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Quantum modeling of the toxicity of selected Anti-*Candida albicans* Schiff bases and their Nickel (II) complexes

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ABSTRACT

A set of 28 anti-Candida albicans Schiff bases and their Ni(II) complexes with their experimental median lethal dose (LD_{50}) were selected for 0D, 1D, 2D and 3D quantitative structure activity relationship (QSAR) analysis by means of Density Functional Theory using the B3LYP version and 6-31G basis set. The computed descriptors were correlated with their experimental LD_{50}. Genetic function approximation (GFA) method and Multi-linear regression analysis (MLR) was used to derive the most statistically significant QSAR model. Among the obtained QSAR models, the most statistically significant one was a tetra parametric linear equation with the squared correlation coefficient R^2 value of 0.9464, adjusted squared correlation coefficient R^2_{adj} value of 0.9321 and Leave one out (LOO) cross validation coefficient (Q^2) value of 0.9010. An external set was used for confirming the predictive power of the model, its R^2_{pred} = 0.7621. It is envisaged that the QSAR results identified in this study will offer important structural insight into designing novel less toxic anti-Candida albicans drugs from Schiff base and their nickel (II) complexes.*

Keywords: QSAR, LD_{50}, GFA, MLR, nickel-schiff base complexes, Candida albicans.

INTRODUCTION

In the past 25 years, the incidence of microbial infections has increased at alarming level over the world as result of antimicrobial resistance. A growing number of immune-compromised patients as a result of cancer chemotherapy, organ transplantation, and HIV infection are the major factors contributing to this increase [1]. There is therefore an urgent need to search and synthesize new class of antimicrobial compounds that are less toxic and effective against pathogenic microorganisms that developed resistance to the antibiotics used in the current regimen.

Of major concern is the pace of resistance of *Candida albicans* to the existing anti-biotics. This fungus can live as harmless commensal in many different body locations, and is carried in almost half of the population. However, in response to a change in the host environment, *C. albicans* can convert from a benign commensal into a disease-causing pathogen, causing infections in the oral, gastrointestinal and genital tracts. It is opportunistic pathogens for some immune-compromised people. It is responsible for painful mucosal infections such as vaginitis in women and oral pharyngeal thrush in AIDS patients. In certain group of vulnerable patients, it causes severe, life-threatening blood stream infections (candidemia) and subsequent infections in the internal organs (37).

For developing potential antibiotics that could curb the emerging trend of *C. albicans* resistance to the existing antibiotics, Schiff base and its complexes with nickel (ii) ion are considered to be among the most important stereo -

chemical models due to their preparative accessibility, structural varieties and high activities against this fungi [2; 3; 4; 9; 11; 12; 13; 14; 15; 16; 17; 29; 30; 31; 32].

However, rational prediction of the toxicities of anti-*Candida albicans* Schiff bases and their nickel (II) complexes after and even prior to their synthesis using QSAR model is undoubtedly a right step in the right direction in the discovery and development of novel anti-Candidal drugs. Novel medicines are typically developed using a trial and error approach, which is time consuming and costly. The application of quantitative structure activity relationship (QSAR) methodologies to this problem has potential to decrease substantially the time and effort required to discover new medicines or to improve current ones in terms of their efficacy. QSAR establishes the mathematical relationship between physical, chemical, biological or environmental activities of interest and measurable or computable parameters such as topological, physicochemical, stereo chemical or electronic indices [5].

The aim of this study is to develop good and rational QSAR mathematical model that can predict to a significant accuracy the toxicity (LD_{50}) of anti-*Candida albicans* Schiff bases and their Ni (II) complexes.

MATERIALS AND METHODS

2.1 Materials

The materials used in this study include; H.P 650 computer system (Intel Pentium), 2.43GHz processor, 4GB ram size on Microsoft windows 7 Ultimate operating system, Spartan 14 V.1.1.0, Chm 3D Pro 12.0.1V, Padel descriptor tool kit and Microsoft office Excel 2013 version + *Analyze it*[@] Statistical software, Material Studio (modeling and simulation software) version 7.0, and Printer.

