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QSAR Studies on Some GSK-3α Inhibitory 6-aryl-pyrazolo (3,4-*b*) pyridines

[1]Ashutosh Jamloki, [1]C. Karthikeyan, [2]S.K. Sharma,
[3]N.S. Hari Narayana Moorthy and [1]P. Trivedi
[1]Department of Pharmacy, Shri G.S. Institute of Technology and Science, 23 Park Road, Indore-452003, Madhya Pradesh, India
[2]Maharaja Surajmal Institute of Pharmacy, New Delhi, India
[3]School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Viswavidyalya, Airport Bypass Road, Gandhi Nagar, Bhopal-462036, Madhya Pradesh, India

Abstract: A quantitative structure-activity relationship study on 6-aryl-pyrazolo (3,4-*b*) pyridines was performed to gain structural insight into the binding mode of the molecules to the glycogen synthase kinase -3α, an enzyme phosphorylate and inhibit Glycogen Synthase (GS) which is the rate limiting enzyme in the glycogen biosynthesis. The molecular modeling studies were performed using CS Chem. Office 2001 molecular modeling software version 6.0. Allinger's MM_2 force field by fixing Root Mean Square Gradient (RMS) to 0.1 Kcal mol^{-1} and semiemperical AM1 Hamiltonian method (MOPAC module) were used to minimize the energy and calculate descriptors. The thermodynamic and steric features of 6-aryl-pyrazolo (3,4-*b*) pyridines are highly correlated with GSK-3α inhibitory activity. The results of the study suggests that introduction of bulky groups at C-5 position of the pyrazolopyridine ring will increase the GSK-3α inhibitory potency as it may involve in hydrophobic interaction with the ATP binding site of the enzyme. The results of the study reveal that the conformational rigidity and orientation of molecule play significant role in the GSK-3α inhibitory activity. Additionally, electronic interactions between molecule and enzyme were found to be crucial for GSK-3α inhibitory activity.

Key words: QSAR, GSK-3α, 6-aryl-pyrazolo (3,4-*b*) pyridines

Introduction

Glycogen Synthase Kinase -3 (GSK-3) is a serine/threonine protein kinase that was first identified in late 1970's, due to its ability to phosphorylate and inhibit Glycogen Synthase (GS) which is the rate limiting enzyme in the glycogen biosynthesis (Embi *et al*., 1980). Two isoforms of GSK-3 exists, GSK-3α and GSK-3β, both share a high homology at their catalytic site but the α form possess an extended N-terminus with respect to the β form (Ali *et al*., 2001). The phosphorylation of proteins by GSK-3 is an important link in signaling pathways, those regulate cell differentiation, cellular growth and proliferation, metabolic processes, apoptosis control, inflammation and mechanisms involved in neuronal function (Martinez *et al*., 2002; Elder-Finkelman, 2002). Therefore GSK-3 inhibiting agents have potential to interfere with many physiological or pathological situations by interference with these signaling processes. Recently a number of publications have appeared suggesting GSK-3 as a target for the treatment of type 2-diabetes (Cohen and Frame, 2001) and Alzheimer's Disease (AD) (Anderton *et al*., 2000). Two characteristic neuropathological hallmarks of AD are Neurofibrillary Tangles (NFT's) and increased production of amyloid beta (Aβ) peptides followed by their aggregation

Corresponding Author: P. Trivedi, Department of Pharmacy, Shri G.S. Institute of Technology and Science, 23 Park Road, Indore-452003, Madhya Pradesh, India

(Caricasole *et al*., 2003). NFT's are composed of highly phosphorylated form of the microtubule associated protein tau (τ) (Lee *et al*., 2001). Total 25 sites of phosphorylation have so far been identified in tau protein, many of which are serine or threonine residues and studies have shown that GSK-3 is one of the main *in vivo* players of phosphorylation of tau protein (Flaherty *et al*., 2000).

