Original Research Article

MOLECULAR MECHANICS-BASED QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP STUDY ON THE INHIBITORY ACTIVITY OF SCHIFF BASES AGAINST *ESCHERICHIA COLI*

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ABSTRACT

Due to their high inhibitory action against Escherichia coli (E. coli), the rise of multidrug-resistant strains of the bacteria necessitates the testing and development of a new set of Schiff bases as anti-E. coli agents worldwide. In this study, the Genetic function approximation (GFA) Quantitative structure-activity relationship (QSAR) analyzes selected Schiff bases with anti-E. coli activity. This was done using different molecular descriptors and Hansch's approach, which results in the production of onestatistically significant hepta parameter model as the strongest model with a squared correlation coefficient (R^2) = 0.828, adjusted squared correlation coefficient (R^2adj) = 0.775, cross-validated correlation coefficient (Q^2) = 0.691, Difference between R^2 and Q^2, Q^2 (R^2 - Q^2) = 0.137, external prediction (R^2pred.) = 0.751 and lack of fit (LOF) of 0.067 value were selected as the best model based on its sound statistical parameters. The development model demonstrated the predominance of the descriptors Minimum H E State (Hmin) and Valence path order 6 (VP-6) in influencing the observed anti-E. coli activity of Schiff bases. Insilico techniques can certainly provide a quick, inexpensive and safe quantitative risk assessment for this class of compounds. It is envisaged that the QSAR results identified in this study will provide important structural insights into the design of the novel anti-E. coli drugs from Schiff bases.

Key words: Escherichia coli, Schiff bases, Hansch's approach, QSAR, Descriptor

INTRODUCTION

Schiff bases are characterized by an imine group **–N=CH**, which helps to elucidate the mechanism of transamination and racemization in biological systems. In terms of biological capabilities, it has an antibacterial and antifungal effect. In recent years, Schiff bases have received considerable attention because of their physiological and pharmacological activities (Misbah, *et al.*, 2013).Antimicrobial medications play a critical role in reducing illness and death caused by infectious diseases in both animals and humans (John, *et al.*, 2015).Selective pressure given to existing antimicrobial medicines has, however, limited the creation and dissemination of drug-resistance traits among disease-causing and commensal bacteria (Aarestrup, *et al.*, 2008). *Escherichia coli* or *E. coli* are gram-negative bacilli of the family Enterobacteriaceae. *E. coli* are normal inhabitants of the human large intestine (it is a bacterium commonly found in the intestines of humans and animals). Of serious concern is the development of resistance

by*Escherichia coli* or*E.coli* strains to the current antibiotics such as ampicillin, sulfonamide, gentamicin, streptomycin, ciprofloxacin, trimethoprim, amoxicillin(L.Blaettler, et al., 2009) (Kronvall, 2010).*E.coli* is commonly a commensal bacterium of humans and animals but Pathogenic variants cause intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia (Von Baum & Marre, 2005) (Sodha, et al., 2011). This trend of resistance exhibited by this organism poses serious threat to human and animals health, necessitating the search for newer antibiotics John, *et al.*, 2015). This class of organic compounds have also demonstrated significant inhibitory activity against the growth of *E. coli*(Hafiz, *et al.*, 2015) (Malik, *et al.*, 2011) (Santhosh & Parthiban, 2011) (Sahu, et al., 2008)making them potential drug candidate in man's quest to curb the dangerous trend of multi-drug resistance posed by this pathogenic micro-organism.

Conventional drug discovery and development is characterized by trial-and-error approach. This is time consuming, costly due to the enormous expense of failures of candidate drugs late in their development and a threat to green chemistry due to enormous waste released into the environment. QSAR offer important structural insight in the design of novel anti-microbial drugs by exploring and harnessing the structural requirements controlling the observed anti-microbial activities as well as providing predictive model for bio-activities of potential drug candidates, reducing the requirement for lengthy, costly and hazardous laboratory test. QSAR is based on the conception that there exists a close relationship between bulk properties of compounds and their molecular structure(John, *et al.*, 2015). Thus, it is the basic tenet of chemistry to identify these assumed relationships and then to quantify them allowing a clear connection between the macroscopic and the microscopic properties of matter(Sanja, *et al.*, 2008).