2.2 Computational Methodology

Chemdraw ultra software was used to draw the structure of the compounds in the data set and each structure was saved as *MDL* file. The Spartan 14 V.1.1.0 soft ware was used for the optimization of the molecules. The molecules were first pre-optimized with the Semi-empirical (AM1) procedure included in Spartan'14 V1.1.0 soft ware and the resulting geometries were further refined by means of Density functional theory (DFT) using the B3LYP version and 6-31G[*] basis set. The lowest energy structure was used for each molecule to calculate their physicochemical properties. The quantum chemical descriptors were calculated using the Spartan'14 V.1.1.0 quantum chemistry package. *Padel descriptor* tool kit was used to calculate 1D, 2D and 3D descriptors as well.

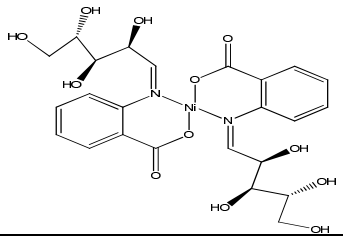
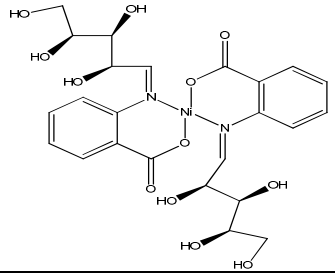
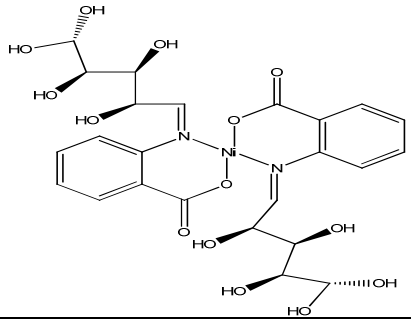
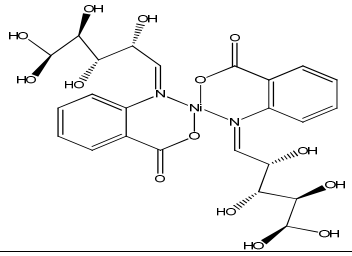
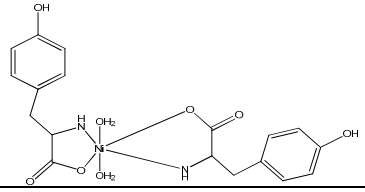
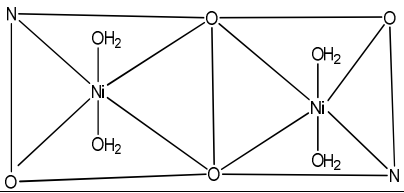
2.3 QSAR methodology

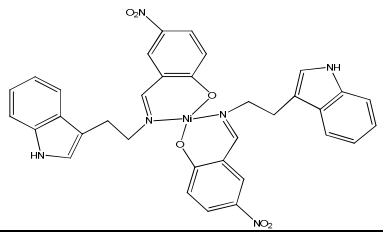
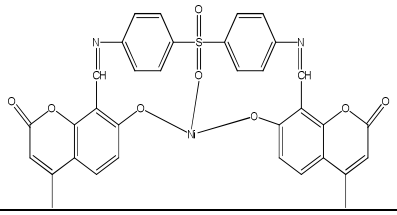
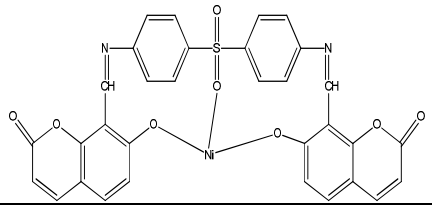
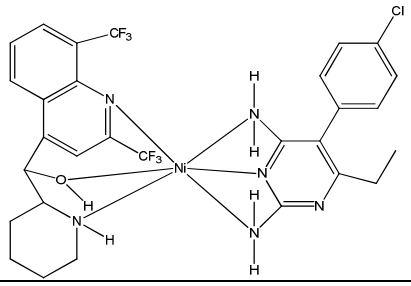
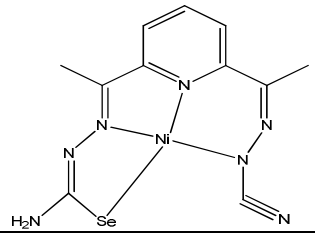
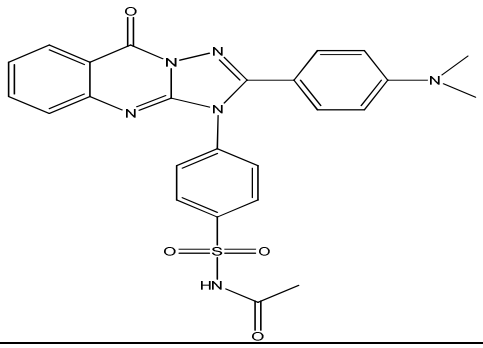
In the present study, we have performed the QSAR studies by Hansch's analysis using the linear free energy relationship (LFER) model described by Hansch and Fujita. In Hansch's approach, structural properties of compounds are calculated in terms of different physicochemical parameters and these parameters are correlated with biological activity through equation using regression analysis [19].

