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ORIGINAL ARTICLE

1st Heterocyclic Update

Cytotoxicity and 2D-QSAR study of some heterocyclic compounds

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KEYWORDS

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LOO

Abstract Herein we have studied the cytotoxicity and quantitative structure–activity relationship (QSAR) of heterocyclic compounds containing cyclic urea and thiourea nuclei. A set of 22 hydantoin and thiohydantoin related heterocyclic compounds were investigated with respect to their LC_{50} values (Log of LC_{50}) against brine shrimp lethality bioassay in order to derive the 2D-QSAR models using MLR, PLS and ANN methods. The best predictive models by MLR, PLS and ANN methods gave highly significant square correlation coefficient (R^2) values of 0.83, 0.81 and 0.91 respectively. The model also exhibited good predictive power confirmed by the high value of cross validated correlation coefficient Q^2 (0.74).

1. Introduction

Hydantoin and thiohydantoin are five-member heterocyclic system containing very reactive cyclic urea and thiourea cores (López and Trigo, 1985; Meusel and Gütschow, 2004). In recent years, heterocyclic compound and its derivatives are very familiar to their anti-inflammatory (Ghate et al., 2003), antimicrobial (Khan et al., 2005), antitubercular (Gupta and Prabhu, 2004), antipyretic (Shastri et al., 2004), analgesic (Ghate et al., 2005), antioxidant (Torres and Faini, 2006) and cytotoxic (Kostava and Momekov, 2008) activities. They are now widely used as drugs, medicines, dyes and raw materials in manufacturing industries etc. Much attention has been paid to the synthesis of nitrogen (N)-, oxygen (O)-, sulfur (S)- containing heterocyclic compounds and their derivatives mainly due to their broad spectrum biological and pharmaceutical activities. In our laboratory, several substituted heterocyclic compounds such as methyl and bromine groups in the benzene ring of isatin, Δ^2-1,3,5-thiadiaozolines were synthesized and found a good cytotoxic activity (Islam et al., 2001a, 2001b; Lingcon et al., 2001). In light of this biological activity, we thought of synthesizing different types of hydantoin and thiohydantoin related heterocyclic compounds and their derivatives which were tested for cytotoxic activity. Though development of drugs is lengthy, laborious and expensive, computer aided drug design (CADD) can help us to increase the pool of interesting structures that can be evaluated. The most important step is to find the possible structural feature of compounds with desired biological activity. Over the years of development many methods, algorithms and techniques have been discovered and applied in QSAR

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studies (He and Jurs, 2005; Eldred et al., 1999). Nowadays QSARs are being applied in many disciplines with much emphasis in drug design. As a well accepted technique, two-dimensional quantitative structure–activity relationship (2D-QSAR) was carried out to study the biological activity. It is a mathematical model that was used to evaluate the toxicity of a compound from its physiochemical properties of molecular structures.

2. Experimental

2.1. General methods

All the chemicals and reagents used in these experiments were pure and purchased from E-Merck (Germany). Purification and drying of reagents and solvents were carried out according to the literature procedure of Armarego and Chai (2003). Thin layer chromatographic analysis was performed on E-Merck 60 F 254 pre-coated aluminum thin layer chromatographic plates. Melting points were determined on a Fisher-John's electrochemical melting point apparatus and uncorrected. The IR spectra were recorded with KBr (Potassium Bromide) disk on DR-8001, Shimadzu FT-IR spectrometer and ^{1}H NMR spectra were also recorded on a WP 200-NMR spectrometer using TMS (Trimethylsilane) as an internal standard.

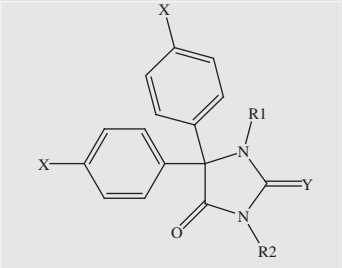
2.2. Synthesis

Twenty two (**22**) compounds were prepared according to the literature procedure of Muccioli et al. (2003) by direct heating instead of micro-wave assistance. All the synthesized compounds illustrated in Table 1 were confirmed with melting point, IR and ^{1}H NMR spectral analysis. The synthetic portion was not included in this manuscript due to the main focus on 2D-QSAR study.

