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STUDY OF BINDING AFFINITIES OF FabZ INHIBITORS: NAS-21 AND NAS-91 ANALOGUES BASED ON RECEPTOR - CENTRIC COMPUTATIONAL METHODS

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ABSTRACT

NAS21, NAS91 and its derivatives belong to class FabZ inhibitors have been focused to develop better anti-malarial drugs. Library of 17 analogues was designed from NAS21, NAS91 scaffold structure, and NAS75, NAS79 was considered for computational study. Their molecular interactions, binding affinities with FabZ was studied using receptor-centric approaches: glide docking, molecular mechanics using generalized Born/surface area solvation model and multi-ligand bimolecular association with energetic. Prediction models were developed between FabZ inhibition activity (pIC_{50}) of these compounds and molecular descriptors like glide score, binding energy and calculated free energy binding. The r^2 value varies from 0.66-0.77 indicating good data fit, and r^2_{cv} was within range of 0.64-0.76, indicating acceptable predictive capabilities of models. A linear correlation was observed between predicted and experimented pIC_{50} with r^2 = 0.66-0.78, suggesting the robustness of models. The ensemble-average free energy estimation including implicit solvation energy terms significantly improves the hit enrichment of virtual screening.

KEY WORDS: FabZ; NAS21; NAS91; Docking and scoring; MM-GB/SA; eMBrAcE.

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INTRODUCTION

Malaria is one of the most severe infectious diseases killing more than three million people each year. The most severe/fatal form of malaria is cerebral malaria, which is caused by *Plasmodium falciparum*. In spite of intense efforts to eradicate malaria, the current situation is very frustrating mostly due to the development of resistance in *P. falciparum* against the most effective and cheapest quinoline and antifolate drugs, which caused the resurgence of malaria in worldwide redoubling the malaria mortality rate[1]. The present grim condition advocates for the development of new and more efficacious antimalarial drug/vaccine against the present drug targets as well as against new targets of malaria. Several pathways unique to parasite have been identified that could culminate the development of innovative antimalarial chemotypes[2]. Recently "apicoplast" (non-photosynthetic plastid in malaria parasite) has drawn maximum attention of the researches[3], because the apicoplast is essential for the survival of *Plasmodium* and other apicomplexan parasites, it was speculated to be involved in various pathways. Further, sequencing of *P. falciparum* genome[4] coupled with detailed analysis of proteins of known function were targeted to apicoplast[5], which allowed the construction of an apicoplast-specific metabolic map[2]. The metabolic pathways predicted to take place in apicoplast are plastid housekeeping pathways (DNA replication, transcription and translation), heme biosynthesis, fatty acid biosynthesis and isoprenoid biosynthesis[2].

The intracellular malaria parasite requires lipids for growth and replication, specifically fatty acids for membrane biogenesis, which is necessary for invasive stage formation. It was assumed that *Plasmodium* lacks the ability to synthesize their own fatty acids, and thus rely on their hosts for lipid scavenging[6]. However, with the discovery of apicoplast, a relict plastid organelle of *Plasmodium*[7], this model came into question. One of the apicoplast pathways is bacterial-like type II fatty acid synthesis (FAS II)[8], which is a *de novo* pathway by which *Plasmodium* can synthesize fatty acids from derivatives of acetate and malonate. The synthesis of fatty acids occurs as iterative elongations of acyl chains utilizing the 2-carbon donor malonyl-CoA. The fatty acid chain extension step of FAS II is catalysed by four enzymes: FabB/F, FabG, FabI, FabZ; and the substrate/product of each reaction are covalently bound to the acyl carrier protein (ACP) cofactor. Deletion of ACP from *T. gondii* has demonstrated that apicoplast fatty acid synthesis is essential for organelle biogenesis and parasite survival in this parasite[9]. Out of the above potential targets, FabZ (β-Hydroxyacyl-acyl carrier protein dehydratase) was only considered in the present study as the receptor for studying the binding affinity of NAS analogues. Experimentally it has been proved that FabZ is utmost important for the survival of *P. falciparum*, and its activity was inhibited by NAS analogues[10]. A significant effort has been undertaken to develop blood stage FAS II inhibitors to treat malaria[11,12,13].

