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STUDY OF HALOGEN SUBSTITUENT ON DOCKING AND 3D QSAR PROPERTIES OF ARYL SUBSTITUTED THIOSEMICARBAZONES AS ANTICONVULSANT

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ABSTRACT

Gamma amino butyric acid (GABA) is a major inhibitory neurotransmitter mainly responsible for action of almost all antiepileptic compounds, Gamma amino butyrate amine transferase (GABA-AT) is one of the most important targets in the design and discovery of successful anti-epileptics. Lysine 329A and Arginine 192A are the most widely used residues of GABA-AT for docking studies. In the present paper, twenty one different aryl substituted thiosemicarbazones (PS_1-PS_{21}) were studied for interaction with Lys-329A & Arg-192A residues of GABA-AT. Docking studies were carried out using Pyridoxal phosphate (PLP) based method by 'Vlife Molecular Design Suite 3.5' software. Grip at docking method, in which both 1OHV and ligand are flexible, was adopted and the results were compared with standard anticonvulsant drug vigabatrin.

In addition, all twenty one molecules were subjected to three dimensional quantitative structure activity relationship (3D QSAR) using Principal Component Regression method (PCR) to design potent anticonvulsant prior to their synthesis. The model gave r^2 & q^2 values of 0.9587 and 0.9327 respectively for forteen compounds in training and seven compounds of test compounds with optimum number of components as 2.

The model was found to be highly predictive and was further applied to set of ten molecules of aryl substituted thiosemicarbazones. Results of docking studies and 3D QSAR studies have shown that halogen substitution in phenyl ring at Meta position plays important role in protection from seizures. Bromo substituent was found to be more effective as compared to chloro and fluoro substituents.

Thus, it would be worthwhile to synthesize bromo substituted aryl thiosemicarbazones and evaluate their anticonvulsant activity.

Keywords: Anticonvulsant, Docking, 3D QSAR, r^2, q^2, cross validation, GABA

INTRODUCTION

1.1 Docking

Docking based drug design by use of structural biology remains one of the most logical and pleasing approaches in drug discovery. The structured knowledge of the binding compatibilities of the active site residues to specific groups as the agonist and antagonist lead to proposals for synthesis of very specific agents with a high probability of

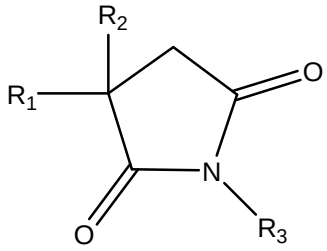

Fig. 1: Fragment 1

biological action[1]. In recent years virtual screening has become important part of modern drug discovery[2]. Two of the most commonly used methodologies in structure based CADD are docking and molecular mechanism[3]. Molecular docking is a very popular to investigate molecular association

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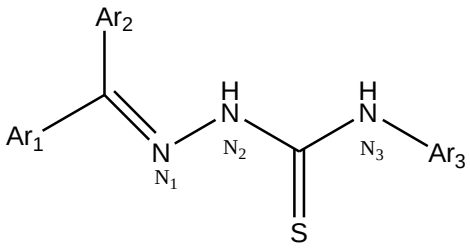
and is particularly useful in the drug discovery arena to study the binding of small molecules (ligands) to macromolecules (receptors)[4]. This involves two key components namely the search algorithm and scoring function; former positions the molecules in orientation and conformation within the active site while latter are determining if orientation chosen by the search algorithm is most energetically favorable[5]. Many algorithm share common methodology with novel extensions and diversity in both their complexity and computational speed provide a base of technique to tackle structure based drug design problems[6] . A vigorous search algorithm exhaustably elucidate all possible binding possible solution via genetic operators to final population, optimizing a predefined fitness function[7].

Vlife MDS 3.5 uses genetic algorithm as a global optimizer combined with energy minimization as a local search method. Three options for docking are available

1. Rigid docking – when a suitable position for ligand in the receptor environment is obtained while maintaining it's rigidity
2. Flexible docking – where a favored geometry for the receptor-ligand interaction is obtained by changing internal torsions of ligand into the