2.3. Cytotoxicity calculation

The median lethal concentration (LC_{50}) with 95% confidence intervals of the test samples in Table 2 and reference standard (Vincristine Sulfate) in Table 3 were calculated using the probit analysis program (Finney, 1971; Rickman et al., 1974) of IBM SPSS Statistics20 software packages. According to the research methodology, all experimental LC_{50} values (μg/mL) were converted to logarithm of LC_{50}, i.e., Log_{10} (LC_{50}) and used as dependent variable in 2D-QSAR study.

Table 1 Synthesized compounds which are employed for 2D-QSAR study.



5,5-Diphenyl-imidazolidine-2,4-dione moiety

Compound ID	*R*1	*R*2	*X*	*Y*
1	H	H	H	O
2	H	C_6H_5 (phenyl)	H	O
3	C_6H_5 (phenyl)	C_6H_5 (phenyl)	H	O
4	H	C_6H_{11} (cyclohexyl)	H	O
5	C_6H_{11} (cyclohexyl)	C_6H_{11} (cyclohexyl)	H	O
6	H	H	Br	O
7	H	CH_3	Br	O
8	H	CH_3–CH_2	Br	O
9	H	CH_3–CH_2–CH_2–CH_2	Br	O
10	H	CH_3–CH_2–CH_2–CH_2–CH_2	Br	O
11	H	H	Cl	O
12	H	CH_3CH_2	Cl	O
13	H	CH_3–CH_2–CH_2–CH_2	Cl	O
14	H	CH_3–CH_2–CH_2–CH_2–CH_2–CH_2–CH_2	Cl	O
15	H	H	H	S
16	H	CH_3	H	S
17	CH_3	CH_3	H	S
18	H	CH_3CH_2	H	S
19	CH_3CH_2	CH_3CH_2	H	S
20	H	$CH_3CH_2CH_2CH_2$	H	S
21	$CH_3CH_2CH_2CH_2$	$CH_3CH_2CH_2CH_2$	H	S
22	H	C_6H_5 (phenyl)	H	S

Table 2 Cytotoxic activity of the synthesized compounds against brine shrimp nauplii.

