



COMPUTATIONAL STUDIES OF SOME BENZOTHAZINONE-PEPIRAZINE DERIVATIVES AND LIPASE B INHIBITOR FOR MYCOBACTERIUM TUBERCULOSIS

Y. Y. Muhammad^{1,*}, A. Uzairu²

¹ Kano University of Science and Technology, Department of Chemistry, Wudil, Kano, Nigeria

² Ahmadu Bello University, Faculty of Science, Department of Chemistry, Zaria, Nigeria

*Corresponding author. Kano University of Science and Technology, Department of Chemistry, Wudil, Kano, Nigeria, Phone: +234 7030929397
e-mail address: yakubuyaum@gmail.com (Y. Y. Muhammad).

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ABSTRACT

Computational technique was employed on Benzothiazinone-pepirazine derivatives as dominant anti-mycobacterium tuberculosis. The compound structures were drawn with the aid of chemdraw 3D Pro 12.1.0V and optimized was employed using DFT method applying B3LYP with the 6-31G/ basis set. Genetic Function Approximation (GFA) was employed to form five models. Model 1 was sorted out based on model validation parameters and found to be significant with R^2 value of 0.948605, R^2_{adj} (adjusted correlation coefficient) value of 0.934329, Q_{Loo} (Cross validation coefficient) value 0.892724 and R^2_{pred} value of 0.658537. The docking studies showed that the ligand 6, 7 and 18 has the highest binding affinities of 10.5, 10.4, 10.3 k/mole are the most vital compounds among the binding scores. Ligand 6 being among the ligands with the highest binding affinity (-10.5 k/mole) was found to be more potent than other compounds. Stability and Robustness the model highlight the way for designing latest Benzothiazinone-pepirazine analogue with better activity against mycobacterium tuberculosis.

1. INTRODUCTION

Tuberculosis (TB) is a lung disorder caused primarily due to *Mycobacterium tuberculosis* and is one of the most dangerous infectious disease in the universe. In line with the World Health Organization (WHO) estimates TB kills more adults now than all other infectious diseases (WHO, 2015). In 2017, (WHO) rated 1.3 million deaths (range, 1.2–1.4 million) among HIV-negative people and there were an additional 300 000 deaths from TB (range, 266 000–335 000) among HIV-positive people. 5.8 million Men, 3.2 million women and 1.0 million children, developed TB disease in 2017, ranging from 9.0–11.1 million people globally (WHO, 2018). Rifampicin, isoniazid ciprofloxacin, and ethambutol are drugs available for treatment of tuberculosis. The development of multidrug resistant (MDR) and extensively drug resistant (XDR) tuberculosis withstand current drugs and this bestow a serious summons towards successful therapy of tuberculosis (Lemichnane et al., 2011).

Meanwhile, the first-line TB drugs presently utilize are at least 40 years old with an increase in drug-resistant strains of myco-bacterium tuberculosis. Similarly, the second-line drugs cast-off mostly for the curing of multidrug-resistant and extensively drug-resistant (XDR) TB incite serious side effects according to WHO (WHO, 20104). To tackle the mycobacterial resistance, there is a demand to recognize novel targets special to tuberculosis which are vague in humans whose hindrance would either prove mortal to the bacterium or render it tremendously to the host immune reaction (Prakash et al., 2005). The high resistances of the M. tuberculosis toward the current drugs led to the progress of QSAR method that is easy, quick and precise to forecast the experimental activities for the latest molecules facing M. tuberculosis (Shola et al., 2019). Computational technique has guide to the assessment of current medication with a less side effect and high therapeutic ability (Abdulfatai et al., 2017).

QSAR modelling alongside with molecular docking approaches were applied to forecast the activities of various inhibitor compounds and interpret the regions of association (steric, hydrogen bond distance, hydrophobic, and hydrogen bond etc) may reduce or escalate the activities (Shola et al., 2018). The investigation was aimed to developed QSAR model and to anticipate the activity of Benzothiazinone-piperazine derivatives with strong anti-tuberculosis means and to performed docking technique between the ligand and the Lipase B enzyme.

2. MATERIALS AND METHOD

2.1 Source of Dataset

Thirty-five molecules of Benzothiazinone-piperazine derivatives as dominant anti-tuberculosis agents were retrieved from the literature (Manoj et al., 2015) and applied in this research. The experimental activity (IC_{50}) expressed in μM were converted to pIC_{50} [$-\log IC_{50} \times 10^6$] to minimize data distribution and enhanced the linearity of the activity

values. The two-dimensional structure (2D) and biological activities of Mycobacterium-tuberculosis used in this study are presented in Table 1 (See Supplementary Information).

2.2 Numerical and Computational Procedures

The 2D structure of each molecules were drawn with aid of chemdraw 3D Pro 12.0.1 V (Becke, 1993). Optimization was taken place using Spartan' 14 V1.1.0 on Hp computer system, using DFT (Density Functional Theory level) with B3LYP (Becke's three-parameter Lee-Yang-Parr hybrid functional) and also in merger with the 6-31G* basis set (Yap, 2011). B3LYP/6-311G* basis set is choose due to the fact that it gives a better result. The paDEL descriptor toolkit was employed to give the molecular descriptors of the optimized compounds. The data sets of the molecules were divided into 26 molecules for developing the model and 7 molecules for model external validation.

The models were generated by applying the GFA (Genetic Function Approximation) method inserted in Material Studio, (a simulation and modelling) software utilize the experimental activities as the dependent variable while the computed descriptors as independent variables.

