

Prediction of toxicity of aliphatic carboxylic acids using adaptive neuro-fuzzy inference system

Vali Zare-Shahabadi*

Young Researchers Club, Mahshahr Branch, Islamic Azad University, Mahshahr, Iran

* E-mail: valizare@gmail.com

Received 05 August 2012

Received in revised form 21 August 2012

Accepted 10 September 2012

Toxicity of 38 aliphatic carboxylic acids was studied using non-linear quantitative structure-toxicity relationship (QSTR) models. The adaptive neuro-fuzzy inference system (ANFIS) was used to construct the nonlinear QSTR models in all stages of study. Two ANFIS models were developed based upon different subsets of descriptors. The first one used $\log K_{ow}$ and E_{LUMO} as inputs and had good prediction ability; for the training set of 28 compounds $R_{Training}^2$ was 0.86 and for the test set of 10 compounds, the corresponding statistic was $R_{Test}^2=0.97$. Two outliers were detected for this ANFIS model and removing them improved the quality of the model. Another ANFIS model was constructed based on $PEOE_VSA_FPNEG$ and $G3u$ descriptors chosen by exhaustive search of all two combinations of calculated descriptors by Dragon and MOE softwares. The later ANFIS model showed better performance than the former ($R_{Training}^2=0.92$ and $R_{Test}^2=0.90$) and no outlier was detected.

Keywords: Quantitative-structure-activity relationship; Adaptive neuro-fuzzy inference system; Aliphatic carboxylic acids; Toxicity; *Tetrahymena pyriformis*

1. INTRODUCTION

Scientific evidence suggests that humans and wildlife species may experience adverse health consequences from exposure to environmental chemicals that interact with their endocrine systems [1]. Reliable assays are needed to identify hazardous chemicals. The experimental determination of toxicological properties is a costly and time-consuming process and, therefore, it is essential to develop mathematical predictive relationships to theoretically quantify toxicity. Quantitative structure-activity relationship (QSAR) studies can provide a useful tool for achieving this goal. Several QSAR [2-6] models have been developed for predicting *Tetrahymena pyriformis* toxicity of some groups of structurally related chemicals, e.g. benzenes, phenols, aromatic compounds, aliphatic compounds, and cyanoacetic acids. However, QSAR models of a majority of chemical groups are yet to be determined. For instance, only a small number of carboxylic acids are found in the QSAR literature [7-10]. QSAR studies on the toxicity of aliphatic carboxylic acids are few and scattered. The aquatic toxicity of aliphatic mono- and dicarboxylic acids and their sodium salts were investigated by Seward and Schultz [9]. They used $\log Kow$ and $ELUMO$ as predictors. Later, higher orders of different molecular descriptors were used by Kompany-Zare to establish predictive QSTR on the same data set [10].

QSAR studies are based on the concept that the activity or toxicity of a substance is a function of its structure and/or physicochemical properties and the activity/toxicity can, therefore, be determined through mathematical relationships developed from architecturally similar compounds. Based on a training database containing measured toxicity potencies of compounds and a number of

molecular descriptors, QSARs can be used to predict the toxicity of chemicals that are not included in the database. Designing of powerful QSAR models requires the selection of a valuable statistical method and an accurate description of the molecules. Different statistical methods, such as multiple linear regression (MLR) [11-14], partial least square analysis (PLS) [15, 16], radial basis function (RBF) neural network [17] and support vector machine (SVM) [18, 19] have been used in QSAR studies.

Neuro-fuzzy as an intelligent computational method uses artificial neural networks theory in order to determine fuzzy inference properties by processing data samples. A specific approach in neuro-fuzzy development is the adaptive neuro-fuzzy inference system (ANFIS), which has shown significant results in modeling complex nonlinear systems with estimation speed, simplicity, error free and capacity to learn from examples [20, 21]. Despite the abilities of ANFIS, this technique was entered to the toxicological studies with some delay, and only one publication based on ANFIS have been reported in the literature [18].

The purpose of this study have been twofold: (1) to explore the structure–toxicity relationships of aquatic toxicity of aliphatic mono- and di-carboxylic acids by means of ANFIS and (2) to compare the developed ANFIS model with the models reported previously [9, 10].