The aim of this work is to build a statistically robust, predictive and rational Genetic function approximation (GFA) based QSAR model for inhibitory activity of Schiff bases against E. coli. by exploring the correlations between the experimental pMIC of the compounds and their calculated molecular descriptors.

MATERIALS AND METHODS

The materials used in this study include; H.P 2000/ computer system (Intel Pentium), 1.30GHz processor, 4GB RAM size on Microsoft windows 13 Ultimate Operating System, Spartan 14 V.1.1.0, chem draw 12.0.1V, Padel descriptor tool kit and Microsoft office Excel 2016 version Statistical software, Material Studio (modeling and simulation software) version 7.0, DTC.In the present study, QSAR studies were performed using Hansch's approach (Amej*et al*., 2017). 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. The various steps are presented in a flowchart in Image.1

DATA COLLECTION

A data set comprising of series of 41 schiff bases *Escherichia coli* derivatives was taken from literature(Hafiz, *et al.*, 2015) (Malik, *et al.*, 2011), (Santhosh & Parthiban, 2011), (Sahu, et al., 2008), and (Karki, *et al.*, 2013). The chemical structures and experimental minimum inhibitory

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concentration (pMIC) values of the inhibitory activity of Schiff bases against*Escherichia coli* is presented in Table 1 below.70% of the data set (31 compounds) was used as training set for building the models while the remaining 30% (14 compounds) was used as test set for external validation of the most statistically significant QSAR model.

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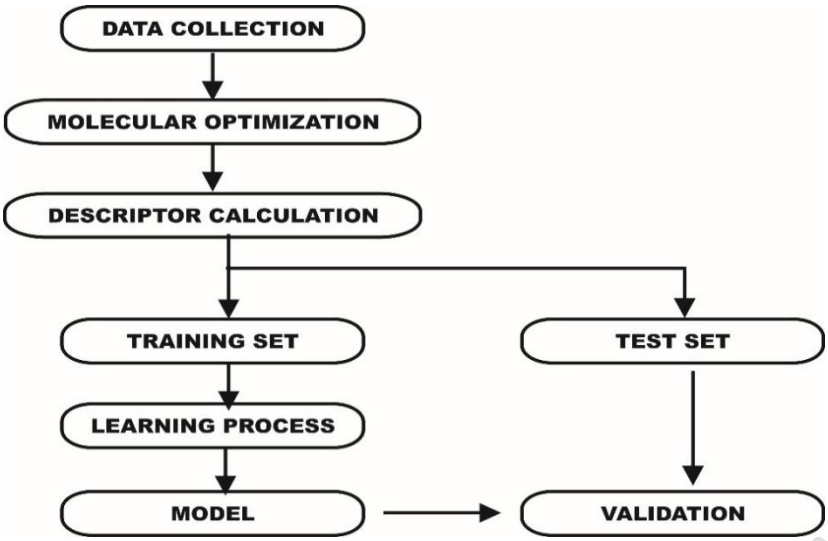

Image 1:QSTR Methodology flow chart (Source: Ameji et al., 2015).

Table 1: Chemical Structures and Experimental Pmic Values of anti-Escherishia coli inhibitory activity.

S/n	Structure	pMIC	S/n	Structure	pMIC
1.		1.342	2.		1.255

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3.		1.556	4.		1.079
5.		1.806	6.		1.415
7.		1.806	8.		2.093
9.		2.301	10.		2.301
11.		2.398	12.		2.000

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13.		2.301	14.		1.362
15.		1.322	16.		1.380
17.		1.623	18.		1.301
19.		1.591	20.		1.342

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21.		1.204	22.		1.602
23.		1.255	24.		1.342
25.		1.301	26.		1.322
27.		1.380	28.		1.230

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29.		1.362	30.		1.301
31.		1.204	32.		1.301
33.		1.279	34.		1.342
35.		1.415	36.		1.204

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37.		1.255	38.		1.230
39.		1.279	40.		1.322
41.		1.431	42.		1.255
43.		1.279	44.		1.279
45		1.000			

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Molecular Optimization

The chemical structure of each compound in the data sets was drawn with Chemdraw ultra V12.0, named and saved as *cdx file. The molecules were optimized with the molecular mechanics (MM) procedure included in Chem 3D Pro. Optimization was done in order to find the equilibrium or lowest energy geometry of molecule. The lowest energy structure was used for each molecule to calculate their physicochemical properties (molecular descriptors).