Compound ID	Concentration tested (μg/mL)	Probit	LC_{50} (μg/mL)	Log_{10} (LC_{50})	95% Confidence interval
1	2, 5, 10, 25, 50, 100	3.72, 3.92, 4.12, 4.48, 4.77, 5.05	95.59	1.98	62.90–177.60
2	2, 5, 10, 25, 50, 100	4.29, 4.48, 4.67, 4.85, 5.15, 5.36	31.50	1.50	21.64–52.65
3	2, 5, 10, 25, 50, 100	5.11, 5.62, 5.77, 6.23, 6.56, 7.33	1.60	0.20	0.89–2.35
4	2, 5, 10, 25, 50, 100	3.82, 4.01, 4.19, 4.56, 4.82, 5.10	86.00	1.93	55.73–156.01
5	2, 5, 10, 25, 50, 100	5.00, 5.50, 5.77, 6.13, 6.41, 7.05	1.90	0.28	1.14–2.77
6	2, 5, 10, 25, 50, 100	3.87, 4.05, 4.23, 4.59, 4.85, 5.10	82.00	1.91	53.64–153.68
7	2, 5, 10, 25, 50, 100	4.23, 4.39, 4.59, 4.80, 5.08, 5.33	40.00	1.6	27.03–69.39
8	2, 5, 10, 25, 50, 100	4.26, 4.45, 4.64, 4.82, 5.13, 5.36	34.00	1.53	23.21–56.31
9	2, 5, 10, 25, 50, 100	4.87, 5.15, 5.33, 5.58, 5.77, 6.04	3.10	0.49	1.53–4.92
10	2, 5, 10, 25, 50, 100	4.98, 5.36, 5.61, 5.92, 6.18, 6.56	2.10	0.32	1.14–3.16
11	2, 5, 10, 25, 50, 100	3.77, 3.96, 4.16, 4.53, 4.77, 5.08	91.25	1.96	59.89–170.43
12	2, 5, 10, 25, 50, 100	4.64, 4.85, 4.95, 5.23, 5.44, 5.67	9.40	0.97	5.76–13.80
13	2, 5, 10, 25, 50, 100	4.82, 5.13, 5.31, 5.52, 5.74, 5.99	3.55	0.55	1.82–5.53
14	2, 5, 10, 25, 50, 100	5.18, 5.74, 6.04, 6.34, 6.75, 7.33	1.30	0.11	0.70–1.96
15	2, 5, 10, 25, 50, 100	4.16, 4.36, 4.56, 4.77, 5.05, 5.28	43.82	1.64	29.59–75.96
16	2, 5, 10, 25, 50, 100	4.08, 4.23, 4.42, 4.67, 4.95, 5.18	63.60	1.8	41.19–122.47
17	2, 5, 10, 25, 50, 100	3.92, 4.08, 4.26, 4.62, 4.92, 5.18	76.30	1.88	50.16–142.11
18	2, 5, 10, 25, 50, 100	4.42, 4.64, 4.80, 5.00, 5.28, 5.52	18.75	1.27	12.93–28.20
19	2, 5, 10, 25, 50, 100	4.72, 4.95, 5.08, 5.33, 5.52, 5.77	6.50	0.81	3.63–9.64
20	2, 5, 10, 25, 50, 100	4.59, 4.80, 4.90, 5.15, 5.41, 5.64	11.20	1.05	7.30–16.61
21	2, 5, 10, 25, 50, 100	4.82, 5.13, 5.31, 5.55, 5.74, 5.99	3.52	0.55	1.81–5.48
22	2, 5, 10, 25, 50, 100	4.85, 5.15, 5.33, 5.58, 5.77, 6.04	3.24	0.51	1.64–5.07

Table 3 Cytotoxicity of the reference standard (Vincristine Sulfate) on brine shrimp nauplii.

Concentration tested (μg/mL)	Probit	LC_{50} (μg/mL)	Log_{10} (LC_{50})	95% Confidence interval
2	4.77	2.99	0.48	2.20–3.80
5	5.31			
10	5.74			
25	6.34			
50	6.75			
100	7.33			

2.3.1. Test animal

Brine shrimps were used as test animal for the investigation of cytotoxic activity (Martin et al., 2008; Islam et al., 2009; Wang et al., 1998) and its scientific name is *Artemia Salina*.

2.3.2. Hatching of shrimp

Brine shrimp (*A. Salina*) eggs were hatched in a vessel containing sterile artificial seawater prepared by dissolving 38 g of table salt in 1L distilled water. The vessel was kept under an inflorescent bulb and facilitated with good aeration for 48 h at room temperature. After hatching, nauplii released from the egg shells were collected at the bright side of the vessel (near the light source) by using micropipette. The larvae were isolated from the eggs by aliquoting them in small beaker containing the seawater.

2.3.3. Brine shrimp lethality bioassay

The brine shrimp lethality bioassay was used to predict the cytotoxic activity (McLaughlin et al., 1998; Middleton et al., 2005) of the compounds. For the experiments, 4 mg of each test sample was dissolved in dimethyl sulfoxide (DMSO) and solutions of varying concentrations (100, 50, 25, 10, 5, and 2 μg/mL) were obtained by the serial dilution technique using simulated seawater. The solutions were then added to the pre-marked glass vials containing 20–25 live brine shrimp nauplii in 10 mL simulated seawater. After 24 h, the vials were inspected using a magnifying glass, and the number of survived nauplii in each vial was counted. The mortality endpoint of this bioassay was defined as the absence of controlled forward motion during 30 s of observation (Meyer et al., 1982). From this data, the percent of lethality of the brine shrimp nauplii for each concentration and control was calculated. Vincristine Sulfate (reference standard) and DMSO were used as positive control and negative control respectively. All the procedures were replicated three times.

