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RESEARCH ARTICLE

QSAR modeling for acute toxicity prediction of fluroquinolone antibiotics by using software

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Abstract

Fluoroquinolones are a class of antibiotics known as antibacterial agents, which kills pathogenic bacteria. Quantitative structure-activity relationship (QSAR) plays an important role in toxicity prediction. The present study deals with acute toxicity prediction as LD_{50} (median lethal dose) values of fluoroquinolone antibiotics in rat by oral exposure through QSAR modeling software package. The comparisons were made between existing LD_{50} values through bioassay (experimental) from PubChem (ChemIDplus) database and predicted LD_{50} values by using T.E.S.T. (Toxicity Estimation Software Tool) for fluoroquinolone antibiotics oral administration in rat. Among the selected 23 fluoroquinolones, experimental data of only 8 fluoroquinolones were obtained and LD_{50} values were predicted of these 8 compounds. The present predicted LD_{50} values from T.E.S.T. for acute toxicity results of six fluroquinolones viz. ciprofloxacilin, ofloxacin, lomefloxacin, fleroxacin, levofloxacin and prulifloxacin were higher while other two fluroqunolones viz. enoxacin and norfloxacin were lower in comparison to experimental values. This software helps to predict the exact LD_{50} values when compared to experimental data were reported in range (>2000 to >5000 mg/kg). This is a preliminary observation as easy screening of LD_{50} values with the particular software package. Further study may be relevant with other softwares to compare the predicted data.

INTRODUCTION

Antibiotics are known as chemotherapeutic agents, which are the combinations of chemical substances. These can kill or inhibit the growth of bacteria by various mechanisms like blocking essential functions of bacteria cell (Davey, 2000). It was known that drugs or medicines have specific mode of actions, may probably exert effects on terrestrial and aquatic ecosystems, when discharged into the medium like soil and water in the environment (Macri et al., 1988; Lanzky et al., 1997; Wollenberger et al., 2000). Soni, (2012) has reviewed that fluoroquinolones are a class of antibiotics with potent bactericidal and broad spectrum inhibitory activity against several pathogens that are responsible for variety of infections including urinary tract infections (UTI), gastrointestinal infections, respiratory tract infections (RTI), sexually transmitted diseases (STD), skin infections etc.

Quantitative structure–activity relationship (QSAR) is a mathematical model that attempt to relate the structure-derived features (molecular descriptors) of a chemical compound to its biological or physicochemical activity. Therefore, this method has been established for the predictive and ultimately diagnostic abilities. This can be used to predict the biological activity viz. IC_{50}, LC_{50}/LD_{50}, EC_{50} etc. or class viz. inhibitor versus non-inhibitor of compounds before the actual bioassay. The molecular descriptors for QSAR are used on the basis of thermodynamic, steric and electronic parameters (Choplin, 2005; Valentina et al., 2009). These parameters include partition coefficient, molecular volume, surface area, molecular refractivity etc. Also, the structural descriptors, which provides information about the various toxicological and pharmacokinetic aspects of the synthesized molecules includes E-state functions, kappa index, Chi index, Lipinski five rules and Wiener index (USEPA, 2012).

An *in silico* method is also based on quantitative structure–activity relationship (QSAR) models, which can be used to understand drug action, design new compounds or drugs and screen chemical libraries (Yap et al., 2006; Guido et al., 2008; Schwaighofer et al., 2009; Valerio, 2009). The experimental measurement as bioassay with animals for compounds is difficult, more expensive and time-consuming, thus a great, facinating effort has been done into attempting to predict biological activity through QSAR along with statistical modeling (Kovalishyn et al., 2014). Recently, the European Chemicals Legislation, Registration, Evaluation and Authorization of Chemicals (REACH) have suggested the use of *in silico* method as a study for reliable toxicological risk assessment (Worth et al., 2007; Lilienblum et al., 2008). According to Singh et al. (2014), multispecies QSARs modeling tools are suitable of predicting the acute toxicity of various chemicals in recommended several test species by Organization for Economic Co-operation and Development (OECD) in different trophic levels such as algae, daphnia, fish and bacteria to help in regulatory toxicology. Some studies on design, synthesis and drug development of antibiotics as antibacterial agents by QSAR modeling (Pil'o et al., 2002; Kumar et al., 2011; Prajapat et al., 2011; Joshi et al., 2012; Kovalishyn et al., 2014; Patil et al., 2014) and few works on toxicity prediction have been reported (Venkataramana et al., 2011). According to Soni, (2012), the growth in understanding of structure activity relationships with fluoroquinolones has been enabled the development of even better chemical compounds. But less works have been reviewed to evaluate acute toxicity prediction of fluoroqunilones antibiotics in mammals through QSAR methods (Chu and Fernandest, 1989; Tillotson, 1996).

There are several recommended toxicity prediction softwares viz. TOPKAT (Toxicity Prediction by Komputer Assisted Technology) (Accelrys, 2004), DRAGON (Talete, 2006), ADMET (Absorption, Distribution, Metabolism, Elimination, and Toxicity) 2 and 3 (User manual, Simulation Plus Inc, 2011), V-life MDS (V-life Technologies, 2006) and ADME (Pallas, Compu Drug International Inc., 2000), T.E.S.T. (Toxicity Estimation Software Tool) (USEPA, 2012), PADEL (Yap, 2011), MDL QSAR (Elsevier MDL, 2006), Molconn-z (Edusoft-LC, 2006) etc. According to USEPA (2012), T.E.S.T. software is a simple QSAR models to calculate the toxicity of chemicals using a simple linear function of molecular descriptors is as follows:

$$\text{Toxicity} = ax_1 + bx_2 + c$$

Where, x_1 and x_2 are the independent descriptor variables and a, b, and c are fitted parameters. The T.E.S.T software provides multiple prediction methodologies, which has greater confidence in the predicted toxicities (as assuming the predicted toxicities are closely similar from different methods). In addition some researchers may have more confidence in particular QSAR approaches based on value added experience.