Descriptors Calculation

The molecular descriptors used in this QSAR modelling were calculated using (Pharmaceutical Data Exploration Laboratory) PaDEL descriptor tool kit. Over 1000 descriptors ranging from 0D,1D,2D and 3D were used for this work.

Data normalization.

The chemical structures and the experimental pMIC of the compounds are presented in Table 1. Data normalization was performed on the dependent variable (MIC) by converting the experimental MIC values to logarithmic scale [pMIC = $\log_{10}$ MIC]. This was done to get a more linear response and reduced data dispersion.

Learning process

During this process, the correlation between biological activities (pMIC) of the compounds and the calculated descriptors were obtained through correlation analysis using the Microsoft excel package in Microsoft office 2016. Pearson's correlation matrix was used as a model, in order to select the suitable descriptors for this regression analysis. The selected descriptors were subjected to regression analysis with experimentally determined activities as the dependent variable using Genetic Function Approximation (GFA) in material studio software to build QSAR models. The models were estimated using the "lack of fit" (LOF) score, which was measured using a slight variation of the original Friedman formula, so that best model received the best fitness score(Wu, et al., 2015). LOF is measured with the aid of the original Friedman formula (Friedman, 1990)shown in equation 1.

LOF = SSE(1-c+dp/m)2----------1

SSE gives the sum of squares of errors, 'c' the number of terms in the model, other than the constant term, 'd' gives the 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. LOF measure cannot always be reduced by adding more terms to the regression model in contrast to the commonly used least squares measure. By limiting the tendency to simply add more terms, the LOF measure resists over fitting (Materia studio, 2016).

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Model validation

The fitting ability, stability, reliability and predictive ability of the best models were evaluated by internal and external validation parameters. The validation parameters were compared with the minimum recommended value for a generally acceptable QSAR model shown in Table 2.

Internal validation parameters

This validation was done using the data that created the model. The various internal validation parameters invoked in this study are; the square of the correlation coefficient (R2), Adjusted R2 (R2adj), Q2 (Leave one out cross validation coefficient, Validation ratio (F value).

External validation parameters

Internal validation is an essential step in QSAR model development. The desired internal validation results show that the model exhibits higher stability and prediction ability. However, it does not show any real prediction ability for external test set of molecules. Therefore, the external predictive ability and extrapolation of the best model should be evaluated(Wu, et al., 2015). The external prediction parameter used in this work is R^2pred.

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Table 2: Validation metrics for a generally acceptable QSAR model

S/N	Symbol	Name	Threshold
1	R^2	Coefficient determination	$\geq$0.6
2	Q^2	Cross validation coefficient	>0.5
3	$R^2_{pred.}$	External test set's coefficient of determination	$\geq$0.6
4	$R^2 - Q^2$	Different between R^2 and Q^2	$\leq$0.3
5	F value	Validation ratio	High
6	P95%	Confidence interval at 95% confidence level.	< 0.05

Source: (Ravichandran, et al., 2011)

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RESULTS AND DISCUSSION

Model 1 Equation;

$$pMIC = 1.186X1 - 3.486X2 - 0.329X3 - 6.116X4 + 0.091X5 - 0.078X6 + 0.540X7 + 3.872.$$

Friedman LOF = 0.073, R^2 =0.828, R^2_{adj} =0.775, Q^2=0.691, S.R = yes, F_{value} =15.777, C.Exp.error =0.069, Minimum error= 0.000.

Table 3: Definition of Various Descriptors Used.

S/N	Descriptor	Symbol	Definition
1	X_1	VPC-4	Valence path cluster, order 4
2	X_2	VP-6	Valence path, order 6
3	X_3	maxsCH3	Maximum atom-type E-State:-CH3
4	X_4	Hmin	Minimum H E-State
5	X_5	ETA_Eta	Composite index Eta
6	X_6	WT.eneg	Non-directional WHIM, weighted by Mulliken atomic electronegativites
7	X_7	WK.eneg	Non-directional WHIM, weighted by Mulliken atomic electronegativites

Plot of Experimental Versus Predicted pMIC of model 1

The agreement between the experimental and predicted pMIC values of molecules used in the training and test set compounds by the optimization model are presented in Fig. 1 and Fig.2, respectively.