TABLE 1 Chemical structure and ClogP data of substituted aryl thiosemicarbazones



Comp. No.	Ar_1	Ar_2	Ar_3	Mol. formula	Mol. wt	ClogP
PS_1	4-bromophenyl	H	4-chlorophenyl	$C_{14}H_{11}BrClN_3S$	368.68	5.536
PS_2	4-bromophenyl	H	4-nitrophenyl	$C_{14}H_{11}BrN_4O_2S$	379.23	4.566
PS_3	4-bromophenyl	H	4-methoxyphenyl	$C_{15}H_{14}BrN_3OS$	364.26	4.742
PS_4	4-bromophenyl	H	4-fluorophenyl	$C_{14}H_{11}BrFN_3S$	352.22	4.966
PS_5	2-bromophenyl	H	4-chlorophenyl	$C_{14}H_{11}BrClN_3S$	368.68	5.536
PS_6	3-bromophenyl	H	4-chlorophenyl	$C_{14}H_{11}BrClN_3S$	368.68	5.536
PS_7	4-chlorophenyl	H	4-nitrophenyl	$C_{14}H_{11}ClN_4O_2S$	334.78	4.416
PS_8	4-chlorophenyl	H	4-chlorophenyl	$C_{14}H_{11}Cl_2N_3S$	324.23	5.386
PS_9	4-chlorophenyl	H	Phenyl	$C_{14}H_{12}ClN_3S$	289.78	4.673
PS_{10}	4-chlorophenyl	H	4-methoxyphenyl	$C_{15}H_{14}ClN_3OS$	319.81	4.592
PS_{11}	4-chlorophenyl	H	4-fluorophenyl	$C_{14}H_{11}ClFN_3S$	307.77	4.816
PS_{12}	Phenyl	Phenyl	4-fluorophenyl	$C_{20}H_{16}FN_3S$	349.42	5.543
PS_{13}	Phenyl	Phenyl	4-nitrophenyl	$C_{20}H_{16}N_4O_2S$	376.43	5.143
PS_{14}	Phenyl	Phenyl	4-methoxyphenyl	$C_{21}H_{19}N_3OS$	361.46	5.319
PS_{15}	Phenyl	Phenyl	4-chlorophenyl	$C_{20}H_{16}ClN_3S$	365.88	6.113
PS_{16}	Phenyl	Phenyl	Phenyl	$C_{20}H_{17}N_3S$	331.43	5.4
PS_{17}	Phenyl	Methyl	4-fluorophenyl	$C_{15}H_{14}FN_3S$	287.36	4.214
PS_{18}	Phenyl	Methyl	4-nitrophenyl	$C_{15}H_{14}N_4O_2S$	314.36	3.814
PS_{19}	Phenyl	Methyl	4-methoxyphenyl	$C_{16}H_{17}N_3OS$	299.39	3.99
PS_{20}	Phenyl	Methyl	4-chlorophenyl	$C_{15}H_{14}ClN_3S$	303.81	4.784
PS_{21}	phenyl	Methyl	phenyl	$C_{15}H_{15}N_3S$	269.36	4.071

notes between ligand and receptor since inception, the genetic algorithm has increased in popularity as an optimization tool. The essence of genetic algorithm in the evolution of the population of

active site while receptor remain fixed

3. Full flexible docking – where the ligand is positioned via its torsional angles as well as the side chain of active residues are flexed.

Gama amino butyric acid (GABA) is a predominant inhibitory neurotransmitter in mammalian CNS modulating central inhibitory tone wise activation of inotropic $GABA_A$ and $GABA_C$ receptor and G-protein coupled $GABA_B$ receptor[8,9]. Gama amino butyrate amino transferase ($GABA_{AT}$) catalyses degradation of GABA to succinylic semi-aldehyde. Depleted levels of GABA have been shown to cause convulsions[10]. Raising GABA levels in brain has an anticonvulsant effect[11]. $GABA_{AT}$ is a validated target for anti-epileptic drugs because of its selective inhibition raises GABA concentration in brain[12]. Numerous strategies exist to elevate GABA levels in the brain. The strategy that we have taken involves inhibition or inactivation of $GABA_{AT}$[13, 14,15,16,17]. GABA itself is not an active anti-convulsant agent, since it does not blood brain barrier[18]. Various semicarbazones, thio-semicarbazones and relative compounds have been found to exhibit a wide range of biological activities including anti-convulsant activities[19]. Numerous semicarbazones and thiosemicarbazones, hydrazones, and amides have been found to possess anti-convulsant activity[20, 21, 22]. Many anticonvulsant agents contain an amide, imide and urea subunit and fragment 1 (Fig.**1**), which is chemically similar to semicarbazones and thio-semicarbazones moieties. The mode of action of semicarbazones and thiosemicarbazones has not yet established considering structural features of

