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Quantitative Structure-Activity Relationship (QSAR) Study of a Series of Chalcone Derivatives Inhibiting Plasmodium Falciparum 3D7

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Abstract: This Quantitative Structure-Activity Relationship (QSAR) study was conducted using a series of twenty (20) chalcone derivatives with inhibitory activities against Plasmodium falciparum 3D7. The molecules were optimized at the B3LYP/LanL2DZ computational level, to obtain the molecular descriptors. This work was performed using the Linear Multiple Regression (LMR) method, the NonLinear Regression (NLMR) and the Artificial Neural Network (ANN) method. These tools allowed us to obtain three (3) quantitative models from the quantum descriptors that are, the overall softness (S), the bond lengths l(c=o) and l(c=c), and the polarizability (α). These models have good statistical performance. Among them, the ANN has a significantly better predictive ability R^2 =0.997; *RMCE* = 0.035; *F*= 3571.499. The external validation tests verify all the criteria of Tropsha *et al.* and Roy *et al.* Also, the applicability domain of this model determined from the levers shows that a prediction of the pIC50 of new chalcone derivatives is acceptable when its lever value is lower than 1.07. For the ANN method, the Ch19 molecule is certainly outside the applicability domain, but it is not an influential point for the model, because this derivative belongs to the validation set, and therefore was not used in the model development. The behavior of this molecule could be explained by its structural diversity.

Keywords: Plasmodium Falciparum 3D7, Chalcone Derivatives, RQSA, RNA, Applicability Domain

1. Introduction

Malaria is a potentially fatal disease caused by parasites of the genus Plasmodium transmitted to humans by the bites of female mosquitoes of the species Anopheles, known as "malaria vectors". There are five species of parasites that cause malaria in humans, two of which, Plasmodium falciparum and Plasmodium vivax, are the most dangerous [1]. According to the latest World Malaria Report, published in December 2020, there were 229 million cases of malaria in 2019, up from 228 million in 2018. There were an estimated 409.000 deaths from the disease in 2019, compared to 411,000 deaths in 2018 [2]. Resistance of malaria parasites belonging to the antimalarial species P. falciparum is a recurrent problem. This resistance is undermining malaria control efforts and reversing progress in child survival. The design of antimalarial drugs that are free of Plasmodium falciparum (pf) resistance remains an unmet challenge for the scientific community. In this context, Kumar et al [3] have shown in their study that a series of chalcone derivatives show inhibition on the Plasmodium falciparum strain 3D7. Chalcones, compounds belonging to the flavonoid family, have proven their efficacy in the therapeutic and medicinal field. The presence of the double bond and the carbonyl group in chalcones gives them several biological activities

[4]. Chalcones and derivatives present biological activities such as anticancer [5, 6], anti-inflammatory [7, 8], antimalarial [9, 10], antioxidant [11, 12], antimicrobial [13, 14], antihyperglycemic [15], antifungal [16], anti-HIV [17]. In our study, the IC50 inhibitory activity of chalcone derivatives against the p. falciparum 3D7 strain was used. Quantitative Structure-Activity Relationship (QSAR) study, is the method that correlates the molecular structure with a well-determined effect such as biological activity or chemical reactivity. It is increasingly used to reduce the excessive number of experiments, sometimes long, dangerous and costly in terms of time and money [18, 19]. It is in this perspective that the present manuscript was written in order to be able to set up a model to fight against the infection caused by malaria. All this contributes to the reduction of drug production costs [20, 21] and contributes to the protection of the environment. In general, the QSAR model is a function of one fifth (1/5) of the initial database. The overall objective of this work is to develop reliable models to explain and predict the antimalarial activity IC50 (median inhibitory concentration in µM) of a series of twenty (20) chalcone derivatives (Figure 1).

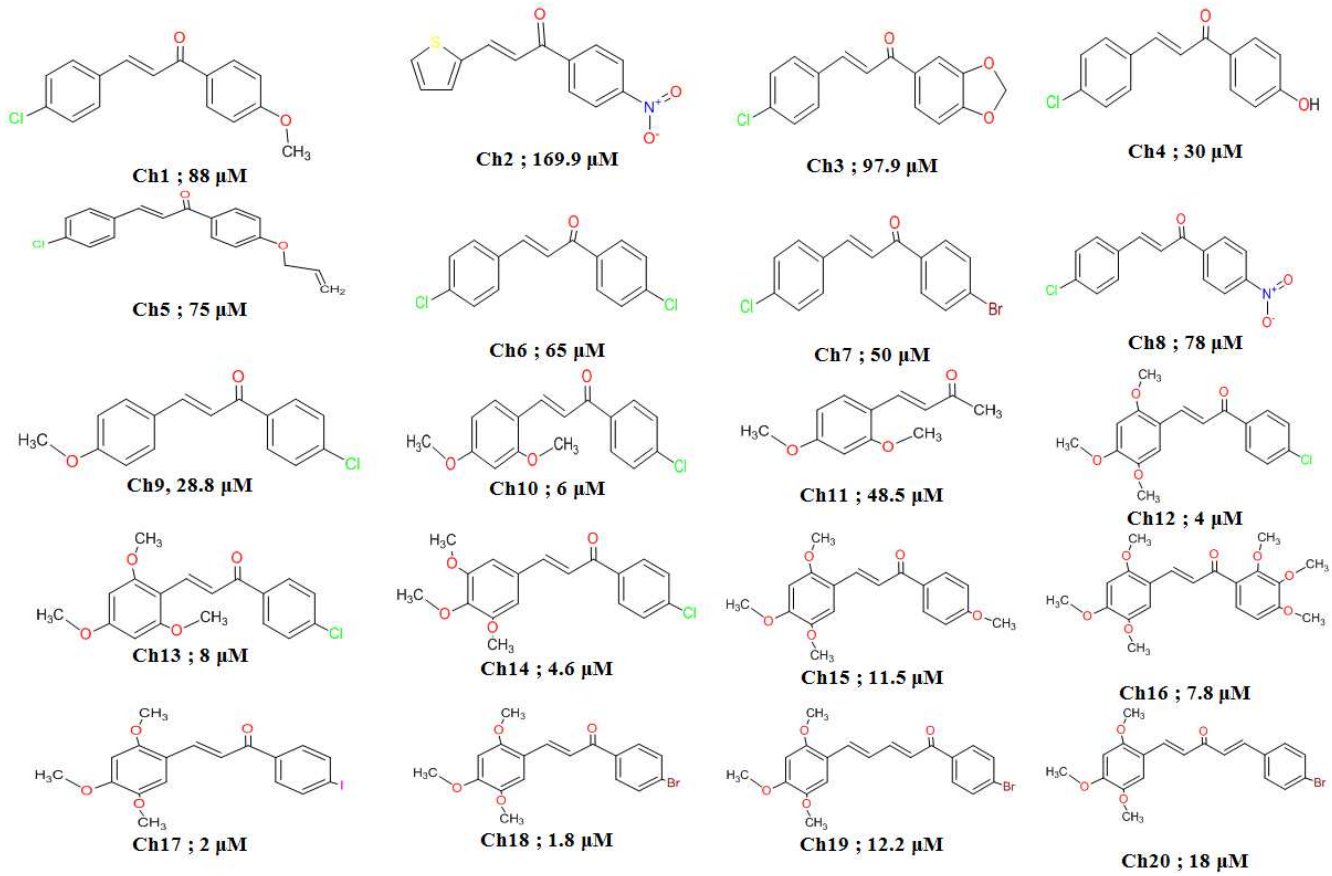

Figure 1. *Molecular structure, code and inhibitory concentration (IC50) of twenty-five (20) chalcone derivatives.*

