



Research Article

QSAR MODEL FOR IDENTIFY NEW ACTIVE MOLECULE AGAINST HUMAN PHASPHOLIPASE A₂ ENZYME

Irfan N, Jothilingam. S, Ramesh Kumar. N,
Department of pharmaceutical chemistry, C I baid Metha College of pharmacy,
Jyothi nagar, Thorapakkam
Chennai-96, TamilNadu, India.

(Received: 12 Dec, 2011; Accepted: 15 March, 2012; Published: 29 April, 2012)

*Corresponding author's email: nirfanahamed1985@live.com

ABSTRACT

The present research work deals with identification of biologically active molecule against human phospholipase A₂ enzyme with the help of QSAR model. Phospholipase A₂ is an enzyme which hydrolyzes the sn-2 position of certain cellular phospholipids. The liberated lysophospholipid and arachidonic acid are precursors in the biosynthesis of various biologically active products. As human pancreatic PLA₂ is present high level in the blood of patients are the responsible for several pathological conditions like septic shock, pancreatitis, trauma, bronchial asthma, gout and other diseases. The potent PLA₂ inhibitors have been suggested to be useful drugs. This QSAR model created based on the 23 physicochemical parameters of 79 indene and indole molecules. The genetic function approximation (GFA) method performs a search over the space of possible QSAR models using lack of fit (LOF) scores to estimate the fitness of each model. These models lead to the discovery of predictive QSAR equations. The correlation coefficient value of this equation was found to be 0.968. With this equation we can easily find the novel biologically active compound against several pathological conditions.

Key words: QSAR model, Phospholipase A₂, Anti-inflammatory

INTRODUCTION

Phospholipase A₂ is an enzyme which hydrolyzes the sn-2 position of certain cellular phospholipids. The liberated Lysophospholipid and Arachidonic acid are precursors in the biosynthesis of various biologically active products. As

human pancreatic PLA₂ is present high level in the blood of patients are responsible for several pathological conditions. The potent PLA₂ inhibitors have been suggested to be useful drugs.

The present study was to create QSAR model based on 23 physicochemical parameters of 79 indene and indole molecules. This model will help the medicinal chemist to target efforts on Analogues which should have improved activity and thus cut down the number of Analogues which have to be made. If an analogue is discovered which does not fit the equation, it implies that some other feature is important and provides a lead for further development.

MATERIALS AND METHODS

Present experimental studies carried out using the tools

Accelrys software

Discovery studio is a complete modeling and simulations environment for life science researchers. Discovery Studio is a single, easy-to-use, graphical interface for powerful drug design and protein modeling research. Discovery Studio 2.1 combines established gold-standard applications such as Catalyst, Modeler, and CHARMM that have years of proven results and utilizes cutting-edge science to address the drug discovery challenges of today. Discovery Studio 2.1 is built on the Pipeline Pilot open operating platform to seamlessly integrate protein modeling, pharmacophore analysis, virtual screening, and third-party applications

Preparation of molecular system:

Macro molecule (protein 1db4)

preparation:

For this QSAR studies, the protein 1DB4 load from RCSB protein data bank (www.rcsb.org/pdb/) and apply the force field .Force field refers to the functional form parameter sets which are used to find out potential energy of a system. It includes parameter which is obtained through experimental works and quantum mechanics calculations.

All molecules in a molecule in a mechanical system are made up of a number of components. Covalently bonded atoms takes into consideration several parameters such as bond length , bond angle , dihedral angles etc., similarly there exists non bonded interactions such as vanderwaals interactions , electrostatic interactions . Thus the total potential energy of the system is calculated as follows $E_1 = [E_{\text{bond}} + E_{\text{angle}} + E_{\text{torsion}} + E_{\text{vandervals}} + E_{\text{electronic}}]$ this summation when given is an explicit form, represents force field, evaluating the potential of a system.

Minimization:

The minimizer uses algorithm to identify the geometrics of the molecule corresponding to the minimum points on the potential surface energy. The minimum reduced the unwanted forces which are present in the molecule and lower the energy level of the molecule. There are

many algorithms available in the minimization process. Some of the minimization methods used in the smart minimizer is steepest decent method, conjugate gradient method, Newton Raphson method and quasi Newton method. From the DS protocols select the minimization and run .the following figure shows the minimized protein with fixed constraint .than save the minimized protein for further studies.

Preparation of bio active molecules:

The 111 bio active compounds are collected from the journals with the activity range 0.005 to >50 μM .