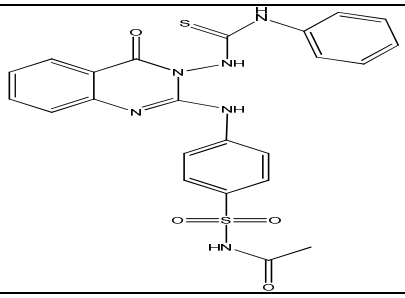
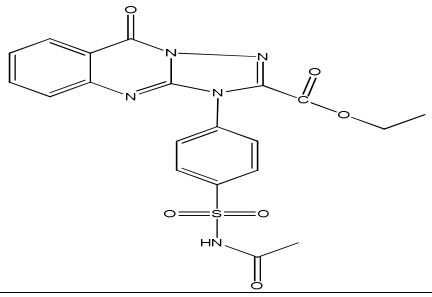
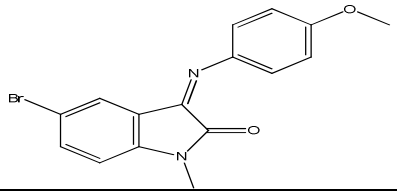
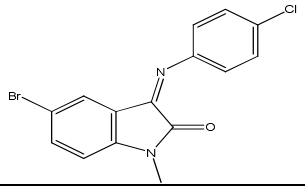
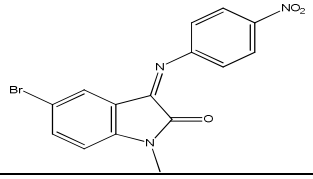
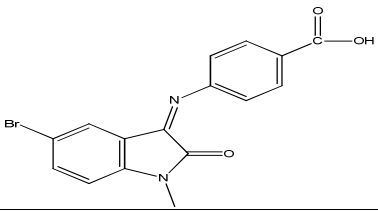
2.3.1 Data collection

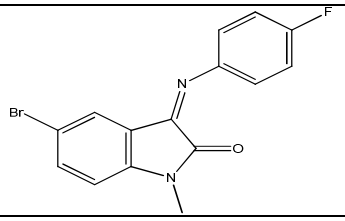
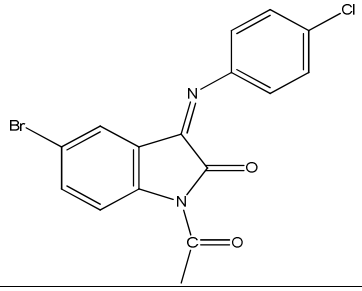
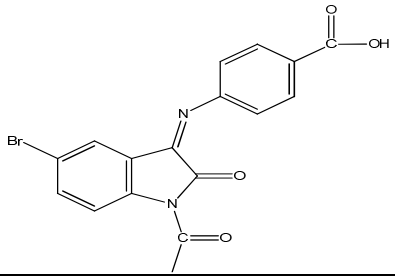
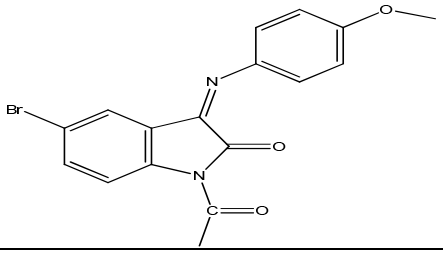
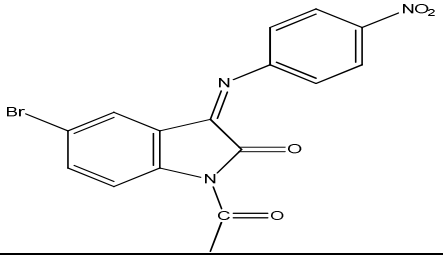
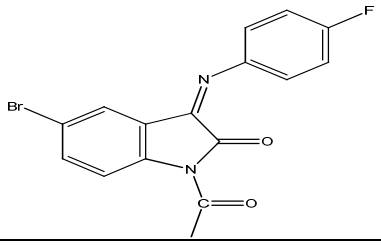
The chemical structures and experimental median lethal those (LD_{50}) values in mg/kg of anti- *Candida albicans* schiff bases and their nickel complexes were taken from literature [20; 21; 22; 23; 24; 25; 26; 27]. The LD_{50} values of the compounds were converted to logarithmic scale (pLD_{50}) in order to reduce the dispersion of data set and to get linear response and well data fitting. The notation, structure, MIC and pMIC values for each member of the training set are presented in Table 1.