The drug molecule binds suitably with specific receptor through drug-receptor interactions to mediate its therapeutic effects. The nature of such interactions is very important and specifically the molecular docking method seems to be an appropriate tool for such analysis, because it provides information on how the chemical structure of drug should be modified to achieve suitable interactions, and subsequently for rapid prediction and virtual prescreening of anti-malarial activity. Availability of crystal structure of FabZ (Pdb ID: 1Z6B) of *P. falciparum* facilitates the understanding of structure-activity relationships of NAS21 (4,4,4-trifluoro-1-(4-nitrophenyl)butane-1,3-dione), NAS91 (4-chloro-2-[(5-chloroquinolin-8-yl)oxy]phenol), NAS75 (1-(4-methoxyphenyl)ethanone[(4-trifluoromethyl)pyrimidine-2-yl]hydrazone),

NAS79 (1-(4-methylphenyl)ethanone[(4-trifluoromethyl)pyrimidine-2-yl]hydrazone), and their derivatives. Of utmost importance in exploring the structure-activity relationships of the analogues is reliable filtering of the putative hits in terms of their predicted binding affinity (scoring problem), which is based on *in silico* generated near native protein-ligand configurations (docking problem). Most of the scoring functions used in docking programs are designed to predict binding affinity by evaluating the compound-receptor interaction. The ligand-receptor recognition is determined by enthalpy as well as entropic effects. The scoring functions have a simplified form of energy function to facilitate high throughput evaluation of a large number of compounds in single docking run. These functions may be problematic when used with contemporary docking programs, and can result in decrease of virtual screening accuracy. To overcome this problem, more precise, but time consuming computational methodologies are necessary. Therefore, attempts were made to evaluate several receptor-centric computational methodologies, which might be applicable in drug discovery using the first leads from a drug development program for 3R-hydroxymyristoyl ACP dehydrase (FabZ) inhibitors as the potent anti- malarial.

MATERIALS AND METHODS

(i) Preparation of the receptor:

The X-ray structure of FabZ (Pdb ID: 1Z6B) from *Plasmodium falciparum* has been used as initial structure in the preparation of NAS binding site. It is a hexameric proteins consists of 6 polypeptide chains. Hydrogen was added to the model automatically *via* the Maestro interface [Schrodinger, Inc.] leaving no lone pair and using an explicit all-atom model. All other water molecules were removed from the complex. The multi step Schrodinger's Protein preparation tool (PPrep) has been used for final preparation of protein. Prep neutralizes side chains that are not close to the binding cavity and do not participate in salt bridges [Schrodinger, Inc.]. This step is then followed by restrained minimization of co-crystallized complexes, which reorients side chain hydroxyl groups and alleviates potential steric clashes. Progressively weaker restraints (tethering force constants 3, 1, 0.3, 0.1) were applied to non-hydrogen atoms only. The complex structure was energy minimized using OPLS_2005 force field and the conjugate gradient algorithm, keeping all atoms except hydrogen fixed. The minimization was stopped either after 1000 steps or after the energy gradient converged below 0.01 kcal/mol. The energy-minimized receptor structures were subsequently used for docking of NAS21, NAS91, NAS75, NAS79 and their derivatives and structure based calculations.

(ii) Preparation of the ligands:

The inhibitory activity (IC_{50}) of all ligands used in the study was determined experimentally against FabZ isolated from *M. smegmatis*[14], which shares the identical binding site with *P. falciparum* FabZ (Figure 1).

