharmacokinetic and pharmacodynamic aspects of drug action. Membrane permeability is a key determinant in pharmacokinetic behaviour of drugs, especially of absorption, distribution, metabolism and excretion. Pharmacodynamics refers to the relationship between drug concentration at the site of action and the resulting effect depends on its ability to bind to the receptor [1–4]. On one side, the lipophilicity of the compound is the result of hydrophobic interactions involving the formation of cavities, hydrophobic and dispersive forces, and on the other side, of the polar interactions involving electrostatic interactions and hydrogen bonds. Depending on the observed system, hydrophobic and polar interactions may have different or even opposite contribution to the total value of lipophilicity. In this way, the lipophilicity of a substance is at least, in part, a system property [5].

The logarithm of *n*-octanol-water partition coefficient, usually notified as log*P*, is the most commonly used for quantitative expression of lipophilicity and can be experimentally determined by direct (shake flask method) and indirect methods (chromatographic and electrochemical techniques) [6].

Shake-flask procedure is a standard method for determination of lipophilicity, but faces problems like poor reproducibility, long time of analysis and limiting of determination log*P* value to 4 [6]. Reversed-phase liquid chromatography (RPLC) is an alternative method which has proven to simulate octanol–water partitioning and is considered as a popular alternative for lipophilicity assessment [7]. Taking into account the same retention mechanism in HPLC and thin-layer chromatography (TLC), in numerous instances the fast, simple, and reliable RP-TLC method can be used for determination of lipophilicity. In RP-TLC a standard descriptor of lipophilicity, the retention factor $R_M^0$, is obtained by extrapolation of plots of $R_M$ values *versus* the concentration of the organic modifier in mobile phase to zero concentration of the modifier (pure

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water), usually conducted by using the Soczewinski–Wachmeister linear Eq. (1) [8]:

$$R_M = R_{M^0} + S\phi \quad (1)$$

where $\phi$ is the volume fraction of organic component of mobile phase.

The mechanisms responsible for the retention of a substance on a lipophilic (hydrophobic) stationary phase against the aqueous mobile phase are those which determine its ability to cross cell membranes as well as its transfer in the blood stream [9]. The advantages of this method for determining the lipophilicity compared to the direct shake-flask method are a broader lipophilicity range application with a small amount of the studied compound which does not have to be pure. Additionally, it is possible a reproductive simultaneous analysis of several compounds with broad range of lipophilicity in a short time [10].

Alternative methodologies such as computational methods can be used for estimation of log*P* values. Most of them add up the log*P* contribution from each fragment (fragment based) or even atoms (atom-based) and then all substructure contributions are added up using contribution terms and correction factors to obtain the final log*P* values. After performing the novel comparison/ranking approaches based on the sum of ranking differences (SRD) and the generalised pair correlation method (GPCM), it was found that chromatographic lipophilicity measures obtained under typical reversed-phase conditions outperform the majority of computationally estimated log*P*s [11]. The computational methods have significant advantages because they do not require expensive instrumentation, reagents and laborious experimental work, but there are some limitations to their use because the calculations are not reliable for log*P* of zwitterionic, tautomeric and charged compounds as well as for strong hydrogen-bonding compounds [11,12].

The lipophilicity is most frequently used parameter in quantitative structure-retention relationship (QSRR), as well as quantitative structure-activity relationship (QSAR) studies [13]. Through these mathematical relationships it is possible to describe how molecular structure, represented by the descriptors, affects the retention of substance, *i.e.* its biological activity. Also, it is possible to predict retention of structurally similar substances, and hence to suggest the synthesis of new compounds with desired characteristics. The construction of predictive QSRR models involves three steps [14] (a) the acquisition of a sufficiently large set of retention data of analytes covering possible structural diversities within a defined group of substances, (b) the calculation of structural descriptors of the analytes, such as topological, three-dimensional (3D; geometrical and electronic) and physicochemical, (c) the correlation of the retention data (dependent variable) with the calculated descriptors (independent variables) using appropriate statistical methods. Partial least square (PLS) is a widely used modelling technique in QSRR and QSAR studies which is helpful for analysis of strongly collinear data. In addition, principal component analysis (PCA) is useful in highlighting similarities among objects and variables.

Developing a new drug from original idea to the launch of a finished product is a complex process which is very expensive and takes many years. A number of early processes are necessary to identify molecules which possess suitable characteristics to make acceptable drugs [15]. QSAR and QSRR studies have crucial importance in medicinal chemistry and are valuable to evaluate and better understand absorption, distribution, metabolism and excretion related properties as well as the pharmacodynamics and toxicological profile of drugs.

Aminoquinolines have been a backbone of antimalarial drugs since the 1940s. Since the emergence of resistance to 4-aminoquinoline drugs, such as chloroquine and amodiaquine, efforts have focused on identifying new aminoquinoline analogues that do not share cross-resistance with chloroquine. These efforts led to the identification of 4-aminoquinoline analogues, wherein their *N*-alkyl side chains provide an excellent site for further modifications [16–18].

Recently, a series of 4-amino-7-chloroquinoline based compounds (4,7-ACQ) were synthesized as a botulinum neurotoxin serotype A light chain inhibitors with significant antiprotozoal activity [19]. The aminoquinoline component was tethered to the benzene, monosubstituted benzenes, pyridine, or monosubstituted pyridines via shorter methylene bridges containing an aliphatic amine. The purpose for evaluating monosubstituted benzene and pyridines species was to probe molecular diversity with respect to the effects of ring substituents on inhibitory potencies. It has been found that these compounds inhibit both the botulinum neurotoxin serotype A light chain (BoNT/A LC) metalloprotease and protozoa *Plasmodium falciparum* (*P.f.*). As the result of this research, phenyl derivative with two carbon atoms in methylene bridge, so as bromophenyl derivative with three carbon atoms in methylene bridge have high potencies and are proposed as good candidates for future *in vivo* testing against drug resistant *P.f.* strains.