2. MATERIALS AND METHODS

2.1. Data set and descriptors

The total data set consisting of the 38 compounds was collected from the published data [9]. For each molecule, the logarithm of reciprocal of the 50% growth inhibition concentration $\log(IGC_{50}^{-1})$ was used as dependent variable [9]. SPXY method [22] was used to partition all molecules to training and prediction sets with size of 28 and 10, respectively. The list of complete set of acids, in addition to aquatic toxicity data and physicochemical descriptors, is provided in Table 1.

Table 1. Experimental and predicted $\log(IGC_{50}^{-1})$ for the training and test sets.

Chemical	$\log(IGC_{50}^{-1})$	$\log K_{OW}$	E_{LUMO}	Predicted $\log(IGC_{50}^{-1})$	
				ANFIS ^a	ANFIS ^b
2-Ethylbutyric acid	-0.15	1.68	1.0000	-0.15	-0.12
Isobutyric acid	-0.33	0.94	0.9800	-0.35	-0.48
Isovaleric acid	-0.34	1.16	0.9600	-0.29	-0.29
2-Propylpentanoic acid	0.03	2.75	1.0000	0.12	0.23
Butyric acid	-0.57	0.79	0.9700	-0.39	-0.50
2-Ethylhexanoic acid	0.08	2.64	1.0100	0.10	0.23
Valeric acid	-0.27	1.39	0.9600	-0.24	-0.31
Trimethylacetic acid	-0.25	1.47	1.0200	-0.20	-0.11
Propionic acid	-0.51	0.33	0.9500	-0.53	-0.46
Heptanoic acid	-0.11	2.42	0.9400	-0.01	0.04
Nonanoic acid	0.35	3.47	0.9700	0.23	0.44
Decanoic acid	0.51	4.09	0.9600	0.33	0.54
Undecanoic acid	0.90	4.42	0.9600	0.40	0.72
3-Methylvaleric acid	-0.23	1.75	0.9800	-0.14	-0.12
4-Methylvaleric acid	-0.27	1.75	0.9700	-0.15	-0.12
Octanoic acid (Caprylic acid)	0.08	3.05	0.9400	0.11	0.22
Hexanoic acid (Caproic acid)	-0.21	1.92	0.9700	-0.11	-0.13
Glutaric acid	-0.64	-0.29	0.8100	-0.68	-0.60
Adipic acid	-0.61	0.08	0.8100	-0.57	-0.56
Succinic acid	-0.94	-0.59	0.6000	-0.90	-0.97
1, 12-Dodecanedicarboxylic acid	0.08	4.13	0.9069	0.26	-0.19
1,10-Decanedicarboxylic acid	-0.09	3.07	0.8757	0.05	-0.17
Malonic acid	-0.71	-0.58	0.1296	-0.79	-0.71
Pimelic acid	-0.58	0.43	0.7474	-0.51	-0.72

Sebacic acid	-0.27	2.01	0.8548	-0.16	-0.41
Suberic acid	-0.51	0.95	0.7951	-0.38	-0.59
Trans-2-pentenoic acid	-0.28	1.41	-0.1600	-0.36	-0.30
4-Pentenoic acid	0.12	0.87	0.9200	-0.37	-0.02
Trans-2-hexenoic acid	-0.13	1.94	-0.1900	-0.10	-0.12
Trans-3-hexenoic acid	-0.22	1.40	0.6500	-0.43	-0.12
Crotonic acid	-0.54	0.72	-0.1900	-0.43	-0.39
Acrylic acid	0.16	0.35	-0.2000	0.08	0.12
2-Octenoic acid	0.21	3.00	-0.1700	0.21	0.22
2-Octynoic acid	-0.06	2.12	0.1200	0.00	0.22
Propiolic acid	-0.29	-0.52	0.0600	-0.20	-0.30
α -Nonynoic acid	0.71	2.65	0.1200	0.67	0.56
2-Nonenoic acid	0.60	3.53	-0.1700	0.60	0.38
3-Butenoic acid	-0.64	0.64	0.8000	-0.44	-0.52

^a The ANFIS used $\log K_{ow}$ and E_{LUMO} as inputs.