1 One molecule was drawn with basic scaffold and the other molecules were constructed with one drawn earlier as the reference model. 2. Drawn compounds are typed with charmm force field. 3. The typed molecule is subjected to the energy minimization using smart minimizer. Minimize a series of ligand poses using CHARMM. 4. Minimized molecule is saved with .sd and .mol2 extension for further study. Following table shows the 2d structure of the molecule and activity

Basic requirement in QSAR studies

1. All analogue belong congeneric series 2. All analogues exert same mechanisms of actions. 3. All analogue bind in a comparable manner. 4. Effect of isosteric

replacement can be predicted 5. Binding affinity correlated to interaction energies 6. Biological activities correlated to binding activity

To set up a Calculate Molecular Properties protocol:

1. Load the QSAR and apply the force field on molecules and Calculate Molecular Properties protocol from the Protocols Explorer. The parameters display in the Parameters Explorer.

2. On the Parameters Explorer, click in the cell for the Input Ligands parameter and click the button to specify the ligand source on the Specify Ligands dialog. On the dialog, select all ligands from a Table Browser, a 3D Window, or a file.

3. Select the properties to calculate by clicking the button in a cell for the Molecular Properties, Semi empirical QM descriptors, or Density Functional QM descriptors, and follow the instructions in the popup dialog window. The Create genetic function approximation can build a Create genetic function approximation model for a dependent property using the selected molecular descriptors.

To set up a Create genetic function approximation Model protocol

1. Load the QSAR | Create genetic function approximation Model protocol from the Protocols Explorer. The

parameters display in the Parameters Explorer.

2. On the Parameters Explorer, click in the cell for the Input Ligands parameter and click the button to specify the ligand source on the Specify Ligands dialog. On the dialog, select all ligands from a Table Browser, a 3D Window, or a file.

3. Set the desired model name using the Model Name parameter. Once created, this model will appear under the other category of the Molecular Properties parameter in the Calculate Molecular Properties protocol and can be used to compute the property for future ligands. 4. Set the initial equation length and remaining parameters as desired. Parameters presented in red are required.

QSAR:

In the present study quantitative structure activity relationship studies were carried out on phospholipase2 inhibitors in order to design selective and potential inhibitors. QSAR models were developed using 1D and 2D-descriptors using discovery studio software. QSAR attempts to model the activity of a series of compounds using measured or computed properties of the compounds. In the equation the term N means the number of data points, r^2 which is the square of the correlation coefficient which describing the binding of the compounds to the QSAR model. $XV r^2$, a

squared correlation coefficient generated during a validation procedure using the equation $XV r^2 = (SD PRESS)/SD SD$ means the sum of squared deviations of the dependent variable values from their mean the predicted sum of squares (PRESS), the sum of overall compounds of the squared differences between the actual and the predicted values for the dependent variables. The PRESS value is computed during a validation procedure for the entire training set. The larger the PRESS value the more reliable is the equation. $XV r^2$ is usually smaller than the overall r^2 for a QSAR equation. It is used as a diagnostic tool to evaluate the predicted power of an equation generated using the multiple learner regression method. GFA work by generating random populations of solution to a problem, scoring the relative quality of the solution, and caring forward the most fit solutions or analogues (generated through mutation and crossover) of other solutions to iteratively generated (and finally converge on) new, more fit solution. In this study GFA analysis was done with following parameters.

- Population size
- Initial equation length
- Final equation length
- Number of generation

Root strap r^2 correlation coefficient calculated during the validation procedure.

79 compounds were included in the training set to generate the primitive QSAR model covering the widest data range of IC₅₀ values 0.005 to 50.01 μM. The predictive characters of QSAR were further assessed using test molecules. To

judge the predictive ability of the QSAR model for new drug candidates the IC₅₀ values for the test and training set were evaluated.

GFA parameters

Number of rows in model	79
Population	100
Maximum generation	50000
Initial terms per equation	20
Scoring function	Friedman LOF
Mutation probability	0.1

RESULT AND DISCUSSION

The GFA method performs a search over the space of possible QSAR models using lack of fit (LOF) scores to estimate the fitness of each model. These models lead to the discovery of predictive QSAR equations.

