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Research Article

Acute Toxicity of Quinoline Derivatives as Antibacterial Drug Candidates: *In Silico* and *In Vivo* Studies

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Abstract
In this work, a series of quantitative structure-toxicity relationship (QSTR) models was developed using the Online Chemical Database and Modelling environment (OCHEM). Two datasets consisting of 1204 and 1330 compounds were used. Three machine learning methods were employed for modelling: the transformer convolutional neural network, the transformer convolutional neural fingerprint, and the associative neural network. Seven QSTR models were developed, and their predictive performance was evaluated using a fivefold cross-validation procedure. This evaluation resulted in q^2 values between 0.74 and 0.75 for the regression models and a balanced accuracy between 81% and 83% for the classification models. The external test sets achieved balanced accuracy scores of 87% for classification and q^2 values between 0.72 and 0.73 for the regression models. The experimental results for the six compounds were consistent with those predicted by the QSTR models and differed slightly between the two compounds. The results of *in vivo* and *in silico* toxicity studies of quinoline derivatives indicated that all compounds have slight or moderate toxicity according to the classification by Passino-Smith and low toxicity according to the GHS categories. A comparative analysis of acute toxicity studies (LD_{50}) of quinoline derivatives on the hydrobiont *Daphnia magna* along with a comparative analysis of the predicted toxicity values of several drugs and quinolines allowed classifying the studied compounds as low-toxic and confirmed their prospects for further study and potential use in the medical field, particularly as effective antimicrobial agents.

Keywords: acute toxicity, *Daphnia magna*, OCHEM, quinoline derivatives, QSTR models

1. INTRODUCTION

Toxicological screening is very important for developing new drugs and expanding the therapeutic potential of existing molecules. Establishing the toxic effects of chemicals, nutrients, and pharmaceuticals is crucial today. Determining the toxicity profiles of new compounds have become a mandatory procedure, especially in the early stages of drug discovery. At the same time, aquatic toxicity is at the forefront of the harmful effects of chemicals on the environment and biological organisms/systems. Although significant interest in aquatic organisms for toxicity testing is relatively recent, science has long noted the close connection and virtual dependence of all living systems on water as an integral part of living particles, organelles, cells, tissues, and organ systems [1]. In acute and chronic toxicity tests, various organisms, such as invertebrates, fish, and phytoplankton are used. The acute toxicity test towards *Daphnia magna* is intended to screen the toxicity of chemicals, wastewater, surface water, and groundwater [2]. *D. magna* is one of the most commonly used model organisms to evaluate the toxicity of a wide range of pharmaceutical drugs [3]. In recent years, many studies have been published on the toxicological effects of drugs belonging to various therapeutic classes, such as analgesics [4], anthelmintics [5], antibiotics [6][7], anticancer drugs [8], antidepressants [9], antidiabetic drugs [10], antiepileptic drugs [11], and anti-inflammatory drugs [12][13] with using *D. magna*. Among various model organisms, *D. magna* is the most sensitive animal to various pharmaceutical drugs of a wide range of therapeutic classes such as paracetamol, carbamazepine, cimetidine and sulfonamide antibiotics [3][14][15].

Among the heterocyclic compounds, quinoline is a valuable building material, which serves as an essential assembly motif for developing new drugs. Quinoline and its derivatives, known for their biological activities, constitute an important class of compounds for drug development. Thus, various scientific communities have developed these compounds as key structures and assessed their biological activities [16][17]. A number of natural

Table 1. Statistical coefficients calculated for the classification models.

Method	Set	Sensitivity (%)	Specificity (%)	AUC[b]	Balanced Accuracy (%)
Trans-CNN	Training	79	83	0.88 ± 0.01	81 ± 1.0
	Test	83	91	0.94 ± 0.02	87 ± 2.0
Trans-CNF	Training	80	86	0.89 ± 0.01	83 ± 1.0
	Test	86	88	0.92 ± 0.02	87 ± 2.0
ASNN	Training	76	85	0.88 ± 0.01	81 ± 1.0
	Test	80	93	0.91 ± 0.02	87 ± 2.0
Consensus[a]	Training	78	87	0.90 ± 0.01	82 ± 1.0
	Test	83	92	0.93 ± 0.02	87 ± 2.0

[a]The consensus model was built by averaging three models. [b]The area under the curve (AUC) is the measure of the ability of a binary classifier to distinguish between classes.

products with a quinoline skeleton are either used as drugs or serve as lead molecules for developing of new and promising therapies. The quinoline-containing structure is crucial for many drugs currently used in clinical practice to treat various diseases [18][19]. Quinolines such as quinine and chloroquine exhibit numerous pharmacological properties, functioning as antimalarial, antiparasitic, antibacterial, antiviral, antifungal, and anticancer agents, and they also play a role in treating autoimmune diseases [20][21]. Additionally, quinolines have been explored as new effective antibacterial agents against multidrug-resistant strains [22][23]. Further research in this domain, including the potential toxicological risk of quinolines, pivotal for exploiting their therapeutic potential.

Machine learning (ML) is becoming an indispensable tool in drug discovery, particularly for predicting molecular properties and toxicity levels [24]. Traditional evaluations of toxicity present challenges due to the complexities and resources required for human and animal studies, which restrict reliable data availability. ML can enhance or replace traditional experimental methods, facilitating the identification of compounds with desirable on-target effects while eliminating those with undesirable properties [25]. However, reliance on ML also carries risks, including biases from unrepresentative training datasets and inadequate model construction. These challenges can lead to inaccurate predictions and misinterpretations of confidence in ML outcomes, ultimately resulting in poor decision-making in drug development. Therefore, a comprehensive understanding of ML model predictive validity is essential for improving decision-making and accelerating drug development, particularly through well-defined datasets that focus on predicting toxicity based on small molecule structures [26]. Our research aimed to evaluate the toxicity of several quinoline derivatives as promising drug candidates based on a rational combination of theoretical and experimental studies.

2. MATERIALS AND METHODS

2.1. Data

The initial set of experimental data on the *D. magna* acute toxicity of various chemical compounds (1600 molecules) used from the OCHEM database [27] included data from the ECOTOX [28], VEGA [29] databases and a list of scientific publications [30]. Two different datasets were used to build the QSTR models. The first dataset **I** (1204 compounds) included various chemical series of compounds chosen from the original data. The data were divided into high-toxicity (526 compounds with $LC_{50} \leq 10$ μM) and low-toxicity (678 compounds with $LC_{50} > 10$ μM) molecules. This dataset was used for the development of classification models, which were used for a preliminary evaluation of the investigated compounds toxicity. A second dataset **II** was created by selecting 1330 compounds from the initial set. The lethal concentration (LC_{50}) values of the molecules ranged from 0.0857 nM to 353.6 mM. The LC_{50} values were converted into log(1/

LC_{50}) values and were used as the target variable to develop regression models. To thoroughly validate the developed models, all datasets were randomly divided into a training set (80% of compounds) and an external test set (20% of molecules).