2. Materials and Methods

2.1. Computational Level of Theory

In order to predict the antimalarial activity of chalcone derivatives quantum chemical calculations were performed using Gaussian 09 software [22]. DFT methods are generally known to generate a variety of molecular properties [23, 24, 25] in QSAR studies. These increase the predictability of QSAR models while reducing the computational time and cost implication in new drug design [26, 27]. The B3LYP/LanL2DZ level of theory was used to determine the molecular descriptors. The twenty (20) molecules used in this study have Inhibitory Concentration (IC) ranging from 1.8 to 169.9 µM. The median inhibitory concentration (IC50) is a measure of the effectiveness of a given compound in inhibiting a specific biological or biochemical function. Biological data are usually expressed as the opposite of the decimal-based logarithm of activity ($-\log_{10}(C)$) to obtain better mathematical values when structures are biologically active [28, 29]. The antibacterial activity will be expressed by the antibacterial potential pIC defined by equation (1):

$$pIC_{50} = -\log_{10}(IC_{50} * 10^{-6}) \quad (1)$$

Where IC_{50}, the inhibitory concentration in µM.

The modeling was done using two methods. The first method is multilinear regression which is implemented in Excel [30] and XLSTAT [31] spreadsheets. The second method is artificial neurons which is included in the JMP Pro software [32].

2.2. Used Molecular Descriptors

In order to develop our QSAR model, some theoretical descriptors were determined. In particular, the global softness, the bond length (l(c=o)), the bond length (l(c=c)), the polarizability (α).

Overall softness is the ability of an atom or molecule to retain an acquired charge. The lower the global softness of a system, the more it resists electron transfer and therefore the more stable it is. Its expression is given by the relation:

$$S = \frac{1}{\eta} \quad (2)$$

With:

$$\eta = \frac{I-AE}{2} = \frac{1}{2}(E_{LUMO} - E_{HOMO}) \quad (3)$$

The geometric descriptors used are the bond length l(c=o) and l(c=c) in Armstrong (A°) (Figure 2). These descriptors are illustrated in the figure below around the chalcone core.

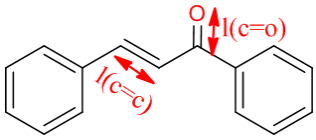

Figure 2. *Geometric descriptors of the chalcone derivatives used: the bond lengths (l(c=o) and l(c=c)) in Armstrong (A°).*

The polarizability designates a phenomenon caused by the moment of the electric charges of the atom. A molecule placed in an electric field E undergoes a deformation and acquires an induced dipole electric moment proportional to the field E, the polarizabilities are expressed in Å3. They have the dimension of a volume. The atomic polarizability increases with the size of the atoms [33].

$$\alpha = \varepsilon_0 \mu E \quad (4)$$

Where:

α: Polarizability coefficient.

ε_0: Dielectric constant.

μ = Induced dipole electric moment.

The calculation of the partial correlation coefficient between each pair of the set of descriptors is less than 0.7 (aij < 0.7), which means that these different descriptors are independent of each other [34, 35]. Table 1 shows the values of the partial correlation coefficients aij of these descriptors.

Table 1. *Correlation matrix between the different physico-chemical descriptors.*

Variables	S	l(c=o)	l(c=c)	α
S	1.0000			
l(c=o)	-0.4277	1.0000		
l(c=c)	0.1802	0.5273	1.0000	
α	-0.0387	0.2905	0.0179	1.0000

2.3. Estimation of the Predictive Capacity of a QSAR Model

The quality of a model is determined based on different statistical criteria of analysis including the coefficient of determination R^2, standard deviation RMCE, cross-validation correlation coefficients Q^2_{CV} and Fischer's F. R^2, S and F relate to the fit of calculated and experimental values. They describe the predictive ability within the limits of the model, and allow us to estimate the accuracy of the calculated values on the test set [36, 37]. As for the cross-validation coefficient Q^2_{CV}, it provides information on the predictive power of the model. This predictive power is called "internal" because it is calculated from the structures used to build the model. The correlation coefficient R^2 gives an evaluation of the dispersion of the theoretical values around the experimental values. The quality of the modeling is better when the points are close to the fitting line [38]. The fit of the points to this line can be evaluated by the coefficient of determination.

$$R^2 = 1 - \frac{\Sigma(y_{i,exp} - \hat{y}_{i,theo})^2}{\Sigma(y_{i,exp} - \bar{y}_{i,exp})^2} \quad (5)$$

Where:

$y_{i,exp}$: Experimental value of antimalarial activity

$\hat{y}_{i,theo}$: Theoretical value of antimalarial activity

$\bar{y}_{i,exp}$: Average value of the experimental values of antimalarial activity.

The closer the R² value is to 1, the more the theoretical and experimental values are correlated

The standard deviation RMCE is another statistical indicator used. It allows to evaluate the reliability and the precision of a model:

$$RMCE = \sqrt{\frac{\Sigma(y_{i,exp} - y_{i,theo})^2}{n-k-1}} \quad (6)$$

The Fisher F test is also used to measure the level of statistical significance of the model, i.e. the quality of the choice of descriptors making up the model.

$$F = \frac{\Sigma(y_{i,theo} - y_{i,exp})^2}{\Sigma(y_{i,exp} - y_{i,theo})^2} * \frac{n-k-1}{k} \quad (7)$$

The coefficient of determination of the cross-validation Q^2_{CV}, allows to evaluate the accuracy of the prediction on the test set. It is calculated using the following relation:

$$Q^2_{cv} = \frac{\Sigma(y_{i,theo} - \bar{y}_{i,exp})^2 - \Sigma(y_{i,theo} - y_{i,exp})^2}{\Sigma(y_{i,theo} - \bar{y}_{i,exp})^2} \quad (8)$$

Criterion of acceptance of a model

The performance of a mathematical model, for Eriksson et al [39], is characterized by a value of $Q^2_{cv} > 0.5$ for a satisfactory model, when for the excellent model $Q^2_{cv} > 0.9$. According to these authors, given a set of tests, a model will perform well if the acceptance criterion $R^2 - Q^2_{cv} < 0.3$ is met.