In this present study an attempt has been made to predict acute toxicity of fluoroquinolone antibiotics in the rat oral exposure for LD_{50} values through QSAR modeling software package. The comparisons were made between existing LD_{50} values through bioassay as experimental and predicted LD_{50} values by using T.E.S.T. (Toxicity Estimation Software Tool) software for fluoroquinolone antibiotics.

MATERIALS AND METHODS:

Name of the compound and its derivatives

There were established 23 types of fluoroquinolone antibiotics selected based on attached fluorine atoms with the central ring system and tabulated their structure, CAS (Chemical Abstracts Services) no. and SMILES (simplified molecular-input line-entry system) string were taken from ChemIDplus of USEPA (Table 1). These fluoroquinolone antibiotics were selected and tabulated on the basis of bacterial resistance with respective three generations viz.

second, third and forth types. According to Lodha, (2008) the essential structure of fluroquinolone with some recent trends in chemical modifications is as follows:

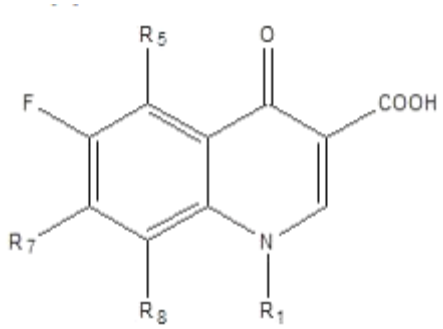


Fig. 1 – Essential scafford structure of fluroquinolone

Where, R1, R5, R7 and R8 are substitution positions in primitive fluoroquinolone moiety.

Name of the software used

In present study, the software was used namely T.E.S.T Verson 4.1.

Meta data used for LD_{50} of rat oral exposure

Meta data i.e. experimental data (mg/kg) for rat oral LD_{50} values were collected from PubChem (ChemIDplus) and converted to Log LD_{50} value for individual antibiotic derivatives.

QSAR modeling by using T.E.S.T. software

The QSAR modeling software package was used to estimate the LD_{50} values of fluroqinolone (Figure 1) and its derivatives. The software used was Toxicity Estimation Software Tool or T.E.S.T., Version 4.1 (US EPA, 2012). The acute toxicity prediction of rat oral LD_{50} values were compared between bioassay results as experimental data collected from PubChem (ChemIDplus) and predicted values were obtained after operating the above mentioned software.

It was reported that T.E.S.T. software package estimates toxicity using a variety of QSAR methodologies (Martin et al., 2008), such as hierarchical clustering, the Food and Drug Administration (FDA) MDL, nearest neighbor and a consensus model, which is simply the average of the predicted toxicities from other QSAR methodologies, considering the applicability domain in each method (Zhu et al., 2009). The required descriptors are calculated without requiring any external programs. A structure of a chemical can easily be shown after entering CAS no of particular chemical. After showing the structure, the chemical's toxicity can be estimated using one of several advanced methodologies. This software calculates LD_{50} values from 7,420 chemicals (Martin et al., 2008). Generally molecular descriptors are physical characteristics of the structure of chemicals viz. the molecular weight or the number of benzene rings of a chemical. The overall pool of descriptors in the software (T.E.S.T.) contains 797 two-dimensional descriptors. The descriptors include the classes of descriptors viz. E-state values and E-state counts, constitutional descriptors, topological descriptors, walk and path counts, connectivity, information content, 2d autocorrelation, Burden eigenvalue, molecular property (such as the octanol-water partition coefficient), Kappa, hydrogen bond acceptor/donor counts, molecular distance edge, and molecular fragment counts. Following important methods were described in instruction manual for the present software (USEPA, 2012):

Hierarchical clustering method

In T.E.S.T., the hierarchical clustering method utilizes a variation of the Ward's Minimum Variance Clustering Method to contribute a series of clusters from the initial training set as per Romesburg, (1984). According to Ruiz et al. (2012), the change in variance caused by combining clusters j and k is in equation follows:

$$\Delta\sigma^2 = \frac{n_j n_k}{n_j + n_k} \sum_{i=1}^{d} (C_{j,i} - C_{k,i})^2 \quad (1)$$

where n_j is the number of chemicals in cluster j, $C_{j,i}$ is the centroid (or average value) for descriptor i for cluster j, and d is the number of descriptors (~800) (Martin et al., 2008). It was noted that the predicted value for a given test chemical is calculated using the equally weighted average of the model predictions from the closest cluster from each step in the hierarchical clustering.

FDA MDL QSAR method

In T.E.S.T., the FDA MDL method is based on the work of Contrera et al. (2003). In this method, it was noted that predictions for each test chemical are made using a unique cluster (constructed at runtime). It contains structurally similar chemicals selected from the overall training set. It is in different to the Hierarchical method, where the predictions are made using one or more clusters, which are constructed *a priori* using Ward's method. For individual test chemical, a cluster is constructed using the 30 most similar chemicals from the training set as defined by the cosine similarity coefficient, $SC_{i,k}$, which is calculated by the equation as follows as per USEPA (2012):

$$SC_{i,k} = \frac{\sum_{j=1}^{\#\,descriptors} x_{ij}\, x_{kj}}{\sqrt{\sum_{j=1}^{\#\,descriptors} x_{ij}^2 * \sum_{j=1}^{\#\,descriptors} x_{kj}^2}} \quad (2)$$

where x_{ij} is the value of the j-th normalized descriptor for chemical i (normalized with respect to all of the chemicals in the original training set) and x_{kj} is the value of the j-th descriptor for chemical k. The entire pool of approximately 800 descriptors is always used to calculate the similarity coefficient in equation (2). A multiple linear regression model is then built for the new cluster using a genetic algorithm-based method, and the toxicity can be easily predicted (Zhu et al., 2009).

Nearest neighbor method

In T.E.S.T. (USEPA, 2012), The nearest neighbor method is a simplification of the variable selection of kNN approach. It was observed in the nearest neighbor method, the toxicity is simply predicted as the average of the toxicity of the three most similar chemicals from the training set. The similarity is defined in terms of the cosine similarity coefficient (Equation 2).