Another protein aggregate, amyloid plaque contains mainly Aβ peptide fibrils, which are derived from amyloid precursor protein (APP) by sequential proteolysis catalyzed by an aspartyl protease, β-site APP cleaving enzyme (BACE) followed by presinelin-dependent γ-secretase cleavage (Wolfe, 2001). Recently it has been reported that Lithium, a GSK-3 inhibitor, block production of Aβ peptides by interfering with APP cleavage at γ-secretase step, where the target for Lithium is GSK-3α, which is required for maximal processing of APP (Phiel *et al*., 2003). Hence GSK-3α inhibitors offer a new therapeutic approach for AD treatment by reducing the formation of both amyloid plaques and NFT's.

So far a few compounds are known with GSK-3 inhibitory potential such as Lithium (Jope, 2003), Azaindolylmaleimides (Shen *et al*., 2004) and Anilinomaleimides (Smith *et al*., 2001), Paullones (Leost *et al*., 2000), Indirubins (Polychonopoulos *et al*., 2004), Aloisines (Mettey *et al*., 2003) and Thiazolidinones (Martinez *et al*., 2002). Recently, Witherington *et al*. (2003) identified and synthesized 6-aryl-pyrazolo (3,4-b) pyridines as potent inhibitors of GSK-3α. A QSAR analysis was performed on the abovementioned series of compounds in order to gain insight into the physicochemical requirements of these inhibitors. The applicability of QSAR studies in mechanistic interpretation of ligand macromolecular interactions has been proved. With the abovementioned rationale, an attempt has been made to propose quantitative models for describing the factors influencing the affinity of the drug molecules towards the enzyme. Additionally efforts have been made to justify the findings of the QSAR study with the results of the molecular modeling studies reported in the research paper chosen for the QSAR study. The results of the study will help in rationalizing the design of 6-aryl-pyrazolo (3,4-*b*) pyridines as GSK-3α inhibitors and predicting the inhibitory activity of the newly designed analogues.

Materials and Methods

The data set of a total of 16 compounds was taken from published literature (Witherington *et al*., 2003), out of which one compound was omitted due to its numerically undefined biological activity. All the values of biological data were expressed as IC_{50} values in nanomolar units. In the present study biological activity data was first converted to its molar unit and subsequently to negative logarithmic units (pIC_{50}) to reduce skewness of data set. The general structure of these analogues is shown in Fig. 1 and compounds with their biological activity data are shown in Table 1. The molecular modeling

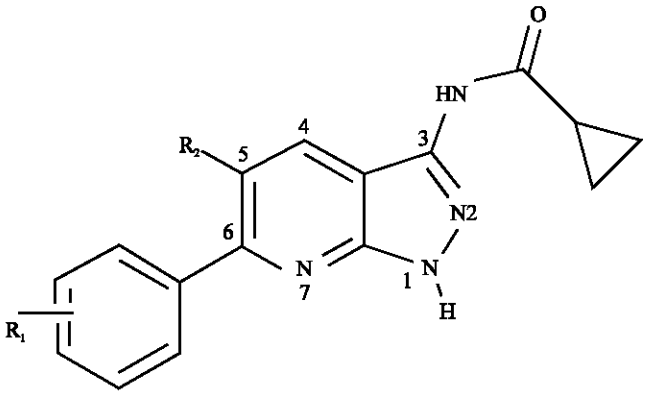

Fig. 1: General structure of 6-aryl-pyrazolo (3,4-*b*) pyridines

Table 1: 6-aryl-pyrazolo (3,4-*b*) pyridines and their GSK-3α inhibitor activities

Compd. No.	Substituents R_1	Substituents R_2	IC_{50} (nM)	pIC_{50} (-Log IC_{50})
1	H	H	425	6.372
2	4-OH	H	8	8.096
3	3,4-di-OH	H	8	8.096
4	3-OH	H	12	7.921
5	3-OMe	H	125	6.903
6	2-OH	H	36	7.445
7	2-OMe	H	1593	5.797
8	4-OH	Ph	24	7.619
9	4-OH	Br	0.8	9.096
10	4-OH	Cl	1	9.000
11	4-OH	Me	6	8.223
12	H	Ph	415	6.382
13	H	Br	75	7.125
14	H	Cl	234	6.631
15	H	CN	87	7.060