LOO (Leave-One-Out) cross-validation is a procedure that consists of excluding a molecule from the training set once, building a new model without this molecule and finally predicting the value of the dependent variable Y_{ipred}. The cross-validation correlation coefficient Q^2_{cv} (8) is calculated between the predicted values and the experimental values of the training set when each molecule has been removed once.

The model is considered to perform well if both acceptance criteria $R^2 > 0.6$ and $Q^2_{cv} > 0{,}5$ [40] are met with special care to the standard deviation σ which should be as small as possible [41].

According to Tropsha et al [42-44], for the external validation set, the predictive power of a model can be obtained from five criteria. These criteria are:

1) $R^2_{Test} > 0.7$,
2) $Q^2_{Cv\ Test} > 0.6$,
3) $|R^2_{Test} - R^2_0| \leq 0.3$,
4) $\frac{|R^2_{Test} - R^2_0|}{R^2_{Test}} < 0.1$ and $0.85 \leq k \leq 1.15$,
5) $\frac{|R^2_{Test} - R'^2_0|}{R^2_{Test}} < 0{,}1$ and $0.85 \leq k' \leq 1.15$

Furthermore, Roy and Roy [45], further refined the predictive ability of a QSAR model. They developed quantities r^2_m and Δr^2_m, called metric values. r^2_m determines the closeness between the observed activity and the prediction. The metric values r^2_m and Δr^2_m are calculated from the observed and predicted activities. Currently, these two different variants r^2_m and Δr^2_m, can be calculated for the test set (internal validation) or for the test set (external validation). A QSAR model is acceptable to these authors, if both of these criteria are met.

$$\overline{r^2_m} = \frac{(r^2_m + r'^2_m)}{2} > 0.5$$

$$\Delta r^2_m = |r^2_m - r'^2_m| < 0.2$$

Où $r^2_m = r^2 * \left(1 - \sqrt{(r^2 - r^2_0)}\right)$ et $r'^2_m = r^2 * \left(1 - \sqrt{(r^2 - r'^2_0)}\right)$

2.4. Statistical Analyses

Linear and Non-Linear Multiple Regressions (LMR and NLMR)

The statistical technique of Linear Multiple Regression (LMR) is used to study the relationship between a dependent variable (Property) and several independent variables (Descriptors). This statistical method minimizes the differences between the actual and predicted values. It was also used to select the descriptors used as input parameters in the multiple nonlinear regression (NLMR). As for the multiple non-linear regression (NLMR) analysis, it also allows to improve the structure-property relationship in order to quantitatively evaluate the property. It is the most common tool for studying multidimensional data. It is based on the following pre-programmed functions of XLSTAT:

$$y = a + (bx_1 + cx_2 + dx_3 + ex_4) + (fx_{12} + gx_{22} + hx_{32} + ix_{42}) \quad (9)$$

Where a, b, c, d,... represent the parameters and et, x_1, x_2, x_3, x_4,... represent the variables.

Artificial Neural Network (ANN)

Artificial neurons are an inspiration of the human biological neuron. To this end, they are made up of cells or neurons linked together by connections that allow them to send and receive signals from other cells. These neurons are mathematical models made up of several neurons, arranged in different layers. Generally, the network consists of three layers; an input layer, a hidden layer and an output layer, connected through a complex network [46, 47]. The most commonly used networks are the Multi-Layer Perceptrons (MLP) whose neurons are generally arranged on layers [48]. In this work, the artificial neural network was obtained using the 4-3-1 multilayer perceptron network, i.e., the network consists of five (4) neurons in the input layer, three (3) neurons in the hidden layer and one (1) neuron in the output layer. The output layer consists of a sigmoid function. The architecture of the applied ANN models is presented in (Figure 3).

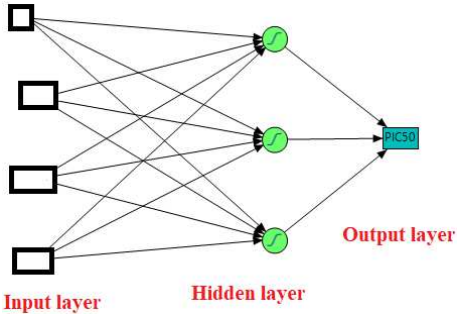

Figure 3. *Schematic of the structure of a multilayer perceptron.*

2.5. Applicability Domain (AD)

The applicability domain of a QSAR model is the physicochemical, structural, or biological space, in which the model equation is applicable to make predictions for new compounds [49]. It corresponds to the region of chemical space including the compounds in the training set and similar compounds, which are close in the same space [50]. Indeed, the model, which is built on the basis of a limited number of compounds, by relevant descriptors, chosen among many others, cannot be a universal tool to predict the activity of any other molecule with confidence. It appears necessary, even mandatory, to determine the AD of any QSAR model. This is recommended by the Organization for Economic Cooperation and Development (OECD) in the development of a QSAR model [51]. There are several methods for determining the domain of applicability of a model [50]. Among them, the approach used in this work is the leverage approach. This method is based on the variation of the standardized residuals of the dependent variable with the distance between the values of the descriptors and their mean, called leverage [52]. The h_{ii} are the diagonal elements of a matrix H called hat matrix. H is the projection matrix of the experimental values of the explained variable Y_{exp} into the space of the predicted values of the explained variable Y_{pred} such that:

$$Y_{pred} = HY_{exp} \quad (10)$$

H is defined by the expression (21):

$$H = X(X^tX)^{-1}X^t \quad (11)$$

The applicability domain is delimited by a threshold value of

the lever noted h*. In general, it is set at $3\frac{p+1}{n}$, where n is the number of compounds in the training set, and p is the number of descriptors in the model [53, 54]. For standardized residuals, the two limit values generally used are ±3σ, σ being the standard deviation of the experimental values of the quantity to be explained [55]: this is the "three-sigma rule" [56].

3. Results and Discussion

This QSAR study was conducted using a series of twenty (20) chalcone derivatives. These compounds were synthesized and tested on Plasmodium falciparum 3D7. The molecules were divided into two groups, fourteen (14) were used for the learning set and six (6) for the validation set. The objective of this part of the work is to model the antimalarial activity of chalcone derivatives from the descriptors. The values of the descriptors as well as those of the experimental biological activities of the molecules are listed in Table 2.