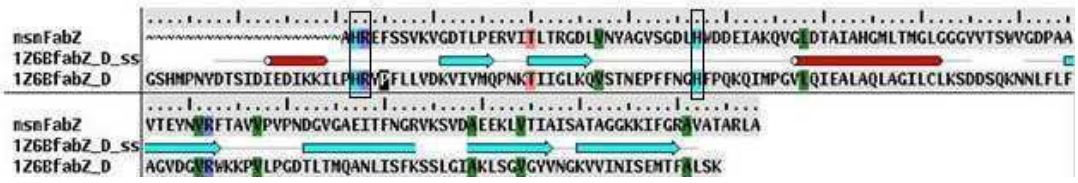
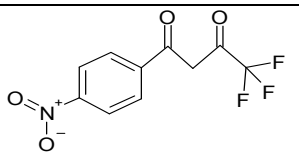
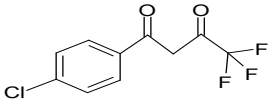
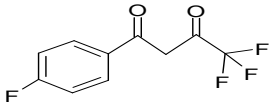
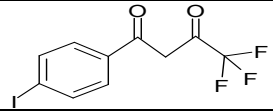
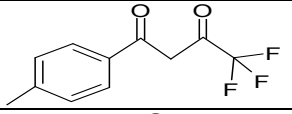
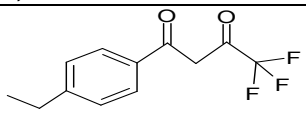
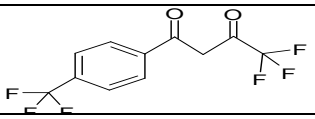
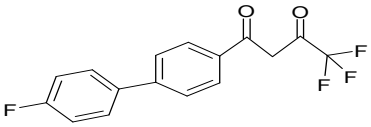
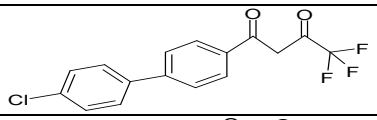
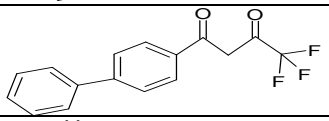
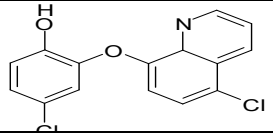
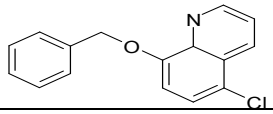


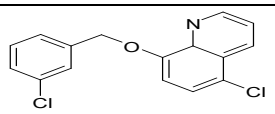
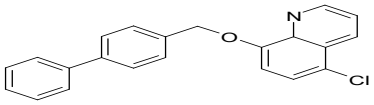
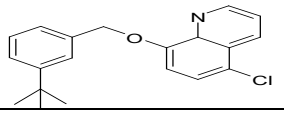
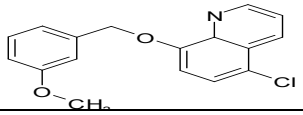
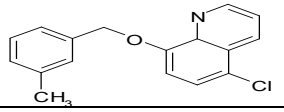
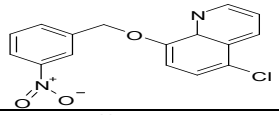
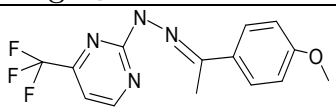
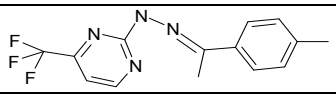
Figure 1

Alignment of P. falciparum FabZ sequence with M. smegmatis FabZ sequence showing perfect alignment of binding site residues.

All these analogues were built from the NAS21 and NAS91 templates by substitution of functional groups (Table 1).

Table 1
FabZ inhibitors used as set of ligands.