2.2. OCHEM Tools

Different techniques and groups of descriptors were tested within OCHEM while constructing the QSTR models. The best models were created by the transformer convolutional neural network (trans-CNN) [31], transformer convolutional neural fingerprint (trans-CNF) [32] and associative neural network (ASNN) [23]. The optimized parameter settings were used for each machine learning method offered by the OCHEM platform. Trans-CNN uses information about molecules based on their SMILES notation to build QSTR models [31]. The method predicts a target value by averaging individual predictions for a batch of non-standard SMILES belonging to a single molecule. Within-batch variance can serve as a measure of the confidence interval of a forecast, and the ability to canonicalize SMILES can be used to determine the uncertainty of forecasts. Trans-CNF is similar to trans-CNN, but instead of using a convolutional neural network, it employs a convolutional neural fingerprint to process the latent representation of the neural network. This network also uses the augmentation technique, which is used in computer vision and has recently been introduced in QSTR studies [32]. The ASNN is a highly effective and efficient algorithm that combines the strength ensemble of feed-forward backpropagation neural networks with the k-nearest neighbors (kNN) method, providing exceptional accuracy and reliability for a wide range of applications. While neural networks create global models, kNN provides local correction of the global model set [33]. This combination helps to correct the bias of the neural network ensemble, thereby increasing its accuracy. The ensemble consisted of 100 neural networks developed using the default parameters provided by OCHEM.

The descriptors calculated by ALogPS [34] and Mold2 [35] software were used to build the ASNN model. The ALogPS program calculates the 1-octanol/water partition coefficient and aqueous solubility. Mold2 software efficiently computes a wide array of descriptors that encode crucial two-dimensional chemical structure information. This software is publicly available and developed by the Bioinformatics Center under the direction of Dr. Wade Tong of the National Center for Toxicology Research (NCTR) [36]. The performance of the QSTR models was evaluated using a fivefold cross-validation technique and external validation sets [37]. To prevent inaccurate model estimations caused by overfitting from variable selection, OCHEM carries out multiple repetitions of all the stages involved in model development within each validation fold. This approach helps ensure that the models created are reliable and can be used to make accurate predictions. The final models' quality was verified using the test sets mentioned earlier.

When evaluating the performance of classification models, two commonly used metrics are sensitivity and specificity. Sensitivity measures the proportion of true positive results, while specificity measures the proportion of true negative results. These metrics are calculated by comparing the predicted and actual values of the target variable and are useful for assessing the accuracy and reliability of a classification model. Sensitivity and specificity were calculated using Formula 1 and 2, respectively.

$$SN = TP/(TP + FN) \quad \textbf{(1)}$$

$$SP = TN/(TN + FP) \quad \textbf{(2)}$$

Table 2. Statistical coefficients of the regression QSTR models.

Method	Training Set			Test Set		
	R^2	q^2	RMSE[c]	R^2	q^2	RMSE
Trans-CNF	0.74 ± 0.02	0.74 ± 0.02	0.84 ± 0.02	0.72 ± 0.04	0.72 ± 0.04	0.80 ± 0.04
Trans-CNN	0.74 ± 0.02	0.74 ± 0.02	0.85 ± 0.02	0.72 ± 0.04	0.71 ± 0.04	0.81 ± 0.05
Consensus[a]	0.76 ± 0.02	0.75 ± 0.02	0.83 ± 0.02	0.73 ± 0.04	0.73 ± 0.04	0.79 ± 0.05

[a]The consensus model as a simple average of the trans-CNF and trans-CNN models.

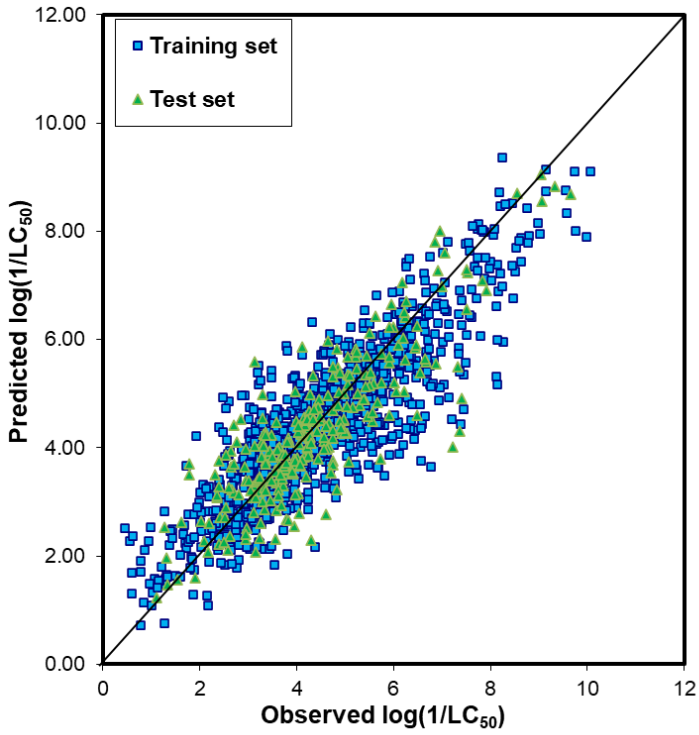


Figure 1. Plots of experimental versus predicted values for the consensus QSTR model.

The quantities TP, FP, TN, and FN represent the number of true positives, false positives, true negatives, and false negatives, respectively. The performance of the models was evaluated by using the balanced accuracy (BA), which is calculated using Formula 3.

$$BA = (SN + SP)/2 \quad \textbf{(3)}$$

The performance of the regression models was assessed using several evaluation metrics. These metrics included the root mean square error (RMSE), which measures the average deviation of the predicted values from the actual values; the mean absolute error (MAE), which measures the average absolute deviation between the predicted and actual values; the squared correlation coefficient, R^2, which is a measure of how well the model fits the data; and the coefficient of determination, q^2, which is a measure of the predictive power of the model [38]. These metrics help determine the accuracy and reliability of regression models and can serve as a guide for further improvements or adjustments to the models. The applicability domain and the accuracy of developed QSTR models predictions were assessed using OCHEM functions [39][40].