Five models were inscribed out and model 1 was sort out as the top for anticipating pIC_{50} of anti- tuberculosis compounds due to it physicochemical parameter. The built QSAR model, was appraised by the external prognostic ability and extrapolation of the best model using the molecules of test set with the aid of equation.

$$R_{pred}^2 = 1 - \left[\frac{\sum(Y_{pred} - Y_{exp})^2}{\sum(Y_{pred} - Y_{train})^2} \right] \quad (\text{Eq. 1})$$

Where $Y_{test(tpred)}$ and $Y_{test(exp)}$ are the predicted and experimental activity of the test sets respectively and Y_{train} is the test set mean activity value (Muhammad et al., 2018).

2.3 Model Applicability Domain

The model applicability domain is utilized to dictate the influential and outliers' molecules of the QSAR model certify the robustness and reliability of the developed model. Evaluation of QSAR model is the predominant technique to verify that the model generate is qualified to build better forecast within the chemical area for which it was generated (Tropsha et al; 2003). The leverage (h_{ii}) of a given compound can be calculated in the equation given below:

$$h_{ii} = x_i^T (X^T X)^{-1} x_i \quad (\text{Eq. 2})$$

Where h_i is the leverage of each molecule, x_i is the descriptor row matrix of the molecule under consideration and X is the calculated molecular descriptors for each training set compound. The warning leverage (h^*) is the limit of normal values for X outliers and is defined as:

$$h^* = \frac{3(y+1)}{n} \quad (\text{Eq. 3})$$

Where n is the total number of training molecules and y is the number of molecular descriptors in the model. The Williams plot was applied to explain the importance region of the model in terms of chemical space. The compound is

said to be an outlier if the leverages value is outside domain.

2.4 Y-Randomization Test

To be guaranteed that the QSAR model generated is well built, the Y- randomization test was executed on the training test data. The lower values of R^2 and Q^2 of the newly developed QSAR models for numerous trials certify that the models are authentic. CR_p^2 is another determination factor for Y-randomization calculated, which should be greater than 0.5 for passing this test.

$$cR_p^2 = R * [R^2 - (R_r^2)]^2 \quad (\text{Eq. 4})$$

3. RESULTS AND DISCUSSION

Model 1 was selected among the five generated QSAR

3.1 QSAR Result

Best model equation

$$\begin{aligned} pIC_{50} = & -4.46799290 * \mathbf{AATS3e} + 4.343967313 \\ & * \mathbf{GATS6m} + 6.138836616 * \mathbf{IC0} \\ & - 0.029770404 * (\mathbf{SpDiam}_{Dt} - 507.60) \\ & + 0.005367093 * (707.04 - \mathbf{ATS2e}) \\ & + 24.49962 \end{aligned}$$

The model descriptors, classes and their definition are present in table 3. An increase in **GATS6m** (Geary autocorrelation-lag6/weighted by mass), **IC0** Information content index (neighbourhood symmetry of 0-order) and **ATS2e** (Broto-Moreau autocorrelation-lag 2/weighted by sanderson electronegativities) descriptors in the model 1 will definitely increase the anti- tuberculosis activities of Benzothiazinone derivatives against lipase B enzyme. Moreover, decrease in descriptors with negative coefficient such as **AATS3e** (Average centered Broto-Moreau autocorrelation – lag 3/weighted by

2.5 Molecular Docking Studies

Initially the optimized molecules were retained as SDF files which transformed to PDB files using Spartan 14 software Version 1.1.4. Lipase B structure employed in this research was recaptured from protein data bank. Docking technique was performed between Lipase B enzyme and Benzothiazinone derivatives using AutoDock vina of PyRx virtual screening software (Trott and Olson 2010). Discovery Studio Visualizer was employed to visualize the docking interaction between the prepared receptor (lipase B) with prepared ligand.

model for predicting the activity (pIC_{50}) of anti- tuberculosis compounds due to its physicochemical parameter. The model validation parameters (internal and external) were proceeded through the minimum standard QSAR model shown in Table 2.

$$R^2_{\text{tmg}}=0.948605, R^2_{\text{adj}}=0.934329, Q^2_{\text{LOO}}=0.892724, N_{\text{tmg}}=26,$$

$$N_{\text{test}}=9, R^2_{\text{test}}=0.658537$$

$$\text{Min expt. error for non-significant } LOF (95\%) = 0.068823$$

sanderson electronegativities) and **SpDiam_Dt** (Spectral diameter from detour matrix) will undoubtedly increase the hydrophobicity and binding ability of the inhibitors.

Figure 1 presents the graph of predicted activities against experimental activities of both the training and test sets. The accuracy of the model indicates the high linearity of the plot which confirmed its high prognostic power of the model. Moreover, the external validation and predictive R^2 value of the model were calculated and present in Table 3A and 3B.

Table 2 - Molecular descriptors, their definition and classes of model (Todeschini et al; 2009).

S/N	Descriptors	Definition	Classes
1	AATS3e	Average centered Broto-Moreau autocorrelation – lag 3 /weighted by sanderson electronegativities	2D
	GATS6m	Geary autocorrelation- lag 6/weighted by mass	2D
3	IC0	Information content index (neighbourhood symmetry of 0-order)	2D
4	SpDiam_Dt	Spectral diameter from detour matrix	2D
5	ATS2e	Broto-Moreau autocorrelation – lag 3/weighted by sanderson electronegativities	2D



Figure 1 - The graph of the biological versus Predicted activity of both the training and test sets of model

Table 3A - External validation of the model 1

AATS3e	GATS6m	IC0	SpDiam_Dt	ATS2e	Ypred	pIC50
7.923033	0.8877	1.651382	465.5386	669.3793	4.547641	4.55
7.968921	0.935414	1.839835	484.946	714.4514	4.887088	4.87
7.871679	0.756033	1.497827	425.1277	637.5031	4.636609	4.74
7.923033	0.779252	1.851382	469.2473	669.3793	5.193908	5.28
7.921361	0.796498	1.939835	484.946	702.8814	5.172122	5.06
7.819505	0.530337	1.597827	425.1277	625.933	4.565291	4.83
7.753038	0.452565	1.670689	425.3415	657.8819	4.79388	4.8
7.801962	0.56632	1.827144	443.5349	702.954	5.24636	5.19
7.594835	0.227875	1.561996	387.4915	663.5103	4.954039	4.61

Table 3B - Assessment of the predictive R2 of model 1.