Because it is considered that the same basic intermolecular interactions determine the behaviour of substance in both biological and chromatographic environment [20] the above mentioned series of eighteen 4,7-ACQ based compounds were analysed using reversed-phase thin layer chromatography. We were interested in determination of their retention parameters, $R_M{}^0$, as measure of lipophilicity and selection of the most influential descriptors regarding retention. Since it has been found that 4,7- ACQ-based compounds inhibit the BoNT/A LC and malaria by different mechanisms of action, *i.e.* inhibition of enzymatic activity (in the case of the presented BoNT/A LC inhibitors) and inhibition of hemozoin formation (in the case of the presented antimalarial agents), we were also interested in determination the most influential descriptors regarding these biological activities. By interpreting the descriptors in the obtained regression models, it is expected to gain some insight into the factors that are likely to govern the retention and biological activity of the investigated 4,7-ACQ and understand which interactions play an important role during these processes which could be used in design strategy to improve compound efficiency. Compounds' 2D and 3D molecular descriptors, in conjunction with corresponding physicochemical parameters and biological data, were used for QSRR and QSAR studies by application of multivariate statistical data analysis (PCA and PLS regression).

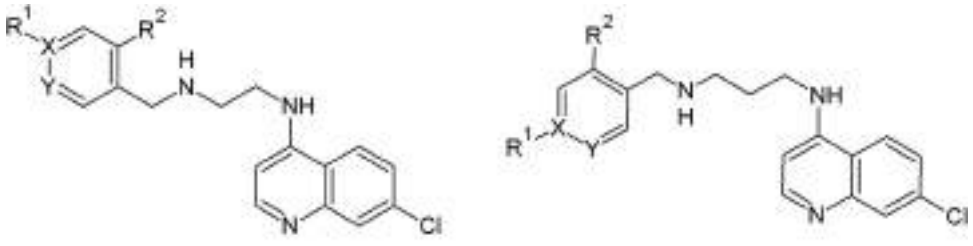
## 2. Materials and methods

### 2.1. Reagents and chemicals

The synthesis and characterization of the studied compounds were previously reported [19]. The structures of the investigated compounds and their biological activities are summarized in Fig. 1 and Table 1. Dimethyl sulfoxide (DMSO) and acetone were of analytical-grade purity and purchased from Merck (Darmstadt, Germany). Water was purified using Millipore Simplicity 185 S.A., 67120, water purification system (Molshem, France).

### 2.2. Thin-layer chromatography

Reversed-phase thin-layer chromatography was performed using the vertical developing chamber (CAMAG Muttenz, Switzerland) on 10 × 10 cm plates covered with octadecyl modified silica. The investigated substances were dissolved in methanol, and the plates were spotted with 0.5 μL aliquots of freshly prepared solutions (C ~0.5 mg/mL). Before development chromatographic chamber was equilibrated for 15 min with vapours of the mobile



**1:** X=C, Y=CH, $R^1$=$R^2$=H
**2:** X=C, Y=CH, $R^1$=$R^2$=H
**3:** X=C, Y=CH, $R^1$=Bu-*t*, $R^2$=H
**4:** X=C, Y=CH, $R^1$=Bu-*t*, $R^2$=H
**5:** X=C, Y=CH, $R^1$=OMe, $R^2$=H
**6:** X=C, Y=CH, $R^1$=OMe, $R^2$=H
**7:** X=C, Y=CH, $R^1$=F, $R^2$=H
**8:** X=C, Y=CH, $R^1$=F, $R^2$=H
**9:** X=C, Y=CH, $R^1$=Br, $R^2$=H
**10:** X=C, Y=CH, $R^1$=Br, $R^2$=H
**11:** X=N, Y=CH, $R^2$=H
**12:** X=N, Y=CH, $R^2$=H
**13:** X=C, Y=N, $R^1$=H, $R^2$=H
**14:** X=C, Y=N, $R^1$=H, $R^2$=H
**15:** X=C, Y=N, $R^1$=H, $R^2$=Cl
**16:** X=C, Y=N, $R^1$=H, $R^2$=Cl
**17:** X=C, Y=N, $R^1$=H, $R^2$=OMe
**18:** X=C, Y=N, $R^1$=H, $R^2$=OMe

**Fig. 1.** The structures of the investigated compounds.

**Table 1**
BoNT/A LC% inhibition, antimalarial activity for 4-amino-7-chloroquinolines.

| Comp. | Biological activity | | | |
|---|---|---|---|---|
| | BoNT/A LC (%)[b] | Antimalarial activity $IC_{50}$, nM | | |
| | | W2[c] | D6[d] | TM91C235[e] |
| **1** | 52.00 | 10.90 | 6.41 | 7.38 |
| **2** | 51.83 | 52.17 | 17.19 | 39.90 |
| **3** | 47.00 | 42.40 | 24.19 | 24.73 |
| **4** | 50.28 | 48.44 | 21.73 | 31.94 |
| **5** | 18.00 | 14.33 | 3.22 | 16.09 |
| **6** | 14.00 | 56.20 | 6.18 | 50.58 |
| **7** | 68.00 | 45.78 | 26.38 | 55.18 |
| **8** | 50.09 | 49.44 | 13.09 | 29.37 |
| **9** | 69.00 | 9.47 | 4.10 | 9.98 |
| **10** | 68.00 | 5.93 | 3.95 | 6.18 |
| **11** | 24.00 | 375.05 | 60.70 | 204.11 |
| **12** | 42.29 | 204.06 | 8.65 | 76.41 |
| **13** | 25.00 | 225.38 | 53.39 | 108.70 |
| **14** | 39.89 | 260.08 | – | 73.43 |
| **15** | 23.00 | 140.81 | 63.75 | 83.34 |
| **16** | 35.87 | 72.97 | 25.67 | 30.97 |
| **17** | 19.00 | 19.00 | 67.09 | 7.29 |
| **18** | 43.25 | 86.87 | 4.76 | 53.24 |

[a] Ref. [19].
[b] % inhibition calculated at 20 μM conc.
[c] *P. falciparum* African D6 clone.
[d] *P. falciparum* Indochina W2 clone.
[e] *P. falciparum* multidrug resistant C235strain (Thailand).

phase being used. All solvents used were of analytical-grade purity. The following isocratic chromatographic systems were used: RP-18 W F254s (Art. 5559, Merck, Darmstadt, Germany) with mobile phase in range of 75–95 Vol% DMSO in water (increment 5%) and the range of 55–95 Vol% acetone in water (increment 10%). Development distance was 5 cm. Detection of individual zones was performed under UV light (254 nm). All experiments were performed at ambient temperature (22 ± 2 °C).