Consensus method

In the consensus method of T.E.S.T., the predicted toxicity is simply the average of the predicted toxicities from the above mentioned QSAR methodologies considering the applicability domain of individual method (Zhu et al. 2008). It was suggested, if only a single QSAR methodology can make a prediction then the predicted value is unreliable and unable to use. This method typically provides the highest prediction accuracy by the predictions from the other above mentioned methods. In addition this method provides the highest prediction coverage because several methods with slightly different applicability domains are used to make a prediction (Ruiz et al., 2012).

Statistical external validation

In T.E.S.T., the predictive ability of each of the QSAR methodologies was evaluated using statistical external validation as per Gramatica and Pilutti (2004). According to Golbraikh et al. (2003), a QSAR model is acceptable on predictive power if the following equations are satisfied:

$$q^2 > 0.5; \quad \textbf{(3)}$$

$$R^2 > 0.6; \quad \textbf{(4)}$$

$$\frac{\left(R^2 - R_o^2\right)}{R^2} < 0.1 \text{ and } 0.85 \leq k \leq 1.15 \quad \textbf{(5)}$$

where q^2 is the leave one out correlation coefficient for the training set, R^2 is correlation coefficient between the observed and predicted toxicities for the test set, R^2_o is correlation coefficient between the observed and predicted toxicities for the test set with the Y-intercept set to zero (where the regression line is given by Y=kX). The prediction accuracy was evaluated in terms of equations (4) and (5). In addition the accuracy will be evaluated in terms of the RMSE (root mean square error), and the MAE (mean absolute error) for the test set. It has been demonstrated that q^2 (the leave one out correlation coefficient for the training set) is not correlated with R^2 for the test set (Golbraikh and Tropsha, 2002).

Applicability domains

A concept of the applicability domain (AD) was created and used to avoid such an incorrect extrapolation of activity predictions in T.E.S.T. According to Ruiz et al. (2012), the QSAR model can predict the potential toxicity of any chemical but the predictive confidence may vary. Generally each model is processed using a training set of chemicals, which cover only a small fraction of the entire chemical world and it was observed that its prediction capability is restricted to its AD, called as its descriptor space. As a result of this, only a certain fraction of chemicals of an external data set can be reasonably predicted. So it is promising to determine the chemical of interest falls within or outside the AD of a particular model. In context, varying degrees of uncertainties could be validated with such a prediction. For model ADs, features and limitations need to be understood thoroughly for the appropriate interpretation of predictive results (Tropsha and Golbraikh, 2007; Golbraikh et al., 2003; Golbraikh and Tropsha, 2002; Netzeva et al., 2005; Schultz et al., 2007; Tetko et al., 2008; Roy et al., 2011; Ruiz et al., 2012).

RESULTS

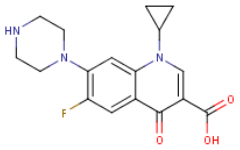
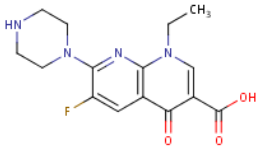
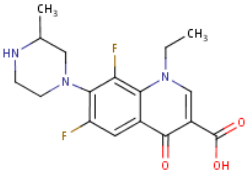
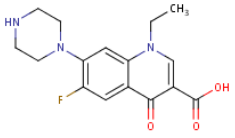
In Table 2, the acute toxicity prediction data were tabulated, out of the 23 selected fluroquinolones, the experimental bioassay as rat oral LD_{50} values of 11 fluroquinolones viz. ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, ofloxacin, fleroxacin, levofloxacin, sparfloxacin, balofloxacin, pazufloxacin and prulifloxacin were reported in PubChem (ChemIDplus) database. Among these antibiotics, experimental LD_{50} values were obtained as the range of minimum (>2000 to >5000 mg/kg) except in 3 compounds such as lomefloxacilin (3800 mg/kg), ofloxacin (3590 mg/kg) and levofloxacin (1478 mg/kg). Other 12 derivatives like nadifloxacin, pefloxacin, rufloxacin, grepafloxacin, tosulfloxacin, temafloxacin, gatifloxacin, gemifloxacin, moxifloxacin, trovafloxacin, clinafoxacin and sitafloxacin, the bioassay data of same test model were not found in the database. Interestingly, it was obtained the exact predicted LD_{50} values for 14 compounds but the T.E.S.T. unable to calculate 9 compounds due to unidentified CAS No. in the software. Out of 14 compounds, 5 compounds were not considered because of unavailability of bioassay data. The prediction of LD_{50} values of rat oral exposure were estimated for 8 derivatives viz. ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, ofloxacin, fleroxacin, levofloxacin and prulifloxacin by using software T.E.S.T. consensus method. The prediction was also evaluated for six derivatives viz. nadifloxacin, pefloxacin, grepafloxacin, tosulfloxacin, temafloxacin and trovafloxacin by using T.E.S.T. but these were not included in comparison between experimental versus predicted data due to unavailability of experimental data. All the predicted and experimental LD_{50} values were calculated in $logLD_{50}$ values. The r^2 value of prediction data of 11 compounds from FDA cluster model fit results for individual compound was tabulated and the residual value of 8 compounds was also calculated in Table 2.

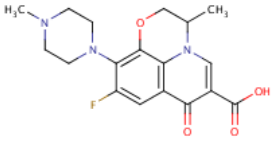
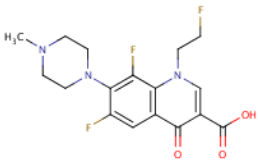
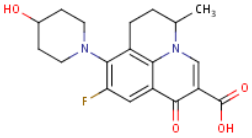
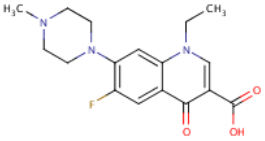
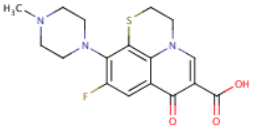
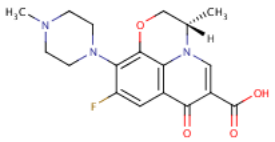
The eight chemicals were very well represented in the model database as assessed by the statistical analysis. The similarity analysis showed that there are several chemicals in the database that have very close similarity distance in T.E.S.T. The confidence in the assessment between experimental and predicted of $-logLD_{50}$ values (mol/kg) were represented graphically for individual fluroquinolone for both test set as well as training set along with Mean