3. Calculating descriptors

All the calculations were performed on Intel core-i5 Fujitsu Laptop Computer with 6–8 GB of memory and 100 GB of scratch disk space. The 3D-structure of 22 molecules was sketched by the Gauss view03 software. The Molecular Mechanics (MM+) force field with the aid of Chemoffice Ultra-5 software package was applied for preliminary geometry optimization. The final geometry optimization of the MM+ force field optimized structures was done by Gaussian98 (Revision A.9) (Frisch et al., 1998) software applying B3LYP/6-31G(d,p) level of theory. Semi-empirical PM3 (Stewart, 1989a, 1989b) method by Gaussian98 software had been used in order to obtain an accurate charge distribution and quantum-chemical descriptors (Net atomic charge, Q_A; mean absolute atomic charge, Q_M; Highest occupied molecular orbital, HOMO; Lowest unoccupied molecular orbital, LUMO; Energy gap, E_{GAP}; Absolute hardness, η; Activation hardness, $\Delta\eta$; σ electron densities, Q_σ; π electron densities, Q_π; Molecular polarizability, α; Ionization energy, I_P; Total energy, E_T; Heat

Table 4 Short explanation of the descriptors used to establish the 2D-QSAR model.

Full name	Description	Abbreviation
Highest occupied molecular orbital	It has been shown Zhou and Parr (1990) that this orbital plays a major role in governing many chemical reactions and determining electronic band gaps in solids; they are also responsible for the formation of many charge transfer complexes Franke (1984), Osmialowski et al. (1985). The energy of the HOMO is directly related to the ionization potential and characterizes the susceptibility of the molecule toward attack by electrophiles. Hard nucleophiles have a low-energy HOMO, soft nucleophiles have a high energy HOMO	HOMO
Heat of formation	The energy liberated or absorbed when one mole of a compound is formed from its constituent elements. In common usage, the heat of formation is used in place of the more precise term the enthalpy of formation, which has the symbol, ΔH_F. The reaction enthalpy can be accounted for by the difference in heats of formation between reactants and products or between conjugated species Gruber and Buss (1989), Shusterman (1991), Debnath et al. (1994)	ΔH_F
Total energy	The total energy has been used as a measure of nonspecific interactions between a solute and stationary phase in gas-chromatography Osmialowski et al. (1986). The energy of protonation, defined as the difference between the total energies of the protonated and neutral forms of the molecule, can be considered as a good measure of the strength of hydrogen bonds (the higher the energy, the stronger the bond) and can be used to determine the correct localization of the most favorable hydrogen bond acceptor site Trapani et al. (1993)	E_T
Steric energy	Molecular mechanics assumes the steric energy of a molecule to arise from a few, specific interactions within a molecule. These interactions include the stretching or compressing of bonds beyond their equilibrium lengths and angles, torsional effects of twisting about single bonds, the Van der Waals attractions or repulsions of atoms that come close together, and the electrostatic interactions between partial charges in a molecule due to polar bonds. To quantify the contribution of each, these interactions can be modeled by a potential function that gives the energy of the interaction as a function of distance, angle, or charge Hehre (2003), Cornell et al. (1995)	E_S

of formation, ΔH_F; Steric energy, E_S; Molecular surface area, MSA; Molecular volume, MV; Molecular dipole moment, MDM etc.) for each compound in the series and some other descriptors (Gibbs's free energy change of solvation, δG°_{solv}; electrostatic Gibb's free energy change in solution, δG_{elec} etc.) were also calculated by Gaussian98 at B3LYP/6-31G(d,p)-CPCM solvation (DMSO) method. Initially, a total of twenty five types of semi-empirical and thermodynamic descriptors were calculated and these descriptors were finally reduced into four (Table 4) to study the 2D-QSAR models. The MLR, PLS and ANN methods were applied to perform the QSAR models and cross validation method (LOO) was also used to validate the predictive ability of the model obtained by MLR method.