### 2.3. Calculation of the structural descriptors and lipophilicity

The lipophilicity of the investigated 4-amino-7-chloroquinoline based compounds was predicted using the *KOWWIN* (EPIWEB 4.1 software) and *mi*logP (www.molinspiration.com). Lipophilicity parameter *KOWWIN* is mixed atom-based and fragment contribution method, while *mi*logP is substructure-based method, based on fragment contribution [21]. The calculated *mi*logP and *KOWWIN* values are given in Table S1 (Supplementary data).

All structures were built using the Maestro 10.1 from Schrödinger Suite 2015-1 (Maestro, version 10.1, Schrödinger, LLC, New York, NY, 2015.). For prediction of protonation state at pH 7.0 in water as a solvent, the results of p$K_a$ prediction using Epik module version 3.1 (Epik, version 3.1, Schrödinger, LLC, New York, NY, 2015.) were used. Since all investigated compounds contain aliphatic and aromatic nitrogen, degree of protonation depends on the environment in which compounds exhibit their activities. Inhibition of BoNT/A LC takes place under physiological conditions and dominant form is monoprotonated. On the other side, in calculations considering malaria, due to low pH in food vacuole [22], all structures were used in their diprotonated form, with protonated nitrogen in aliphatic and quinoline moiety.

QikProp version 4.3 (QikProp, version 4.3, Schrödinger, LLC, New York, NY, 2015.) was used for the calculation of physically significant molecular descriptors and pharmaceutically relevant properties. Single point calculations using the RM1 method [23] from Semiempirical NDDO module of Scrodinger Suite 2015-1 was used for semiempirical parameters. The calculated structural descriptors are given in Table S2 and Table S3 (Supplementary data).

### 2.4. Multivariate statistical analysis and modelling

Principal component analysis and PLS regression were performed by a PLS_Toolbox software package (v. 5.7 Eigenvectors Inc.) for MATLAB (v. 7.8.0 R2009) (MathWorks, Natick, MA, USA).

PCA was carried out as an exploratory data analysis by using a singular value decomposition algorithm (SVD) and a 0.95 confidence level for $Q$ and $T^2$ Hotelling limits for outliers. Before the PCA, autoscaling was performed on chromatographic data and structural descriptors. Projection of objects on the PCs defines new coordinates (scores), while other informative quantities are the loadings representing the PC directions relative to the original variables. Scores and loading plots in the space of the significant PCs permit easy visualization of similarities/dissimilarities within the group of objects and relationship among the variables [24].

The PLS method was employed by means of SIMPLS algorithm without forcing orthogonality constrains to the model which are

supposed to condense Y-block variance into the first few latent variables.

In the QSRR analysis the validation of the models was performed with leave-one out procedure.

In the QSAR analysis the validation of the models was performed by the venetian blinds procedure, with seven splits for BoNT inhibition, for activity against D6 by the venetian blinds procedure, with eight splits, for activity against W2 by the venetian blinds procedure, with four splits, and for activity against TM91C235 strain by the venetian blinds procedure, with four splits.

Some compounds having large residuals were excluded as regression outliers. In BoNT/A PLS model excluded molecules share common structural characteristics, the presence of metoxy-group (**5**, **6**, **17**, **18**). Compounds **12** and **18** were excluded as outliers in PLS model with activity against D6 strain. In PLS model with W2 strain, only compound **17** is excluded as outlier. When activity against TM91C235 strain was used as dependent variable, compounds **7** and 1**7** were excluded as outliers in PLS model.

The quality of the regression fits was monitored with the $R^2_{cal}$, (cumulative sum of squares of the *Ys* explained by all extracted components) $R^2_{CV}$ (cumulative fraction of the total variation of the *Ys* that can be predicted by all extracted components), and $R^2_{pred}$ (cumulative fraction of the total variation of the *Ys* that can be predicted by test components). These values have to be as high as possible, and with root mean-square errors of calibration, cross-validation, and prediction, *RMSEC*, *RMSECV* and *RMSEP*, respectively, have to be as low as possible, with the lowest difference between them. Low value of *RMSEC* is desirable but if the high values of *RMSECV* are present at the same time, it indicates the poor predictability of the calibration model. It is considered that QSRR model is predictive, if the following conditions are satisfied: $R^2_{cal} > 0.6$, $R^2_{CV} > 0.5$ [25].

The predictive power of the QSRR models were checked by dividing the entire data set of 18 compounds into two subsets: training set consisting of the 13 randomly selected solutes (**1**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **12**, **13**, **14**, **17**, **18**) and test set composed of the rest of the studied compounds (**2**, **10**, **11**, **15**, **16**).

## 3. Results and discussion

### 3.1. Chromatographic behaviour of 4-amino-7-chloroquinoline

The investigated compounds (Table 1) possess 4-amino-7-chloroquinoline (4,7-ACQ) component tethered via a short nitrogen containing methylene bridge to a single aromatic ring (either benzene, substituted benzenes, pyridine, or substituted pyridines).

The chromatographic behaviour of 4,7-ACQ derivatives was studied in two chromatographic systems (RP-18 silica – DMSO/water and RP-18 silica – acetone/water).

The obtained results are depicted in Tables S4 and S5 (Supplementary data) which show the dependence of $R_F$ values on the composition of the mobile phase used. The $R_M{}^0$ values of the 4,7-ACQ derivatives obtained by extrapolation of $R_M$ values to 0 Vol% of organic modifier are summarized in Tables S6 and S7 (Supplementary data).