$Y_{pred} - Y_{exp}$	$(Y_{pred} - Y_{exp})^2$	Y_{trng}	$(Y_{exp} - Y_{trng})$	$(Y_{exp} - Y_{trng})^2$
0.002359	5.56418E-06	5.01153	-0.46153	0.21301
-0.01709	0.000292012	5.01153	-0.14153	0.020031
0.103391	0.010689598	5.01153	-0.27153	0.073729
0.086092	0.007411748	5.01153	0.26847	0.072076
-0.11212	0.012571252	5.01153	0.04847	0.002349
0.264709	0.070070855	5.01153	-0.18153	0.032953
0.00612	3.7452E-05	5.01153	-0.21153	0.044745
-0.05636	0.00317647	5.01153	0.17847	0.031852
-0.34404	0.118363107	5.01153	-0.40153	0.161226
$\Sigma(Y_{pred} - Y_{exp})^2 = 0.2226$			$\Sigma(Y_{exp} - Y_{trng})^2 = 0.6519$	

$$R^2 = (1 - 0.2226/0.6519) = 0.65853$$

Table 5 - Y randomization test result

Model	R	R ²	Q ²
Original	0.985622	0.97145	0.828461
Random 1	0.982428	0.965164	0.828164
Random 2	0.781438	0.610646	-1.3712
Random 3	0.756959	0.572986	-0.93703
Random 4	0.372437	0.13871	-3.84808
Random 5	0.473695	0.224387	-2.92478
Random 6	0.799852	0.639763	-0.84577
Random 7	0.920143	0.846664	-0.40989
Random 8	0.699882	0.489835	-2.75574
Random 9	0.445727	0.198672	-1.89996
Random 10	0.963859	0.929024	0.566911
Random Models Parameters			
Average r :	0.719642		
Average r ² :	0.561585		
Average Q ² :	-1.35974		
cRp ² :	0.663789		

Meanwhile, Figure 2 present the Williams plot (standardized residual against leverages). From the plot only one compound is discovered to be an outlier compound which is part of the test set. This compound has the leverages value high than the warning leverages ($h^*=0.69$) is attribute because the compound structure is different from other compounds in the dataset. The Y- randomization test was presented in table 6. The minimal values of R² and Q² for the numerous trials confirm that the generated QSAR model is reliable and steady. Moreover, the cRp² value greater than 0.5 guaranteed that the developed models is well built.

3.2 Molecular Docking Result of Benzothiazinone-piperazine Derivatives

Docking technique of Benzothiazinone-piperazine derivatives was performed against lipase B (receptor) to find

out their interactions and their docking affinities. Compounds with best docking scores are shown in table 6. The docking study revealed clearly that the ligand number 6 has the highest binding score (-10.5) kcal/mole and formed hydrogen bonding with SER124 (2.23929Å), MET125 (2.85231 Å) and TRP262 (2.24581 Å) and is also surrounded by hydrophobic residues such as LEU40, PHE150, TRP262, ARG41, LEU161, ILE162, TRO158, ALA165, LEU227, ALA165 and LEU227, electrostatic interaction with LEU161. This computational study disclosed that ligand number 6 showed good binding affinity toward the Lipase B enzymes than another co-ligand. Figure 3 shows the ligand 6 and 7, 3D and 2D structure of respectively.

Table 6 - Binding energy, Interaction residues, Hydrogen bonds and Hydrogen bond distance of ligand with highest docking scores

Ligand	Binding Energy	Hydrophobic Interaction	Electrostatic/ Other Interaction	Hydrogen Bond	Hydrogen Bond Distance
6	-10.5	LEU40,PHE150,TRP262,ARG41,LEU161,ILE162,PHE150,TRP158,ALA165,LEU227,ALA165, and LEU227	LEU161	SER124,MET125,TRP262	2.23929, 2.86942, 2.24581
7	-10.4	LEU40,PHE150,TRP262,ARG41,LEU161,ILE162,PHE150,TRP158,ALA165,LEU227,ALA165,LEU227	LEU161	SER124,MET125,TRP262	2.0823, 2.71691, 1.98299
18	-10.3	LEU40,PHE150,ARG41,ALA165,ILE162,ILE51,PHE150,ALA165,LEU227,LEU40,ALA165,LEU227,ARG41	LEU161,TRP262	SER124,MET125,TRP262	1.91458, 2.54996, 3.02782