Table 2. *Physicochemical descriptors and experimental pIC_{50} of the learning and validation sets.*

MOLECULES	S	l(c=O)	l(c=c)	α	IC_{50}	pIC_{50}
Training Set						
Ch1	0.49610	1.26518	1.36033	206.06567	88.00000	4.05552
Ch2	0.61033	1.26277	1.36622	191.16400	169.90000	3.76981
Ch3	0.52656	1.26394	1.36064	204.86367	97.90000	4.00922
Ch5	0.49537	1.26510	1.36033	231.01400	75.00000	4.12494
Ch6	0.48363	1.26300	1.36126	196.45933	65.00000	4.18709
Ch7	0.48344	1.26303	1.36120	203.60867	50.00000	4.30103
Ch8	0.57505	1.26183	1.36241	207.88367	78.00000	4.10791
Ch11	0.50317	1.26131	1.36370	158.27400	48.50000	4.31426
Ch12	0.51986	1.26473	1.36526	239.17900	4.00000	5.39794
Ch13	0.51726	1.26671	1.36796	240.87567	8.00000	5.09691
Ch15	0.50382	1.26652	1.36410	248.05200	11.50000	4.93930
Ch16	0.50726	1.26644	1.36279	277.51567	7.80000	5.10791
Ch17	0.51916	1.26479	1.36521	254.10067	2.00000	5.69897
Ch19	0.56023	1.25706	1.35747	258.57933	12.20000	4.91364
Validation Set						
Ch4	0.48107	1.26485	1.36048	192.02100	30.00000	4.52288
Ch9	0.52103	1.26471	1.36405	209.34833	28.80000	4.54061
Ch10	0.52716	1.26588	1.36498	225.55667	6.00000	5.22185
Ch14	0.50594	1.26371	1.36162	237.98133	4.60000	5.33724
Ch18	0.51960	1.26478	1.36527	246.32200	1.80000	5.74473
Ch20	0.58774	1.27158	1.36403	314.78300	18.00000	4.74473

3.1. Multilinear Regression (LMR)

The equation of the QSAR model is presented below. The statistical indicators are given in Table 3.

$$pIC_{50}^{exp} = -10.16787 * \mathrm{S} - 179.04454 * \mathrm{l(C=O)} + 170.45944 * \mathrm{l(C=C)} + 0.01726 * \alpha$$

The negative sign of the coefficient of overall softness and C=O bond length reflects that antimalarial activity will be improved for low values of both descriptors. In contrast, the positive sign of the coefficient of the polarizability α and the C=C bond length indicates that high values of these descriptors improve antimalarial activity.

Table 3. *Statistical analysis report of IC_{50} inhibitory potential of chalcone derivatives in RML model.*

Number of observations N	14
Coefficient of determination R^2	0.901
Standard deviation RMCE	0.216
Test de Fischer F	109.104
Cross-validation correlation coefficient Q^2_{CV}	0.901
Confidence level α	> 95%

The value of the coefficient of determination R^2 which is 0.901, shows that the estimated values of pIC_{50} contain 90.1% of the experimental values. The value of Fisher's test (F = 109.104) is relatively high compared to the critical value, from Fisher's table Fcr = 3.06 [57]. This value 109.104 of Fisher's test, higher than the critical value, shows that the error committed is less than what the model explains [57]. The standard deviation (RMCE = 0.216) expresses the small variation of the predicted values from the experimental mean. For this model, the cross-validation correlation coefficient Q^2_{cv} is equal to $Q^2_{cv} = 0.9009$. This value, greater than 0.9, reflects a model said to be excellent according to Erikson et al [39]. This model is acceptable because it agrees with the acceptance criterion of these authors $R^2 - Q^2_{cv}$ =0.901-0.901= 0.000 < 0.3. All these statistical indicators show that the model developed explains the antimalarial activity in a statistically significant and satisfactory way.

The regression plot of the LMR model showing the theoretical antimalarial activity versus the experimental activity is shown in Figure 4.

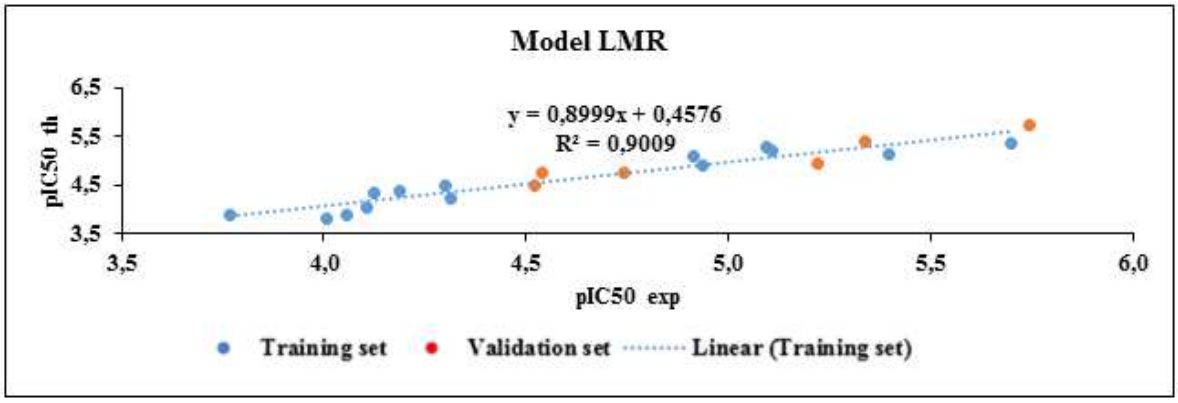

Figure 4. *Regression line of the LMR model.*

The analysis of the regression curve of the RML model shows that all points are around the regression line. This result indicates that there is a small difference (RMCE =0.2157) between the values of pIC50exp and pIC50th, thus a good similarity in these values. This similarity is illustrated in figure 5.