Although the both used organic modifiers, DMSO and acetone, are polar and aprotic solvents, some differences between them are evident: DMSO belongs to the third group while acetone is in sixth group of Snyder's triangle of selectivity, acetone is less polar ($P' = 5.1$) then DMSO ($P' = 7.2$) and acetone is localized ($m = 0.87$) while DMSO is not.

The reversed-phase retention behaviour of the investigated compounds is based on several types of interactions. The presence of highly polarized π electronic system in the aminoquinoline part of molecule and in single aromatic ring offers the possibility for dipolar interactions between the molecules and both stationary and mobile phase. The methylene bridge can be involved in hydrophobic interactions with stationary phase, while nitrogen atom in methylene bridge and in aminoquinoline part act as proton acceptor with proton donating molecules of the mobile phase. Since the all of the investigated substances possess the identical aminoquinoline part of molecule, the difference in their retention behaviour is based on both the number of methylene groups in the compounds' methylene bridge (two or three) and the structure of aromatic ring.

Increasing the number of C-atoms in methylene bridge led to the substance's stronger retention due to the stronger hydrophobic interactions with the stationary phase and increasing lipophilicity.

The observed retention generally indicated that compounds containing phenyl- and substituted phenyl substructure were more retained on the stationary phase in relation to their pyridine analogues. In group of *N*-benzyl substituted derivatives, introducing the *tert*-butyl group at *para*-position of the phenyl ring drastically reduced polarity of compounds **3** and **4** with regard to compounds **1** and **2** without substituent at this position. As a consequence, the retention of *tert*-butyl derivatives strengthened due to their stronger interactions with the non-polar stationary phase. In both groups, benzene and pyridine derivatives, the introduction of metoxy-group influenced the increasing polarity and the weaker retention of compounds due to the existence of new centre for hydrogen bonding with the molecules of polar mobile phase. The introduction of halogen atom into the structure of the parent compound resulted in an increase of lipophilicity of the analogues. Also, bromo-group as more voluminous substituent contributed to the stronger retention compared to the fluoro-group.

It was observed that the retention of the investigated compounds decreased with increasing concentration of the organic modifier in the mobile phase. The greatest $R_M{}^0$ values have compounds **3** and **4** in both systems used. In general, it was observed that the $R_M{}^0$ values show linear dependence on the concentration of an organic component in the mobile phase. The existence of high linear dependence indicate a possibility of using the $R_M{}^0$ values for lipophilicity assessment of 4,7-ACQ. This assumption is also supported by the existence of statistically significant linear correlation between $R_M{}^0$ and log*P* values calculated by the use of different software packages. For chromatographic system containing DMSO, linear relationship between $R_M{}^0$ and $\log P_{KOWWIN}$, *i.e. mi*log*P* was characterized with $r = 0.9512$ ($t = 12.32$, $t_{cr(0.05;16)} = 2.12$), *i.e.* $r = 0.9463$ ($t_{(0.05;16)} = 11.71$), respectively. For chromatographic system containing acetone, linear relationship between $R_M{}^0$ and $\log P_{KOWWIN}$, *i.e. mi*log*P* was characterized with $r = 0.9859$ ($t = 23.54$, $t_{cr(0.05;16)} = 2.12$), *i.e.* $r = 0.9840$ ($t = 22.10$), respectively.

The greater values of lipophilicity parameters determined by TLC are obtained with DMSO, which is more polar solvent in comparison to acetone. Since the retention in reversed-phase chromatography is partly based on adsorption where the molecules of an analyte are competing with molecules of the mobile phase on the surface of the stationary phase, the more polar mobile phase is, the less it can occupy active centres on the surface of the stationary phase. As a consequence, the retention of the analytes increases. Concerning the strength of the mobile phase, in reversed-phase chromatography an increase of the polarity of mobile phase results in a decrease of the eluting strength.

### 3.2. Exploratory data analysis

In order to obtain some basic insight into the chromatographic behaviour of the studied compounds, a PCA was first performed on chromatographic data ($R_M$ values) and resulted in four-component model that explains 99.21% of the total variance. The first principal component (PC1) accounted for 90.67% of data variance, the