^b PEOE_VSA_FPNEG and G3u were used by ANFIS.

The structures of the compounds under study were drawn in 2D ChemDraw. The drawn structures were then converted into 3D modules using the default conversion procedure implemented in CS Chem3D Ultra. The generated 3D structures of the compounds were subsequently subjected to energy minimization in the MOE (Molecular Operating Environment, Chemical Computing Group), using the AM1 procedure (AM1: RHF; gradient: 0.05). For each compound, 251 descriptors were calculated using the MOE software. Moreover, all of the energy minimized structures were ported to Dragon software (Milano Chemometrics and QSAR research group, <http://www.disat.unimib.it/chm/>) to compute of 1481 descriptors. Two descriptors, i.e. $\log K_{ow}$ and E_{LUMO} , were also extracted from ref. [9]. Auto-scaling and mean-centering were used as data pretreatments.

2.2. Adaptive neuro-fuzzy interference system (ANFIS)

Among various fuzzy inference systems, Takagi–Sugeno (TS) systems have been successfully applied for data-driven fuzzy modeling [23]. The TS model consists of a set of local input-output relations that describe the overall system. The rules in a first-order TS model have the following structure:

Rule i : if x_1 is A_{i1} , ..., and x_n is A_{in} , then:

$$Y_i = a_i^T x + b_i \quad (i = 1, 2, 3, \dots, k) \quad (1)$$

where $x = [x_1, x_2, \dots, x_n]^T$ is the input (antecedent) vector, and $A_{i1}, A_{i2}, \dots, A_{in}$ are the fuzzy sets defined for the respective antecedent variables. The rule consequent Y_i is an affine combination of the inputs with parameters a_i and b_i . [24-26].

An ANFIS system can be considered to be an implementation of a TS system in neural-network architecture. In the following, we briefly sketch an outline of the ANFIS system by using a model with two inputs as an example (see Figure 1). Let the inputs of the fuzzy system be x_1 and x_2 , and let the output be z . We consider a TS system with first-order consequents [27] and two rules, as follows:

Rule 1: If x_1 is A_1 and x_2 is B_1 , then $f_1 = p_1 x_1 + q_1 x_2 + r_1$.

Rule 2: If x_1 is A_2 and x_2 is B_2 , then $f_2 = p_2 x_1 + q_2 x_2 + r_2$.

To construct the adaptive system, five layers are used, as shown in Figure 1. Each layer involves several nodes described by a node function. The circles in the network represent nodes that possess no variable parameters, while the squares represent nodes that possess adaptive parameters to be determined by network during training. The node function in each layer is described below.

Layer:

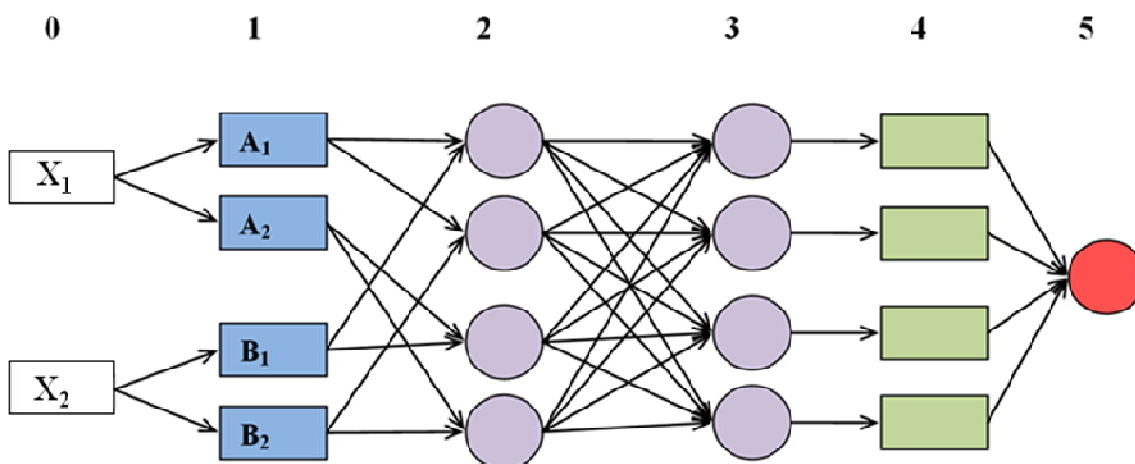


Fig. 1. Basic ANFIS structure.