2.3. D. magna Acute Toxicity Test

Standard testing for OECD 202 [41] is performed by exposing young daphnids to the test compounds at a range of concentrations of 0.001, 0.1, 1.0, 10.0, and 20.0 mg/L for 48 h. Seven individuals in 50-mL glass participated in each testing. Immobilization is recorded at 24 and 48 ho and compared with control values. Lethal concentration LC_{50} and the respective 95 % confidence intervals (CI) were calculated using the Statistica 7 program. Animals not able to motion within 15 s after nutation of the test glass were considered to be immobilized. All samples of compounds studied in the work were synthesized in the Department of Chemistry of Zaporizhzhya National University (Ukraine).

3. RESULTS AND DISCUSSION

3.1. QSTR

*3.1.1. Classification Models (Dataset **I**)*

The initial set of 1204 compounds was randomly split into training (963) and test (241) sets. The numerical values of activity were discretized as described in the experimental section. Three machine learning methods were used for modeling:

Table 3. Quinolone derivatives acute toxicity calculated using the consensus classification QSTR model.

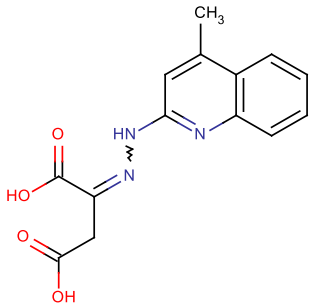
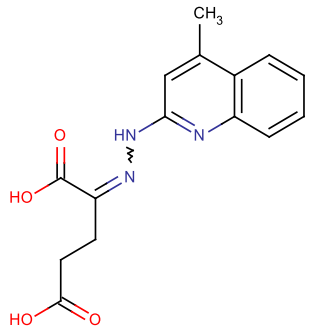
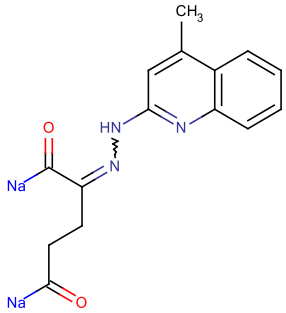
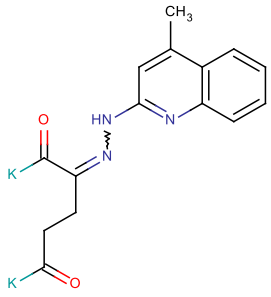
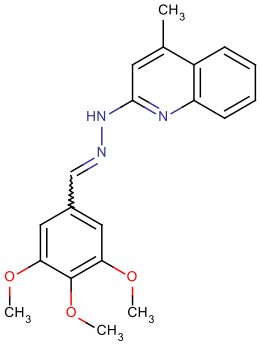
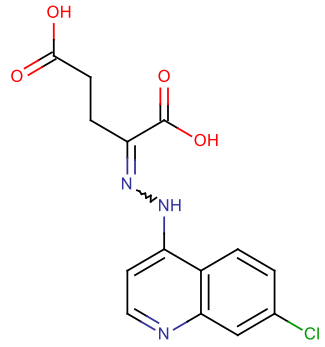
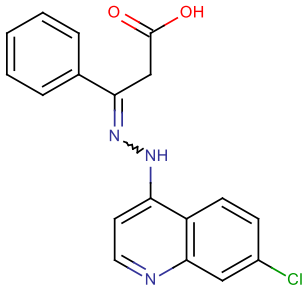
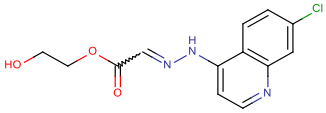
No	Structures	Predicted Toxicity	Estimated Accuracy	Applicability Domain
1		low	0.93	[a]TRUE
2		low	0.81	TRUE
3		low	0.65	TRUE
4		high	0.80	TRUE
5		high	0.62	[b]FALSE

Table 3. *Cont*.

No	Structures	Predicted Toxicity	Estimated Accuracy	Applicability Domain
6		low	0.85	TRUE
7		low	0.94	TRUE
8		low	0.81	TRUE

[a]TRUE - the forecast is reliable; FALSE - the forecast is unreliable.

the trans-CNN [31], trans-CNF [32], and ASNN [33]. We preliminarily investigated all descriptor sets available at the OCHEM website. The final ASNN models with the highest prediction accuracies were calculated using descriptors calculated by ALogPS [34] and Mold2 [35] software as described in the experimental section.

The developed models are summarized in Table 1. All the models showed similar results in terms of sensitivity, specificity, AUC and balanced accuracy (BA). The cross-validated BAs for the training sets were in the range of 81–83%. Similar accuracies were also calculated for the *BA* equals to 87% test sets. The consensus model achieved the highest performance. This model was applied to preliminarily evaluate the toxicity of quinolone derivatives, as described in the next section.

*3.1.2. Regression Models (Dataset **II**)*

The initial dataset of 1330 compounds was split by chance into training (1064) and test (266) sets as described. The regression models built by the trans-CNF [31] and trans-CNN [32] methods (see Table 1) achieved the best performances. The q^2 values were 0.74–0.75 and 0.72–0.73 for the training and test sets, respectively. Other statistical parameters of the models are summarized in Table 2. A consensus model, which was an average of both models, achieved the best performance. This method was used to quantitatively evaluate the toxicity of quinolone agents, as described in the next section.

Fig. 1. shows the regression line plotting the values predicted by the consensus QSTR model for dataset **II**. Most predictions do not differ from the experimental values by more than 2 log units. Only 35 chemicals in the training set had residuals between the experimental and predicted log LC_{50} values higher than 2 log units but lower than 3 log units. Notably, in the test set, only 5 chemicals had residue values between 2 and 3 log LC_{50} units. This highlights

Table 4. Prediction of the studied compounds acute toxicity by the regression consensus QSTR models.

No.	LC_{50} (mg/L)	lg(1/LC_{50})	CONSENSUS-STD	AD	Toxicity Class According to the Classification of Passino-Smith
1	36.08	3.91	0.38	TRUE	Slightly
2	17.34	4.24	0.38	TRUE	Slightly
3	7.74	4.61	0.30	TRUE	Moderately
4	1.70	5.31	0.25	TRUE	Moderately
5	0.36	5.99	0.07	TRUE	Highly
6	16.20	4.29	0.60	FALSE	Slightly
7	8.88	4.58	0.36	TRUE	Moderately
8	7.01	4.62	0.20	TRUE	Moderately

[a]Danger rating according to the classification of Passino-Smith [42]: practically harmless (100–1000 mg/L), slightly toxic (10–100 mg/L), moderately toxic (1–10 mg/L), highly toxic (0.1–1.0 mg/L); TRUE – the forecast is reliable; FALSE – the forecast is unreliable.

the robustness of the proposed consensus model (see Fig. 1, Table 2).