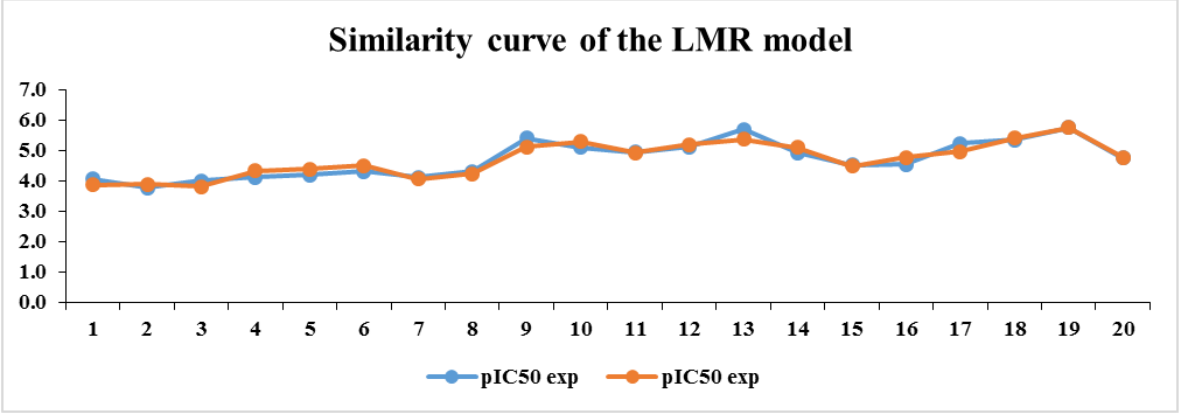

Figure 5. *Similarity curve of the experimental and predicted values of the RML model.*

External Validation Criteria

Verification of Tropsha's criteria

The external validation of the model was performed with the chalcone derivatives (Ch4, Ch9, Ch10, Ch14, Ch18, Ch20) respectively. The Tropsha criteria checks for the external validation sets are presented in Table 4.

Table 4. *Tropsha criteria checks for the external validation set of the RML model.*

Statistical parameters	Tropsha criteria [42-44]	
R^2	> 0.7	0.90
Q^2_{CV}	> 0.6	0.90
$\lvert R^2 - R_0^2 \rvert$	≤ 0.3	0.082
$\frac{\lvert R^2 - R_0^2 \rvert}{R^2}$	< 0.1	0.091
k	$0.85 \leq k \leq 1.15$	0.999
$\frac{\lvert R^2 - R_0'^2 \rvert}{R^2}$	< 0.1	0.114
k'	$0.85 \leq k' \leq 1.15$	1.000

All values meet Tropsha's criteria, so the model is acceptable for predicting antimalarial activity.

Verification of Roy's criteria

The statistical indicators of Roy, Paul and Roy [58], were calculated for this model.

Table 5. *Roy criteria checks of the external validation set of the RML model.*

Indicators	r_m^2	r'^2_m	$\overline{r_m^2} = \frac{(r_m^2 + r'^2_m)}{2}$	$\Delta r_m^2 = \lvert r_m^2 - r'^2_m \rvert$
Values	0.714	0.681	0.697	0.033

The analysis of this table shows that, the r_m^2 is greater than 0.5 and the Δr_m^2 is less than 0.2. Therefore, it can be stated that the model is robust and has good predictive power.

3.2. Non-Linear Multiple Regression (NLMR)

The equation of the QSAR model is presented below. The statistical indicators are given in Table 6.

$$pIC_{50}^{exp} = -25522 + 107.90365 * S + 21842 * l(c = O) + 17168 * l(c = c) + 0.02069 * \alpha - 108.05366 * S^2 - 8720 * l(c = O)^2 - 6234 * l(c = c)^2 - 7.96211 * 10^{-6} * \alpha^2$$

Table 6. *Statistical analysis report of the IC_{50} inhibitory potential of chalcone derivatives.*

Statistical indicators of multilinear regression	Model NLMR
Number of observations N	14
Coefficient of determination R^2	0.9621
Standard deviation RMCE	0.1886
Test de Fischer F	304.7514
Cross-validation correlation coefficient Q_{CV}^2	0.9621
Confidence level α	> 95%
R^2 - Q_{CV}^2	0.0000

The value of the coefficient of determination R^2 which is 0.9621, shows that the estimated values of pIC_{50} contain 96.21% of the experimental values. The value of Fisher's test (F = 304.7514) is relatively high compared to the critical value, from Fisher's table Fcr = 3.06 [57]. This value 304.7514 of Fisher's test, higher than the critical value, shows that the error committed is less than what the model explains [57]. The standard deviation (RMCE = 0.1886) expresses the small variation of the predicted values from the experimental mean. For this model, the cross-validation correlation coefficient Q_{cv}^2 is equal to $Q_{cv}^2 = 0.9621$. This value, higher than 0.9, reflects a so-called excellent model according to Erikson et al [39]. This model is acceptable because it agrees with the acceptance criterion of these authors $R^2 - Q_{cv}^2$=0.9621- 0.9621= 0.000 < 0.3. All these statistical indicators show that the model developed explains the antimalarial activity in a statistically significant and satisfactory manner.

The regression plot of the NLMR model showing the theoretical antimalarial activity versus the experimental activity is shown in Figure 6.

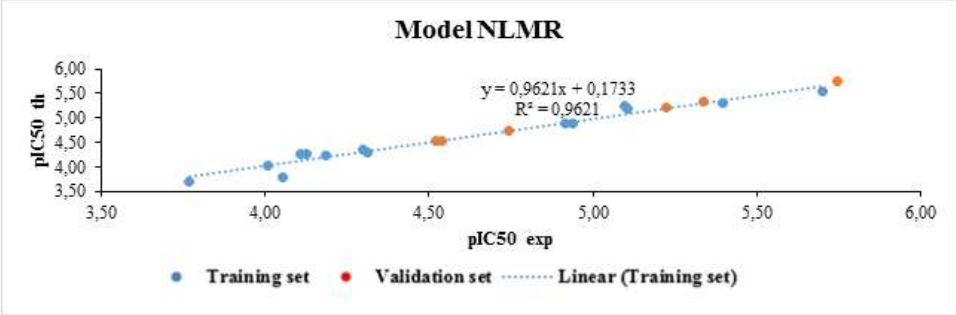

Figure 6. *Regression line of the NLMR model.*

The analysis of the regression curve of the NLMR model shows that all points are around the regression line. This result indicates that there is a small difference (RMCE =0.1886) between the values of pIC50exp and pIC50th, thus a good similarity in these values. This similarity is illustrated in figure 7.

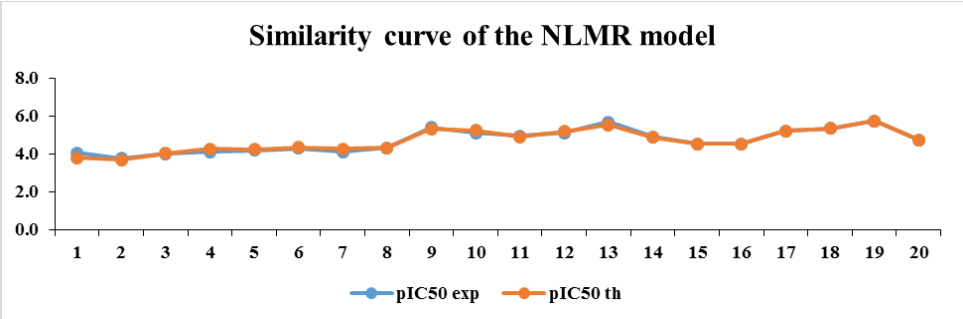

Figure 7. *Similarity curve of the experimental and predicted values of the NLMR model.*

External Validation Criteria

Verification of the Tropsha criteria

The external validation of the model was performed with the chalcone derivatives (Ch4, Ch9, Ch10, Ch14, Ch18, Ch20) respectively. The Tropsha criteria checks for the external validation sets are presented in Table 7.