S No.	Label	Structure	*p*IC50 (µg/*ml*)
1	NAS-21		2.82
2	NAS-21_1		2.56
3	NAS-21_2		2.56
4	NAS-21_3		2.50
5	NAS-21_4		2.48
6	NAS-21_5		2.47
7	NAS-21_6		2.69
8	NAS-21_7		2.70
9	NAS-21_8		2.63
10	NAS-21_9		2.61
11	NAS-91		2.42
12	NAS-91_10		2.78

13	NAS-91_11		2.79
14	NAS-91_12		2.76
15	NAS-91_13		2.76
16	NAS-91_14		2.79
17	NAS-91_15		2.82
18	NAS-91_16		2.81
19	NAS-75		2.77
20	NAS-79		2.82

Maestro-molecular builder was used for building these analogues, and LigPrep was used for final preparation of ligands. LigPrep is a utility of Schrödinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation searching for tatutomers and steric isomers, and performing a geometry minimization of ligands. The ligands were energy minimized by molecular mechanics force fields (MMFFs) with default setting.

(iii) Glide docking protocol

All the ligands were docked to FabZ binding site using Glide version 4.0[15]. The binding site residues of FabZ includes His 133, His 98 and Arg 99, which are proved to be very essential for interaction with the NAS inhibitors using site directed mutagenesis studies[10]. After ensuring that both the proteins and ligands are in correct form for docking, the receptor-grid file was generated using grid receptor generation program. The default size was used for the bounding and enclosing boxes. The ligands were docked initially using the ‘‘standard precision’’ method and further refined using ‘‘extra precision’’ Glide algorithm. For the ligand docking stage, Vander Waals scaling of the ligand was set at 0.5. Out of the 50,000 poses that were sampled, 4000 were taken through minimization (conjugate gradients 1000) and the 30 structures having the lowest energy conformations were further evaluated for the favorable Glide docking score[16]. A single best conformation for each ligand was considered for further analysis.

(iv)Ligand and structure-based descriptors (LSBD) protocol

The eMBrAcE and Prime MM-GBSA calculations were performed using LSBD application of the Schrödinger software package (Schrödinger, LLC: Portland, OR). These calculations were applied on ligand-receptor complex structures obtained from Glide docking.

(v) Multi-ligand bimolecular association with energetics (eMBrAcE)

The eMBrAcE (MacroModel v9.1) program calculates binding energies between ligands and receptors using molecular mechanics energy minimization for docked conformations. eMBrAcE applies multiple minimizations, during which each of the specified pre-positioned ligands is minimized with the receptor. For the energy-minimized structures, the calculation is performed first on the receptor, then on the ligand and finally on the complex. The program calculates ligand-receptor interaction energies (ΔG_{ele}, ΔG_{vdW}), surface generalized Born solvation model (GBSA) for electrostatic part of solvation energy ($\Delta G_{solvation}$). The non-polar solvent-accessible surface area (SASA) of solvation energy was calculated using Qikprop program. The approach is simple, fast and straightforward. It benefits the calculation of relative binding affinity needed to evaluate the activity of large set of molecules in rational drug design. The total free energy of binding (FEB) is calculated using linear optimized multiple regression analysis as follows:

$$FEB = C + \alpha(\Delta G_{vdW}) + \beta(\Delta G_{ele}) + \gamma(\Delta G_{solv}) + \delta(SASA)$$

Where; α, β, γ and δ are the coefficients for vander Walls, electrostatic, solvation energy terms and SASA respectively; C is a constant.

(vi) Prime MM-GBSA

This application is used to predict the free binding energy between a receptor and a ligand by combining the OPLS molecular mechanics energies (E_{MM}), surface generalized Born solvation model for polar solvation (G_{SGB}), and a nonpolar solvation term (G_{NP}). The G_{NP} term comprises the non-polar solvent accessible surface area and Vander Waals interactions. The total free energy of binding is calculated as:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$
$$G = E_{MM} + G_{SGB} + G_{NP}$$

Prediction error sum of squares (PRESS) is a standard index to measure the accuracy of a modeling method based on the cross validation technique. The r^2_{cv} was calculated based on the PRESS and SSY (sum of squares of deviations of experimental values from their mean).