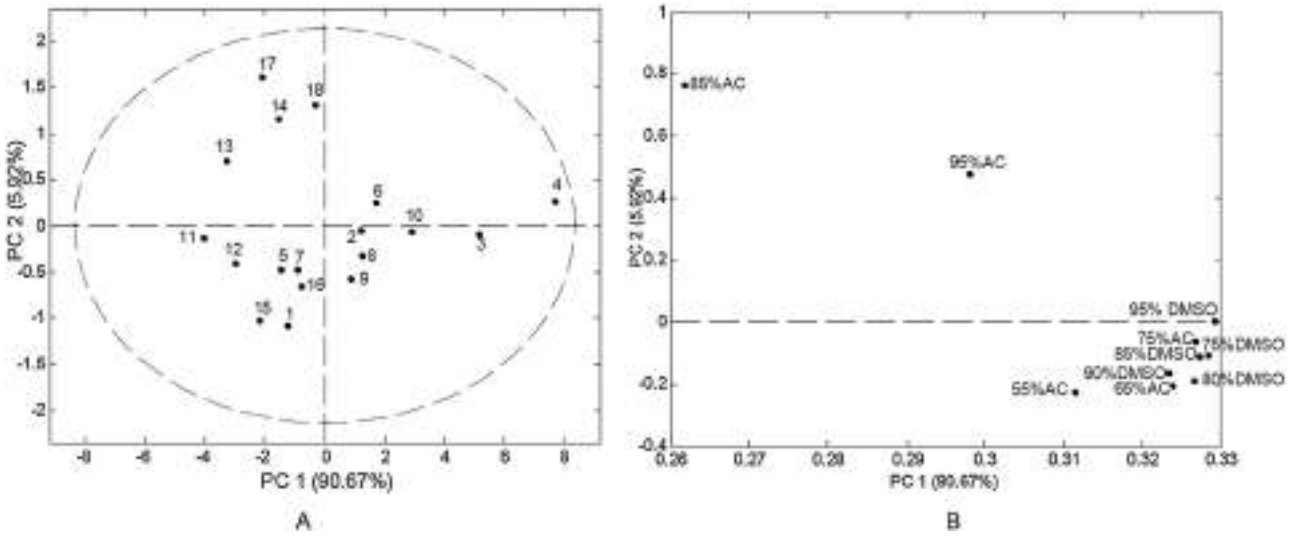



**Fig. 2.** Score (A) and loading plots (B) of retention parameters.

second (PC2) 5.92%, the third 1.64%, and the fourth 0.99% of data variance. Percent variance captured by PCA model for $R_M$ values is presented in Table S8 (Supplementary data). Score values, *i.e.* their mutual projections, for PC1 and PC2 are shown in Fig. 2A and the mutual projections of the loading vectors in Fig. 2B. All the data lay inside the Hotelling $T^2$ ellipse, suggesting that there were no outliers. The obtained results show that the separation of the investigated compounds is based on their lipophilicity which increases along the PC1 axis. Compounds containing pyridine (**11–14**) or substituted pyridine ring (**15–18**) as well as compound with benzene (**1**) or substituted benzene ring (**5**, **7**) are located on the negative segment of the axis. The rest of the compounds, with more pronounced lipophilic features, containing benzene (**2**) or substituted benzene ring (**3**, **4**, **6**, **8**, **9**, **10**) are positioned on the positive segment of the graph. The most negative value of PC1 has compound **11**, and the most positive value of PC1 has compound **4**.

The graph of loadings (Fig. 2B), does not reveal any significant influence of the mobile-phase composition along the PC1 direction. Chromatographic systems containing 85 and 95% acetone in mobile phase have the highest positive impact on PC2, while the rest of chromatographic systems are positioned close to zero PC2.

The PCA was calculated on set of structural descriptors for pH 7 with structures of 4,7-ACQ with only aliphatic nitrogen protonated, and on set of structural descriptors which are calculated for pH 7 with structures of 4,7-ACQ with aliphatic, as well as chloroquine moiety nitrogen protonated. In the first case the PCA resulted in two-component model that explains 67.62% of total variance, while in second case PCA resulted in four-component model that explains 86.71% of total variance. Percent variance captured by PCA model is given in Table S9 (Supplementary data). Corresponding score and loading plots are presented in Fig. 3A, B, C and D, respectively. According to the Hotelling $T^2$ ellipse all the data lay inside ellipse, suggesting that there were no outliers. Based on such results it can be concluded that PC2 is discriminative for clustering of the investigated 4,7-ACQ according to their polarity in case of calculated structural descriptors which correspond to the monoprotonated 4,7-ACQ, while PC1 is discriminative for their clustering in case of calculated structural descriptors which correspond to the diprotonated structure of 4,7-ACQ. This clustering is very similar to those performed on retention data.

The graph of loadings for set of descriptors calculated for monoprotonated 4,7-ACQ (Fig. 3B) indicates that descriptors which encode polar properties of compounds like FISA, QPlogPw, acceptHB, CIQPlogS and ACxDN^.5/SA exhibit the highest positive impact on the PC2 score. They are followed by quantum-chemical descriptors with positive (EA, IP, Dipole, Dipole y, HOMO) and negative impact on PC2 (Dipole z, Dipole x, Dipole, dip^2/V, Jm, LUMO). The most of descriptors which encode hydrophobic (dispersive) interactions have negative impact on PC2 and the highest negative influence on the PC2 score has QPPCaco. The graph of loadings for set of descriptors calculated for diprotonated 4,7-ACQ (Fig. 3D) indicates that structural descriptors which encode polar properties of compounds have negative impact on PC1, and descriptors which encode hydrophobic (dispersive) interactions have positive impact on PC1. The quantum-chemical descriptors are positioned between these two groups of descriptors. Percent variance captured by this PCA model is given in Table S10 (Supplementary data).

### 3.3. Modelling of retention and biological activity

Retention parameters and biological activity of the studied 4-amino-7-chloroquinolines were used in QSRR and QSAR analysis in order to determine the factors affecting their retention and biological activities. The structural descriptors are calculated for pH 7, when only aliphatic nitrogen is protonated and for pH 7 with protonated aliphatic and quinoline nitrogen. External validation data set provides real predictive ability, but independent and representative, external validation set is rare [26]. In the absence of the external validation, the predictive power of the QSRR models can be performed by cross-validation, which simulates how well the model predicts new data, or by dividing the investigated set of compounds into training and test set. It was not possible to form a unique training and test set to perform this procedure in QSAR analysis for accurate prediction of biological activity. The proposed QSAR models may qualitatively indicate descriptors that play an important role in the activity exhibited by 4,7-ACQ derivatives.

According to Wold [26] the descriptors that have the greatest influence on retention and biological activity were determined using variable importance in the projection (VIP) scores. The variables with VIP scores higher than 1.5 are of highest importance and are included in the final model. These descriptors are summarized in Tables 2 and 3 in descending order of their coefficient values with notification of the sign of their contribution on the dependent variable.