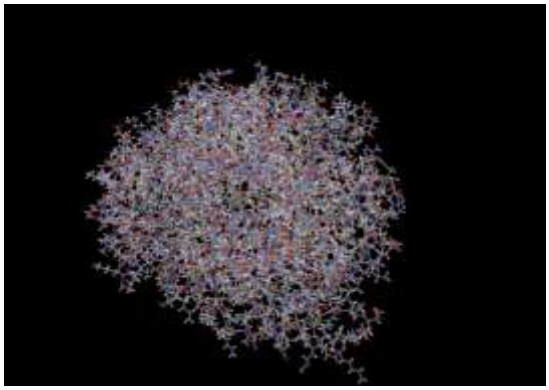


Fig. 2: Crystal structure of 1OHV

$GABA_{AT}$ receptors, semicarbazones and thiosemicarbazones are proposed to interact with active residues i.e. Lysine 329A and Arginine 192A. In view of these features a series of halogen substituted aryl thiosemicarbazones has been studied for its interaction with $GABA_{AT}$ active residues and based on it a 3D QSAR model has been developed for evaluation of anticonvulsant activity.

1.2 3D QSAR

This is a module for generation of 3D QSAR equation using various statistical regression methods. The facilities for molecular alignment and generation of steric and electrostatic interactive energies are also provided. This module helps in designing the novel ligands, based on generated QSAR model. It consist of following steps –

1. Building of 3D QSAR equation using following statistical method with stepwise genetic algorithm and simulated annealing method for variable selection.
   - Multiple regression
   - Partial least square regression
   - Principal component regression
2. Capturing non linear relationship using artificial intelligence method
   - Neural network (Back propagation and pruning neural network)
3. Building 3D QSAR equation using K nearest neighbor principle (kNN) with stepwise, genetic algorithm and simulated annealing methods for variable selection. This method allows generation of automated QSAR models that optimize k values with stepwise, genetic algorithm and simulated annealing methods.
4. Pattern recognition of descriptor via different graphs in worksheet
   - Pattern plots
   - X-Y plots
   - Fitness plot

MATERIALS AND METHODS

2.1 Docking

$GABA_{AT}$ receptor modeling: The receptor model was build by using VLife MDS 3.5 software running on Microsoft window XP. It consists of several steps.

1. The 3D crystal structure of $GABA_{AT}$ (PDB code 1OHV, Fig. **2**)[23, 24, 25] was downloaded from Brookhaven protein data bank (PDB, http://www.rcsb.org/pdb) and loaded to Biopredicta tools of VLife MDS. The non-bonded oxygen atoms of waters present in the crystal structure were removed. After assigning bond order, missing H atoms were added and partial atomic charge was calculated using MMFF (Merck molecular force field). The receptor file was converted to .mol2 file. $GABA_{AT}$ has been observed as a tetramer containing 4 chains A, B, C, and D. Lysine and Arginine are reported to be

active residues present in chain A (LYS 329A & ARG 192A). Chain B, C and D were then removed from the receptor by Biopredicta tools. The incomplete residues were corrected by mutations. The co-crystal ligand i.e. pyridoxal phosphate (PLP) was extracted and the receptor file was saved as .mol2 file. In the similar way, structures of all 21 ligand molecules (PS_1 to PS_{21}) were drawn in 2D format and then were imported to VLife MDS 3.5. All structures were optimized by using MMFF force field and saved as .mol2 file.

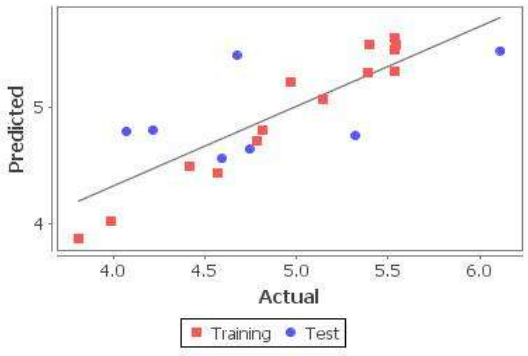


Fig. 4: Fitness plot for training and test set substituted aryl thiosemicarbazones

2. Grip docking was performed between receptor molecule and each and every ligand molecule and the binding energies of 10 different poses were noted, considering PLP as a reference ligand. Details of binding energy data (kcal/mole) are given in Table **2**.

Hydrogen bonding interactions, hydrophobic interactions, Ionic bonding, π stacking interactions were studied for the most favourable pose and receptor molecule active residues. Details are given in Table **2**.