3.2. Forecasting Compound Toxicity by QSTR Models

In the first stage, the toxicity of potentially active compounds was predicted using classification QSTR models. For a more detailed assessment, numerical/quantitative toxicity was predicted using the proposed QSTR regression models. The results of the research are presented in Tables 3 and 4.

By analyzing the classification models presented in Table 3 to predict the toxicity of the quinoline derivatives, it was concluded that all compounds except compound **4** had low toxicity. Compound **4** is moderately toxic (LC_{50} > 10 mol/L), which was confirmed by regression models (Table 4).

After analyzing the results of toxicity predicting of the quinoline derivatives to *D. magna*, based on the classification of Passino-Smith, it can be concluded that the investigated compounds fall into three categories: slightly toxicity (compounds **1**, **2** and **6**), moderate toxicity (compounds **3, 4, 7,** and **8**), and high toxicity (only compound **5**). In accordance with modern requirements for the safety of pharmacologically active compounds, a comparative *in vivo* analysis of the toxicity profile of the studied quinolines and known drugs was carried out using models published on the OCHEM server [43], which take into account a wide range of values/types of toxicity depending on the type of organism and method administration of the drug (Table 5).

Table 5 shows that the calculated LD_{50} values of the reference drugs and the studied quinoline derivates have similar toxicity levels - for drugs an average of 2.35 -log(M) and for quinolines an average of 2.27 -log(M) to 2.75 -log(M). To analyze the toxicity profile of the studied derivatives, *in silico* toxicity LD_{50} values expressed as -log(M) for guinea pigs, mice, rats, and rabbits were transformed into absolute LD_{50} values under oral and dermal testing conditions (Table 6).

3.3. Acute Toxicity Test with D. magna

Following the absolute LD_{50} toxicity values in accordance with Globally Harmonized System of Classification Labeling of Chemicals (GHS) [46], all studied quinolines can be classified as

Table 5. Comparative analysis of toxicity predictions (-log(M)) for known drugs [44] and quinoline derivatives using OCHEM models [45].

Species	Administration Route	Toxicity Endpoint	Drugs	Quinoline Derivates							
				1	2	3	4	5	6	7	8
Guinea pig	Oral	LD_{50}	**2.57**	**2.48**	**2.55**	**2.95**	**3.01**	**2.68**	**2.57**	**2.79**	**2.69**
Mammal	Unreported	LD_{50}	2.64	2.55	2.60	3.02	3.06	2.68	2.63	2.75	2.67
Man	Oral	TDL	4.17	3.54	3.75	4.31	4.27	4.55	3.96	4.30	4.42
Mouse	Intraperitoneal	LD_{50}	2.90	2.83	2.83	3.46	3.55	2.88	2.82	2.90	2.87
Mouse	Intraperitoneal	LDL	2.97	2.87	2.87	3.53	3.64	2.99	2.85	2.93	2.94
Mouse	Intraperitoneal	TDL	4.29	3.76	3.88	4.66	4.73	4.53	3.98	4.25	4.37
Mouse	Intravenous	LD_{50}	3.45	3.19	3.21	3.95	4.06	3.62	3.21	3.38	3.46
Mouse	Oral	LD_{50}	**2.40**	**2.41**	**2.47**	**2.62**	**2.62**	**2.51**	**2.46**	**2.63**	**2.52**
Mouse	Oral	LDL	2.51	2.48	2.54	2.75	2.74	2.63	2.55	2.71	2.64
Mouse	Oral	TDL	4.20	3.69	3.83	4.30	4.33	4.52	3.94	4.26	4.35
Mouse	Subcutaneous	LD_{50}	2.64	2.64	2.66	3.16	3.24	2.63	2.62	2.73	2.64
Mouse	Subcutaneous	LDL	2.79	2.63	2.69	3.15	3.21	2.90	2.70	2.88	2.88
Mouse	Unreported	LD_{50}	2.73	2.66	2.68	3.20	3.28	2.77	2.67	2.79	2.71
Rat	Intraperitoneal	LD_{50}	2.91	2.84	2.87	3.44	3.52	2.92	2.86	2.96	2.92
Rat	Intraperitoneal	LDL	3.01	2.94	2.97	3.48	3.54	3.05	2.99	3.09	3.04
Rat	Intraperitoneal	TDL	4.40	3.89	4.01	4.79	4.85	4.71	4.12	4.39	4.54
Rat	Intravenous	LD_{50}	3.41	3.07	3.11	3.99	4.09	3.65	3.14	3.36	3.45
Rat	Intravenous	TDL	5.12	4.50	4.59	5.69	5.78	5.34	4.70	4.97	5.27
Rat	Oral	LD_{50}	**2.34**	**2.34**	**2.42**	**2.57**	**2.56**	**2.44**	**2.42**	**2.62**	**2.49**
Rat	Oral	LDL	2.56	2.50	2.60	2.75	2.73	2.71	2.62	2.85	2.76
Rat	Oral	TDL	4.03	3.54	3.70	4.28	4.32	4.36	3.79	4.13	2.56

Table 5. *Cont.*

Species	Administration Route	Toxicity Endpoint	Drugs	Quinoline Derivates							
				1	2	3	4	5	6	7	8
Rat	Subcutaneous	LD_{50}	2.54	2.55	2.60	3.05	3.13	2.44	2.61	2.68	4.83
Rat	Subcutaneous	TDL	4.75	3.95	4.11	4.87	4.96	5.16	4.26	4.59	2.14
Rat	Skin	LD_{50}	**2.03**	**2.01**	**2.11**	**2.14**	**2.17**	**2.02**	**2.20**	**2.22**	**2.75**
Rabbit	Unreported	LD_{50}	2.69	2.58	2.64	2.92	2.95	2.79	2.70	2.87	3.80
Rabbit	Intravenous	LD_{50}	3.70	3.28	3.35	4.21	4.33	4.00	3.37	3.67	2.55
Rabbit	Oral	LD_{50}	**2.43**	**2.41**	**2.48**	**2.78**	**2.79**	**2.53**	**2.47**	**2.67**	**1.99**
Rabbit	Skin	LD_{50}	**2.06**	**2.01**	**2.02**	**2.36**	**2.43**	**1.96**	**2.12**	**2.14**	**4.29**
Woman	Skin	TDL	4.06	3.42	3.62	4.22	4.24	4.38	3.75	4.10	4.29

LD_{50} - lethal dose fifty; TDL - toxic dose low; LDL - lethal dose low.

substances in the low toxicity hazard categories. The *in silico* and *in vivo* toxicity results of studied quinolines to *D. magna* are summarized in Table 7.