Both QSRR models have high statistical performances, indicating their high predictive power. The PLS model which refers to the DMSO resulted in four latent variables, while the model which refers to the acetone resulted in five latent variables. In Tables S11

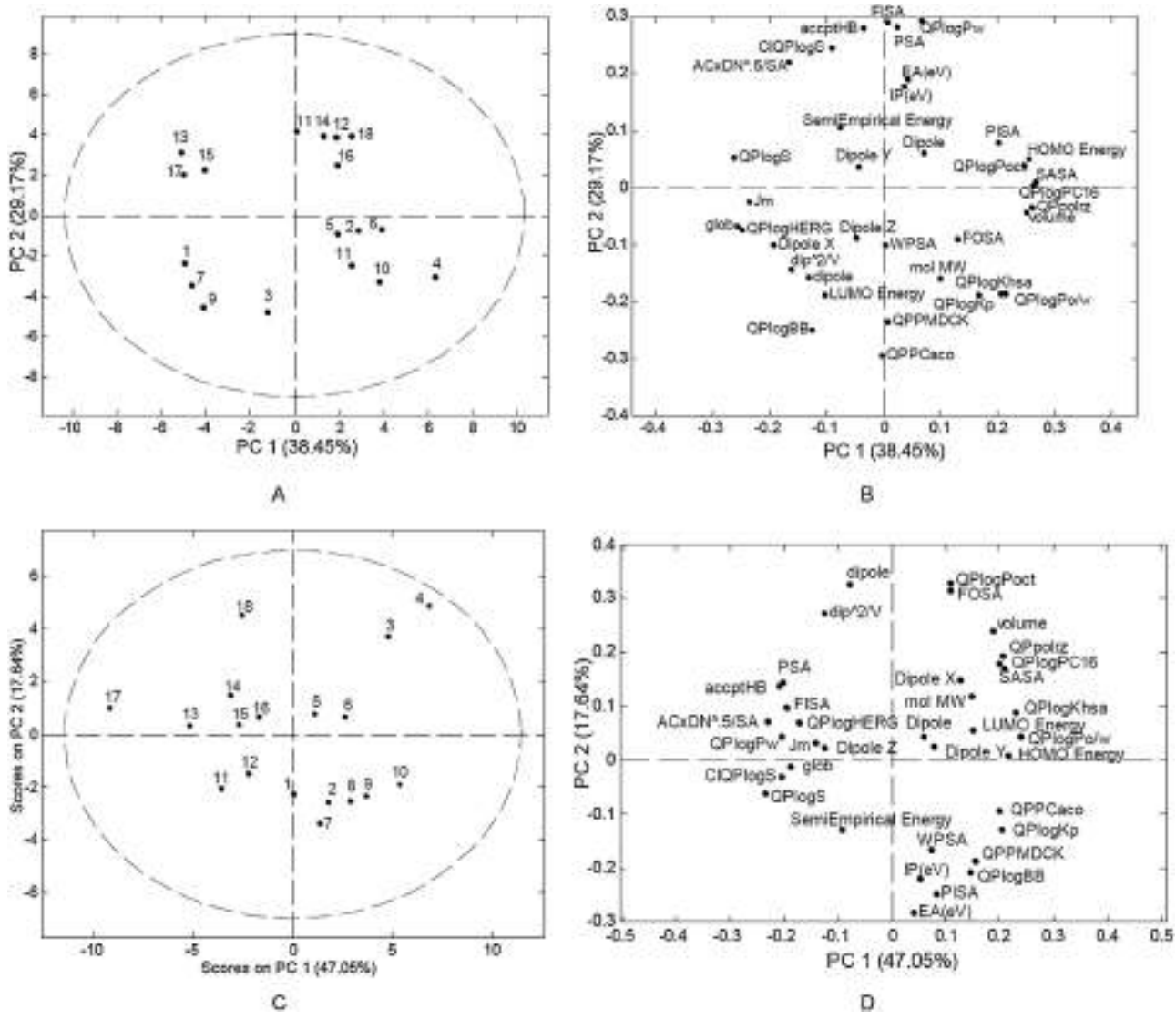



**Fig. 3.** Score (A) and loading plots (B) of structural descriptors calculated for monoprotonated, and score (C) and loading plots (D) of structural descriptors calculated for diprotonated structures of 4,7-ACQ derivatives.

**Table 2**
Results obtained *via* PLS analysis of retention data.

| Mobile phase | *RMSEC* | *RMSECV* | *RMSEP* | $R^2_{cal}$ | $R^2_{CV}$ | $R^2_{pred}$ | Descriptors |
|---|---|---|---|---|---|---|---|
| DMSO–water | 0.102 | 0.340 | 0.265 | 0.993 | 0.926 | 0.977 | ClQPlogS(−), QPlogKhsa(+), QPlogpo/w(+), molMW(+), ACxDN^.5/SA(−), FOSA(+), QPPCaco(+), acceptHB(−), QPlogKp(−) |
| Acetone water | 0.019 | 0.117 | 0.231 | 0.999 | 0.978 | 0.967 | ClQPlogS(−), acceptHB(−), ACxDN^.5/SA(−), QPPCaco(+), FISA(−), QPlogKhsa(+), QPlogPw(−), QPlogpo/w(+), PSA(−), molMW(+), QPlogKp(−), QPPMDCK(+) |

**Table 3**
Results obtained *via* PLS analysis of biological activities.

| Dependent variable | Statistical performance of the model | | | | Molecular descriptors |
|---|---|---|---|---|---|
| | *RMSEC* | *RMSECV* | $R^2_{cal}$ | $R^2_{CV}$ | |
| BoNT/A LC% | 0.049 | 0.083 | 0.903 | 0.735 | acceptHB(−), ACxDN^.5/SA(−), dipole(+), QPPCaco(+), QPPMDCK(+), FISA(−), ClQPlogS(−), dip^2/V(+), QPlogKp(+), QPlogPw(−), $QPlogP_{o/w}$(+) |
| D6 $IC_{50}$ | 0.108 | 0.277 | 0.941 | 0.645 | HOMO(−), QPlogKp(−), dip^2/V(−), QPPCaco(−), PSA(−)FISA(+), |
| W2 $IC_{50}$ | 0.089 | 0.252 | 0.970 | 0.768 | HOMO (−), dip^2/V(−), QPlogBB(−), QPPMDCK(−), QPPCaco(−), FISA(+), dipole (−), ClQPlogS(+), QPlogKp(−) |
| TM91C235 | 0.063 | 0.203 | 0.976 | 0.807 | dip^2/V(−), dipole (−), QPPMDCK(−), QPlogBB (−) |

and S12 are given cumulated $R^2X$ and $R^2Y$ values. It can be seen that first latent variable has the highest percent variance captured by PLS regression, with a slight increase upon extracting the next latent variable. A more detailed view of the results of PLS modelling in QSRR analysis, which include plots of the measured versus predicted retention parameters, plots of the variables versus VIP scores, and plots of the coefficients of parameters in model is given in Figs. S1 and S2 (Supplementary data).