2.2 3D QSAR

3D QSAR were performed using VLife 3D QSAR (VLife, 2002) installed on Core 2 Duo workstation. 3D structures were drawn for each molecule and molecular geometry optimized using Monte Carlo conformational search[26]. Merck molecular force field MMFF and charges. Monte Carlo simulation explores the conformational search scale of molecule using random moves are accepted such that a different region of search space is sampled at each step. The most commonly used output form a conformational search in the set of torsion angle values, each of which produces a visible conformation, this together with the definition of all rotatable bonds and the starting coordinates for the molecules allow all valid conformation to be generated. In all its uses the metropolis condition and RMS deviation to accept or discard generated conformers, dielectric properties were keeping constant at 1.0 using distance dependent functions. Optimized molecule were aligned by template based method (Fig. **3**) using the most active molecule, PS_{15} and PS_{22} as a template.

2.2.1 Descriptor calculation

A common rectangular grid around molecule was generated. The steric and electrostatic energies were computed at lattice points of the grid using the methyl probe using charge +1. The term descriptor is utilized to indicate field value at lattice point 2080, 2080. The 3D descriptors were calculated setting charge type as Gasteiger-Marseli (GM) and dielectric constant value at 1.0. The descriptors with no variation in values were rejected. Descriptor with constant values will not contribute to QSAR, selection of training and test set and variable selection and model building

Optimal training and test set was generated using RS algorithm. A training set of 14 molecules were generated. Forward method with PCR variable selection method was employed for selection of

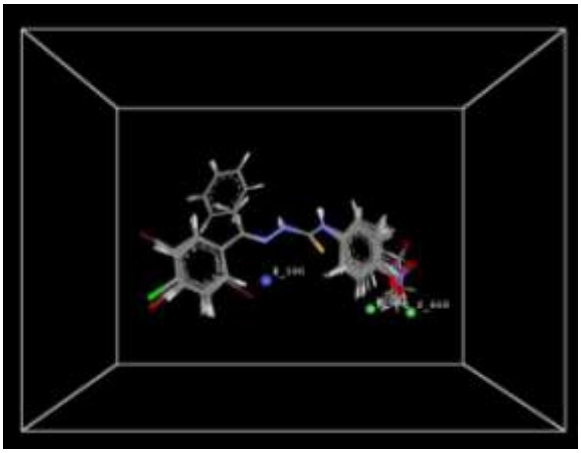


Fig. 5: Relative position of steric and electronic field around aligned molecules

variable to obtain QSAR model. The step by step procedure begins by developing a trial model with single independent variable and adds independent variable, one step a time examining the fit of the model at each step, the method continues until

there are no more significant variables remaining outside the model.

2.2.2 *Cross validation*

The standard leave-one-out (LOO) procedure was implemented that is a molecule in the training set was eliminated and its biological activity was predicted as the weighted average activity of the k most similar molecules (eq. 1)

$$\hat{Y}_i = £\ wiy_i \qquad (i)$$

The similarities were evaluated as the inverse of Euclidean distance between molecules (eq. 2) using only the subset of descriptors

$$d_{ij} = [\sum_{k=1}^{vn} (x_{i,k} - x_{j,k})]^{1/2}$$

This step was repeated until every molecule in the training set has been eliminated and its activity predicted once. The cross validated r^2 (q^2) values was calculated using equation 3 where y_i and $\hat{y}_i$ are actual and predicted of the i^{th} molecule, respectively and y_{mean} is the mean of observed activity of all molecule in the training set

$$q^2 = 1 - \Sigma (y_i - \hat{y}_i)^2 / \Sigma (y_i - y_{mean})^2$$

Since the calculation of the pairwise molecules similarities enhance the prediction was based upon current training set, the q^2 values obtained (0.9327) is the indicative power of the PCR model.

2.2.3 *The external validation*

The external validation i.e. predicted r^2(pred_r^2) value was calculated using the following equation , where y_i and y_i are the actual and predicted ClogP values of the ith molecules in the test set respectively and y_{mean} is the mean of observed ClogP values of all molecules in the training set

TABLE 2: Binding energy and interaction data of substituted aryl thiosemicarbazones