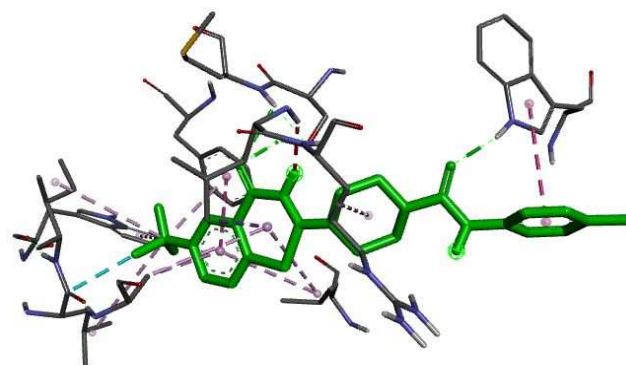
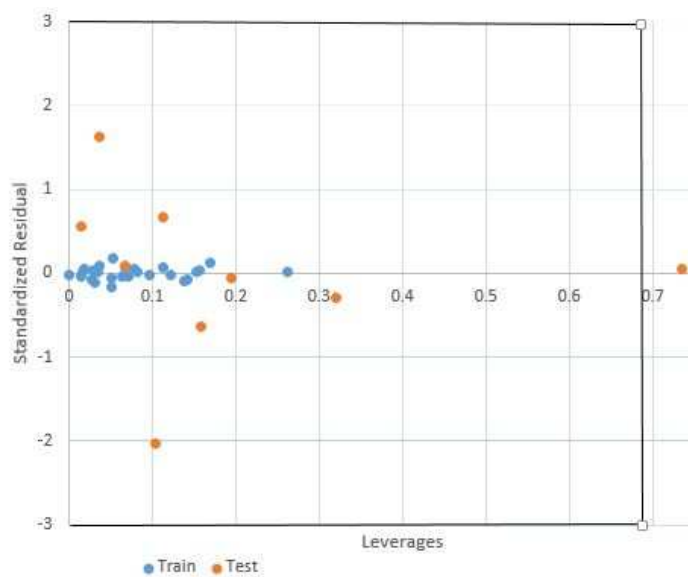


Figure 2 - Williams plot of the best model

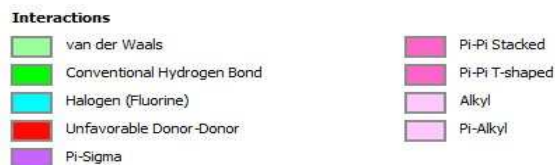
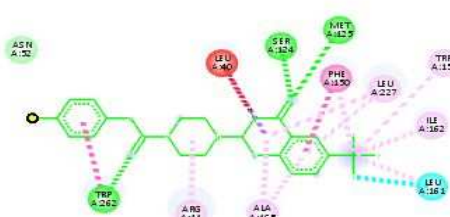
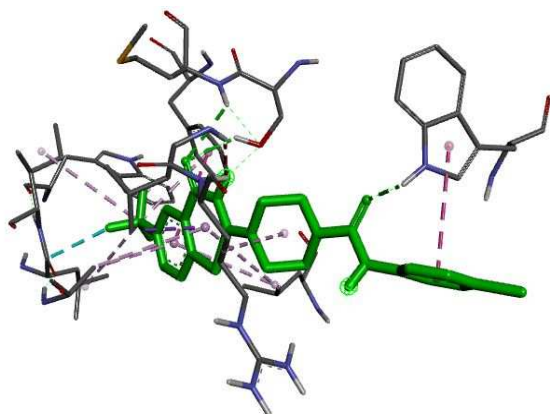


Figure 3B- 3D and 2D interaction diagrams of predicted complexes of Lipase B with compound 7

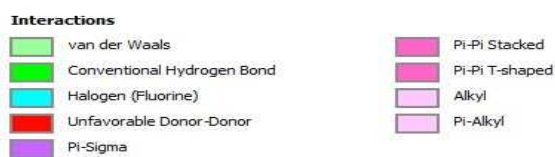
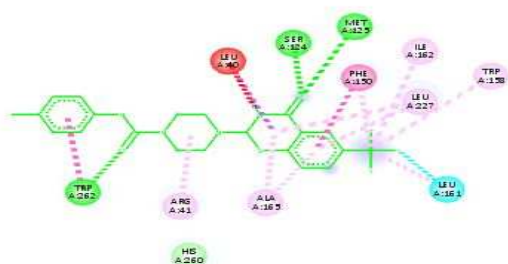


Figure 3A- 3D and 2D interaction diagrams of predicted complexes of Lipase B with compound 6

4. CONCLUSION

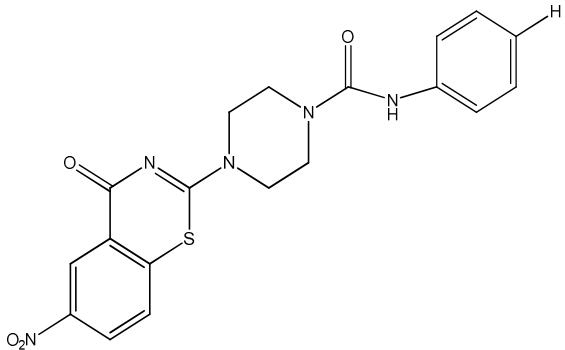
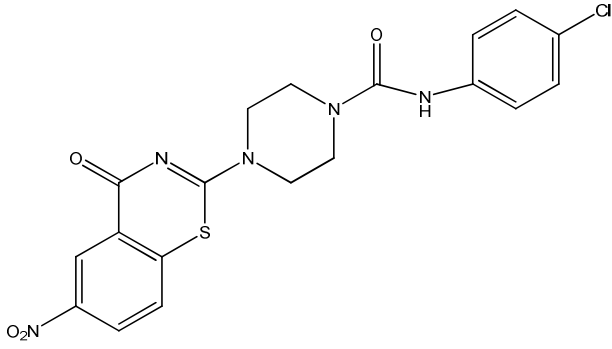
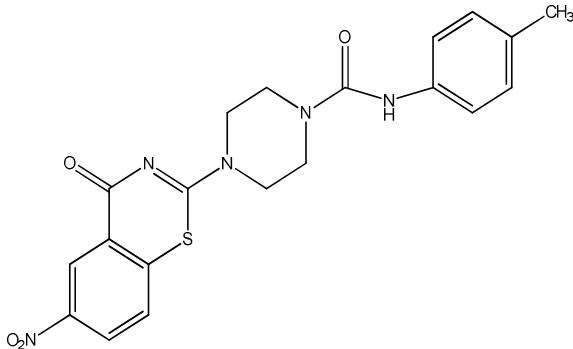
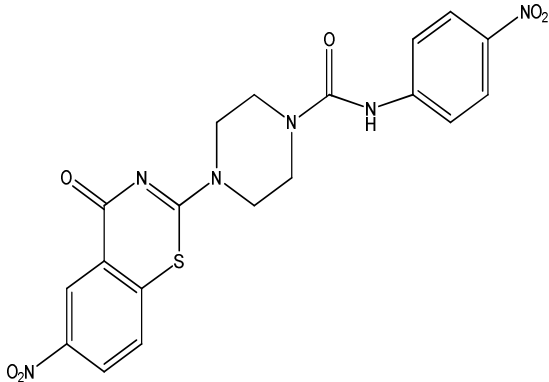
An insilico method was performed on 35 Benzothiazinone-piperazine derivatives as anti-tuberculosis molecules. The Model was evaluated internally and externally due to its physicochemical parameter of square correlation coefficient (R^2) of 0.948605, adjusted squared correlation coefficient (R^2_{adj}) value of 0.934329, Leave one out (LOO) cross-validation coefficient (Q^2_{cv}) value of 0.892724 and the external validation (R^2_{pred}) of 0.658537. From the molecular docking studies carried out it was shown that all the ligands were binding with receptor (Lipase B) favourably. The applicability, stability and robustness of the generated QSAR model have been confirmed by internal and external validation assessment. The docking analysis indicated that the ligand 6, 7 and 18 with docking energy of 10.5, 10.4, and 10.3 kcal/mole are among the compounds with the highest docking scores. Ligand 6 being among the ligands with the highest binding affinity (-10.5 k/mole) was found to be more potent than its co-ligands. Stability and Robustness of the model highlight the way for designing latest Benzothiazinone-piperazine analogue with better activity against mycobacterium tuberculosis.