The results of *in vivo* and *in silico* toxicity studies of quinolines (Table 7) indicate that all compounds are in the range of slight to moderate toxicity by Passino-Smith classification and low toxicity by GHS Categories. Comparative analysis of *in vivo* and *in silico* studies of the acute toxicity (LD_{50}) of quinolines on the *D. magna* hydrobiont and comparative analysis of the predicted toxicity values of several drugs and quinolines made it possible to classify the studied compounds as low toxicity and confirmed their promise for study and further potential use in the medicinal field including as effective antimicrobial agents.

In recent decades, quinoline derivatives have occupied an important place in scientific research due to the significance of the quinoline skeleton in various natural and pharmaceutical products [47]. Since the discovery of quinoline compounds in the 19th century, many of their medicinal properties have been identified. Today, several biologically important agents have been described among quinoline derivatives. Thus, diarylurea and diarylamide derivatives including quinoline core armed with dimethylamino or morpholino side chain were selected and described by the National Cancer Institute (NCI, USA) as cytostatics against 60 cancer cell lines of nine cancer types [48]. New 1,3-oxazolo[4,5-c]quinoline derivatives demonstrated antibacterial and antituberculosis properties [49]. Antimicrobial activity has been established for quinoline derivatives containing a 1,2,4-triazole fragment [50] and for 2(4)-hydrazone derivatives of quinoline [22]. Numerous authors have indicated the presence of insecticidal activity in new quinoline derivatives containing a perfluoropropanyl group [51]. Many quinoline derivatives present a promising opportunity for further biomedical research and therapeutic development due to, for example, antioxidant properties [52].

It is important to note that the perspective for further studies of quinolines is largely determined by their potential toxicity, including water toxicity, as a medium for supporting life in organisms of various functional and organizational levels. For instance, several authors have reported the results of

studies of the aquatic toxicity of numerous quinolones using the marine bacterium *Vibrio fischeri* as a test organism. EC_{50} values were recorded in the range from 14 μg/L for ofloxacin to 1020 μg/L for pipemidic acid. Following the approach outlined in the EEC directive 93/21/EEC, the authors classified almost all quinolones as belonging to the group categorized as "very toxic to aquatic organisms" (EC_{50} below 1 mg/L) [53]. Adverse effects of quinoline and isoquinoline were observed in the algae *V. fischeri*, while in the *D. magna* immobilization test, the authors recorded the toxicity of only hydroxylated quinoline derivatives. In conclusion, it was stated that hydroxylation of quinoline leads to detoxification of genotoxic potential, whereas quinolines, isoquinolines and their metabolites have ecotoxicological significance in groundwater [54].

It is also known that the testing of the aquatic toxicity of various azaarenes (quinoline, acridine) was conducted using hybrobionts of the embryo-larval stages of rainbow trout (*Salmo gairdneri*) and largemouth bass (*Micropterus salmoides*). The authors demonstrated a correlation between the number of rings and the level of toxicity of the studied azaarenes - LC_{50} of acridine (three rings) and quinoline (two rings) were 11.0 and 0.32 mg/L, respectively. A compound with a large number of aromatic rings has exhibited high toxicity [55]. In our previous work, the acute toxicity results of studied (quinolin-4-ylthio)carboxylic acids as promising antibacterial agents against MDR *E. coli* strains indicated that most of the studied compounds are classified as slightly toxic and practically harmless [23].

4. CONCLUSIONS

It is known that toxicological research strategies in the drug discovery field involve a multi-level approach using *in silico*, *in vitro* and *in vivo* safety experiments to assess the risks of a drug candidate. Computational *in silico* approaches and experimental *in vivo* studies aimed at predicting toxicity are used to optimize the safety aspect of drug development and candidate selection so that subsequent preclinical programs/protocols and clinical trials can be successful. Predictive classification and regression models based on

Table 6. Comparative analysis of absolute LD_{50} values for the tested quinolones.

Species	Administration Route	Tested Quinolines							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Guinea pig	Oral	955.6	853.1	353.9	340.0	730.8	865.9	551.1	596.9
Mouse	Oral	1112.0	1026.0	751.5	828.7	1091.0	1121.0	803.9	887.0
Rat	Oral	1319.0	1156.0	852.9	947.2	1264.0	1223.0	822.6	941.7
Rat	Skin	2794.0	2339.0	2269.0	2336.0	3387.0	2030.0	2038.0	2128.0
Rabbit	Oral	1112.0	997.7	525.9	564.2	1037.0	1090.0	729.8	831.6
Rabbit	Skin	2820.0	2877.0	1367.0	1275.0	3871.0	2418.0	2439.0	2978.0

Table 7. Comparative analysis of *in vivo* and *in silico* studies of the acute toxicity (LD_{50}) of quinolines **1–8** on the *D. magna* hydrobiont model.

No.	*In vivo* Acute Toxicity, mg/L (for *D. magna*)	Class of toxicity by Passino-Smith (for *D. magna*)		Category of Toxicity by GHS[3]
		In vivo[1]	*In silico*[2]	
1	29.4 ± 1.6	Slightly	Slightly	Low
2	22.5 ± 1.4	Slightly	Slightly	Low
3	9.7 ± 0.2	Moderately	Moderately	Low
4	8.2 ± 0.3	Moderately	Moderately	Low
5	3.7 ± 0.1	Moderately	Highly	Low
6	15.3 ± 0.8	Slightly	Slightly	Low
7	9.9 ± 0.2	Moderately	Moderately	Low
8	12.5 ± 0.7	Slightly	Moderately	Low

[1] *In vivo* LC_{50} range according to the classification of Passino-Smith; [2] LC_{50} range according to the built QSTR models classification; [3] Categories of toxicity based on drugs acute toxicity by the oral and dermal administration route expressed as LD_{50}/LC_{50}.

different machine-learning methods were built using the OCHEM platform. The created QSTR models demonstrated good stability, robustness and predictive power, as verified by cross-validation and prediction of a randomly formed test set. Our newly developed QSTR models, freely published on the OCHEM server, can be extremely useful for a diverse range of drug design researchers working to create novel therapeutic antimicrobial agents. In particular, we found that the studied quinolines show great promise as scaffolds for developing these agents. The *in vivo* and *in silico* toxicity results of quinoline derivatives not only allowed us to classify them as low-toxic compounds but also to recommend them as antibacterial drug candidates based on the outcomes of computer-aided drug design. With the help of our QSTR models, researchers will be able to predict the toxicity of their drug candidates more accurately and optimize their designs accordingly, ultimately leading to the development of safer and more effective treatments for various infectious diseases.