Layer 1: The nodes in this layer represent the fuzzy sets in the antecedents of the fuzzy rules. It has parameters that control the shape and the location of the center of each fuzzy set. In this study, we choose $\mu_{A_i}(x)$ to be Gaussian with the height equal to 1. The membership function is given by

$$\mu_{A_i}(x) = e^{-(x-c_i)^2/2\sigma_i^2} \tag{2}$$

where c_i represents the center of the Gaussian function and σ_i represents the spread of the membership function. The outputs of this layer are the values of the antecedent membership functions corresponding to the fuzzified inputs of the system.

Layer 2: Every node in this layer computes the product of its inputs. The output of the layer is given by

$$w_i = \mu_{A_i}(x_1) \times \mu_{B_i}(x_2) \tag{3}$$

where μ_{A_i} and μ_{B_i} are the fuzzy sets defined for the variables x_1 and x_2 , respectively. The aim of this layer is to compute the degrees of activation (firing strength) of particular fuzzy rules.

Layer 3: The nodes in this layer normalize the firing strength of the rules by calculating the ratio of the i th rule's firing strength to the sum of all rules firing strengths by

$$w_i^* = \frac{w_i}{w_1 + w_2} \quad (i = 1, 2, \dots) \tag{4}$$

Layer 4: Nodes in this layer are adaptive, where each node function represents a first-order model with consequent parameters. Thus, the output from this layer is expressed by

$$O_i^4 = w_i^* f_i = w_i^* (p_i x_1 + q_i x_2 + r_i) \tag{5}$$

where w_i^* is the output of Layer 3, and $\{p_i, q_i, r_i\}$ is the parameter set.

Layer 5: This is the output layer where each node is fixed. The single node labeled Σ computes the overall output as the summation of all the inputs from the previous layer by

$$O_i^5 = \sum_i w_i^* f_i = \frac{\sum_i w_i f_i}{w_i} \quad (6)$$

Optimizing the values of the adaptive parameters is of vital importance for the performance of the adaptive system. In particular, the premise parameters in Layer 1 and the consequent parameters in Layer 4 need to be determined. Layer 1 parameters define the center and the spread of the antecedent membership function. Layer 4 parameters defined by the set $\{p_i, q_i, r_i\}$ corresponding to the consequent coefficients in Eq. (5). A hybrid-learning algorithm, which is a combination of the gradient descent and least-square techniques, was used to determine the parameters of an ANFIS model [28].

3. RESULTS AND DISCUSSION

Seward and Schultz [9] have shown that distinct class-based relationships exist for different sub-classes aliphatic carboxylic acids including saturated mono-acids and saturated di-acids. They could build a response surface using two molecular descriptors (i.e. $\log K_{ow}$ and E_{LUMO}) that encompassed all considered acids with exception of four outliers. The best reported R^2 in their report was 0.848. The main goal in this work was to use ANFIS and develop a QSAR model for all considered sub-classes of carboxylic acids, with better quality, and without any outlier elimination.

In the present study, first of all, $\log K_{ow}$ and E_{LUMO} were used as inputs for ANFIS model as did Seward and Schultz [9] in their work. For each input two membership functions (Gaussian MF) were assigned, this led to $2^2 = 4$ fuzzy rules. The statistical parameters of the resulted model are as follows: $R_{Training}^2 = 0.86$ and $R_{Test}^2 = 0.97$. Figure 2 shows a plot of observed versus calculated $\log (ICG_{50}^{-1})$ values for both training and prediction sets. A good correlation between the observed and predicted values of $\log (ICG_{50}^{-1})$ can be seen in this figure. However, two components had unusual errors in their predicted values. The outliers were representative of size extremes; undecanoic acid and 4-pentenoic acid. These outliers were belong to the training set and removing them improved the $R_{Training}^2$ from 0.86 to 0.93.