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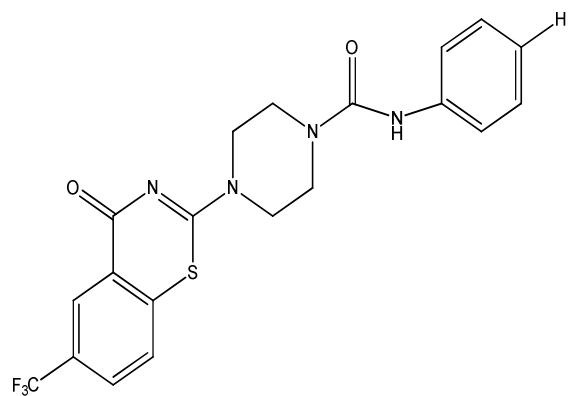
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APPENDIX

Table 1- Compounds structure and their activities

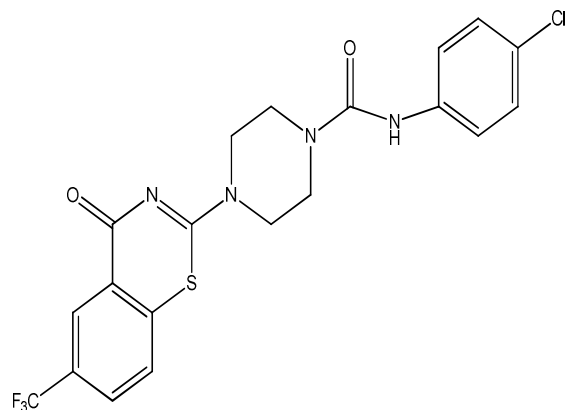
S/N	Compounds	pIC ₅₀
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2		4.55
3		4.83
4		4.86