GFA equation = 4.7849 + 0.00716121 *
 -In-Situ Starting Energy - 2.0176 * Activ
 + 0.10343 *
 Dipole_Mag_Propgen_VAMP - 0.610585
 * Local_polarity_Propgen_VAMP +
 0.26681 * Mean_Polarizability_VAMP +
 0.633171 * Num_H_Acceptors -
 0.149947 * Num_RotatableBonds -
 0.000507116 * Octupole_XYY_VAMP +
 0.0647933 *
 RIJestateSumHal_Propgen_VAMP +
 8.73998e-05 * -In-Situ Final Energy *

-In-Situ Final Energy + 5.40146e-05 *
 ESP_point_count__3_Propgen_VAMP *
 ESP_point_count__3_Propgen_VAMP +
 17.3371 *
 Molecular_FractionalPolarSurfaceArea *
 Molecular_FractionalPolarSurfaceArea -
 7.29063e-07 *
 No._of_surface_points_with_-ve_ESP_Pr
 opgen_VAMP *
 No._of_surface_points_with_-ve_ESP_Pr
 opgen_VAMP + 86.4313 *
 QsumHal_Propgen_VAMP *
 QsumHal_Propgen_VAMP -
 0.000148821 * Quadrupole_YY_VAMP *
 Quadrupole_YY_VAMP - 2.65319e-07 *
 Total_Energy_VAMP *
 Total_Energy_VAMP + 13.6832 * Activ *
 Covalent_hydrogen_bond_acidity_Propge
 n_VAMP - 0.000694297 * Activ *
 No._of_surface_points_with_+ve_ESP_Pr

opgen_VAMP + 1.85589e-06 *

DMol3_Mol_ID *

Electronic_Energy_VAMP + 0.0165753 *

Num_AromaticRings *

RIJestateSumO_Proppen_VAMP

specific group at that point and increase the activity of molecule and the negative values indicate the presence of ionic group which reduce the activity.

From the above equation, the positive values are the reference for the presence of

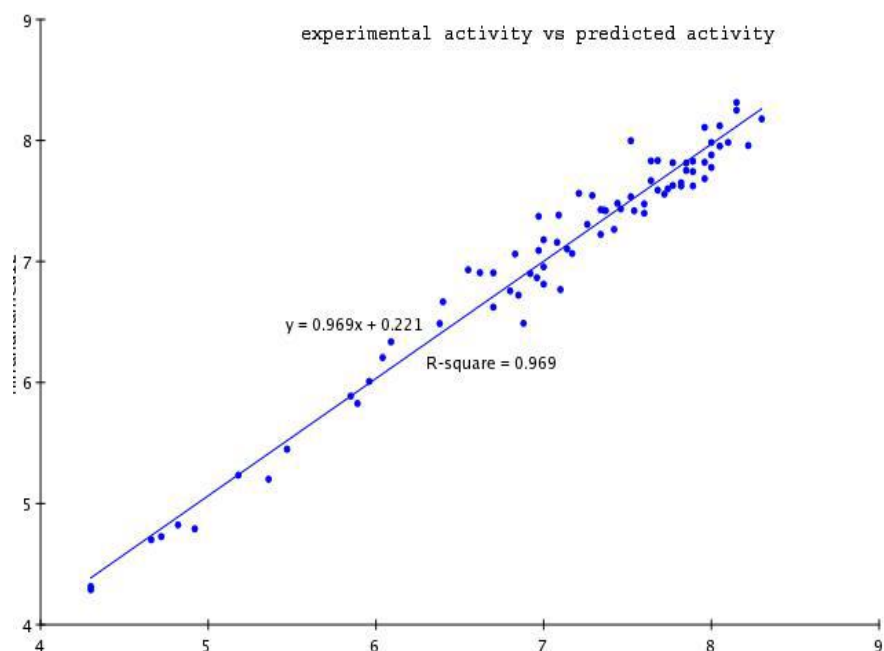
The validation statistics for the model

Fridman LOF	0.323
R-squared	0.968
adjusted R-squared	0.957
cross validated R-squared	0.941
lack of fit points	58
error for non-significant LOF	0.176
significance of regression F value	89.789