4. Statistical methods

For the QSAR study, model selection was performed by Build QSAR (version 2.1.0.0) (De Oliveira and Gaudio, 2001) software program. Statistical calculations allowed for the selection of the models with the following characteristics: high squared correlation coefficient (R^2), high Fischer's value (F-test), low standard error of estimate (SEE), correlation matrix (Table 5) among the parameters and the least number of descriptors involved. Next, the IBM SPSS Statistics20 software packages were applied for detailed statistical analysis of the QSAR models.

Table 5 Correlation matrix among the descriptors.

	HOMO	ΔH_F	E_T	E_S
HOMO	1			
ΔH_F	0.786	1		
E_T	0.235	0.502	1	
E_S	0.885	0.848	0.565	1

5. Results and discussions

The correlation between various descriptors (Kier and Hall, 1986, 1999) with biological activity is the most important means of structure–activity relationship (SAR) study. Thus the equation should use the minimum number of descriptors to obtain the best fit. To achieve this, a popular procedure is used to find out the saturation point, a point beyond which there is no considerable improvement in regression coefficient (R^2) values has been observed. By interpreting the resulting descriptors, it is possible to gain some insight into factors that are likely to govern the cytotoxic activity. The best QSAR model built using multiple linear regression (MLR) method is represented by the following equation:

Table 6 The R^2_{train} and Q^2_{LOO} values after several *Y*-randomization tests.

No of Y_{rand}	R^2_{train}	Q^2_{LOO}
1	0.1325	0.0236
2	0.0259	0.0116
3	0.0502	0.0092
4	0.0552	0.0217
5	0.1190	0.0145
6	0.0376	0.0221
7	0.1082	0.0359
8	0.1169	0.0475
9	0.05182	0.0154
10	0.0488	0.0123

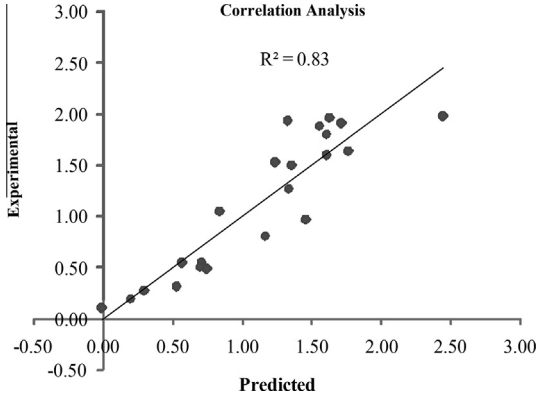


Figure 1 Predicted cytotoxic activities by MLR in comparison with experimental.

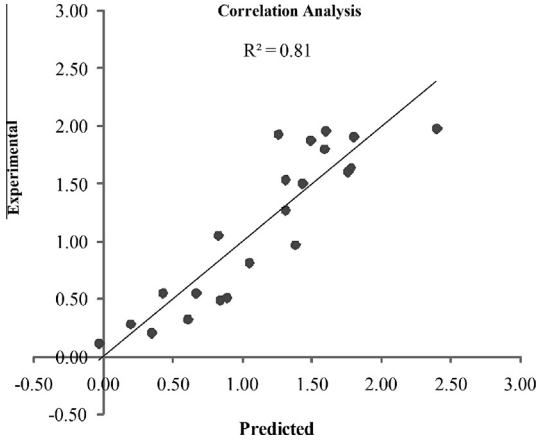


Figure 2 Predicted cytotoxic activities by PLS in comparison with experimental.