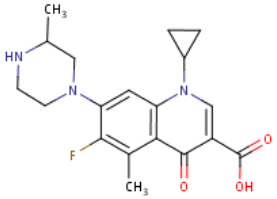
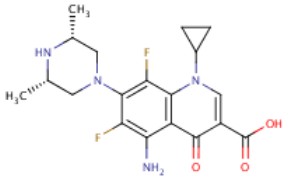
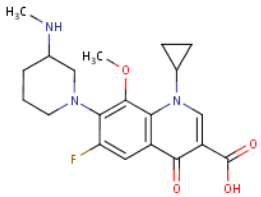
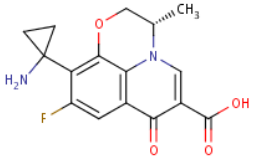
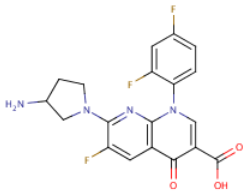
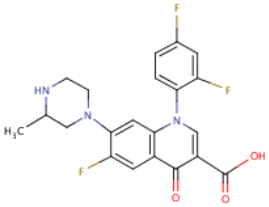
absolute error (MAE) value calculated by software itself (Fig. 2 A & A1; B & B1; C & C1; D & D1; E & E1; F & F1; G & G1 and H & H1). If the MAE was lower than the value for the entire test and training set then the predicted value increases the confidence. The present predicted values from acute toxicity results of four fluroquinolones viz. ciprofloxacilin, enoxacin, norfloxacilin, levofloxacin were higher while other four fluroqunolones viz. ofloxacin, fleroxacin, lomefloxacin and prulifloxacin were lower comparing to experimental values (Table 2).

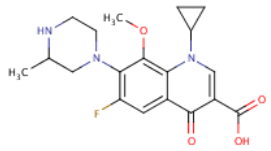
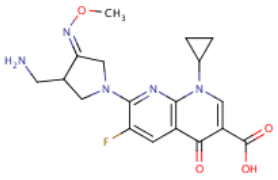
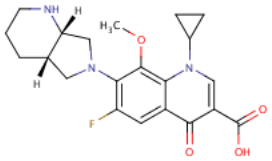
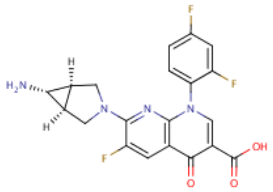
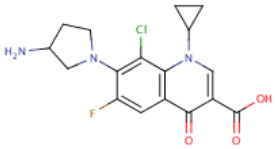
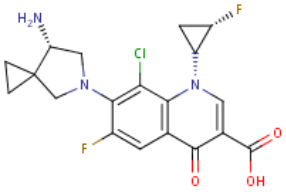
Several fluroquinolone antibiotic and its related compounds are available in numbers. The selected fluroquinolones were only 23 types in the present study. Moreover, acute toxicity studies with special reference to oral LD_{50} values in rat were found very less in number i.e. only 11 types of fluroquinolones. These fluoroquinolone compounds are ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, ofloxacin, fleroxacin, levofloxacin, sparfloxacin, balofloxacin, pazufloxacin and prulifloxacin. The experimental data were obtained from PubChem (ChemIDplus) database (Table 2) but in the software it was unable to predict LD_{50} value of 3 compounds viz. sparfloxacin, balofloxacin and pazufloxacin due to unidentified CAS No. Besides these, rest derivatives even lack rat oral LD_{50} values. QSAR modeling was carried out with the help of T.E.S.T. software (USEPA, 2012).

Table 1 – List of fluoroquinolone class of antibiotics

Sl no.	Generic Name	CAS No.	SMILES	structure
Second generation				
1.	Ciprofloxacin	85721-33-1	C1CC1N2C=C(C(=O)C3=CC(=C(C=C32)N4CCNCC4)F)C(=O)O	
2.	Enoxacin	74011-58-8	Fc1c(nc2c(c1)C(=O)C(\C(=O)O)=C/N2CC)N3CCNCC3	
3.	Lomefloxacin	98079-51-7	Fc1c(c(F)c2c(c1)C(=O)C(\C(=O)O)=C/N2CC)N3CC(NCC3)C	
4.	Norfloxacin	70458-96-7	O=C(O)\C2=C\N(c1cc(c(F)cc1C2=O)N3CCNCC3)CC	

5.	Ofloxacin	82419-36-1	Fc4cc1c2N(/C=C(\C1=O)C(=O)O)C(COc2c4N3CCN(C)CC3)C	
6.	Fleroxacin	79660-72-3	CN1CCN(CC1)C2=C(C=C3C(=C2F)N(C=C(C3=O)C(=O)O)CCF)F	
7.	Nadifloxacin	124858-35-1	CC1CCC2=C3N1C=C(C(=O)C3=CC(=C2N4CCC(CC4)O)F)C(=O)O	
8.	Pefloxacin	70458-92-3	O=C(O)\C2=C\N(c1cc(c(F)cc1C2=O)N3CCN(C)CC3)CC	
9.	Rufloxacin	101363-10-4	CN1CCN(CC1)C2=C(C=C3C4=C2SCCN4C=C(C3=O)C(=O)O)F	
Third generation				
10.	Levofloxacin	100986-85-4	C[C@H]1COc2c3n1cc(c(=O)c3cc(c2N4CCN(CC4)C)F)C(=O)O	