Table 7. *Tropsha criteria checks for the external validation set of the NLMR model.*

Statistical parameters	Tropsha's criteria [42-44]	
R^2	> 0.7	1
Q^2_{CV}	> 0.6	1
$\lvert R^2 - R_0^2 \rvert$	≤ 0.3	0.000
$\frac{\lvert R^2 - R_0^2 \rvert}{R^2}$	< 0.1	0.000
k	$0.85 \leq k \leq 1.15$	1
$\frac{\lvert R^2 - R_0'^2 \rvert}{R^2}$	< 0.1	0.000
k'	$0.85 \leq k' \leq 1.15$	1.000

All values meet the Tropsha criteria, so the model is acceptable for predicting antimalarial activity.

Verification of the Roy criteria

The statistical indicators of Roy [58], have been calculated for this model. The different values are listed in Table 8.

Table 8. *Roy criteria checks of the external validation set of the NLMR model.*

Indicators	r_m^2	r'^2_m	$\overline{r_m^2} = \frac{(r_m^2 + r'^2_m)}{2}$	$\Delta r_m^2 = \lvert r_m^2 - r'^2_m \rvert$
Values	1	1	1	0

The analysis of this table shows that, the r_m^2 is greater than 0.5 and the Δr_m^2 is less than 0.2. Therefore, it can be stated that the model is robust and has good predictive power.

3.3. Applicability of the LMR and NLMR Models

The graph of the standardized residuals as a function of the h_{ii} levers in Figure 8, allows us to visualize the applicability domain of the LMR and NLMR models.

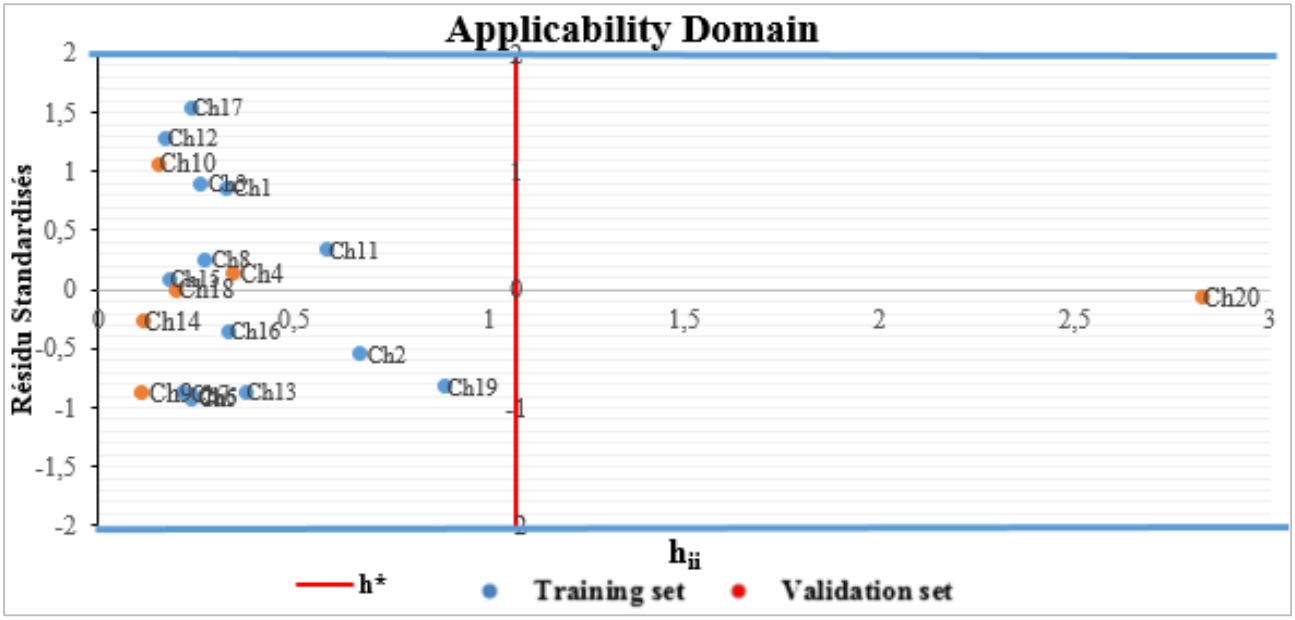

Figure 8. *Standardized residuals of antimalarial activity plotted against the levers of the LMR and NLMR models.*

For the 14 compounds in the training set and the 4 descriptors in the model, the threshold value of the levers h* is 1.07. The extreme values of the standardized residuals are ±2 according to the "three sigma rule" [56]. These different values delimit the domain of applicability [59] of the model as shown on the graph in figure 6. The derivative Ch20, from the validation set, has a leverage value h_{ii} = 2.827, higher than the threshold value h*. However, this derivative has a very low standardized residual, i.e. -0, "0687" greater than -2. Thus, Ch20 is certainly outside the applicability domain, but it is not an influential point for the model. There is no need to remove Ch20 from the set of molecules, as this derivative

belongs to the validation set, so was not used in the development of the models. The behavior of this molecule could be explained by its structural diversity. Indeed, the Ch20 derivative has two double bonds symmetrical to the oxo group.

3.4. Artificial Neural Network (ANN)

The values of the descriptors as well as those of the experimental biological activities of the molecules used for the development of the ANN model are listed in Table 9.