$$\log_{10}(LC_{50}) = 0.7752(\pm 1.0023)\mathrm{HOMO} + 0.0023(\pm 0.0069)\Delta H_F + 0.0016(\pm 0.0005)E_T + 0.0096(\pm 0.0062)E_S + 11.5616(\pm 9.1645) \quad (1)$$

$$(n = 22, R = 0.91, R^2 = 0.83, \mathrm{SEE} = 0.31, F = 20.70, p < 0.0001, Q^2 = 0.74, S_{\mathrm{PRESS}} = 0.38, \mathrm{SDEP} = 0.34)$$

where, n is the number of observations, R is the correlation coefficient, R^2 is the squared correlation coefficient, SEE is the standard error of estimate, p is the statistical significance >99.9% with Fisher's statistic F, S_{PRESS} is the standard deviation of sum of squared error of prediction and SDEP is the standard deviation of error of prediction.

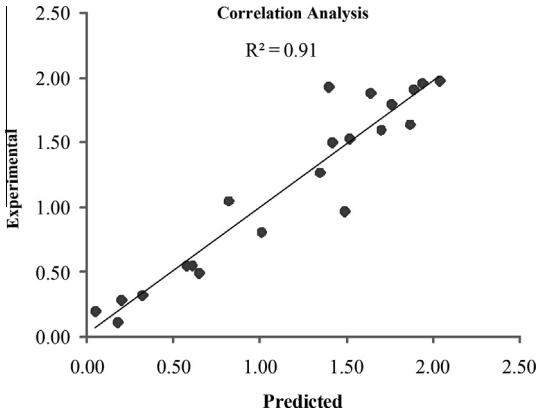


Figure 3 Predicted cytotoxic activities by ANN in comparison with experimental.

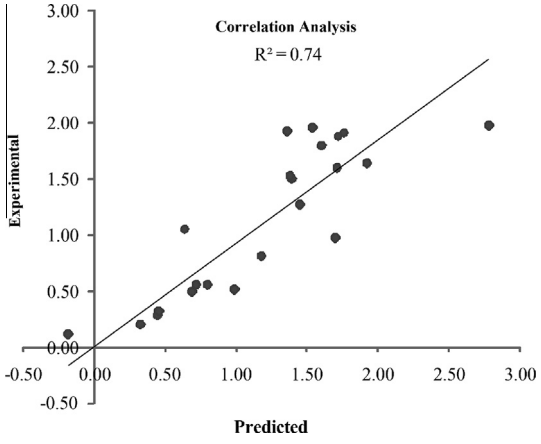


Figure 4 Predicted cytotoxic activities by cross validation (LOO) in comparison with experimental.

Table 7 Prediction of cytotoxic activity of test set compounds in five cross validation cycles (leave-5-out) based on the descriptors set of Eq. (1).

Cycle	Test set	Training set	$R^2_{cv(ext)}$
1	1, 6, 11, 16, 21	Rest of the compounds, $n = 17$	0.66
2	2, 7, 12, 17, 22	Rest of the compounds, $n = 17$	0.71
3	3, 8, 13, 18, 1	Rest of the compounds, $n = 17$	0.73
4	4, 9, 14, 19, 2	Rest of the compounds, $n = 17$	0.75
5	5, 10, 15, 20, 3	Rest of the compounds, $n = 17$	0.70

Table 8 Experimental cytotoxic activities and predicted activities by MLR, PLS, ANN and cross validation (LOO) methods.