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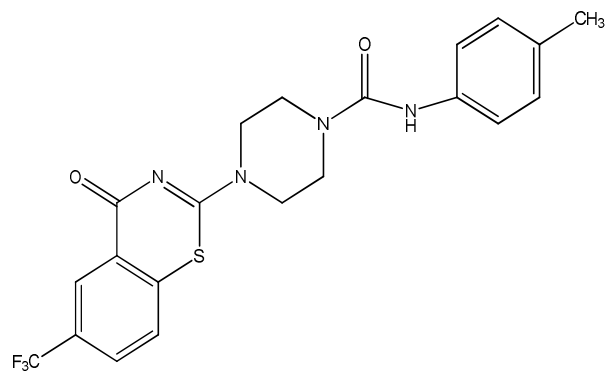
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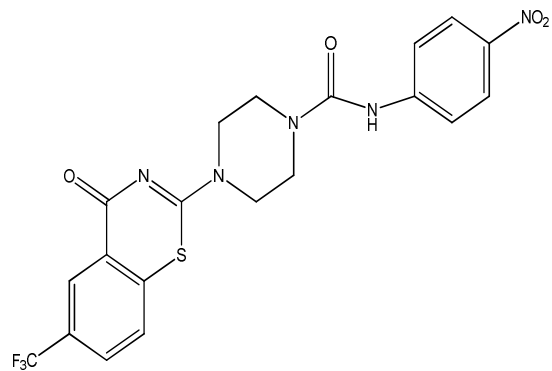
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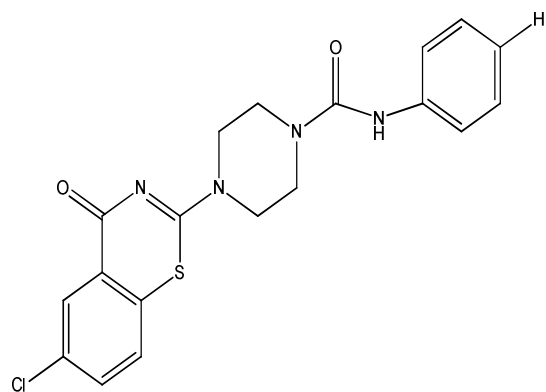
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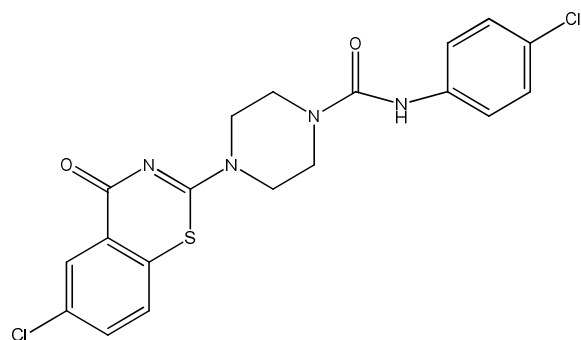
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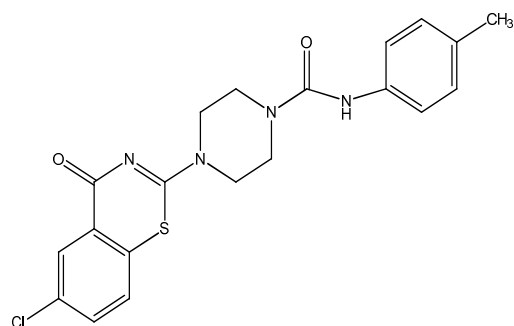
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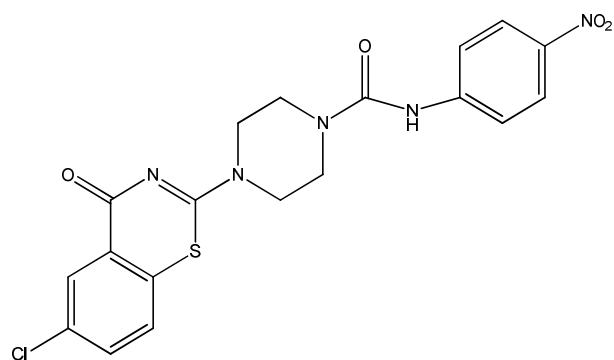
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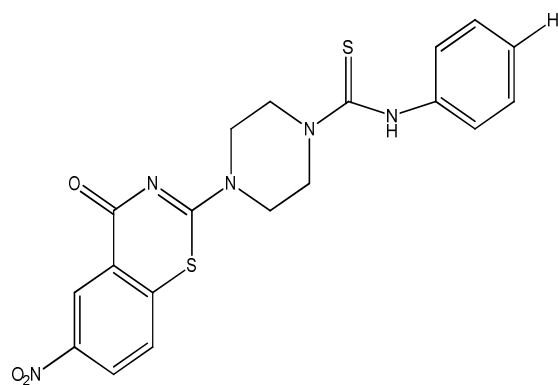
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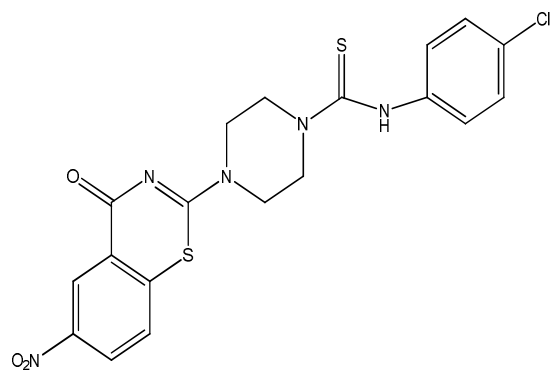


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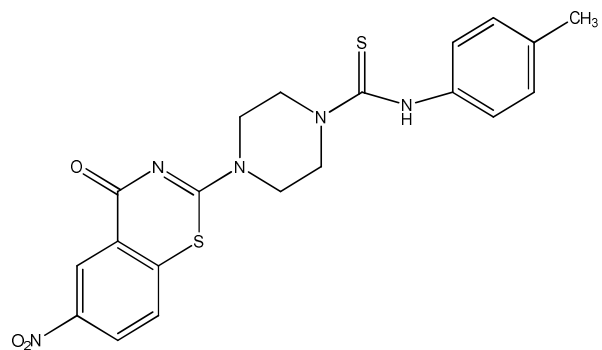


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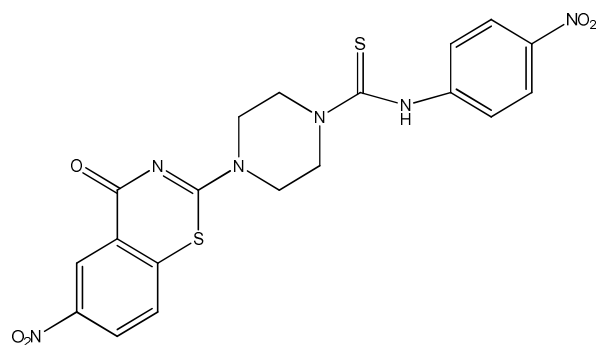
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15