Experimental and predicted values of Training set compounds using GFA

Molecule Name	Activity	GFA Predicted Value	Molecule Name	Activity	GFA Predicted Value	Molecule Name	Activity	GFA Predicted Value
1	7.89	7.829	26	7.96	7.82	28xii	7.21	7.563
10	6.97	7.091	12d	4.72	4.727	28xiii	7.77	7.628
11	8	7.983	15b	7.85	7.813	28xix	8	7.88
12	7.14	7.103	15c	7.82	7.651	28xi	4.3	4.315
13	8.05	7.953	15e	7.89	7.624	28xv	5.18	5.235
14	6.88	6.489	15g	7.52	7.998	28xvi	7.29	7.545
16	6.09	6.336	27ii	7.77	7.816	28xviii	7.96	8.108
17	7.09	7.383	28i	8.1	7.984	28xx	7.68	7.59
18	6.97	7.373	28ii	8.22	7.958	28xxi	7.44	7.481
19	7.89	7.743	28iv	8.05	8.121	28xxii	7.6	7.476
20	8.15	8.314	28ix	7.68	7.834	28xxiii	6.92	6.9
21	6.96	6.866	28v	8.3	8.178	28xxix	7.85	7.752
23	7	6.813	28viii	7.74	7.6	28xxv	7.34	7.428

24	7.96	7.684	28x	7.72	7.556	28xxvii	8	7.777
25	6.83	7.062	28xi	7.64	7.831	28xxviii	7.54	7.419
28xxx	7.37	7.42	43d	5.47	5.449	49h	7.36	7.424
28xxxix	7.46	7.434	43e	5.85	5.888	50b	7.52	7.535
28xxxix	7	7.179	43f	5.89	5.827	51a	4.3	4.288
28xxxiii	6.55	6.931	43g	6.62	6.907	65a	6.04	6.206
28xxxv	7.42	7.265	48b	4.66	4.701	65b	6.38	6.487
28xxxvii	7.64	7.668	49b	8.15	8.25	65c	6.8	6.757
28xxxviii	7.82	7.623	49c	7.34	7.224	65d	7	6.956
43a	6.7	6.906	49d	7.08	7.158	67a	4.82	4.823
43b	5.36	5.201	49e	7.26	7.307	67b	4.92	4.791
43c	6.85	6.722	49g	7.1	6.768	6b	5.96	6.009



Graph 1: Correlation between experimental and predicted activities by QSAR equation using GFA method

CONCLUSION

The result generated from QSAR equation using GFA method, the values observed r^2 and XV r^2 are in specific range and there is a good correlation between experimental

and GFA predicted activity. Good correlation is observed between the experimental IC_{50} and computational predicted IC_{50} values. It has been

suggested as since the predictive ability of equations is good, they can be used to develop new analogs.

REFERENCE

1. T. J. Perun. Computer aided drug design, First edition **1989**; 369, 453-455.
2. Hagishita S, Yamada M, Shirahase K, Okada T, Murakami Y, Ito Y, Matsuura T, Kato T, Ueno M, Chikazawa Y, Yamada K, Ono T, Teshirogi I, Ohtani M, Potent inhibitors of secretory phospholipase A2: synthesis and inhibitory activities of indolizine and indene derivatives, *Journal of medicinal chemistry* **1996**, 39, 3636-33658.
3. Sawyer JS, Beight DW, Smith EC, Snyder DW, Chastain MK, Carlson DG, Carbocyclic [g]indole inhibitors of human Nonpancreatic sPLA2, *Journal of medicinal chemistry* **2005**, 48: 893-896.
4. Rob C. Oslund, Nathan Cermak and Michael H. Gelb, Highly specific and broadly potent inhibitors of mammalian secreted phospholipase A2, *Journal of medicinal chemistry* **2008**, 51:4708-4714.
5. Kakehi, A.; Ito, S.; Yamada, N.; Yamaguchi, A facile synthesis of 2-indolizinethiols using new protecting groups. *Bull. Chem. Soc. Jpn.* **1990**, 63: 829-834.
6. Mobilio, D.; Marshall, L. A. Recent Advances in the Design and Evaluation of Inhibitors of PLA2, *Annu. Rep. Med. Chem.* **1989**, 24, 157-166
7. Sofia, M. J.; Silbough, S. A. Novel Approaches to Anti-Inflammatory Agents as Therapeutics For Pulmonary Disease. *Annu. Rep. Med. Chem.* **1993**, 28, 109-118.
8. Wilkerson, W. W. Anti-Inflammatory Phospholipase A2 Inhibitors. *Drugs Future* **1990**, 15, 139-148.
9. Vadas, P.; Pruzanski, W. Role of Secretory Phospholipase A2 in the Pathobiology of Disease. *Lab. Invest.* **1986**, 55, 391-404.
10. Pruzanski, W.; Vadas, P. Secretory Synovial Fluid Phospholipase A2 and its Role in the Pathogenesis of Inflammation in Arthritis. *J. Rheumatol.* 1988, 15, 1601-1603.