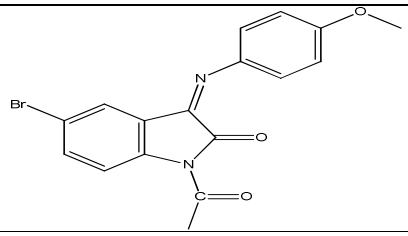
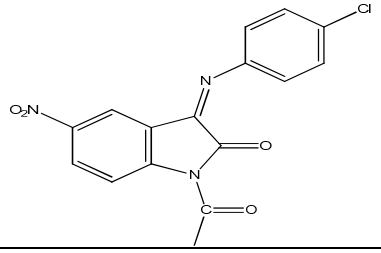
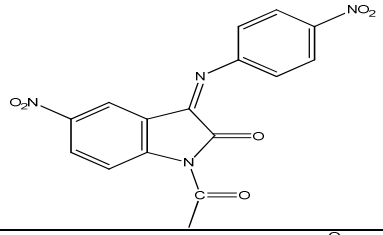
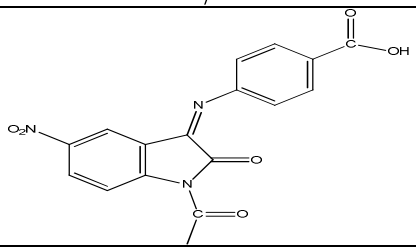
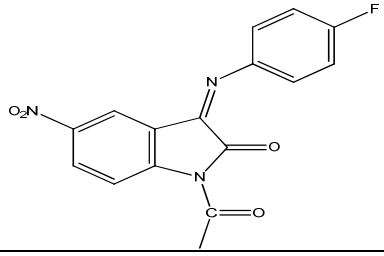
TABLE 1: Experimental LD_{50} values of anti-*Candida albicans* molecules

Cpd.	Structure	LD_{50}(mg/kg)	pLD_{50}
C1		600	2.78
C2		680	2.83
C3		620	2.79
C4		650	2.81
C5		50	1.70
C6		55	1.74

C7		5000	3.70
C8		400	2.60
C9		400	2.60
C10		25	1.40
C11		2000	3.30
C12		3700	3.57

C13		3380	3.53
C14		2950	3.47
C15		27	1.43
C16		600	2.78
C17		100	2.00
C18		300	2.48
C19		300	2.48

			
C20		27	1.43
C21		200	2.30
C22		300	2.48
C23		300	2.48
C24		300	2.48

C25		300	2.48
C26		27	1.43
C27		150	2.18
C28		42	1.62
C29		28	1.45

2.3.2 Descriptor selection

Over 1000 descriptors comprising of 0D, 1D, 2D, and 3D types were generated for each molecule. The descriptors were correlated with the LD_{50} values of the molecules using Pearson's correlation matrix. Pearson's correlation matrix was used to select the suitable descriptors for Genetic Function Approximation (GFA) and multi-linear regression (MLR) analysis based on the correlation coefficients.

2.3.3 Training set and data set.

The data set for the biological activity was split into training set and test set. At least 70% of the data set was used as training set and the rest as test set in line with the optimum splitting pattern of data set in QSAR study [33]. Consequently, the data set of 28 complexes was split into 20 training set and 8 test set. The training set was used to generate the model while the test set was used to evaluate the model's external prediction ability.