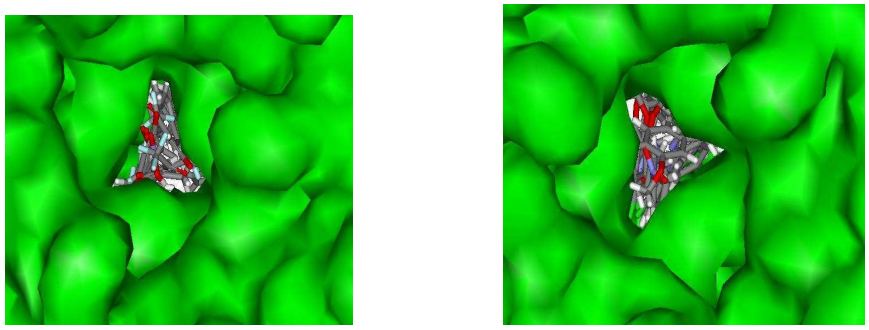
$$r^2_{cv} = 1 - \frac{PRESS}{SSY} = 1 - \frac{\sum_{i=1}^{n} (y_{exp} - y_{pred})^2}{\sum_{i=1}^{n} (y_{exp} - \bar{y})^2},$$

Where; y_{exp}, y_{pred} and y are the predicted, observed, and mean values of the relative activities of the artemisinin analogues. The cross validation analysis performed by using the leave one out (LOO) method in which one compound is removed from the data set and its activity is predicted using the model derived from the rest of the data points.

RESULTS AND DISCUSSION

One of the key challenges in computer-aided drug discovery is to maximize the capabilities of the method in use for predicting and rank-ordering the binding affinities of compounds for a given target protein. The efficiency of a

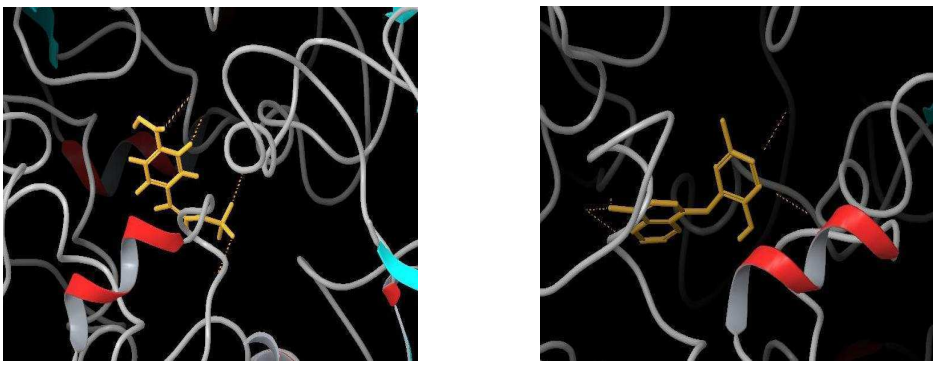
prediction method is predominantly determined by these capabilities. Various descriptors extracted from the structural information of ligand-receptor complex may provide an advantageous solution for creating a reliable binding-affinity-prediction model. The results obtained from a standard docking protocol combines the data from three different structure-based descriptors, and then investigated the utility of these descriptors on the prediction accuracy of the binding affinity of NAS21, NAS91, NAS75, NAS79 and their derivatives. Same of the docking protocol was used for docking of all the analogues with FabZ, which revealed good binder with FabZ. Reasonable binding modes for the analogues derived from NAS21 and NAS91 were generated by docking of the ligands into the binding site of FabZ. By visually checking the docking positions and orientations, it was observed that all these analogues from docking bind in the same orientation and at very similar positions (Figure 2a & 2b).