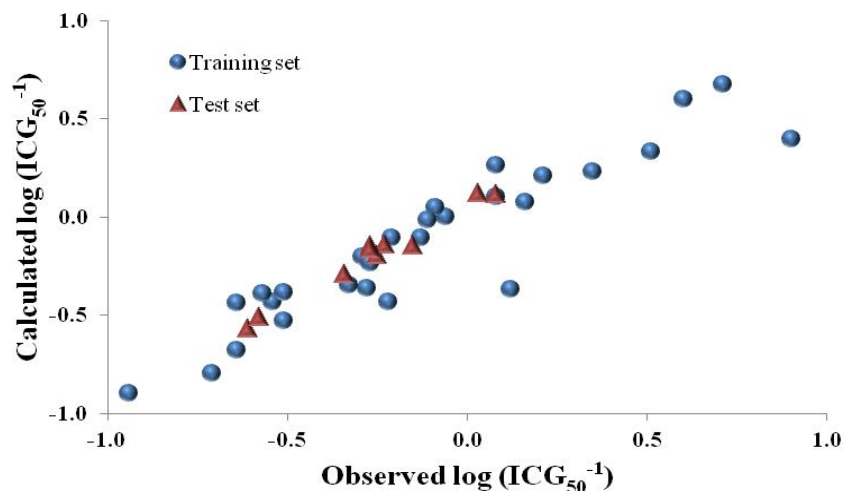


Fig. 2. A plot of observed versus predicted aquatic toxicity of carboxylic acids from the ANFIS model used $\log K_{ow}$ and E_{LUMO} as inputs.

Undecanoic acid is extremely large among the molecules in the data set and very hydrophobic. Toxicity of this highly hydrophobic acid may be affected by low aquatic solubility [9]. Other outlier was a small carboxylic acid, i.e., 4-pentenoic acid. This molecule with unsaturated carbon-carbon bond has two positions for potential electrophilic attack (i.e., C=C and C=O) [9]. Such attraction results in a stable intermediate ion and affects the toxicological activity of the chemical. As the distance between the terminal carbon and the carbonyl group increases, the potential for such attacks decreases.

In the next step, other descriptors calculated by Dragon and MOE softwares were used to examine whether there were molecular descriptors with better performance than $\log K_{ow}$ and E_{LUMO} . Since the number of data point in the training set is small, i.e., 28, only two descriptors can be used as inputs, provided that for each input only two MF are assigned. In other words, if three descriptors is chosen as inputs, each input has at least two MFs, this leads to $2^3 = 8$ fuzzy rules, which results in $(3+1) \times 8 = 32$ consequent parameters; but there are only 28 data points for training, so the ANFIS is not able to tune consequent parameters.

An exhaustive search was employed to select the best subset of molecular descriptors containing only two descriptors. The chosen descriptors were: PEOE_VSA_FPNEG and G3u. The G3u belongs to WHIM (weighted holistic invariant index/weighted by atomic masses) descriptors. It is a geometrical descriptor based on statistical indices, calculated on the projections of the atoms along principal axes. WHIM descriptors are built in such a way as to capture relevant molecular 3D information regarding molecular shape, size, symmetry and atom distribution with respect to invariant reference frames. Interpretability of these descriptors is relatively complex but they encode refined structural information for the activity. The PEOE_VSA_FPNEG belongs to partial charge descriptors category and refers to fractional negative polar van der Waals surface area. There is a high correlation between $\log K_{ow}$ and PEOE_VSA_FPNEG (i.e., $R^2=0.73$). However, constructing an ANFIS model based on only PEOE_VSA_FPNEG was more predictive than that used $\log K_{ow}$ as input.

To show that the ANFIS model was reliable, a permutation test based on the repetitive randomization of the response vector was also used. In each cycle of the test, the response vector (toxicity values) was randomly rearranged; an ANFIS model was built using the selected descriptors and new response vector; the resulted ANFIS model was used to predict the toxicity of the chemicals in the training set and the corresponding squared correlation coefficient (R^2) was recorded. The results of this test are graphically represented in Figure 3. Obviously, the models with randomized activities represent very low correlation coefficients compared with the original model. These findings confirm the significance of the proposed ANFIS model for predicting the $\log(IGC_{50}^{-1})$ of the aliphatic carboxylic acids.