Table 2: Descriptors used in present study

Descriptor	Type	Descriptor	Type
Heat of Formation (HF)	Thermodynamic	Standard Gibbs Free Energy (SGFE)	Thermodynamic
Boiling Point (BP)	Thermodynamic	Charge-Dipole Energy	Thermodynamic
Torsion Energy (E_t)	Thermodynamic	Stretch Energy	Thermodynamic
Total Energy (E)	Thermodynamic	Stretch-Bend Energy (E_{sb})	Thermodynamic
Dipole-dipole Energy (E_d)	Thermodynamic	Bend Energy (E_b)	Thermodynamic
Non-1, 4 VDWE Energy (E_v)	Thermodynamic	Charge-Charge Energy	Thermodynamic
VDWE 1,4 Energy (VDWE)	Thermodynamic	Molar Refractivity (MR)	Thermodynamic
Melting Point (MP)	Thermodynamic	Partition Coefficient (Octanol/Water)	Thermodynamic
Critical Pressure (CP)	Thermodynamic	Connolly Accessible Area (CAA)	Steric
Critical Temperature (CT)	Thermodynamic	Connolly Molecular Area (CMA)	Steric
Critical Volume (CV)	Thermodynamic	Principal Moment of Inertia-Z (PMI-Z)	Steric
Henry's Law Constant (HLC)	Thermodynamic	Connolly Solvent-Excluded Volume (CSEV)	Steric
Ideal Gas Thermal Capacity (IGTC)	Thermodynamic	Principal Moment of Inertia-X (PMI-X)	Steric
LogP	Thermodynamic	Principal Moment of Inertia-Y (PMI-Y)	Steric
Ovality	Steric	Electronic Energy	Electronic
Dipole Moment	Electronic	HOMO Energy	Electronic
Dipole Moment-X Axis (DX)	Electronic	LUMO Energy	Electronic
Dipole Moment-Y Axis (DY)	Electronic	Repulsion Energy (RE)	Electronic
Dipole Moment-Y Axis (DZ)	Electronic		

studies were performed using CS Chem. office, 2001 molecular modeling software ver. 6.0, supplied by Cambridge Software Company (CS, Chemoffice, 2001). The structure of each compound was drawn in ChemDraw Ultra ver. 6.0 and copied to Chem3D ultra ver. 6.0 in order to create its 3-D model. Each model was cleaned up and energy minimization was performed using Allinger's MM_2 force field by fixing Root Mean Square Gradient (RMS) to 0.1 Kcal mol^{-1} Å. Further geometry optimization was done using semiemperical AM1 (Austin Model 1) Hamiltonian method, closed shell restricted wave function available in the MOPAC module until the RMS value becomes smaller than 0.001 Kcal mol^{-1} Å. Energy minimized geometry was used for calculation of various thermodynamic, steric and electronic descriptors which are mentioned in Table 2.

Stepwise multiple linear regression was performed using SYSTAT software (SYSTAT 10.2 version, 2003) in order to obtain QSAR models. Statistical quality of the models was judged by correlation co-efficient (r), squared correlation co-efficient (r^2), which is relative measure of quality of fit, adjusted squared correlation co-efficient (r^2_{Adj}) i.e. explained variance in biological activity, Standard Error of Estimate (SEE) representing absolute measure of quality of fit and Fischer's value (F), which represents F-ratio between the variance of calculated and observed activity. Chance statistics (evaluated as the ratio of the equivalent regression equations to the total number of randomized sets; a chance value of 0.001 corresponds to 0.1% chance of fortuitous correlation) was performed in order to assure that the results are not merely based upon chance correlation.

Studentized-Residual method was adopted for the detection of outliers. A compound was considered as an outlier for deriving a particular model when the residual value exceeded twice the standard error of estimate of the model. In order to validate the generated QSAR models Leave One Out (LOO) method was used. Squared cross- correlation co-efficient (q^2), standard deviation of sum of square of difference between predicted and observed values (S_{PRESS}) and standard deviation of error of prediction (S_{DEP}) were also calculated for each model to estimate the predictive potential of models with the help of VALSTAT Software (VALSTAT, 2004).