(a) **(b)**

Figure 2
Docking solutions of FabZ-inhibitors, superimposed in the binding pocket of receptor. (a) NAS21 inhibitor and its analogues; (b) NAS91 inhibitors and its analogues.

The results of a standard docking run of NAS21 and NAS91 inhibitor of FabZ along with their ligand-receptor interactions were shown in figure 3a & 3b.



(A) **(B)**

Fgure 3
Standard docking of best boses of (a) NAS21 inhibitor; (b) NAS91 inhibitor along with their receptor ligand interaction.

Since the experimental binding affinity of these analogues was known, it was feasible to develop the structure-activity relationship models, which in turn helps in designing more potent analogues against FabZ.

Building models for prediction of pIC_{50} using structure based approaches

Prediction models for prediction of inhibitory activity (pIC_{50}) of NAS21 and NAS91 analogues were developed based on the given set of compounds using Glide score and binding energies (ΔG_{bind}) as molecular descriptors. Besides, a scheme similar to linear response of energy (LRE) was adopted to develop the prediction model by optimizing the different energy terms. Results for the prediction models were represented in Table 2 and Table 3.

Table 2

Predicted pIC_{50} of NAS21 and NAS91 inhibitors using Glide score (XP) and Prime/MM-GBSA energy as a descriptor and experimental activity.

Ligand	Glide score	ΔG_{bind} (kcal/mol)	Expt. pIC_{50}	Pred.pIC_{50} (Gscore)	Pred.pIC_{50} (ΔG_{bind})
nas21	-9.35	-62.87	2.82	2.76	2.81
nas21_1	-6.71	-39.19	2.56	2.57	2.59
nas21_2	-7.79	-46.60	2.56	2.65	2.66
nas21_4	-6.84	-34.81	2.50	2.58	2.55
nas21_5	-5.94	-37.13	2.48	2.51	2.57
nas21_6	-5.91	-34.27	2.47	2.51	2.54
nas21_7	-9.89	-38.10	2.69	2.79	2.58
nas21_8	-9.23	-56.60	2.70	2.74	2.75
nas21_9	-9.00	-37.13	2.63	2.73	2.57
nas75	-5.85	-31.50	2.61	2.51	2.51
nas79	-5.51	-26.37	2.42	2.48	2.46
nas91	-8.04	-63.40	2.78	2.66	2.81
nas91_10	-10.07	-53.50	2.79	2.81	2.72
nas91_11	-7.79	-57.19	2.76	2.65	2.76
nas91_12	-8.28	-57.19	2.76	2.68	2.76
nas91_13	-8.17	-66.20	2.79	2.67	2.84
nas91_14	-10.00	-56.30	2.82	2.81	2.75
nas91_15	-9.17	-61.74	2.81	2.75	2.80
nas91_16	-9.53	-51.40	2.77	2.77	2.70

Table 3

Calculated Energies and estimated binding free energy (ΔG_{cald}) of NAS21 and NAS91 analogues.

Ligand	Expt. pIC_{50}	ΔG_{vdW} (kcal/mol)	ΔG_{ele} (kcal/mol)	ΔG_{solv} (kcal/mol)	SASA	*Pred. pIC_{50}
nas21	2.82	26.17	-120.86	-759.71	441.486	2.71
nas21_1	2.56	20.38	-102.61	-236.56	431.864	2.59
nas21_2	2.56	39.26	-65.37	-514.91	423.382	2.60
nas21_4	2.50	2.71	-87.25	-528.07	445.617	2.56
nas21_5	2.48	26.19	-58.29	-475.94	474.105	2.59