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Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

[1] P. Ball. (2017). "Water is an active matrix of life for cell and molecular biology". *Proceedings of the National Academy of Sciences.* **114** (51): 13327-13335. 10.1073/pnas.1703781114.

[2] S. V. Kergaravat, S. R. Hernandez, and A. M. Gagneten. (2021). "Second-, third- and fourth -generation quinolones: Ecotoxicity effects on Daphnia and Ceriodaphnia species". *Chemosphere.* **262** : 127823. 10.1016/j.chemosphere.2020.127823.

[3] A. Tkaczyk, A. Bownik, J. Dudka, K. Kowal, and B. Slaska. (2021). "Daphnia magna model in the toxicity assessment of pharmaceuticals: A review". *Science of the Total Environment.* **763** : 143038. 10.1016/j.scitotenv.2020.143038.

[4] L. H. Heckmann, A. Callaghan, H. L. Hooper, R. Connon, T. H. Hutchinson, S. J. Maund, and R. M. Sibly. (2007). "Chronic toxicity of ibuprofen to Daphnia magna: Effects on life history traits and population dynamics". *Toxicology Letters.* **172** (3): 137-45. 10.1016/j.toxlet.2007.06.001.

[5] A. Puckowski, S. Stolte, M. Wagil, M. Markiewicz, P. Lukaszewicz, P. Stepnowski, and A. Bialk-Bielinska. (2017). "Mixture toxicity of flubendazole and fenbendazole to Daphnia magna". *International Journal of Hygiene and Environmental Health.* **220** (3): 575-582. 10.1016/j.ijheh.2017.01.011.

[6] R. Dionisio, D. Daniel, G. D. Alkimin, and B. Nunes. (2020). "Multi-parametric analysis of ciprofloxacin toxicity at ecologically relevant levels: Short- and long-term effects on Daphnia magna". *Environmental Toxicology and Pharmacology.* **74** : 103295. 10.1016/j.etap.2019.103295.

[7] L. Lomba, D. Lapena, N. Ros, E. Aso, M. Cannavo, D. Errazquin, and B. Giner. (2020). "Ecotoxicological study of six drugs in Aliivibrio fischeri, Daphnia magna and Raphidocelis subcapitata". *Environmental Science and Pollution Research.* **27** (9): 9891 -9900. 10.1007/s11356-019-07592-8.

[8] D. Mielecki, E. Grzesiuk, A. Bednarska, D. Garbicz, B. Swiderska, and M. Grzesiuk. (2023). "Contamination of aquatic environment with anticancer reagents influences Daphnia magna - Ecotoxicogenomics approach". *Ecotoxicology and Environmental Safety.* **249** : 114372. 10.1016/j.ecoenv.2022.114372.

[9] P. A. Evangelista, F. Z. Vilca, R. F. Pimpinato, F. Periotto, F. M. de Oliveira Lourenço, and V. L. Tornisielo. (2023). "Presence and ecotoxicity of residual antidepressants in environmental samples". *Environmental Nanotechnology, Monitoring & Management.* **20**. 10.1016/j.enmm.2023.100847.

[10] K. O'Rourke, B. Engelmann, R. Altenburger, U. Rolle-Kampczyk, and K. Grintzalis. (2023). "Molecular Responses of Daphnids to Chronic Exposures to Pharmaceuticals". *International Journal of Molecular Sciences.* **24** (4). 10.3390/ijms24044100.

[11] M. Nkoom, G. Lu, and J. Liu. (2022). "Chronic toxicity of diclofenac, carbamazepine and their mixture to Daphnia magna: a comparative two-generational study". *Environmental Science and Pollution Research.* **29** (39): 58963-58979. 10.1007/s11356-022-19463-w.

[12] S. Lee, G. T. Jung, M. Cho, J. W. Lee, K. Eghan, J. Lee, S. Yoon, K. P. Kim, and W. K. Kim. (2023). "Plausibility of Daphnia magna as an alternative experimental model to evaluate effects on eicosanoid synthesis". *Ecotoxicology and Environmental Safety.* **262** : 115119. 10.1016/j.ecoenv.2023.115119.

[13] M. Nkoom, G. Lu, J. Liu, H. Dong, and H. Yang. (2019). "Bioconcentration, behavioral,

and biochemical effects of the non-steroidal anti-inflammatory drug diclofenac in Daphnia magna". *Environmental Science and Pollution Research.* **26** (6): 5704-5712. 10.1007/s11356-018-04072-3.

[14] M. De Liguoro, A. Riga, and P. Fariselli. (2018). "Synergistic toxicity of some sulfonamide mixtures on Daphnia magna". *Ecotoxicology and Environmental Safety.* **164** : 84-91. 10.1016/j.ecoenv.2018.08.011.

[15] H. Chen, X. Gu, Q. Zeng, and Z. Mao. (2019). "Acute and Chronic Toxicity of Carbamazepine on the Release of Chitobiase, Molting, and Reproduction in Daphnia similis". *International Journal of Environmental Research.* **16** (2). 10.3390/ijerph16020209.

[16] B. S. Matada, R. Pattanashettar, and N. G. Yernale. (2021). "A comprehensive review on the biological interest of quinoline and its derivatives". *Bioorganic & Medicinal Chemistry.* **32** : 115973. 10.1016/j.bmc.2020.115973.

[17] O. F. Elebiju, O. O. Ajani, G. O. Oduselu, T. A. Ogunnupebi, and E. Adebiyi. (2022). "Recent advances in functionalized quinoline scaffolds and hybrids-Exceptional pharmacophore in therapeutic medicine". *Frontiers in Chemistry.* **10** : 1074331. 10.3389/fchem.2022.1074331.

[18] A. N. S. da Gama and M. N. C. Soeiro. (2021). "Quinoline-based Compounds as Key Candidates to Tackle Drug Discovery Programs of Microbicidal Agents". *Current Pharmaceutical Design.* **27** (15): 1757-1762. 10.2174/1381612826666201006125644.

[19] P. Yadav and K. Shah. (2021). "Quinolines, a perpetual, multipurpose scaffold in medicinal chemistry". *Bioorganic Chemistry.* **109** : 104639. 10.1016/j.bioorg.2021.104639.