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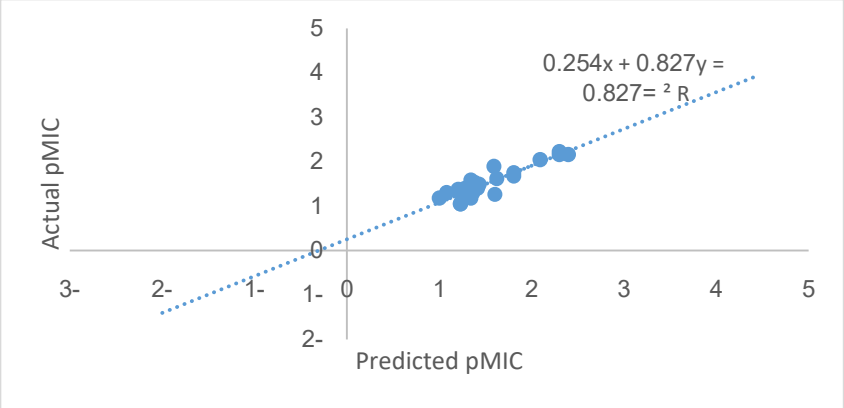

Figure 1: Plot of Actual Versus Predicted pMIC (Training set)

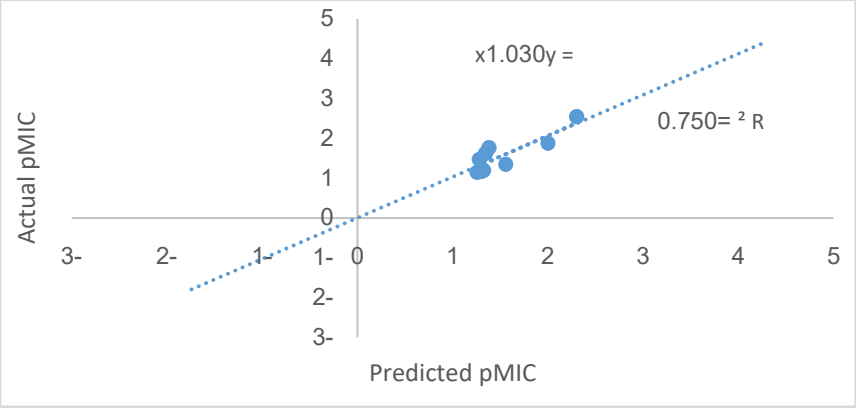

Figure21:Plot of Actual Versus Predicted pMIC (Test set)

Residual Plot of Model

The measure of the dispersion of residual pMIC values from the predicted pMICvalues is shown in Fig.3.

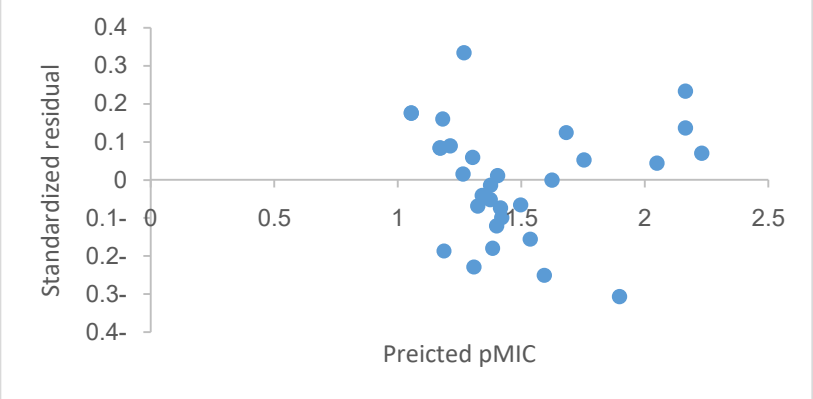

Figure 3: Residual Plot of Model.

Comparison of Actual and Predicted pMIC

The comparison of the predicted pMIC of the model with their experimental values is presented in Table 4.