nas21_6	2.47	2.48	-83.37	-557.57	441.587	2.56
nas21_7	2.69	5.67	-85.24	-556.9	538.973	2.65
nas21_8	2.70	29.22	-104.62	-517.13	526.417	2.73
nas21_9	2.63	16.7	-98.7	-570.23	502.426	2.68
nas75	2.61	8.47	-120.62	-157.07	572.85	2.70
nas79	2.42	6.87	-86.97	-15.32	529.966	2.58
nas91	2.78	26.35	-124.77	-19.41	520.962	2.69
nas91_10	2.79	19.71	-129.18	-93.56	498.015	2.68
nas91_11	2.76	20.63	-138.75	-83.66	498.03	2.69
nas91_12	2.76	19.45	-141.23	-91.88	579.225	2.76
nas91_13	2.79	38.43	-122.39	-124.7	547.526	2.76
nas91_14	2.82	41.53	-139.26	-105.54	532.48	2.78
nas91_15	2.81	20.45	-139.52	-68.06	526.69	2.71
nas91_16	2.77	-4.1	-126.88	-607.98	560.905	2.7

The quality of fit between the docking score and ΔG_{bind} with the experimentally determined affinity data was shown in Figure 4 and 5 respectively.

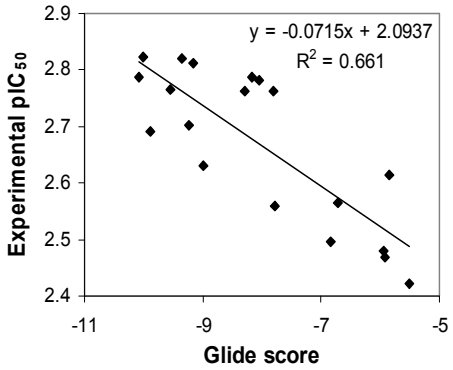


Figure 4
Model for predicting pIC_{50} of the NAS21 and NAS91 analogues based on Glide score.

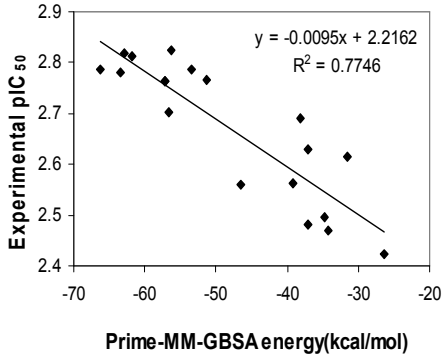


Figure 5
Model for predicting pIC_{50} of the NAS21 and NAS91 analogues based on Prime/MM-GBSA energy (ΔG_{bind}).

The r^2 value for the prediction model was found to be 0.66 and 0.77, whereas the r^2_{cv} value was 0.64 and 0.76 respectively using Glide score and ΔG_{bind} as molecular descriptors. The prediction equations of the models and the corresponding statistics were given below:

$$pIC_{50} = 2.09\ (\pm 0.1018) - 0.0715\ (\pm 0.01242)^*\ \text{G-score} \quad (1)$$
$$(N = 20,\ r^2 = 0.66,\ s = 0.08100,\ F = 33.15,\ r^2_{cv} = 0.64,\ PRESS = 0.135831)$$
$$pIC_{50} = 2.22(\pm\ 0.06128) - 0.00946(\pm 0.001238)^*\ \Delta G_{bind} \quad (2)$$
$$(N = 20,\ r^2 = 0.77,\ s = 0.06605,\ F = 58.42,\ r^2_{cv} = 0.76,\ PRESS = 0.091747)$$