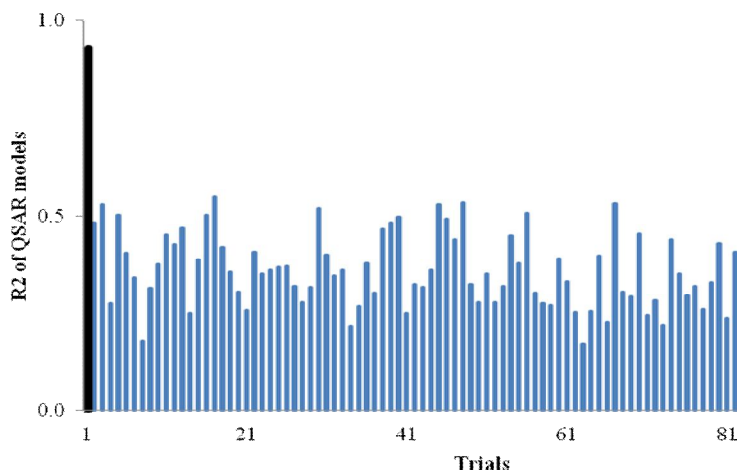


Fig. 3. Y-randomization test; the first bar shows the R^2 value for the model based on the actual data. The other 100 bars show the R^2 values for 100 models based on permuted data.

Figure 4 shows a plot of observed versus calculated $\log(\text{IC}_{50}^{-1})$ values for both training and prediction sets. Figure 5 represents a plot of the standardized residuals versus the indices of all of the observations for the new ANFIS model. As can be seen from these figures, the predictive ability of the ANFIS model was improved. Statistical parameters of the model are given in Table 2.

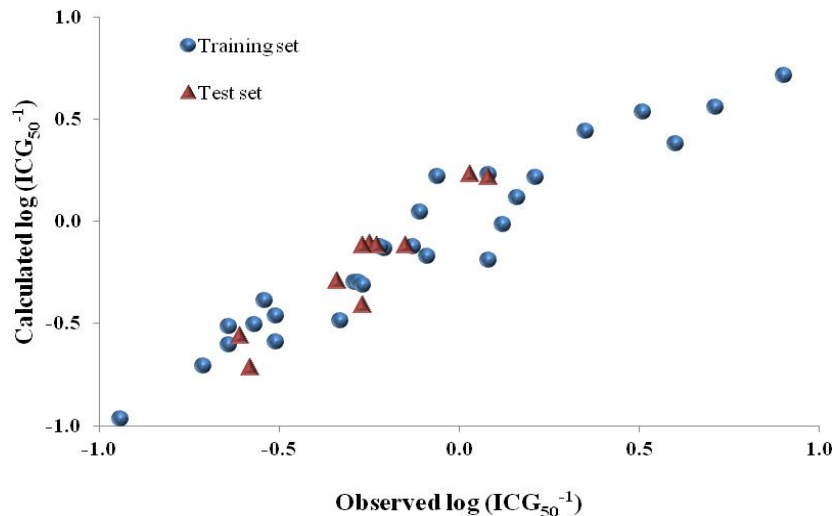


Fig. 4. A plot of observed versus predicted aquatic toxicity of carboxylic acids from the ANFIS model used PEOE_VSA_FPNEG and G3u as inputs.