11.	Grepafloxacin	119914-60-2	O=C(O)\C2=C\N(c1cc(c(F)c(c1C2=O)C)N3CC(NCC3)C)C4CC4	
12.	Sparfloxacin	110871-86-8	C[C@@H]1CN(C[C@@H](N1)C)c2c(c(c3c(c2F)n(cc(c3=O)C(=O)O)C4CC4)N)F	
13.	Balofloxacin	127294-70-6	CNC1CCCN(C1)C2=C(C=C3C(=C2OC)N(C=C(C3=O)C(=O)O)C4CC4)F	
14.	Pazufloxacin	127046-18-8	C[C@H]1COC2=C3N1C=C(C(=O)C3=CC(=C2C4(CC4)N)F)C(=O)O	
15.	Tosulfoxacin	100490-36-6	NC1CCN(C1)c1nc2n(cc(C(O)=O)c(=O)c2cc1F)-c1ccc(F)cc1F	
16.	Temafloxacin	108319-06-8	Fc1ccc(c(F)c1)N\3c2cc(c(F)cc2C(=O)C(/C(=O)O)=C/3)N4CC(NCC4)C	

Forth generation				
17.	Gatifloxacin	112811-59-3	Fc1c(c(OC)c2c(c1)C(=O)C(\C(=O)O)=C/N2C3CC3)N4CC(NCC4)C	
18.	Gemifloxacin	175463-14-6	Fc2c(nc1N(/C=C(/C(=O)O)C(=O)c1c2)C3CC3)N4C/C(=N\OC)C(C4)CN	
19.	Moxifloxacin	354812-41-2	COc1c2c(cc(c1N3C[C@@H]4CCCN[C@@H]4C3)F)c(=O)c(cn2C5CC5)C(=O)O	
20.	Trovafloxacin	147059-72-1	O=C(O)C2=CN(c1nc(c(F)cc1C2=O)N3C[C@H]4[C@H](N)[C@H]4C3)c5ccc(F)cc5F	
21.	Clinafoxacin	105956-99-8	Fc2c(c(Cl)c1N(/C=C(/C(=O)O)C(=O)c1c2)C3CC3)N4CCC(N)C4	
22.	Sitafloxacin	127254-12-0	F[C@H]5C[C@H]5N2/C=C(/C(=O)O)C(=O)c1cc(F)c(c(Cl)c12)N4C[C@@H](N)C3(CC3)C4	

23.	Prulifloxacin	123447-62-1	CC1N2C3=CC(=C(C=C3C(=O)C(=C2S1)C(=O)O)F)N4CCN(CC4)CC5=C(OC(=O)O5)C

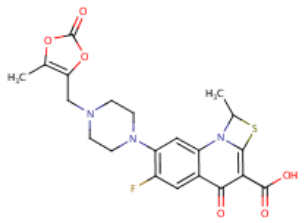
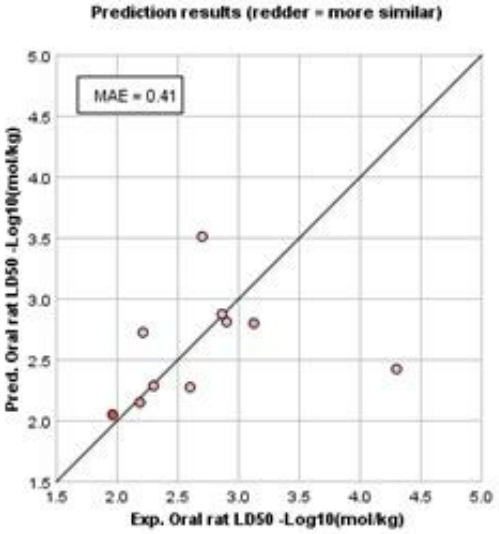


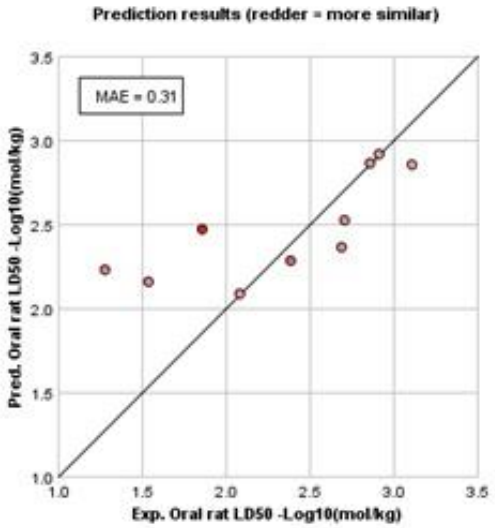
Table 2 – Prediction of LD_{50} values in rat by fluoroquinolone antibiotics in comparison to available and unavailable bioassay metadata

Sl no.	Name	Estimation by bioassay experiment		Estimation by T.E.S.T (Consensus method)		Statistical data validation by T.E.S.T	
		LD_{50} values (mg/Kg)	Log LD_{50} values (mg/Kg)	Predicted LD_{50} value (mg/kg)	Predicted Log LD_{50} value (mg/kg)	r^2 value of individual predicted data from FDA model	Residual
1.	Ciprofloxacin	>2000[a]	3.301	3506.65	3.545	0.911	-0.244
2.	Enoxacin	>5000[b]	3.699	5287.39	3.723	0.817	-0.024
3.	Lomefloxacin	3800[c]	3.580	3104.79	3.492	0.916	0.088
4.	Norfloxacin	>4000[d]	3.602	5016.64	3.700	0.889	-0.098
5.	Ofloxacin	3590[e]	3.555	1975.66	3.296	0.743	0.259
6.	Fleroxacin	>4000[f]	3.602	2903.66	3.462	0.881	0.140
7.	Nadifloxacin	n.a.	n.a.	1234.17	3.091	0.841	n.d.
8.	Pefloxacin	n.a.	n.a.	3446.93	3.537	0.817	n.d.
9.	Rufloxacin	n.a.	n.a.	n.f.	n.f.	n.f.	n.d.
10.	Levofloxacin	1478[g]	3.170	1975.66	3.296	0.743	-0.126
11.	Grepafloxacin	n.a.	n.a.	2828.79	3.451	0.894	n.d.
12.	Sparfloxacin	>5000[h]	3.699	n.f.	n.f.	n.f.	n.d.
13.	Balofloxacin	>5000[i]	3.699	n.f.	n.f.	n.f.	n.d.
14.	Pazufloxacin	>5000[j]	3.699	n.f.	n.f.	n.f.	n.d.
15.	Tosulfloxacin	n.a.	n.a.	3153.17	3.499	0.804	n.d.
16.	Temafloxacin	n.a.	n.a.	2295.92	3.361	0.835	n.d.
17.	Gatifloxacin	n.a.	n.a.	n.f.	n.f.	n.f.	n.d.
18.	Gemifloxacin	n.a.	n.a.	n.f.	n.f.	n.f.	n.d.
19.	Moxifloxacin	n.a.	n.a.	n.f.	n.f.	n.f.	n.d.
20.	Trovafloxacin	n.a.	n.a.	1472.14	3.168	0.907	n.d.
21.	Clinafoxacin	n.a.	n.a.	n.f.	n.f.	n.f.	n.d.
22.	Sitafloxacin	n.a.	n.a.	n.f.	n.f.	n.f.	n.d.
23.	Prulifloxacin	>5000[k]	3.699	1526.32	3.184	0.973	0.515