The QSRR models with VIP variables higher than 1.5 indicate that the most relevant descriptors which influence the retention are: the conformation-independent predicted aqueous solubility (CIQPlogS), prediction of binding to human serum albumin (QPlogKhsa), predicted octanol/water partition coefficient (QPlogpo/w), predicted water/gas partition coefficient (QPlogPw), predicted skin permeability (QPlogKp), predicted apparent Caco-2 cell permeability (QPPCaco), predicted brain/blood partition coefficient (QPlogBB), index of cohesive interaction in solids (ACxDN^.5/SA), estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (acceptHB), hydrophobic component of the total solvent accessible surface area (FOSA), hydrophilic component of the total solvent accessible surface area (FISA), molecular weight of the molecule (molMW), predicted apparent MDCK cell permeability in nm/sec, (QPPMDCK), total solvent-accessible volume (volume) and van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms (PSA). Descriptor FOSA is included only in PLS model where the dependent variable is obtained with mobile phase DMSO-water and descriptors FISA, PSA and QPPMDCK are included only in model containing acetone.

Considering the sign of the regression coefficients, it can be concluded that descriptors QPlogpo/w, QPlogKhsa, QPPCaco, FOSA, QPPMDCK, molMW and volume, which encode the hydrophobic (dispersive) interactions of the investigated compounds with stationary phase, have positive influence on $R_M{}^0$ values. Positive influence of the volume and molMW on retention indicate that bulky compounds are strongly retained on the stationary phase. An increase of molecular volume leads to increasing cavity formation energy in water, the larger the solute, the greater the energy demand to make cavity and the lower the solubility in water. These parameters determine transport characteristics of the molecules, such as intestinal absorption or blood-brain barrier penetration [27].

Descriptors which have negative sign of regression coefficients (CIQPlogS, ACxDN.5/SA, QPlogPw, acceptHB, FISA, QPlogKp) are related with the ability of compounds to participate in hydrogen bonding and accepting abilities while the polar surface area (PSA) reflects electrostatic and polarization interactions between the solute and the solvent. Polar interactions which encode these descriptors are based on tendency of a molecule to be dissolved in the polar mobile phase and therefore to less retain. For molecules to penetrate the blood-brain barrier, these parameters should be small [28]. They are important when substance passes through the cell membrane and are crucial for drug absorption, including intestinal absorption, bioavailability and blood-brain barrier penetration.

Since the investigated 4,7-ACQ have proved to be potent dual BoNT/A LC and *P. f.* inhibitors (Table 1), quantitative-structure–activity relationship (QSAR) was performed.

The structure–activity data shown in Table 1 indicates that polar substituent on aromatic C(4) atom reduced BoNT/A LC inhibitory efficacy (**5** and **6** *versus* **1** and **2**, respectively). The unfavourable electrostatic interactions of enzyme residue(s) and polar groups on aromatic components were additionally confirmed for pyridine derivatives (**11–18**) which are less potent than benzene derivatives. Among investigated compounds bromophenyl derivatives **9** and **10** have proved to be most potent inhibitors of BoNT/A LC. Additionally, *in vitro* antimalarial activities of tested ACQs revealed that compounds **9** and **10** were the most potent.

In investigated set of compounds was not found any regularity between number of carbon atoms in methylene bridge and biological activity.

In the QSAR analysis PLS model for BoNT inhibition resulted in two latent variables, For antimalarial activity against D6, PLS model resulted in four latent variables, for activity against W2 with six ones, and for TM91C235 strain with seven latent variables. Cumulated $R^2X$ and $R^2Y$ values are given in Tables S13 and S16 (Supplementary data).

In QSAR analysis, the best statistical performances has PLS model which refers to the activity against TM91C235, while the model relating to the activity against D6 cell line has the worst performances. A more detailed view of the results of PLS modelling which refer to the biological activities is given in Figs. S3–S6 (Supplementary data).

In contrast to the parameters which influence the retention and can be useful for elucidating interactions that occur during the transport of compounds to their sites of action, QSAR models indicate molecular descriptors that account for the interactions of compounds with specific sites within the cells. The logC values of minimal inhibitor concentration were used as dependent variables, and calculated structural descriptors were used as independent variables in PLS models. For BoNT/A LC inhibition were used only structural descriptors calculated at pH 7 when only aliphatic nitrogen is protonated. Due to low pH in food vacuole, the structural descriptors for *P.f.* strains D6, W2 and TM91C235 were calculated from the diprotonated structures with aliphatic as well as quinoline moiety nitrogen protonated.

The descriptors which are included in the final model for BoNT/A LC are almost the same and with the same sign of regression coefficients as descriptors affecting retention, *i.e.* lipophilicity of the investigated compounds. Molecular weight (mol MW) and volume are not included in this model, while descriptor which represents the square of the dipole moment divided by the molecular volume (dip^2/V) and dipole with positive contribution are included in PLS model for BoNT/A LC. It means that hydrophobic interactions influence positively on the mechanism of BoNT inhibition, while polar interactions are less favoured. These results indicate that less polar substituents in molecular structure of 4,7-ACQ are favourable for higher inhibition of BoNT/A LC according to their possibility to take part in hydrophobic interactions. Therefore, compounds (**5**, **6**, **17**, **18**) with polar metoxy-group, exhibited low inhibition of BoNT/A LC and had to be removed as the outliers from the PLS model.