2.3.4 Regression analysis

Different possible combinations of descriptors were subjected to Genetic Function Approximation (GFA) and multiple linear regressions (MLR) analysis with the experimentally determined LD_{50} as dependent variable and the descriptors as independent variable. Out of the three statistically significant generated GFA models, the best (model-1) was selected based on the one with the smallest LOF score. The MLR equation was generated in stepwise manner by forward selection method starting with best single variable and adding further significant variable according to their contribution to the model that leads to the smallest P-value at 95 percent confidence level, until there is no other variable outside the equation that satisfies the selection criteria [28]. The P-values of the model was provided by the *Analyze it®* statistical software at 95% confidence level. The p-value is a probability that measures the evidence against the null hypothesis. Lower probabilities provide stronger evidence against the null hypothesis. The null hypothesis implies that there is no association between the descriptors and the pMic of the molecules. Model (1 and 2) gives the best QSAR equations using GFA and MLR analysis respectively.

Use of the Friedman lack-of-fit (LOF) measure has several advantages over the regular least square error measure. In Materials Studio, LOF is measured using a slight variation of the original Friedman formula [18]. The revised formula is:

$$LOF = SSE / (1 - \frac{C+dp}{M})^2 \quad \ldots\ldots (1)$$

Where SSE is the sum of squares of errors, c is the number of terms in the model, other than the constant term, d is a user-defined smoothing parameter, p is the total number of descriptors contained in all model terms (ignoring the constant term) and M is the number of samples in the training set. Unlike the commonly used least squares measure, the LOF measure cannot always be reduced by adding more terms to the regression model. While the new term may reduce the SSE, it also increases the values of c and p, which tends to increase the LOF score. Thus, adding a new term may reduce the SSE, but actually increases the LOF score. By limiting the tendency to simply add more terms, the LOF measure resists over fitting better than the SSE measure (Materials Studio 5.0 Manual).

2.3.5 Model validation

Validation is a crucial aspect of any QSAR modeling. It is the process by which the reliability and relevance of a procedure are established for a specific purpose [6]. It is the process of establishing the reliability and predictivity of a QSAR model. Both external and internal validations were carried out on the model. The validation parameters were compared with the minimum recommended value for a generally acceptable QSAR model proposed by Ravinchandran et al. [34] shown in Table 2.

Table 2: Minimum recommended value of Validation Parameters for a generally acceptable QSAR model
(Source: Ravinchandran et al., 2011)

	Validation Parameter		
S/n.	Symbol	Name	Value
1.	R^2	Coefficient of determination	≥ 0.6
2.	$P_{(95\%)}$	Confidence interval at 95% confidence level.	< 0.05
3.	Q^2	Cross validation coefficient	< 0.5
4.	$R^2_{ext.}$	Coefficient of determination for external test set	≥ 0.6
5.	$R^2 - Q^2$	Difference between R^2 and Q^2	≤ 0.3
6.	$N_{ext.\ test\ set}$	Minimum number of external test set	≥ 5

2.3.5.1 Internal validation

This is the validation done using the data that created the model. The QSAR models were internally validated using the methods of least squares fit (R^2), cross validation coefficient (Q^2), adjusted R^2 (R^2adj), difference between R^2 and Q^2 ($R^2 - Q^2$) and its confidence interval of all regression coefficient at 95% significant level (α value). The values of these parameters were compared with the minimum criterion for robust QSAR models proposed by Ravichandran *et al*. in Table 2.

R^2 value is interpreted as the proportion of variation in Y that is explained by the model. It is given by the formulae:

$$R^2 = \frac{SSR}{SST} = \frac{SST-SSE}{SSE} \quad \ldots\ldots (2)$$

Where SST = total sum of squares, SSR = regression sum of squares, and SSE = minimum sum of squared residuals of any linear model.

R^2 value varies directly with the increase in number of regressors i.e. descriptors, thus, R^2 cannot be a useful measure for the goodness of model fit. Therefore, R^2 is adjusted for the number of explanatory variables in the model. The adjusted R^2 is defined as:

$R^2_{adj} = 1 - (1 - R^2)\frac{n-1}{n-p-1} = \frac{(n-1)R^2-P}{n-p+1}$ …….. (3)

Where p = number of independent variables in the model. [7].

The LOO cross validated coefficient (Q^2) is given by;

$Q^2 = 1 - \frac{\sum(Yp-Y)^2}{\sum(Y-Ym)^2}$ ……. (4)

Where Yp and Y represent the predicted and observed activity respectively of the training set and Y_m the mean activity value of the training set.