External validation of the model

The actual, predicted and residual pMIC values of the test set compounds is presented in the Table 5.

Table 2. Actual, Predicted and Residual pMIC of model(training set)

Compound	Actual pMIC	Equation 1: predicted values	Equation 1: residual values
1	1.342	1.182	0.160
2	1.255	1.324	-0.069
4	1.079	1.308	-0.229
5	1.806	1.754	0.052
7	1.806	1.682	0.124
8	2.093	2.049	0.044
10	2.301	2.165	0.136
11	2.398	2.165	0.233
13	2.301	2.231	0.070
14	1.362	1.303	0.059
16	1.380	1.536	-0.156
17	1.623	1.624	-9.090e-004
19	1.591	1.898	-0.307
20	1.342	1.593	-0.251
22	1.602	1.268	0.334
23	1.255	1.171	0.084
25	1.301	1.342	-0.041
26	1.322	1.421	-0.099
28	1.230	1.054	0.176
29	1.362	1.376	-0.014
31	1.204	1.384	-0.180
32	1.301	1.212	0.089
34	1.342	1.416	-0.074
35	1.415	1.404	0.011
37	1.255	1.172	0.083
38	1.230	1.055	0.175
40	1.322	1.374	-0.052
41	1.431	1.497	-0.066
43	1.279	1.400	-0.121
44	1.279	1.264	0.015
45	1.000	1.187	-0.187

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Compound	pMIC	VPC-4	VP-6	Maxs CH3	hmin	ETA_ Eta	WT. eneg	WK. eneg	pred. Pmic
3	1.556	0.854	0.541	2.083	0.097	11.356	16.271	-0.247	1.348
6	1.415	0.846	0.615	0	0.072	15.826	12.251	-0.150	2.693
9	2.301	0.412	0.298	0	0.144	7.991	10.182	0.327	2.546
12	2	0.890	0.648	0	0.129	11.982	13.415	-0.082	1.879
15	1.322	0.964	0.720	1.575	0.100	13.230	16.858	-0.125	1.196
18	1.301	1.383	1.039	0	0.065	16.168	13.818	-0.072	1.848
21	1.204	1.522	1.064	0	0.076	17.585	14.045	-0.071	1.968
24	1.342	1.349	1.049	0	0.100	16.126	15.912	0.349	1.620
27	1.38	1.830	1.330	2.078	-5.95E-04	28.200	18.322	-0.181	1.766
30	1.301	1.730	1.427	1.715	0.028	27.871	21.432	0.171	1.170
33	1.279	2.177	1.562	2.084	-0.017	32.487	15.928	0.009	2.144
36	1.204	1.830	1.330	2.078	-5.95E-04	28.201	18.322	-0.181	1.766
39	1.279	1.191	0.899	0	0.069	16.509	20.789	-0.250	1.472
42	1.255	0.610	0.442	2.065	0.105	10.737	18.325	-0.246	1.146

Table5: Actual, Predicted and ResidualpMIC of model (test set).

Molecular Optimization and Descriptor Calculation

The molecules used for this study were successfully optimized at each stage. The optimization time for each level of theory follows Molecular mechanics. The molecular properties (descriptors) computed from each optimized structure include theChemdraw 12.0.1V software, PaDEL descriptor toolkit listed above. The successful optimization of the studied molecules indicates that their structures correspond to their real or natural geometry. Thus, all the descriptors derived from these structures are reliable.

GFA derived QSAR model for Schiff bases against *Escherichia coli*.

Based on the model with the best statistical parameters, model-1 was identified as the best model for predicting the pMIC of Schiff bases molecules. Model-1 was developed to predict the inhibitory activity ofSchiff bases against*E. coli.* The result of the GFA QSAR model is in conformity with the standard shown in Table 2, as R^2 = 0.828, R^2_{adj} =0.775, Q^2 = 0.691, $R^2_{pred.}$ = 0.751. This confirms the robustness of the model.