Traditional antimalarial drug chloroquine [17], (CQ) exerts its antimalarial effect by using pH gradient difference between red blood cells and parasite digestive vacuoles. Chloroquine is a diprotic weak base and, at physiological pH (~7.4), can be found in its unprotonated (CQ), monoprotonated ($CQ^+$) and diprotonated ($CQ^{++}$) forms. The uncharged chloroquine is the only membrane permeable form of the molecule and it freely diffuses into the erythrocyte up to the digestive vacuole (DV). In this compartment, chloroquine molecules become protonated and, since membranes are not permeable to charged species, the drug accumulates into the acidic digestive vacuole where it is believed to bind haematin, a toxic byproduct of the haemoglobin proteolysis, preventing its incorporation into the haemozoin crystal. The free haematin seems to interfere with the parasite detoxification processes and thereby damage the plasmodium membranes [29]. Chloroquine sensitive parasites (CQS) accumulate much more chloroquine in the DV than chloroquine resistant strains (CQR). It has been suggested that 4,7-ACQ-based drugs may also act as inhibitors of oxidative [30] and gluthatione-mediated [31] heme degradation. However, it is important to note that the heme detoxification pathway is not

directly involved in CQ resistance. Rather, it has been reported that CQ resistance is associated with mutations in drug transporters (PfCRT, Pgh1, and PfMRP) that affect drug accumulation in the parasite, either by reducing drug uptake or increasing drug efflux or both [32,33].

According to the PLS models the activity against *P.f.* strains D6 and W2 are affected by descriptors which encode polar interactions in the positive manner (FISA, CIQPlogS) and descriptors which encode hydrophobic interactions in the negative manner (HOMO, QPlogBB, QPCaco, QPlogKp, QPPMDCK, dip^2/V). Based on the calculated VIP scores for both PLS models, HOMO descriptor is the most significant. Model descriptors for activity against TM91C235 strain include only quantum-chemical descriptors (dip^2/V and dipole) and descriptors which encode hydrophobic interactions (QPPMDCK and QPlogBB). Both groups of descriptors have negative influence on activity against this pathogen. The regression vectors of descriptors which are included in PLS models of retention and activity against all three *P.f.* pathogens are with the opposite sign.

Recently we studied the most relevant descriptors influencing antimalarial activity of mixed 1,2,4,5 tetraoxanes which exhibited impressive *in vitro* and some of them *in vivo* antimalarial activity. PLS models on qualitative level indicate that lipophilicity and energy of HOMO orbitals influence the antimalarial activities in a positive manner, while descriptors which encode polar properties of mixed 1,2,4,5 tetraoxanes influence their activities in a negative manner [34].

The obtained results are also distinguished from results obtained in QSRR/QSAR study [35] of series of thirteen 1,7-bis(aminoalkyl) diazachrysene (1,7-DAAC) derivatives which inhibit three unrelated pathogens (BoNT/A LC, *P.f.* malaria, and Ebola filovirus) [36] *via* three different mechanisms of action. The resulted QSR(P)R indicated the importance of descriptors related to the hydrophobicity of the molecules (*e.g.* predicted partition coefficients and hydrophobic surface area) and QSAR models for describing inhibition of BoNT/A LC and antimalarial activity against three *P.f.* strains include overall compound polarity, electron density distribution, and proton donor/acceptor potential [35]. According to the PLS models, the structural descriptors which encode these characteristics of 1,7-DAAC derivatives are with the opposite sign of regression coefficients in comparison to the investigated 4,7-ACQ. These results, and the fact that in final PLS models for 4,7-ACQ are included descriptors which are related to the polarity and quantum-chemical descriptors indicate on different binding mode of 1,7-DAAC and 4,7-ACQ derivatives.

## 4. Conclusion

In the present paper two RP-TLC systems containing RP-18 silica gel and binary mixtures of water with acetone or DMSO as mobile phase were used in order to investigate the retention mechanism and to establish lipophilicity of series of eighteen 4-amino-7-chloroquinolines. The results obtained in this study let us conclude that retention mechanism of investigated compounds is based on hydrophobic interactions of the substances with non-polar components of stationary phase.

High values of correlation coefficients between $R_M{}^0$ values and calculated partition coefficients indicate that $R_M{}^0$ values can be a reliable alternative for determination of lipophilicity by classic shake-flask method.

According to PLS models, the descriptors which encode hydrophobic (dispersive) interactions (QPlogpo/w, QPlogKhsa, QPPCaco, FOSA, QPPMDCK, molMW and volume) of the investigated compounds with stationary phase have positive influence on $R_M{}^0$ values, while negative influence on $R_M{}^0$ values have structural descriptors related with the ability of compounds to participate in hydrogen bonding and accepting abilities, so as in electrostatic and polarization interactions between the solute and the stationary phase (CIQPlogS, ACxDN^.5/SA, QPlogPw, acceptHB, FISA, QPlogKp).

The proposed QSAR models may qualitatively indicate descriptors that play an important role in the activity exhibited by 4,7-ACQ derivatives. The descriptors which are included in the final model for inhibition BoNT/A LC are almost the same and with the same sign of regression coefficients as descriptors affecting retention, *i.e.* lipophilicity of the investigated compounds. Besides these, quantum-chemical characteristics of investigated 4,7-ACQ derivatives expressed as dip^2/V and dipole are important for inhibition BoNT/A LC. According to their possibility to take part in hydrophobic interactions, less polar substituents in molecular structure of 4,7-ACQ are favourable for higher inhibition of BoNT/A LC.

The PLS models of activity against all three *P.f.* pathogens also indicate the importance of quantum-chemical characteristics of 4,7-ACQ on their antimalarial activity.

HOMO descriptor is the most significant for activity against D6 and W2 *P.f.* strains. Besides this, it is noteworthy that according to the PLS models, the activity against these strains are affected by descriptors which encode hydrophobic interactions in the negative manner (QPlogBB, QPCaco, QPlogKp, QPPMDCK, dip^2/V) and descriptors which encode polar interactions in the positive manner (FISA, CIQPlogS).

Model descriptors for activity against TM91C235 strain include only quantum-chemical descriptors (dip^2/V and dipole) and descriptors which encode hydrophobic interactions (QPPMDCK and QPlogBB). Both groups of descriptors have negative influence on activity against this pathogen. Contrary to the BoNT inhibition, these results indicate that higher polarity of 4,7-ACQ contribute to their higher antimalarial activity.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2016.01.033.

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