[20] O. O. Ajani, K. T. Iyaye, and O. T. Ademosun. (2022). "Recent advances in chemistry and therapeutic potential of functionalized quinoline motifs - a review". *RSC Advances.* **12** (29): 18594-18614. 10.1039/d2ra02896d.

[21] A. A. Aly, M. Ramadan, G. E.-D. A. Abuo-Rahma, Y. A. M. M. Elshaier, M. A. I. Elbastawesy, A. B. Brown, and S. Bräse. (2021). In: "Advances in Heterocyclic Chemistry". 147-196. 10.1016/bs.aihch.2020.08.001.

[22] D. Hodyna, V. Kovalishyn, Y. Romanenko, I. Semenyuta, V. Blagodatny, M. Kachaeva, O. Brazhko, and L. Metelytsia. (2023). "Quinoline Hydrazone Derivatives as New Antibacterials against Multidrug Resistant Strains". *Chemistry & Biodiversity.* **20** (9): e202300839. 10.1002/cbdv.202300839.

[23] L. Metelytsia, D. Hodyna, I. Dobrodub, I. Semenyuta, M. Zavhorodnii, V. Blagodatny, V. Kovalishyn, and O. Brazhko. (2020). "Design of (quinolin-4-ylthio)carboxylic acids as new Escherichia coli DNA gyrase B inhibitors: machine learning studies, molecular docking, synthesis and biological testing". *Computational Biology and Chemistry.* **85** : 107224. 10.1016/j.compbiolchem.2020.107224.

[24] Y. Gao, L. Guo, Y. Han, J. Zhang, Z. Dai, and S. Ma. (2023). "A Combination of In Silico ADMET Prediction, In Vivo Toxicity Evaluation, and Potential Mechanism Exploration of Brucine and Brucine N-oxide-A Comparative Study". *Molecules.* **28** (3). 10.3390/molecules28031341.

[25] E. N. Muratov, J. Bajorath, R. P. Sheridan, I. V. Tetko, D. Filimonov, V. Poroikov, T. I. Oprea, Baskin, II, A. Varnek, A. Roitberg, O. Isayev, S. Curtarolo, D. Fourches, Y. Cohen, A. Aspuru-Guzik, D. A. Winkler, D. Agrafiotis, A. Cherkasov, and A. Tropsha. (2020). "QSAR without borders". *Chemical Society Reviews.* **49** (11): 3525-3564. 10.1039/d0cs00098a.

[26] S. Seal, M. Mahale, M. Garcia-Ortegon, C. K. Joshi, L. Hosseini-Gerami, A. Beatson, M. Greenig, M. Shekhar, A. Patra, C. Weis, A. Mehrjou, A. Badre, B. Paisley, R. Lowe, S. Singh, F. Shah, B. Johannesson, D. Williams, D. Rouquie, D. A. Clevert, P. Schwab, N. Richmond, C. A. Nicolaou, R. J. Gonzalez, R. Naven, C. Schramm, L. R. Vidler, K. Mansouri, W. P. Walters, D. D. Wilk, O. Spjuth, A. E. Carpenter, and A. Bender. (2025). "Machine Learning for Toxicity Prediction Using Chemical Structures: Pillars for Success in the Real World". *Chemical*

Research in Toxicology. **38** (5): 759-807. 10.1021/acs.chemrestox.5c00033.

[27] I. Sushko, A. K. Pandey, S. Novotarskyi, R. Körner, M. Rupp, W. Teetz, S. Brandmaier, A. Abdelaziz, V. V. Prokopenko, V. Y. Tanchuk, R. Todeschini, A. Varnek, G. Marcou, P. Ertl, V. Potemkin, M. Grishina, J. Gasteiger, Baskin, II, V. A. Palyulin, E. V. Radchenko, W. J. Welsh, V. Kholodovych, D. Chekmarev, A. Cherkasov, J. Aires-de-Sousa, Q. Y. Zhang, A. Bender, F. Nigsch, L. Patiny, A. Williams, V. Tkachenko, and I. V. Tetko. (2011). "Online chemical modeling environment (OCHEM): web platform for data storage, model development and publishing of chemical information". *Journal of Cheminformatics.* **3** (S1): 10.1186/1758-2946-3-s1-p20.

[28] US EPA. ECOTOX Database, Version 4.0

[29] IRCCS. VEGA HUB

[30] I. Sushko, S. Novotarskyi, R. Korner, A. K. Pandey, M. Rupp, W. Teetz, S. Brandmaier, A. Abdelaziz, V. V. Prokopenko, V. Y. Tanchuk, R. Todeschini, A. Varnek, G. Marcou, P. Ertl, V. Potemkin, M. Grishina, J. Gasteiger, C. Schwab, Baskin, II, V. A. Palyulin, E. V. Radchenko, W. J. Welsh, V. Kholodovych, D. Chekmarev, A. Cherkasov, J. Aires-de-Sousa, Q. Y. Zhang, A. Bender, F. Nigsch, L. Patiny, A. Williams, V. Tkachenko, and I. V. Tetko. (2011). "Online chemical modeling environment (OCHEM): web platform for data storage, model development and publishing of chemical information". *Journal of Computer-Aided Molecular Design.* **25** (6): 533-54. 10.1007/s10822-011-9440-2.

[31] P. Karpov, G. Godin, and I. V. Tetko. (2020). "Transformer-CNN: Swiss knife for QSAR modeling and interpretation". *Journal of Cheminformatics.* **12** (1): 17. 10.1186/s13321-020-00423-w.

[32] I. V. Tetko, P. Karpov, E. Bruno, T. B. Kimber, and G. Godin. (2019). In: "Artificial Neural Networks and Machine Learning – ICANN 2019: Workshop and Special Sessions, (Lecture Notes in Computer Science, ch. Chapter 79". 831-835. 10.1007/978-3-030-30493-5_79.

[33] I. V. Tetko. (2008). "Associative neural network". *Methods in Molecular Biology.* **458** : 185-202. 10.1007/978-1-60327-101-1_10.

[34] I. V. Tetko and V. Y. Tanchuk. (2002). "Application of associative neural networks for prediction of lipophilicity in ALOGPS 2.1 program". *Journal of Chemical Information and Computer Sciences.* **42** (5): 1136-45. 10.1021/ci025515j.

[35] H. Hong, Q. Xie, W. Ge, F. Qian, H. Fang, L. Shi, Z. Su, R. Perkins, and W. Tong. (2008). "Mold(2), molecular descriptors from 2D structures for chemoinformatics and toxicoinformatics". *Journal of Chemical Information and Modeling.* **48** (7): 1337-44. 10.1021/ci800038f.