Results and Discussion

GSK-3α inhibitory activity data and various molecular descriptors were taken as dependent and independent variables respectively and correlations were established between them by employing stepwise multiple linear regression method. The orthogonality of descriptors in the selected correlations were confirmed by the calculation of overall correlation matrix and tolerance level of each descriptor (Table 3 and 4). Tolerance is defined as 1- R squared, where R squared is the multiple R of a given independent variable regressed on all other independent variables. Tolerance value ranges from 0.0 to 1.0. If tolerance value of an independent variable equals 1.00, it is totally independent of the other predictor variables, whereas if it equals 0.0 it is totally collinear with other independent variables. Further the selected models were checked for any autocorrelation by calculating Durbin-Watson (D-W) Statistics.

Among many correlations generated, statistical significant models were selected for further discussion. The most significant biparametric QSAR model selected was:

$$pIC_{50} = [0.002\ (\pm 0.000)]\ PMI\text{-}Y + [-0.522\ (\pm 0.053)]\ VDWE + [2.626\ (\pm 0.737)] \quad \text{(Model 1)}$$

N is 15, r is 0.965, r^2 is 0.931, r^2_{Adj} is 0.919, SEE is 0.274,
F is 80.900, Chance is < 0.001, D-W Statistics is 2.091,
$q^2 = 0.892$, S_{PRESS} is 0.343, S_{DEP} is 0.306.

Where, N is the number of data points, r is correlation coefficient, r^2 is squared correlation coefficient, r^2_{Adj} is the Explained Variance (EV) calculated as $r^2_{Adj} = r^2 (1\text{-}1/F)$ that accounts in percentage when multiplied by 100 for the variance in activity, SEE is standard error of estimate and other terms bear the meaning as described earlier.

This biparametric model explains 91.9% variance in the GSK-3α inhibitory activity. The F-statistics of the model is significant at 99.9% level, as shown by greater calculated value of F than the tabulated $F_{(2, 12\ \alpha = 0.001)} = 8.51$. t-tests were performed to assess significance of individual coefficients. The calculated t-values as given in Table 4, exceeded tabulated t = 3.012, at 99.5% confidence interval, showing significance of regression coefficients at this level. Tolerance level of each descriptor is close

Table 3: Correlation matrix for descriptors in the selected models

	pIC_{50}	PMIY	VDWE	E_d
pIC_{50}	1.000			
PMIY	0.616	1.000		
VDWE	-0.526	0.297	1.000	
E_d	0.277	-0.219	-0.357	1.000

Table 4: T-statistics and tolerance values for the descriptors in the selected models

Model No.	Constant/descriptor	Tolerance	t-value
	Constant	-	3.561
Model 1	PMI-Y	0.912	10.660
	VDWE	0.912	-9.789
	Constant	-	5.083
	PMI-Y	0.898	15.949
Model 2	E_d	0.858	3.830
	VDWE	0.822	-12.391

to 1 as shown in Table 4, thus confirming absence of collinearity among predictor variables. Magnitude of D-W coefficient shows independence of observation. High q^2 value and low S_{PRESS} and low S_{DEP} values show good predictive power of the model.

This model establishes the correlation between principal moment of inertia (PMI-Y), Van der Waals 1,4 Energy (VDWE) and GSK-3α inhibitory activity. PMI-Y is a spatial descriptor, which explains the significance of orientation and molecular distribution along Y-axis for GSK-3α inhibitory potency. The positive coefficient of PMI-Y in the model suggests the presence of bulky substituents oriented towards Y-axis of the molecule will enhance the GSK-3α inhibitory activity. The observation supports the hypothesis that the presence of the bulky substituents like bromine with inherent hydrophobic character may involve in nonspecific interaction with the ATP binding site. However phenyl substituents that possess good hydrophobicity exhibits lower inhibitory potency. This may be attributed to the strain exerted by the two adjacent phenyl rings on the planar pyrazolo (3,4-*b*) pyridine ring thereby partly disrupting the hydrogen bonding interaction between nitrogen in the pyrazolo group and the complementary group in the enzyme.