Table 9. *Physico-chemical descriptors and experimental pIC_{50} of the learning and validation sets of the RNA model.*

MOLECULES	S	l(c=o)	l(c=c)	α	pIC_{50}
Training Set					
Ch1	0.49610	1.26518	1.36033	206.06567	4.05552
Ch2	0.61033	1.26277	1.36622	191.16400	3.76981
Ch3	0.52656	1.26394	1.36064	204.86367	4.00922
Ch5	0.49537	1.26510	1.36033	231.01400	4.12494
Ch7	0.48344	1.26303	1.36120	203.60867	4.30103
Ch11	0.50317	1.26131	1.36370	158.27400	4.31426
Ch13	0.51726	1.26671	1.36796	240.87567	5.09691
Ch15	0.50382	1.26652	1.36410	248.05200	4.93930
Ch16	0.50726	1.26644	1.36279	277.51567	5.10791
Ch17	0.51916	1.26479	1.36521	254.10067	5.69897
Ch4	0.48107	1.26485	1.36048	192.02100	4.52288
Ch10	0.52716	1.26588	1.36498	225.55667	5.22185
Ch14	0.50594	1.26371	1.36162	237.98133	5.33724
Ch20	0.58774	1.27158	1.36403	314.78300	4.74473
Validation Set					
Ch6	0.48363	1.26300	1.36126	196.45933	4.18709
Ch8	0.57505	1.26183	1.36241	207.88367	4.10791
Ch12	0.51986	1.26473	1.36526	239.17900	5.39794
Ch19	0.56023	1.25706	1.35747	258.57933	4.91364
Ch9	0.52103	1.26471	1.36405	209.34833	4.54061
Ch18	0.51960	1.26478	1.36527	246.32200	5.74473

The equation of the QSAR model is presented below. The statistical indicators are given in Table 10.

$$pIC_{50}^{pred} = -2.12706476174042 * X_1 + 1.47111601937638 * X_2 - 2.48057666904236 * X_3 + 4.42784050891397$$

with:

$$X_1 = TanH(0{,}5 * (21.4228987189848 * S - 4.2278442473982 * l(c=o) + 74.1349469461423 * l(c=c) + 0.00792918776536016 * \alpha(ua) - 108.405359647331))$$

$$X_2 = TanH(0{,}5 * (-19.4866213931623 * S + 1187.38157127176 * l(c=o) - 1051.49797587843 * l(c=c) + 0.0201126355264715 * \alpha(ua) - 61.5851767967757)$$

$$X_3 = TanH\ (0{,}5 * (-39.0004676014179 * S + 872.946233132494 * l(c=o) - 763{,}600349581977 * l(c=c) + -0.0342102058320001 * \alpha(ua) + -35.2800958226289))$$

Table 10. *Statistical analysis report of IC50 inhibitory potential of chalcone derivatives.*

Statistical indicators of the neural network	Training set
Number of observations *N*	14
Coefficient of determination R^2	0.997
Test de Fischer *F*	3571.499
Standard deviation *RMCE*	0.035
Cross-validation correlation coefficient Q^2_{CV}	0.997
R^2 - Q^2_{CV}	0.000
Confidence level α	> 95%

The value of the coefficient of determination R^2 which is 0.997, shows that the estimated values of pIC_{50} contain 99.70% of the experimental values. The value of Fisher's test (F = 3571.499) is very high compared to the critical value, from Fisher's table Fcr = 3.06 [57]. This value 3571.499 of Fisher's test, higher than the critical value, shows that the error committed is less than what the model explains [57]. The standard deviation (RMCE = 0.035) expresses the small variation of the predicted values from the experimental mean. For this model, the cross-validation correlation coefficient Q^2_{cv} is equal to $Q^2_{cv} = 0.997$. This value, higher than 0.9, reflects a so-called excellent model according to Erikson et al [39]. This model is acceptable because it agrees with the acceptance criterion of these authors $R^2 - Q^2_{cv}$ =0.997- 0.997= *0.000* < 0.3 All these statistical indicators show that the model developed

explains the antimalarial activity in a statistically significant and satisfactory manner.

The regression plot of the RNA model showing the theoretical antimalarial activity versus the experimental activity is shown in Figure 9.

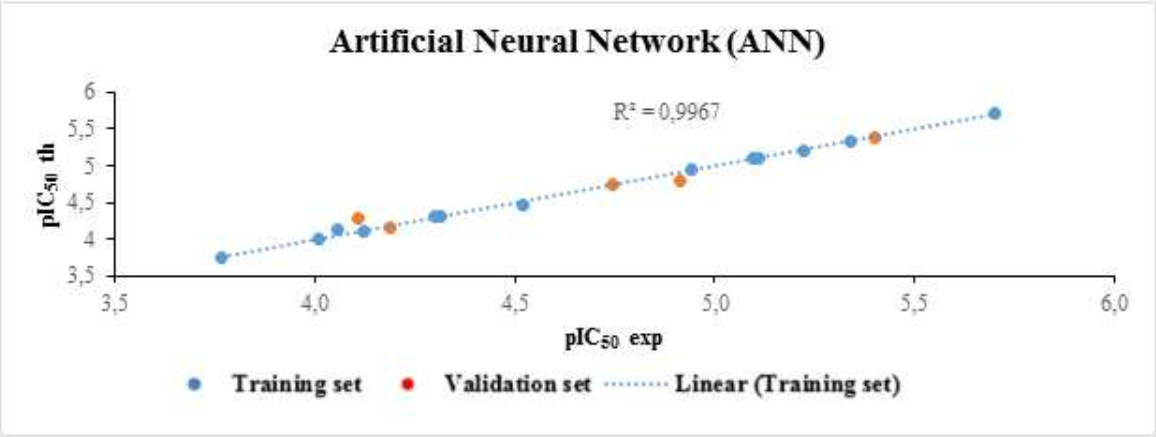

Figure 9. *Regression line of the ANN model.*

The analysis of the regression curve of the RNA model shows that all points are around the regression line. This result indicates that there is a small difference (RMCE =0.035) between the values of pIC_{50}^{exp} and pIC_{50}^{th}, thus a good similarity in these values. This similarity is illustrated in Figure 10.

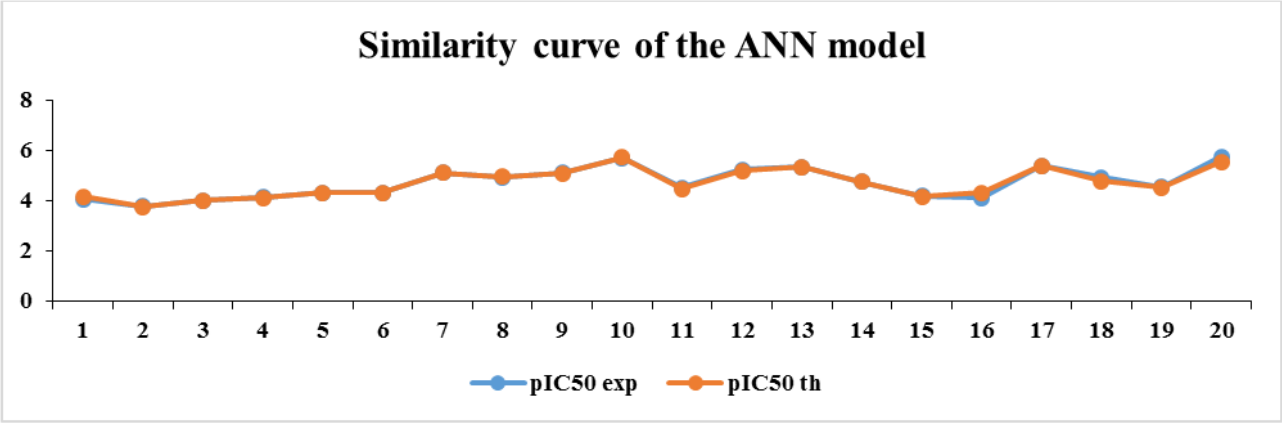

Figure 10. *Similarity curve of experimental and predicted values of the ANN model.*

The similarity curve shows that the experimental and predicted pIC_{50} values overlap perfectly.