Comp. No.	Binding energy (Kcal/mol)	Interactions	interactions
		LYS 329A (hydrogen bond)	ARG 192A (hydrophobic)
PS_1	-63.282293	___	___
PS_2	-69.063951	___	___
PS_3	-68.097051	✓	___
PS_4	-64.831334	___	___
PS_5	-75.449313	✓	___
PS_6	-71.038563	___	___
PS_7	-66.484923	✓	___
PS_8	-59.590290	___	___
PS_9	-76.250782	___	___
PS_{10}	-63.825758	___	___
PS_{11}	-66.600334	___	___
PS_{12}	-83.081955	___	___
PS_{13}	-64.927986	___	___
PS_{14}	-74.079470	___	___
PS_{15}	-73.802302	___	___
PS_{16}	-86.156093	___	___
PS_{17}	-79.148334	___	✓
PS_{18}	-87.615591	___	✓
PS_{19}	-63.359368	___	___
PS_{20}	-78.458768	___	✓
PS_{21}	-84.087620	___	___
Vigabatrin	-45.996000	✓	___
Pyridoxal phosphate	-76.287683	___	✓

___ indicates no interaction.

$$Pred\_r^2 = 1 - \Sigma (y_i - \hat{y}_i)^2 / \Sigma (y_i - y_{mean})^2$$

Both the simulations are overall molecules in the test set, the pred_r^2 value is indicative of all the predicted power of current PCR model for external test set. For the given test set, this value was found to be 0.9587. No. of maximum and minimum neighbor were 5 and 2 respectively as a result steric descriptor S_595 (-0.0273105), S_668 (-0.00952076) and electrostatic descriptor E_390 (-0.0797175) were used to build the model and to generate the equation. The relative position of steric field and the electrostatic field around aligned molecules, these are important for ClogP variations in the model shown by the Fig. **4.**

2.2.4 ***Evaluation of model***

The statistical significance of model was evaluated one tail hypothesis[27]. The robustness of the QSAR model for experimental training set was examined by comparing. This model to those derived from random data set.

The QSAR model was evaluated only the following statistical measurements, no. of observation i.e. molecules in data set (n = 21); number of descriptors (V= 3); cross validated r^2 (q^2 = 0.9327); predicted r^2 for the external test set (pred_r^2 = 0.9587); standard error (SE=0.605) (Fig. **5**).

RESULTS AND DISCUSSION

All chemical structures of 21 compounds (PS1-PS21) were drawn with chemdraw ultra8.0 software and their ClogP values were also calculated. All these molecules were subjected to docking and interaction studies with Vlife MDS 3.5 software.

2.1 Docking studies:

Docking studies were performed on $GABA_{AT}$ receptor (PDB code 1 OHV) and interaction studies were carried out with active residues of $GABA_{AT}$ i.e. Lysine 329A and Arginine 192A. Pyridoxal phosphate (PLP) based grip docking was performed and the results were compared with vigabatrin. All 21 molecules showed better binding energies than vigabatrin (-45.31 Kcal/mole) while 6 compounds namely PS_{12}, PS_{16}, PS_{17}, PS_{18}, PS_{20}, PS_{21} showed better binding energies than the co-crystal ligand PLP (-76.2876 Kcal/mole). The binding interactions with LYS 329A and ARG 192A were carried out with respect to hydrogen bonding and hydrophobic interactions. 3 molecules namely PS_3, PS_5 and PS_7 showed hydrogen bonding with LYS 329A, while 3 molecules namely PS_{17}, PS_{18} and PS_{20} displayed hydrophobic interactions with ARG 192A. Hydrogen bonding and hydrophobic interactions were studied only since these two interactions are supposed to contribute in majority to drug action.

3.2 3D-QSAR studies:

3D-QSAR studies were carried out using 3D QSAR module of VLife MDS software, considering chemical structures as one variable and ClogP value as another variable. The results obtained have shown the q^2 and r^2 value of (0.9327 and 0.9587, Fig. 4) with 14 compounds in training set and 7 compounds in test set. The model was obtained with Random selection method with PCR (Principal component regression) method of analysis. Values of q^2 and r^2 have indicated that the model is highly predictive in nature. The model has shown 3 descriptors (2 steric and 1 electronic) contributing to hydrophobicity namely S_595, S_668 and E_390. Here steric descriptors have been found to play major role as compared to electronic descriptor. The steric descriptor has been contributed by presence of aryl ring at both ends of molecules where as the electronic descriptor has been contributed by thiosemicarbazone pharmacophore. The 'show point' result displayed 2 steric descriptors namely S_595 and S_668 near the aryl ring Ar_3. Most importantly various substituents on para position of phenyl ring contributed to the hydrophobicity, while the electronic descriptor i.e E_390 has been displayed near N_3 i.e N_3 electronegativity has also contributed to the activity.

Thus it would be better to synthesize thiosemicarbazones having para substituted phenyl ring with unsubstituted N_3.

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