The predicted r^2 value is calculated as follows;

Pred-R^2 $= 1 - \frac{\sum[Ypred(te)-Y(te)]^2}{\sum[Y(te)-Ym(tr)^2}$ ..... (5)

Ypred.(test) and Y(test) indicate predicted and observed activity values respectively of the test set compounds and Ym(tr) indicates mean activity value of the training set.

3.3.5.2 External validation

The real predictive ability of any *QSAR* model cannot be judged solely by using internal validation, it has to be validated on the basis of predictions of activities of molecules not used in the models [34]. Prior to the development of the models, each data set was split into training and test set. QSAR models were built using the training set while the tests set were used for externally validating the models. The predicted R^2 was computed in each case using the formulae in equation (5).

4.0 QSAR Study Results and Discussion

The best performing QSAR model for the LD_{50} of the complexes using GFA and MLR is represented by model 1 and 2 respectively. The name and symbol of the descriptors used in the QSAR model is shown in table 3.

Table 3: The symbol and definition of the descriptors used in the model

s/n	Descriptor symbol	Definition
1	nBase	Number of basic groups
2	WA.polar	Non-directional WHIM, weighted by atomic polarizabilities
3	WV.polar	Non-directional WHIM, weighted by atomic polarizabilities
4	WV mass	Non-directional WHIM, weighted by atomic masses

4.2 Model- 1: GFA Derived Model for pLD_{50} of Anti-Candidal Complexes.

pLD_{50} = 0.585842864nBase - 0.027523548WA.polar - 0.006247287WV.polar + 0.028007450WV.mass +1.578832691

n = 20, Friedman LOF = 0.05427800, R^2 = 0.94637700, R^2adj. = 0.93207700, Q^2 = 0.90104700
F-value = 66.18260200, Min. expt.error for non-significant LOF (95) = 0.11884300