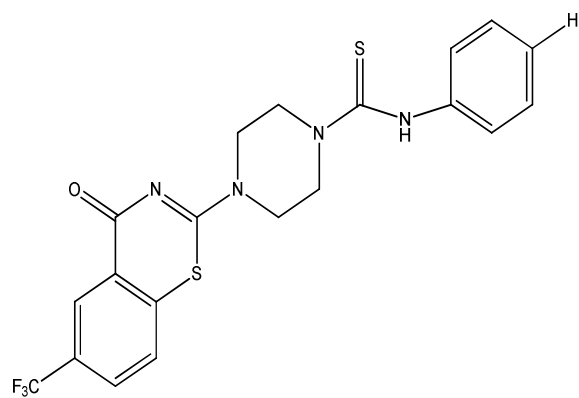
5.54

16



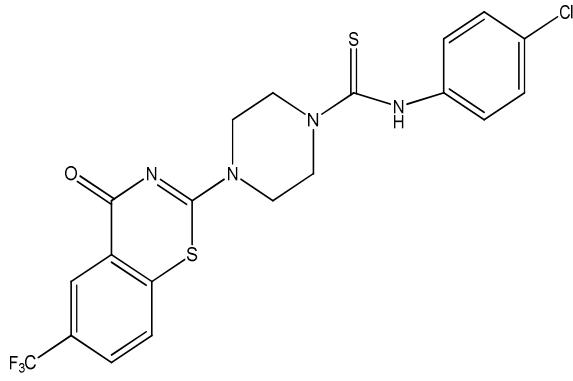
5.27

17



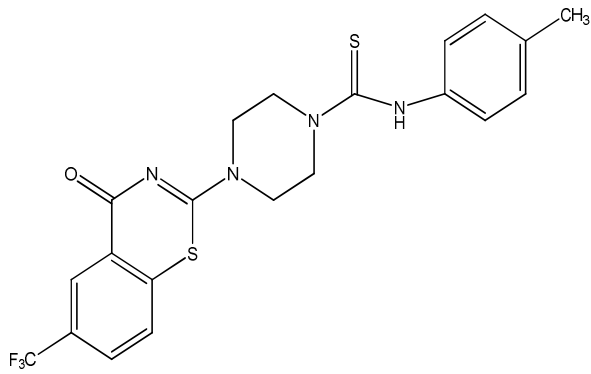
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18



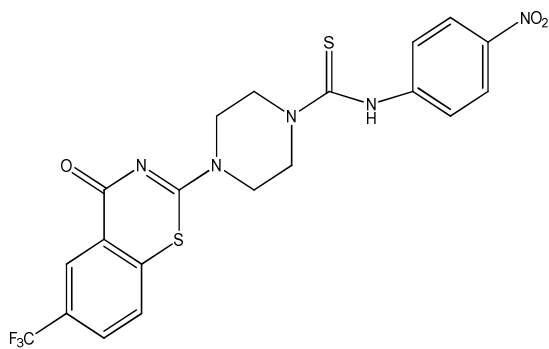
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19



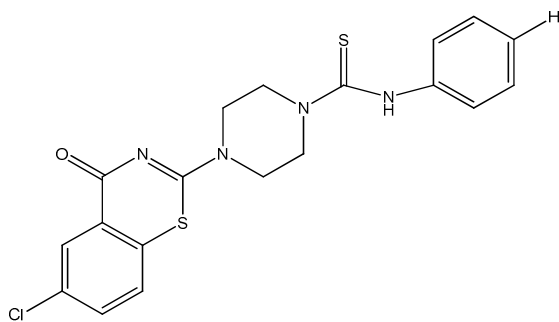
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20



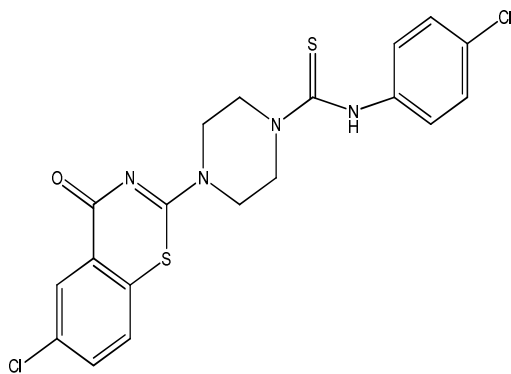
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21



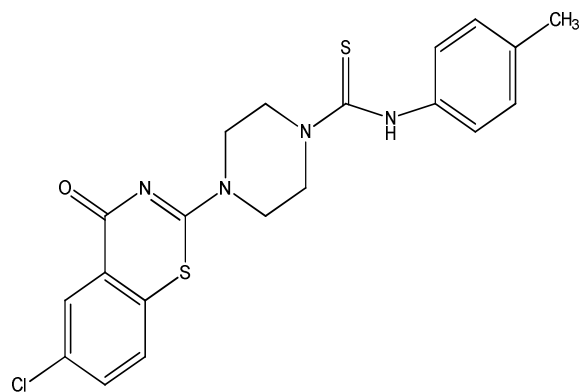
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22



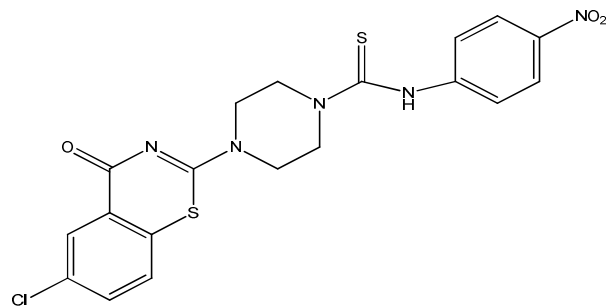
4.83

23



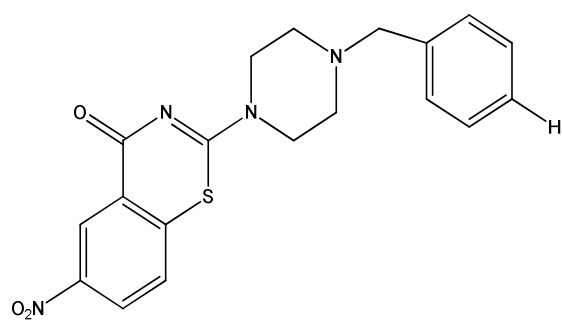
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24



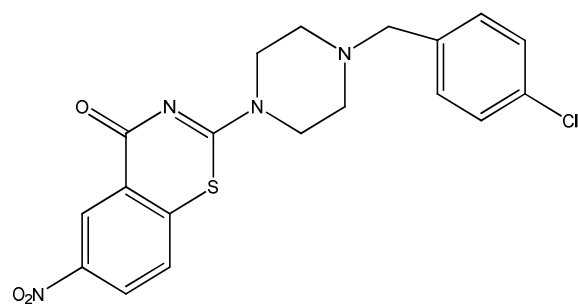
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25