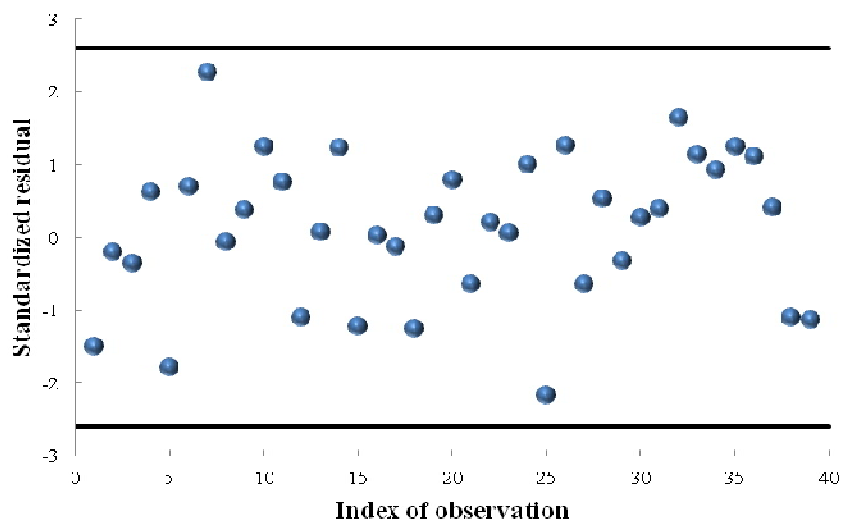


Fig. 5. A plot of standardized residuals obtained from the ANFIS model versus indices of all the carboxylic acids.

Whilst the prediction ability of the ANFIS model obtained for the aquatic toxicity of the aliphatic carboxylic acids was confirmed, comparison with the previous QSAR models for the same compounds will be beneficial. The first QSAR model on this data set was reported by Seward and Schultz [9]. They applied MLR regression on the 38 carboxylic acids using two molecular descriptors, $\log K_{ow}$ and E_{LUMO} and no external test set was considered. The discovered QSAR model had R^2 value of 0.727 and quality of the model was improved by removing three outliers. The R^2 value of the final model approached to 0.848. Another QSAR study on the same data set was reported by Kompany-Zareh [10]. He used nine descriptors and second to fourth orders of them as

inputs for genetic algorithm to choose the best combination of variables in the QSAR study. A QSAR model using five variables was developed. The reported R^2 values for training and test sets were 0.95 and 0.73, respectively. Referring to Table 2 reveals that the proposed ANFIS model has better performance. Whilst the calibration quality of the later work is better to what found in this work, ANFIS had much better performance on the external set. Moreover, ANFIS used only two variables.

Table 2. Comparison of some reported models on the same data set of aliphatic carboxylic acids.

Reference	Model	Number of used variables	Number of compounds in data set		R^2_{Training}	R^2_{Test}	Number of detected Outliers
			Training set	Test set			
9	MLR	2	38	---	0.848	---	3
10	Nonlinear-MLR	5	28	10	0.95	0.73	---
<i>Current work</i>	<i>ANFIS</i>	2	28	10	0.923	0.902	---

4. CONCLUSION

In this paper, non-linear quantitative structure–toxicity relationships (QSTR) were developed for the prediction of $\log(\text{IGC}_{50}^{-1})$ values of aliphatic carboxylic acids based on two subsets of molecular descriptors. The first one, which was used previously by Seward and Schlutz [9], contained $\log K_{ow}$ and E_{LUMO} . ANFIS as a powerful nonlinear tool was used to develop a model between these descriptors and $\log(\text{IGC}_{50}^{-1})$ values. The resulted ANFIS model had better quality compared the MLR model developed previously [9]. The other set of molecular descriptors contained $PEOE_VSA_FPNEG$ and $G3u$, which was selected by exhaustive search of all two combinations of calculated descriptors (more than 1700 descriptors). The ANFIS model developed based on these descriptors had better performance. No outlier was detected. Both of ANFIS models were validated using external test set. As final conclusion, ANFIS was able to produce substantially better models than the models reported recently for predicting $\log(\text{IGC}_{50}^{-1})$ values of aliphatic carboxylic acids [9, 10].

ACKNOWLEDGMENT

This paper was extracted from a research project entitled “Prediction of toxicity of aliphatic carboxylic acids using ANFIS”. Financial assistance from the Islamic Azad University-Mahshahr Branch is gratefully acknowledged.