Compound ID	Log (LC_{50})				
	Exp.	MLR	PLS	ANN	Cross validation (LOO)
1	1.98	2.45	2.40	2.04	2.78
2	1.50	1.36	1.43	1.42	1.39
3	0.20	0.20	0.35	0.05	0.33
4	1.93	1.33	1.26	1.40	1.36
5	0.28	0.30	0.20	0.20	0.45
6	1.91	1.72	1.80	1.89	1.76
7	1.60	1.61	1.76	1.70	1.71
8	1.53	1.24	1.31	1.52	1.38
9	0.49	0.75	0.84	0.65	0.69
10	0.32	0.53	0.61	0.32	0.46
11	1.96	1.63	1.60	1.94	1.54
12	0.97	1.46	1.38	1.49	1.70
13	0.55	0.71	0.67	0.58	0.80
14	0.11	−0.01	−0.03	0.18	−0.18
15	1.64	1.77	1.78	1.87	1.92
16	1.80	1.61	1.59	1.76	1.60
17	1.88	1.56	1.49	1.64	1.72
18	1.27	1.34	1.31	1.35	1.45
19	0.81	1.17	1.05	1.01	1.18
20	1.05	0.84	0.83	0.82	0.64
21	0.55	0.57	0.43	0.61	0.72
22	0.51	0.70	0.89	0.71	0.99

Exp.: Experimental, MLR: Multi Linear Regression, PLS: Partial Least Square, ANN: Artificial Neural Network, LOO: Leave One Out.

The high correlation coefficient R (0.91) indicates the susceptibility of descriptors (HOMO, ΔH_F, E_T and E_S) to form the above model (1). Squared correlation coefficient (R^2) of 0.83 explains 83% variance in biological activity of the tested compounds. It also indicates the statistical significance >99.9% with F-values (20.70). Cross-validated square correlation coefficient (Q^2) by LOO technique was 0.74 which showed a good internal predictive ability of the model. The model was also validated by applying the Y-randomization test. Several random shuffles of the Y vector were performed and the obtained results are in good agreement with the suggested limits (Eriksson et al., 2003). The low R^2_{train} and Q^2_{LOO} values shown in Table 6 indicate that there is no chance of correlation or structural dependency in the proposed model. Consequently Eq. (1) can be considered as a perfect model with both high statistical significant and excellent predictive ability. The predictive ability of the model was further confirmed by leave-5-out cross validation with $R^2_{\text{cv(ext)}}$. The $R^2_{\text{cv(ext)}}$ values are shown in Table 7. It is observed that HOMO, ΔH_F, E_T and E_S are the best descriptors in the establishment of the QSAR model for heterocyclic derivatives such as hydantoin and thiohydantoin related compounds. The correlation of the experimental activities with the MLR calculated ones is illustrated in Fig. 1. Partial Least Square (PLS) was also applied to generate a model (Geladi and Kowalski, 1986; Cramer et al., 1988) for quantitative structure–activity relationship (QSAR) between a set of molecular descriptors used in the MLR method and experimental activity. The correlation of the experimental and calculated activities with the PLS method is shown in Fig. 2. The correlation coefficient R (0.90), squared correlation coefficient R^2 (0.81), standard error of estimate SEE (0.32), and Fischer Statistics F (18.55) obtained with the PLS method indicate that the model proposed to predict activity is significant and pertinent to that of MLR method. In addition the architecture 5-5-1 of three layer artificial neural networks (ANN) shown in Fig. 5 is used to calculate the biological activities with the help of a set of molecular descriptors and experimental activity. The correlation between experimental and calculated activities with the ANN method is shown in Fig. 3. The correlation coefficient R (0.95), squared correlation coefficient R^2 (0.91), standard error of estimate SEE (0.23), and Fischer Statistics F (41.94) obtained with the neural network show that model proposed to predict activity by ANN was good and relevant to that of MLR method. The correlation of the experimental values with the calculated values in LOO procedure shown in Fig. 4 was reliable due to the high values of the correlation coefficient R_{CV} (0.86), squared correlation coefficient R^2_{cv} (0.74), standard error of estimate SEE (0.40) and Fischer Statistics F (14.65).