External Validation Criteria

Verification of the Tropsha criteria

External validation of the model was performed with the chalcone derivatives (Ch6, Ch8, Ch12, Ch19, Ch9, Ch18) respectively. The Tropsha criteria checks for the external validation sets are presented in Table 11.

Table 11. *Tropsha criteria checks for the external validation set of the ANN model.*

Statistical parameters	Tropsha's criteria [42-44]	
R^2	> 0.7	0.975
Q_{CV}^2	> 0.6	0.954
$\lvert R^2 - R_0^2 \rvert$	≤ 0.3	0.0293
$\frac{\lvert R^2 - R_0^2 \rvert}{R^2}$	< 0.1	0.0301
k	$0.85 \leq k \leq 1.15$	0.990
$\frac{\lvert R^2 - R_0'^2 \rvert}{R^2}$	< 0.1	0.0159
k'	$0.85 \leq k' \leq 1.15$	1.009

All values meet Tropsha's criteria, so the model is acceptable for predicting antimalarial activity.

Verification of Roy's criteria

The statistical indicators of Roy, Paul and Roy [58], were calculated for this model. The different values are shown in Table 12.

Table 12. *Roy criteria checks of the external validation set of the RNA model.*

Indicators	r_m^2	r'^2_m	$\overline{r_m^2} = \frac{(r_m^2 + r'^2_m)}{2}$	$\Delta r_m^2 = \lvert r_m^2 - r'^2_m \rvert$
Values	0.83	0.88	0.85	0.05

The analysis of this table shows that, the r_m^2 is greater than 0.5 and the Δr_m^2 is less than 0.2. Therefore, it can be stated that the model is robust and has good predictive power.

3.5. Domain of Applicability of ANN Models

The graph of standardized residuals as a function of the levers h_{ii} in Figure 11, allows us to visualize the domain of applicability of the model.

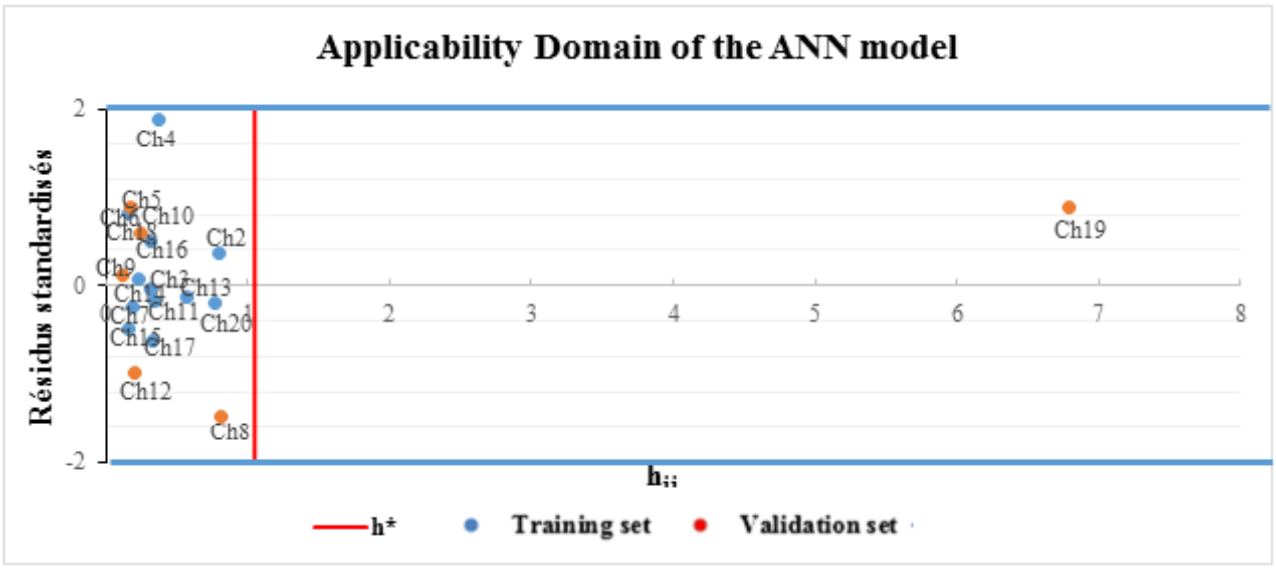

Figure 11. *Plot of standardized residuals of antimalarial activity as a function of the levers of the ANN model.*

For the 14 compounds of the training set and the 4 descriptors of the model, the threshold value of the levers h* is 1.07. The extreme values of the standardized residuals are ±2 according to the "three sigma rule" [56]. These different values delimit the applicability domain [59] of the model as shown on the graph in figure 6. The derivative Ch19, from the validation set, has a leverage value hii = 6.783, higher than the threshold value h*. However, this derivative has a very low standardized residual, i.e. 0. "882" less than 2. Thus, Ch19 is certainly outside the applicability domain, but it is not an influential point for the model. There is no need to remove Ch19 from the set of molecules, as this derivative belongs to the validation set, so was not used in the development of the model. The behavior of this molecule could be explained by its structural diversity. Indeed, the Ch19 derivative has two conjugated double bonds.

4. Conclusion

This work found mathematical relationships between the inhibitory concentration IC50 of Plasmodium falciparum 3D7 and the physicochemical descriptors of chalcone derivatives. Overall softness (S), bond lengths l(c=o) and l(c=c), and polarizability (α) are the descriptors that best explain the antimalarial activity of chalcone derivatives. Statistical tools such as multilinear regression (LMR), nonlinear multiple regression (NLMR) as well as the artificial neural network (ANN) method were used. The statistical indicators of the 3 models (LMR, NLMR, ANN) show that they are acceptable, robust and have good predictive power. In this study, the artificial neuron method (ANN) (R^2 =0.997; *RMCE* = 0.035; *F*= 3571.499 was found to be the best statistical tool for predicting the antimalarial activity of chalcone derivatives. Moreover, the applicability range of this model determined from the levers shows that a prediction of the pIC_{50} of new chalcone derivatives is acceptable when its lever value is less than 1.07. Otherwise the biological activity of this compound could not be predicted with reliability. In a future work, the obtained models will be used for the prediction of new chalcone derivatives.

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