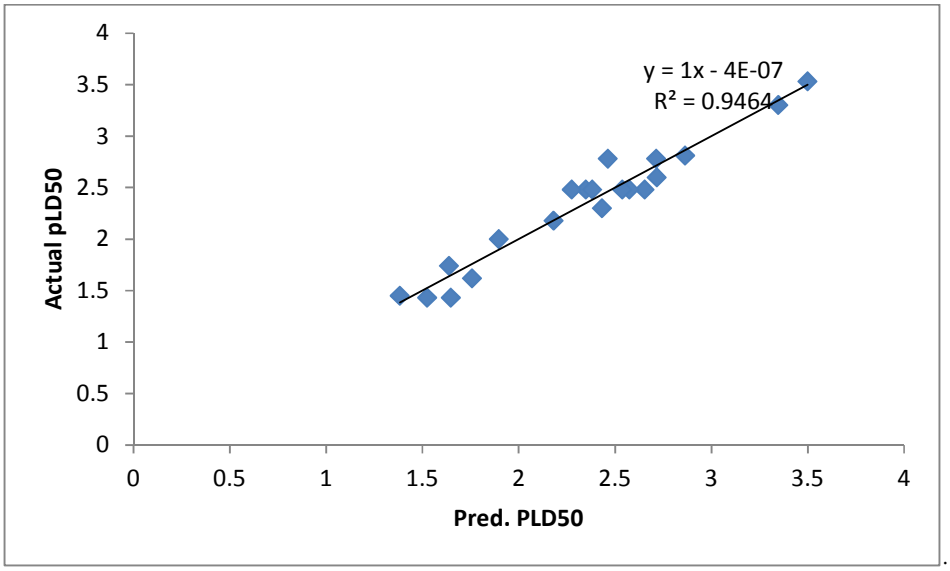


Figure 1: **Effect of model 1**

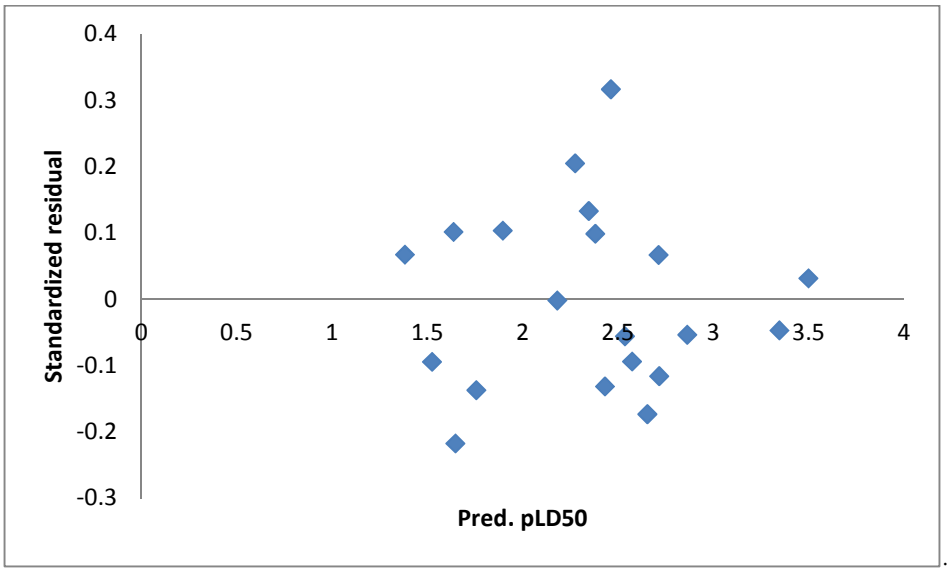


Figure 2: Residual plot of model 1

Table 5: Comparison of observed LD_{50} and predicted LD_{50} of model 1

cpd	Observed values	predicted values	residual values
c1	2.78	2.915194	-0.13519
c11	3.3	3.335877	-0.03588
c13	3.53	3.506082	0.023918
c15	1.43	1.604798	-0.1748
c16	2.78	2.537789	0.242211
c17	2	1.913506	0.086494
c18	2.48	2.644023	-0.16402
c19	2.48	2.615787	-0.13579
c21	2.3	2.362829	-0.06283
c22	2.48	2.328314	0.151686
c23	2.48	2.462673	0.017327
c24	2.48	2.269462	0.210538
c25	2.48	2.362534	0.117466
c26	1.43	1.621528	-0.19153
c27	2.18	2.064732	0.115268
c28	1.62	1.651027	-0.03103
c29	1.45	1.34241	0.10759
c4	2.81	2.90935	-0.09935
c6	1.74	1.888276	-0.14828
c8	2.6	2.493808	0.106192

Table 6a: External validation of Model-4a

test cpd	nBase	WA.polar	WV.polar	WV.mass	Act.pLD50	pred. pLD50	Residual
c10	1	51.02399	124.9024	60.70434	1.4	1.680187	-0.28019
c12	4	117.9509	207.3046	150.6553	3.57	3.600156	-0.03016
c14	4	136.8415	267.2913	201.8686	3.47	4.13982	-0.66982
c20	0	47.46302	66.86961	91.59443	1.43	2.420055	-0.99005
c3	0	109.2176	315.7378	220.2636	2.79	2.769294	0.020706
c5	0	63.36333	137.6757	140.3717	2.78	2.906203	-0.1262
c7	0	148.0742	405.0315	301.0607	3.7	3.4049	0.2951
c9	0	41.12801	96.12118	97.57831	2.6	2.579267	0.020733

Table 6b: External validation of Model-4a

test cpd	Ym(tr)	Y(te)	Ypre(te)	$[Ypred.(te) – Y(te)]^2$	$[Y(te) – Ym(tr)]^2$
c10	2.3415	1.40	1.680683	0.078505	0.886422
c12	2.3415	3.57	3.601014	0.000909	1.509212
c14	2.3415	3.47	4.140893	0.448659	1.273512
c20	2.3415	1.43	2.420643	0.980208	0.830832
c3	2.3415	2.79	2.770501	0.000429	0.201152
c5	2.3415	2.78	2.906992	0.015927	0.192282
c7	2.3415	3.70	3.406476	0.087084	1.845522
c9	2.3415	2.60	2.579857	0.00043	0.066822
				$\sum = 1.612151$	$\sum = 6.805758$

Since Pred-r^2 =1 - $\sum[Ypred(te) – Y(te)]^2 / \sum[Yte – Ym(tr)]^2$
Pred-r^2 = 1-(1.612151/6.805758) = 0.7631

4.4.5 Model-2: MLR Derived Model for pLD_{50} of Anti-Candidal Complexes.

pMIC = 1.579 + 0.5858 nBase - 0.02752 WA.polar - 0.006247 WV.polar + 0.02801 WV.mass

n = 20, R^2 = 0.946, R^2_{adj} = 0.932, RMSE = 0.1536, $P < 0.0001$ at 95% Confidence Level.