Over all, the biological activities predicted by MLR, PLS, ANN and LOO procedure with respect to their experimental

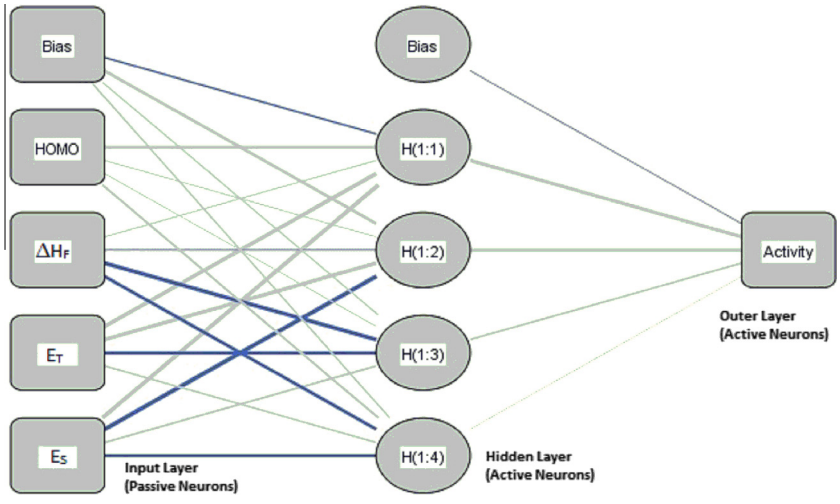


Figure 5 Schematic representation of architecture (5-5-1) of the three layer neural network (ANN).

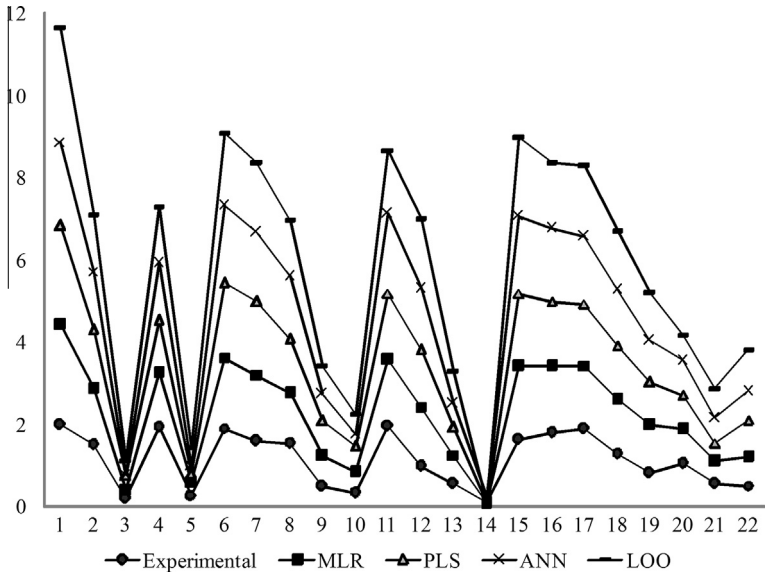


Figure 6 Graphical representation of biological activities predicted by MLR, PLS, ANN, LOO as well as experimental activities.

values are shown in Table 8. The predicted activities in Fig. 6 show the approximately same approaches to the experimental values.

6. Conclusion

The brine shrimp lethality bioassay is considered as a useful tool for rapid and preliminary assessment of toxicity of the compounds. Further studies are required to calculate the more accurate bioactivity and, to find the mode of pharmacological activities. Significant regression equations were obtained by MLR, PLS and ANN methods with respect to their experimental cytotoxic activities. The best regression equation was obtained on the following descriptors: Highest occupied molecular orbital (HOMO), heat of formation (ΔH_F), total energy (E_T) and steric energy (E_S). These variables allowed physical explanation of electronic molecular properties contributing to the cytotoxic activity as the electronic character relates directly to the electron distribution of interacting molecules. The predicted biological activities by MLR, PLS and ANN showed a good agreement with experimental values but the activities obtained from ANN were relatively better among them. The LOO, Leave-5-out cross validation and the *Y*-randomization techniques indicate that the model is significant, robust and has a good predictive ability.

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