REFERENCES

- [1] M. Jorgensen, B. Vendelbo, N.E. Skakkebaek, H. Leffers, Environ. Health Perspect. 108 (2000) 403-412.
- [2] J. Devillers, SAR QSAR Environ. Res. 15 (2004) 237-249.
- [3] B. Hemmateenejad, M. Elyasi, Anal. Chim. Acta 646 (2009) 30-38.
- [4] A. Mohajeri, M.H. Dinpajooh, Journal of Molecular Structure: THEOCHEM 855 (2008) 1-5.
- [5] B. Hemmateenejad, A.R. Mehdipour, R. Miri, M. Shamsipur, Chemical Biology & Drug Design 75 (2010) 521-531.
- [6] Z. Bayat, S. Vahdani, J. Chem. Pharm. Res. 3 (2011) 93-102.
- [7] C.L. Russom, S.P. Bradbury, S.J. Broderius, D.E. Hammermeister, R.A. Drummond, Environ. Toxicol. Chem. 16 (1997) 948-967.

- [8] H. Könemann, A. Musch, *Toxicology* 19 (1981) 223-228.
- [9] J.R. Seward, T.W. Schultz, *SAR QSAR Environ. Res.* 10 (1999) 557-567.
- [10] M. Kompany-Zareh, *Med. Chem. Res.* 18 (2009) 143-157.
- [11] X.J. Cui, Z.Y. Zhang, X. Yuan, J.P. Zhang, S.D. Liu, L.P. Guo, P.D.B. Harrington, *Chem. Res. Chinese. U.* 22 (2006) 439-442.
- [12] S. Kar, K. Roy, *J. Hazard. Mater.* 177 (2010) 344-351.
- [13] F. Abbasitabar, V. Zare-Shahabadi, *SAR QSAR Environ. Res.* 23 (2011) 1-15.
- [14] V. Zare-Shahabadi, F. Abbasitabar, *J. Comput. Chem.* 31 (2010) 2354-2362.
- [15] A. Niazi, S. Jameh-Bozorgh, D. Nori-Shargh, *Chin. Chem. Lett.* 18 (2007) 621-624.
- [16] S. Funar-Timofei, D. Ionescu, T. Suzuki, *Toxicol. In Vitro* 24 (2010) 184-200.
- [17] B. Xia, K. Liu, Z. Gong, B. Zheng, X. Zhang, B. Fan, *Ecotoxicol. Environ. Saf.* 72 (2009) 787-794.
- [18] M. Jalali-Heravi, A. Kyani, *Chemosphere* 72 (2008) 733-740.
- [19] A. Niazi, S. Jameh-Bozorgh, D. Nori-Shargh, *J. Hazard. Mater.* 151 (2008) 603-609.
- [20] A. Khajeh, H. Modarress, *Expet. Syst. Appl.* 37 (2010) 3070-3074.
- [21] P. Shahbazikhah, M. Asadollahi-Baboli, R. Khaksar, R. Fareghi Alamdari, V. Zare-Shahabadi, *J. Braz. Chem. Soc.* 22 (2011) 1446-1451.
- [22] R.K.H. Galvão, M.C.U. Araujo, G.E. José, M.J.C. Pontes, E.C. Silva, T.C.B. Saldanha, *Talanta* 67 (2005) 736-740.
- [23] J. Gan, S.M. Zhou, A New Fuzzy Membership Function with Applications in Interpretability Improvement of Neurofuzzy Models Computational Intelligence, in: DS Huang, K Li, G Irwin (Eds.), Springer Berlin / Heidelberg 2006, pp. 183-194.
- [24] M. Setnes, R. Babuska, H.B. Verbruggen, *International Journal of Human-Computer Studies* 49 (1998) 159-179.
- [25] M. Sugeno, G.T. Kang, *Fuzzy Sets and Systems* 28 (1988) 15-33.
- [26] M. Jalali-Heravi, P. Shahbazikhah, A. Ghadiri-Bidhendi, *QSAR & Combinatorial Science* 27 (2008) 729-739.
- [27] H. Takagi, M. Sugeno, IFAC Symposium on Fuzzy Information, Knowledge Representation and Decision Analysis 1983, p. 55.
- [28] J.S.R. Jang, *IEEE Trans. Syst. Man Cybern.* 23 (1993) 665-685.