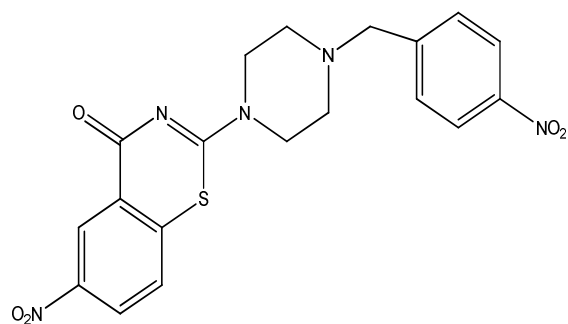
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26



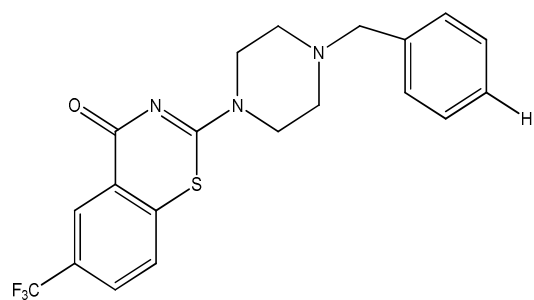
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27



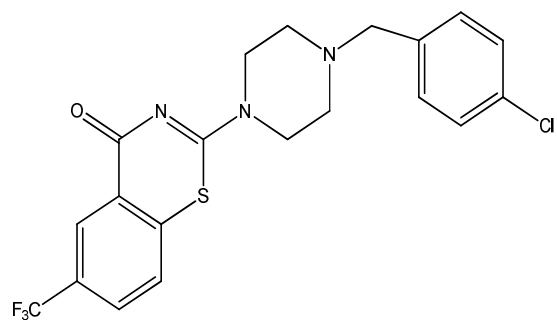
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28



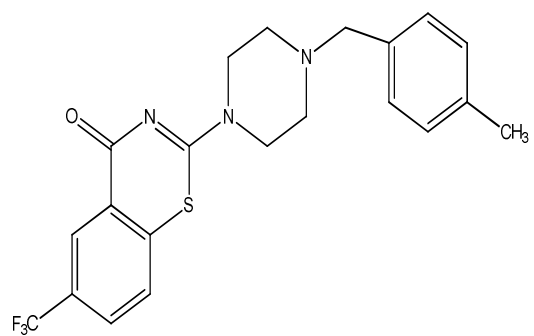
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29



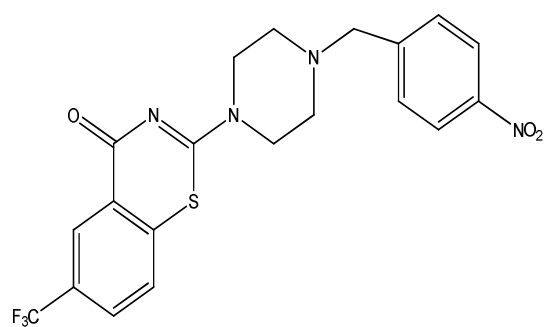
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30



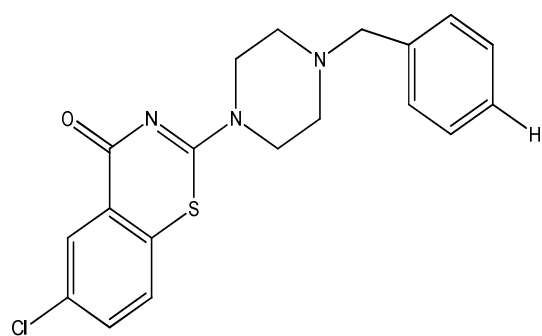
4.77

31



5.28

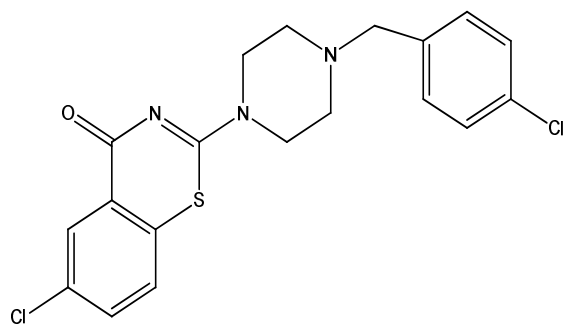
32



4.47

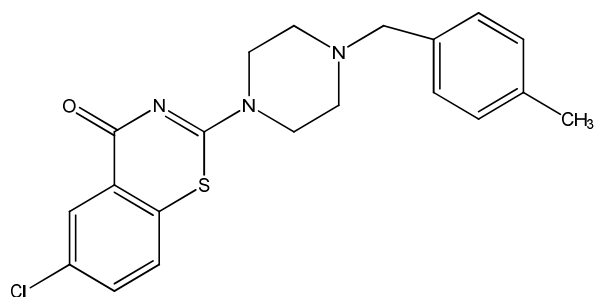
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4.81



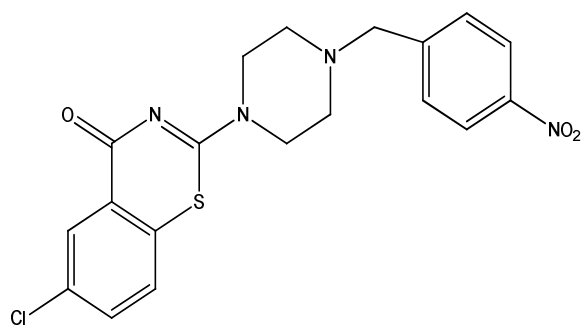
34

4.61



35

4.82



Where superscript **t** represent the test set