One docking structure from each molecule docking result was picked up as final docked structure in the protein and was imported into eMBrAcE for further calculations. As the Glide treats a receptor rigidly during docking simulation, an energy minimization was performed to the docked complex. A vdW energy and electrostatic energy between ligand and receptor as well as solvation energy were calculated for each minimized complex. Also solvent accessible surface area (SASA) change was calculated using Qikprop. A scheme similar to Linear Response was used to develop a free energy of binding (FEB) relationship based on these energies, which can express the activity of these analogues. A multiple regression was performed using Minitab statistical package. The energy components and activity (pIC_{50}) of these analogues were listed in Table 3. The calculated activity has good correlation to the actual activity. Although these energy components were added directly together in most of these applications, it was still a challenge to apply these methods into large set of ligands. Normally, these different energy components (vdW, electrostatic, solvation) were calculated using more than one method. To same set of structure, different force field or different methods will produce different values of energy. This suggested that these energy components need to be scaled before an equation was obtained to get a better expression for these energy components. A set of weights can be used to scale these energies to get free energy expression by linearly combining these energies. Some scoring functions[17] used these strategies, which were optimized using a test set of molecules. In the work, a linear combination strategy was used to express FEB by four energy components calculated from different methods. An expression of free energy, whose weight coefficients were optimized by a multiple regression was obtained and successfully predicted the activity of ligands. The equation of the model for calculation of pIC_{50} and the corresponding statistics were represented below:

$$pIC_{50} = 1.77 + 0.00390^*\Delta GvdW - 0.00473^*\Delta Gele\ -0.000223^*\Delta Gsolv\ 0.000478^*SASA \quad (3)$$
$$(N = 20,\ r^2 = 0.87,\ s = 0.054,\ F = 25.14,\ r^2_{cv} = 0.84,\ PRESS = 0.068)$$

The statistical significance of various prediction models developed in this study were evaluated by the correlation coefficient r^2, standard errors, F-test value, leave-one-out cross-validation coefficient r^2_{cv} and predictive error sum of squares (PRESS). The regression models developed in this study were statistically best fitted, and can be consequently used for prediction of pIC_{50} of the NAS21 and NAS91 analogues as reported in Table 2 and Table 3. The quality of fit between the predicted pIC_{50} of the NAS21 and NAS91 analogues based on FEB with the experimentally determined affinity data was shown in Figure 6.

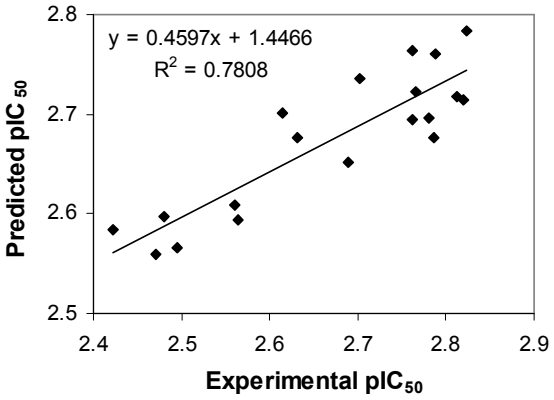


Figure 6
Model for predicting pIC_{50} of the NAS21 and NAS91 analogues based on FEB.

The root mean square error (RMSE) between the experimental pIC_{50} values and the predicted pIC_{50} were within the range between 0.11- 0.10, which was an indicator of the robustness of the fit and suggested that the calculated pIC_{50} based on above structure based approaches were reliable.

CONCLUSION

Attempts were made in the present study to introduce several advanced computer-aided drug discovery methodologies for receptor-centric virtual screening. The magnitude of the binding affinity can be a key factor that decides the activeness of an individual inhibitor. An energetic evaluation of the binding affinity will provide a way to estimate the activity of inhibitors. In any binding energy calculation, the correct binding structure of each ligand has to be determined first prior to binding energy estimation. The binding structures of FabZ with NAS21 and NAS91 are not available. So, flexible docking was used to determine the binding structure of the NAS21 and NAS91 analogues with FabZ. The calculated docking scores and binding free energy value of a set of structural analogues demonstrates excellent linear correlation to the experimental activity. These models could be useful to predict the range of activities for new NAS21 and NAS91 analogues. The information that we have expressed in this study may lead to the designing (synthesis) of more potent NAS21 and NAS91 derivatives for inhibition of both FabZ. Although the current study does not involve a large number of test set compounds, but the evaluation data should add valuable information that may enhance the practice of computerized drug discovery.

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