The closeness of coefficient of determination (R^2) to its absolute value of 1.0 is an indication that the model explained a very high percentage of the response variable (descriptor), high enough for

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a robust QSAR model. The high adjusted R^2 (R^2_{adj}) value and its closeness in value to the value of R^2 implies that the model has excellent explanatory power to the descriptors in it. Also, the high and closeness of Q^2 value to R^2 revealed that the model was not over fitted. The high R^2_{pred}. is an indication that the model is capable of providing valid predictions for new molecules that falls within its applicability domain. F value judges the overall significance of the regression coefficients. The high F value of the model is an indication that the regression coefficients are significant.The comparison of observed and predicted inhibitory activities of the molecules is presented in Table 3. The predictability of model 1 is evidenced by the low residual values observed in the Table. Also, the high linearity (R^2 = 0.828) of the plot of observed pMIC against predicted pMIC(Figure 1) also shows the high predictability of the model. To ascertain whether there exists a systematic error in the model development, observed pMIC was plotted against residual pMIC (Figure 3). The propagation of residuals on both sides of zero indicated that there was no systemic error in model development (Heravi-Jalali & Kyani, 2004).

Significance of the descriptors in model 1

The positive coefficient of the descriptors; X_1, X_5 and X_7, indicate that the magnitude of the pMICof these compounds against *E.coli*increases with increase in the values of these descriptors. Hence, the higher the values of these descriptors in these molecules, the lower the biological activity of the molecules against *E.coli*and vice versa.

X_6 and X_7 (Non-directional WHIM, weighted by Mulliken atomic electronegativites(WT.eneg), Non-directional WHIM, weighted by Mulliken atomic electronegativites (WK.eneg)), are descriptors of molecular electronegativity. The result of the QSAR optimization model shows that the *E. coli* inhibitory activity of the compounds increases with decrease in electronegativity of the compounds.

X_1 and X_2 (Valence path cluster, order 4(VPC-4), Valence path, order 6(VP-6))is a descriptor of molecular size. Its correlation with pMIC of the molecule as shown in the model indicate that the biological activity of the studied compounds against *E. coli*increases with decrease in the size of the compounds. Therefore, for an enhancedbiological activity from Schiff bases against *E. coli*,the size of molecules should be minimal.

Summary of Findings

The generated optimum QSAR models performed to explore the structural requirements controlling the observed biological activities of Schiff bases are represented by the model above. This Model gives the best predictive model for pMICof Schiff bases against *E. coli*. The observed pMIC of the compounds against *E. coli*was found to be heavily influenced by X_2 and X_4. These descriptorscontribute about 61.16% of the observed anti-*E. coli*inhibitory activity of the molecules. The negative coefficients of the descriptors as shown in the model implies that the

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lower the value of these descriptors in a molecule, the higher the pMIC, and the lower the biological activity of the molecule against *E. coli*and vice versa.

CONCLUSION

The generated QSAR models, was performed to explore the structural requirements controlling the observedantibacterial properties. The research has achieved the stated objectives. The robustness and applicability of the QSAR models has been established by internal and external validation techniques. It has been established that; The dominant structural features responsible for the inhibitory activity of Schiff bases against *E. coli*were X_1, X_2, X_3, X_4, X_5, X_6, and X_7(Valence path cluster, order 4(VPC-4),Valence path, order 6(VP-6), Maximum atom-type E-State:-CH3(maxsCH3), Minimum H E-State(Hmin), Composite index Eta(ETA_Eta), Non-directional WHIM, weighted by Mulliken atomic electronegativites(WT.eneg) and Non-directional WHIM, weighted by Mulliken atomic electronegativites (WK.eneg)), were the dominant structural features responsible for the observed inhibitory activity of the molecules against *E. coli.* Also,it is envisaged that the wealth of information in these models will provide a fast, economical and more environmentally friendly insight to designing novel and less toxic bioactive Schiff base compounds that will curb the emerging trend of multi-drug resistant strain of *E.coli.*

RECOMMENDATIONS

In the future design of novel Schiff bases as anti-*E. coli* drug, it is recommended based on this research that;The compounds should be made less bulky as possible since molecular size is negatively correlated to the bioactivity of the compounds as shown in the GFA derived model. And the number of hydrogen atoms in the moiety or parent structure should be high in order to achieve a reasonable anti-*E. coli* activity.Also, further QSAR work should be done on the pharmacokinetic properties of these compounds. Since safe and effective drug treatment is not only a function of its activity and but also a function of how the human body responds to the administration of the medication.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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