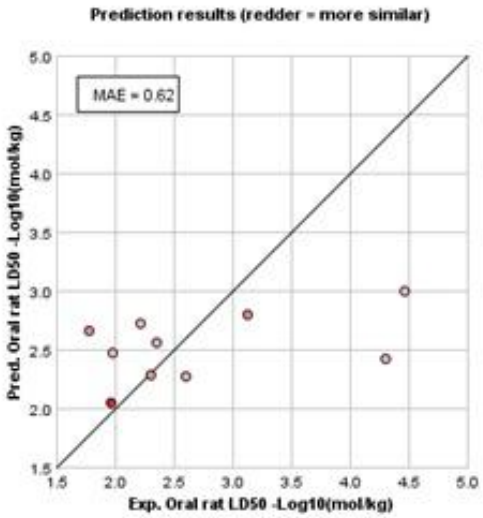
a to k = data from ChemIDplus; n.a. = Not Available; n.f. = Not found in T.E.S.T. software; n.d. = Not done



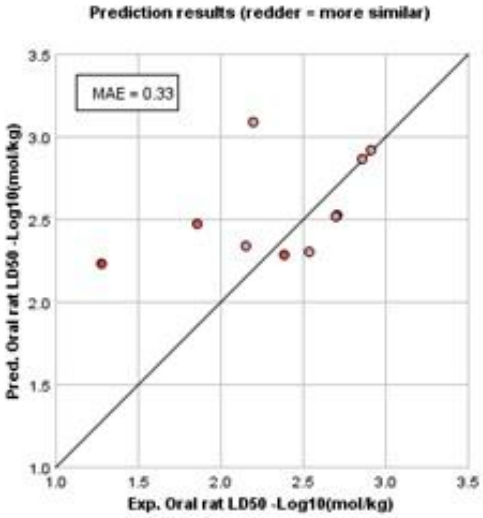
A= Prediction of ciprofloxacin with most similar chemicals in external test set



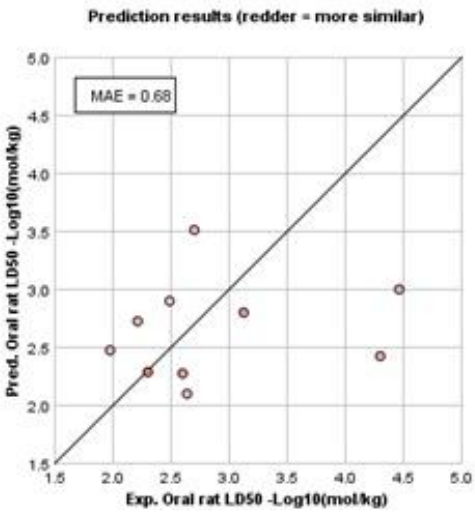
A1= Prediction of ciprofloxacin with most similar chemicals in training set



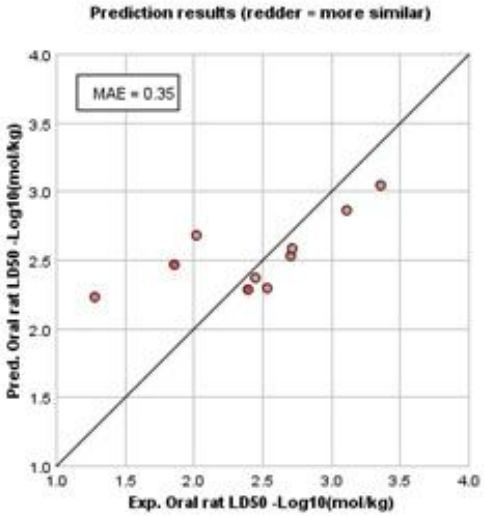
B= Prediction of enoxacin with most similar chemicals in external test set



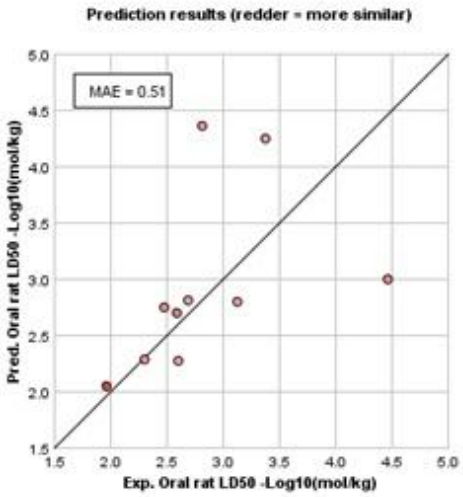
B1= Prediction of enoxacin with most similar chemicals in training set



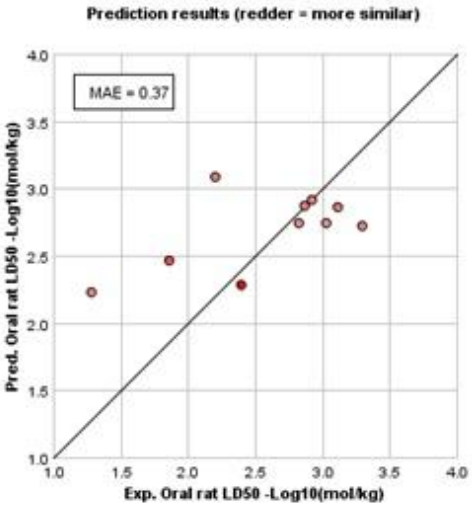
C= Prediction of lomefloxacin with most similar chemicals in external test set