[36] H. Hong. Mold2

[37] I. V. Tetko, I. Sushko, A. K. Pandey, H. Zhu, A. Tropsha, E. Papa, T. Oberg, R. Todeschini, D. Fourches, and A. Varnek. (2008). "Critical assessment of QSAR models of environmental toxicity against Tetrahymena pyriformis: focusing on applicability domain and overfitting by variable selection". *Journal of Chemical Information and Modeling.* **48** (9): 1733-46. 10.1021/ci800151m.

[38] N. N. Jonhos and F. G. Leone.(1977)." Statistic and Experimental Design in Engineering and the Physical Sciences". John Wiley & Sons, New York.

[39] I. Sushko, S. Novotarskyi, R. Körner, A. K. Pandey, V. V. Kovalishyn, V. V. Prokopenko, and I. V. Tetko. (2010). "Applicability domain for in silico models to achieve accuracy of experimental measurements". *Journal of Chemometrics.* **24** (3-4): 202-208. 10.1002/cem.1296.

[40] I. Sushko. OCHEM User's Manual

[41] OECD. (2004). "Test No. 202: Daphnia sp. Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals, Section 2". OECD Publishing, Paris. 10.1787/9789264069947-en.

[42] D. R. M. Passino and S. B. Smith. (1987). "Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish". *Environmental Toxicology and*

Chemistry. **6** (11): 901-907. 10.1002/etc.5620061111.

[43] S. Sosnin, D. Karlov, I. V. Tetko, and M. V. Fedorov. (2019). "Comparative Study of Multitask Toxicity Modeling on a Broad Chemical Space". *Journal of Chemical Information and Modeling.* **59** (3): 1062-1072. 10.1021/acs.jcim.8b00685.

[44] C. Knox, M. Wilson, C. M. Klinger, M. Franklin, E. Oler, A. Wilson, A. Pon, J. Cox, N. E. L. Chin, S. A. Strawbridge, M. Garcia-Patino, R. Kruger, A. Sivakumaran, S. Sanford, R. Doshi, N. Khetarpal, O. Fatokun, D. Doucet, A. Zubkowski, D. Y. Rayat, H. Jackson, K. Harford, A. Anjum, M. Zakir, F. Wang, S. Tian, B. Lee, J. Liigand, H. Peters, R. Q. R. Wang, T. Nguyen, D. So, M. Sharp, R. da Silva, C. Gabriel, J. Scantlebury, M. Jasinski, D. Ackerman, T. Jewison, T. Sajed, V. Gautam, and D. S. Wishart. (2024). "DrugBank 6.0: the DrugBank Knowledgebase for 2024". *Nucleic Acids Research.* **52** (D1): D1265-D1275. 10.1093/nar/gkad976.

[45] I. Oprisiu, S. Novotarskyi, and I. V. Tetko. (2013). "Modeling of non-additive mixture properties using the Online CHEmical database and Modeling environment (OCHEM)". *Journal of Cheminformatics.* **5** (1): 4. 10.1186/1758-2946-5-4.

[46] L. Persson, S. Karlsson-Vinkhuyzen, A. Lai, Å. Persson, and S. Fick. (2017). "The Globally Harmonized System of Classification and Labelling of Chemicals—Explaining the Legal Implementation Gap". *Sustainability.* **9** (12). 10.3390/su9122176.

[47] L. M. Ferreira, P. Garcia-Garcia, P. A. Garcia, and M. A. Castro. (2025). "A review on quinolines: New green synthetic methods and bioactive potential". *European Journal of Pharmaceutical Sciences.* **209** : 107097. 10.1016/j.ejps.2025.107097.

[48] S. O. Zaraei, N. N. Al-Ach, H. S. Anbar, R. El-Gamal, H. Tarazi, R. T. Tokatly, R. R. Kalla, M. A. Munther, M. M. Wahba, A. M. Alshihabi, M. K. Shehata, R. M. Sbenati, A. I. Shahin, R. El-Awady, T. H. Al-Tel, and M. I. El-Gamal. (2022). "Design and synthesis of new quinoline derivatives as selective C-RAF kinase inhibitors with potent anticancer activity". *European Journal of Medicinal Chemistry.* **238** : 114434. 10.1016/j.ejmech.2022.114434.

[49] S. Eswaran, A. V. Adhikari, and R. Ajay Kumar. (2010). "New 1,3-oxazolo[4,5-c] quinoline derivatives: synthesis and evaluation of antibacterial and antituberculosis properties". *European Journal of Medicinal Chemistry.* **45** (3): 957-66. 10.1016/j.ejmech.2009.11.036.

[50] S. Eswaran, A. V. Adhikari, and N. S. Shetty. (2009). "Synthesis and antimicrobial activities of novel quinoline derivatives carrying 1,2,4-triazole moiety". *European Journal of Medicinal Chemistry.* **44** (11): 4637-47. 10.1016/j.ejmech.2009.06.031.

[51] L. Cheng, P. P. Cai, R. R. Zhang, L. Han, C. X. Tan, J. Q. Weng, T. M. Xu, and X. H. Liu. (2019). "Synthesis and Insecticidal Activity of New Quinoline Derivatives Containing Perfluoropropanyl Moiety". *Journal of Heterocyclic Chemistry.* **56** (4): 1312-1317. 10.1002/jhet.3502.

[52] I. R. Rao, P. Punitha, B. Premalatha, T. S. Prasad, and M. Suresh. (2024). "Synthesis, structure identification, antioxidant and antimicrobial activities of some novel quinoline derivatives". *Discover Chemistry.* **1** (1). 10.1007/s44371-024-00068-w.

[53] T. Backhaus, M. Scholze, and L. H. Grimme. (2000). "The single substance and mixture toxicity of quinolones to the bioluminescent bacterium Vibrio fischeri". *Aquatic Toxicology.* **49** (1-2): 49-61. 10.1016/s0166-445x(99)00069-7.

[54] J. Neuwoehner, A. K. Reineke, J. Hollender, and A. Eisentraeger. (2009). "Ecotoxicity of quinoline and hydroxylated derivatives and their occurrence in groundwater of a tar-contaminated field site". *Ecotoxicology and Environmental Safety.* **72** (3): 819-27. 10.1016/j.ecoenv.2008.04.012.

[55] J. A. Black, W. J. Birge, A. G. Westerman, and P. C. Francis. (1983). "Comparative aquatic toxicology of aromatic hydrocarbons". *Fundamental and Applied Toxicology.* **3** (5): 353-8. 10.1016/s0272-0590(83)80004-9.