Table 7: Effect of terms in model – 2 at 95% confidence level

Term	SS	DF	MS	F	p-value
nBase	1.681	1	1.681	71.26	<0.0001
WA.polar	0.981	1	0.981	41.60	<0.0001
WV.polar	0.225	1	0.225	9.53	0.0075
WV.mass	3.005	1	3.005	127.33	<0.0001

The result of the GFA QSAR model is in conformity with the standard shown in Table 2 as R^2 = 0.9464, R^2_{adj} = 0.9321, Q^2 = 0.9010, $R^2_{pred.}$ = 0.7631. This confirms the robustness of the model.

The comparison of observed and predicted antibacterial activities of the complexes is presented in Table 5. The predictability of model-1 is evidenced by the low residual values observed in Table 5. Also, the plot of predicted LD_{50} against observed LD_{50} shown in Fig.1 confirms the robustness of the model. Further, the plot of observed pLD_{50} versus residual pLD_{50} (Fig.2) indicated that there was no systemic error in model development as the propagation of residuals was observed on both sides of zero [8].

The *P*-value of the model at 95% confidence level shown in model-2 is < 0.0001. This reveals that the alternative hypothesis that there is an association between the descriptors used in the model and the LD_{50} of the molecules takes preference over the null hypothesis.

The effect of terms shown in section Table 7 reveals that at 95% confidence level, all the descriptors in the model contribute significantly as their P-values are less than 0.05, a requirement at this confidence limit.

The closeness of the values of R^2, R^2_{adj}, Q^2 of model obtained from GFA to that obtained through MLR, further reveals the reliability and robustness of the GFA model.

QSAR derivation indicated that the toxicity of Schiff bases and their Ni (II) complexes is strongly correlated with number of basic group (nBase), non-directional WHIM weighted by atomic polarizabilities (WV polar and WA.polar), and non-directional WHIM weighted by atomic masses (WV. mass). This suggests that the toxicity of this class of compounds depended on their hydrophilicity, polarity as well as their molecular sizes.

However, the toxicity of a molecule varies inversely with its LD_{50}, thus the higher the LD_{50}, the lower the toxicity and vice versa.

The decrease in toxicity with increase in the number of basic groups as shown in the models may be rationalized thus; as the number of basic group increases in a molecule, the molecule become more hydrophilic enough to dissolve in aqueous gastric juice and blood stream [35]

The decrease in toxicity with increase in the number of basic group might be as a result of the increasing solubility of the molecule which consequently leads to increase in volume of distribution of the molecule in the biological system.

WV.polar and WA.polar are WHIM descriptors which describe the polarity of a molecule. The decrease in toxicity with decrease in polarity of the molecule as shown in the models may be due to the increase in lipophilicity of the molecules due to reduced polarity, enhancing its penetration into the lipophilic biological membranes.

Also, WV.mass is non-directional WHIM descriptor which describes the size of the molecule, The decrease in toxicity with increasing size of the molecules may be due to the possibility of the molecule to be largely confined to the plasma compartment because of their large size [36] affecting its distribution via out the body.

CONCLUSION

The generated QSAR models, performed to explore the structural requirements controlling the observed toxicity (LD_{50}) of Schiff bases and their nickel (II) complexes, hinted that the toxicity of the molecules were affected by directional WHIM descriptors weighted by atomic polarizability and the number of basic group. The robustness and applicability of QSAR equation has been established by internal and external validation techniques. It is envisaged that the wealth of information in this QSAR model and its predictive power will provide an insight to designing less toxic novel bioactive anti-*Candida albicans* molecules.

5.0 Recommendation

In the future design of novel less toxic Schiff bases and their Ni (II) complexes as anti-*Candida albicans* drug, it is recommended based on this research that the molecules should be made less polar as possible. Also, the molecules should be made slightly hydrophilic by incorporating basic functional groups into the parent moeities. The size of

the molecule should be made optimal by substituting bulkier ligands in the parent moieties with a less bulky ligand or functional groups.

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