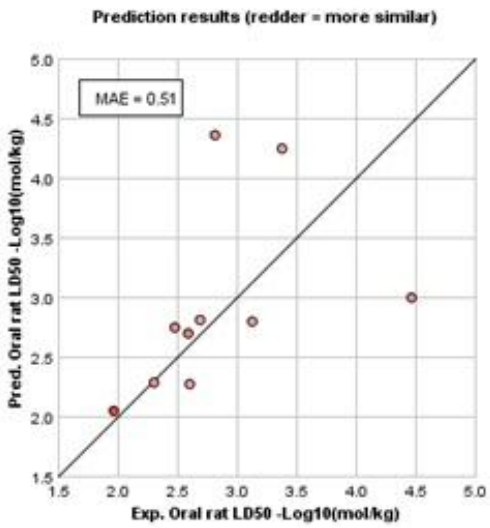
C1= Prediction of lomefloxacin with most similar chemicals in training set



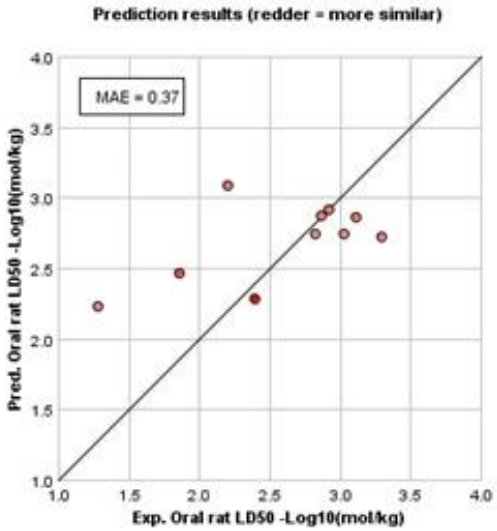
D= Prediction of norfloxacin with most similar chemicals in external test set



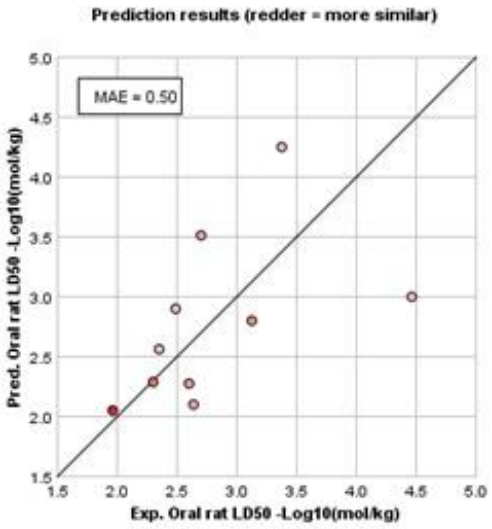
D1= Prediction of norfloxacin with most similar chemicals in training set



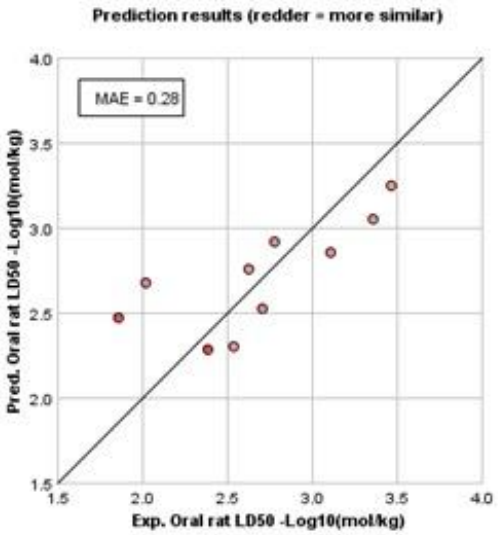
E= Prediction of ofloxacin with most similar chemicals in external test set



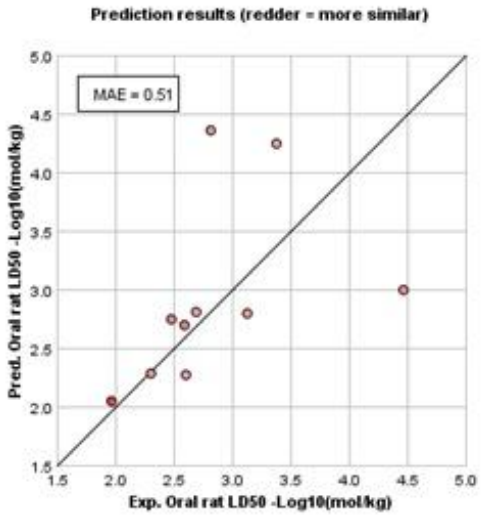
E1= Prediction of ofloxacin with most similar chemicals in training set



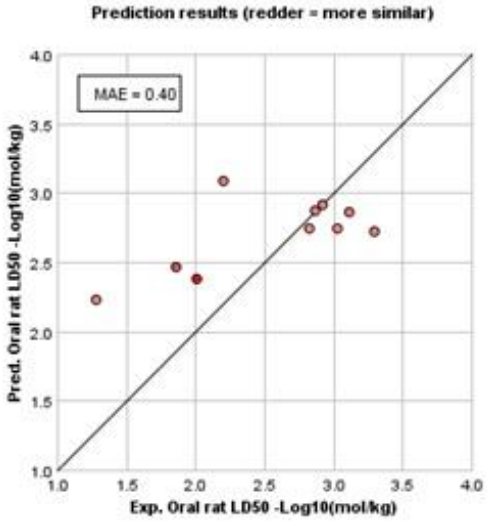
F= Prediction of fleroxacin with most similar chemicals in external test set



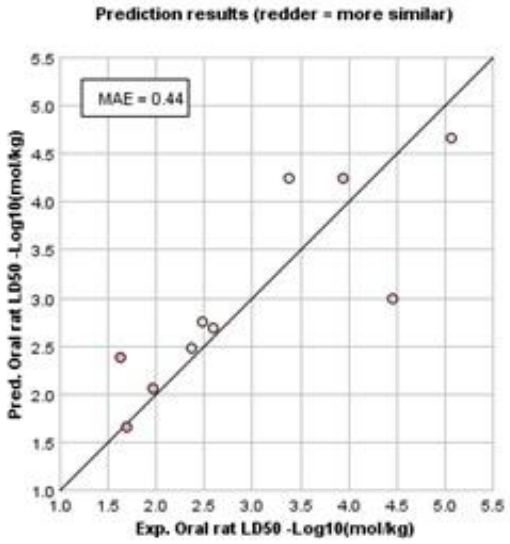
F1= Prediction of fleroxacin with most similar chemicals in training set



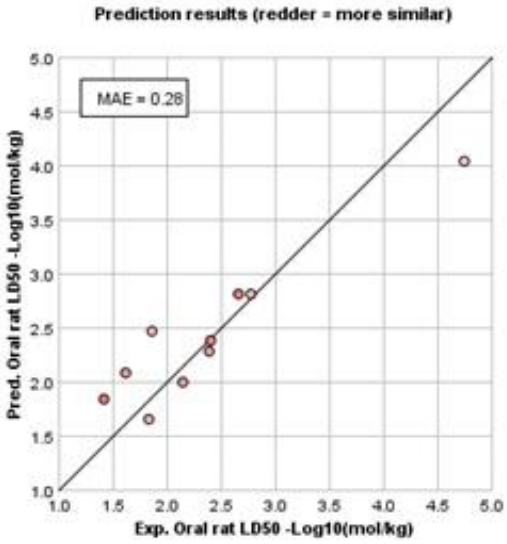
G= Prediction of levofloxacin with most similar chemicals in external test set



G1= Prediction of levofloxacin with most similar chemicals in training set



H= Prediction of prulifloxacin with most similar chemicals in external test set



H1= Prediction of prulifloxacin with most similar chemicals in training set

Fig. 2. Predicted rat oral LD_{50} values (mol/kg) of fluroquinolones by T.E.S.T. QSAR modeling. MAE = Mean absolute error in -Log10(mol/kg)

DISCUSSION

The present prediction results were supported by Wang et al. (2010). According to them, QSAR technique can be advantageous on discovering the relationship between the fluroquinolone molecular structures and their acute toxicities. It was known that the T.E.S.T. software estimates the predicted rat oral LD_{50} values by calculating several molecular descriptors viz. Constitutional descriptors, Chi Connectivity Indices, Kappa Shape Indices, Electrotopological State Indices, Fragments for each atom, 2D Molecular properties, Information Indices, Burden eigenvalue descriptors, Topological descriptors, Walk and Path counts, 2D Autocorrelation Descriptors, Molecular Properties and Molecular Distance Edge Descriptors. These molecular descriptors have used to detect toxicity prediction in mammals by QSAR modeling (Gombar and Jain, 1987; Gombar and Enslein, 1990; Hall et al., 1991; Ruiz et al., 2012). Linear regression analysis is a widely useful quantization method at the end of QSAR study (Xu, 2004**)**.

In this present study, the LD_{50} predicted values were compared with the available experimental data of fluroquinolones from ChemIDplus for QSAR modeling by using T.E.S.T. software. It was reported that T.E.S.T. software has better performance than other softwares like ADMET and TOPKAT for the prediction of sulfur mustard and its breakdown products on mammal (Ruiz et al., 2012). According to studies by Canadian Center for Occupational Health & Safety (2012) and Ruiz et al. (2012), the toxicity ranges were determined as super toxic (<5 mg/kg), extremely toxic (5–50 mg/kg), very toxic (50–500 mg/kg), moderately toxic (500–5,000 mg/kg), slightly toxic (5,000–15,000 mg/kg) and practically non-toxic (>15,000 mg/kg). The present predicted acute toxicity results with special reference to LD_{50} values of 6 fluroquinolones viz. ciprofloxacilin, ofloxacin, fleroxacin, levofloxacin and prulifloxacin were within range of 1500 to >3500 mg/kg as moderately toxic and other 2 fluroqunolone viz. enoxacin and norfloxacin was higher value of >5000 mg/kg as slightly toxic. According to Li et al. (2014), the quinolones showed limited acute toxicity, the coexistence of multiple quinolones in environmental media like water etc. may lead to severe overall toxicity. The present results suggested to predicting toxicity with other aquatic test models viz. daphnia, fish etc.

CONCLUSION

The acute toxicity with special reference to LD_{50} value in rat after oral exposure for 8 fluroquinolone antibiotics out of 23 was predicted in comparison to experimental available LD_{50} data. The result suggested that predicted acute toxicity of six fluroquinolones viz. ciprofloxacilin, ofloxacin, lomefloxacin, fleroxacin, levofloxacin and prulifloxacin were within range of 1500 to >3500 mg/kg as moderately toxic and other two fluroqunolones viz. enoxacin and norfloxacilin were within range of >5000 mg/kg as slightly toxic. The QSAR model was used through T.E.S.T. software (USEPA, 2012) for the prediction of LD50 values of fluroquinolones. This software has a potent capability to predict rat oral LD_{50} value with suitable programming of QSAR modeling for molecular descriptors and similar test chemicals by calculating test sets and training set (Ruiz et al., 2012) and the present study was evaluated the exact value of LD_{50} in rat exposed orally, which was lacking in experimental data as only obtained of minimum range (>2000 to >5000 mg/kg). Although the quinolones showed limited acute toxicity, the coexistence of multiple quinolones in environmental matrices may lead to severe overall toxicity. Limited research work on QSAR modeling has been carried out on fluroquinolone antibiotics (Li et al., 2014) with other test species. This present prediction work was based on only single QSAR modeling software but should need further investigation by using other softwares. The future prediction should be done in other aquatic test models with this software to know exact impact on non mammals.

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