Van der Waals 1,4 energy, a thermodynamic descriptor, can be defined as the sum of pairwise Van der Waals interaction energy terms for atoms separated by exactly three chemical bonds. Negative coefficient of this descriptor in the model indicates the necessity of conformational flexibility to allow proper orientation of the pharmocophoric groups in the molecule for GSK-3 inhibition.

On addition of a thermodynamic descriptor Dipole-Dipole energy (E_d) to the model 1 significant improvement in the statistical parameters was observed.

$$pIC_{50} = [0.002\ (\pm 0.000)]\ \text{PMI-Y} + [0.203\ (\pm 0.053)]\ E_d + [-0.476\ (\pm 0.038)]\ \text{VDWE} + [2.564\ (\pm 0.504)] \quad \text{(Model 2)}$$

N is 15, r is 0.985, r^2 is 0.970, r^2_{Adj} is 0.962, SEE is 0.187, F is 120.256, Chance is < 0.001, q^2 is 0.945, S_{PRESS} is 0.256, S_{DEP} is 0.219 and Durbin-Watson (D-W) Statistics is 2.325.

This model accounts for 96.2% of GSK-3α inhibitory activity. F-statistics proves it to be statistically highly significant (more than 99.9 %), as the calculated variance ratio i.e. Fischer value (F) exceeds the tabulated $F_{(3,\ 12\ \alpha\ =\ 0.001)} = 12.7$. Low standard error of estimate suggests a high degree of confidence in the analysis. D-W statistics affirm absence of serial autocorrelation. The values of intercorrelation coefficient indicate lack of multicollinearity (Table 3), which was further reaffirmed

Table 5: Descriptors contributing to the GSK-3α inhibitory activity of 6-Aryl-Pyrazolo (3,4-b) pyridines

PMI-Y	VDWE	E_d
4452.91	5.92479	-3.05852
5418.1	4.59481	-3.09596
6346.36	8.59255	-2.72937
5173.39	4.5809	-2.62678
5637.45	8.37295	-2.65263
4690.86	4.84573	-2.35605
4939.37	7.96979	-3.06989
5835.04	7.07243	-3.71392
5741.09	5.13774	-1.15337
5603.42	5.03322	-1.04112
5502.55	5.33326	-3.25312
5207.09	8.25357	-3.72862
4920.27	6.29807	-1.18003
4706.74	6.19835	-1.07488
4672.78	5.87088	-0.925399

Table 6: Observed and predicted activities of 6-Aryl-Pyrazolo (3,4-b) pyridines

Compd. No.	Observed activity (pIC_{50})	Predicted activity	
		Model 1	Model 2
1	6.372	6.401	6.126
2	8.096	8.694	8.466
3	8.096	7.775	7.930
4	7.921	8.264	8.112
5	6.903	6.952	7.030
6	7.445	7.297	7.184
7	5.797	6.162	6.065
8	7.619	7.981	7.749
9	9.096	8.706	8.970
10	9.000	8.549	8.807
11	8.223	8.335	8.081
12	6.382	6.328	6.064
13	7.125	6.896	7.152
14	6.631	6.644	6.960
15	7.060	6.706	6.989

by checking the tolerance level of each descriptor (Table 4). t-statistics shows that regression coefficient are highly significant (Table 4). The model has remarkable predictive power, which is mirrored in its high q^2 value and low S_{PRESS} and S_{DEP} values. None of the compound was found to be outlier in any model.

Higher correlation coefficient value and low standard error of estimation indicates that this model is better than the preceding one. Moreover the model exhibits more predictive power than abovementioned model, as suggested by its higher q^2 value. In view of all the statistical parameters model 2 seems to be superior in theoretical prediction of GSK-3α inhibitory activity.

Dipole-Dipole energy, which is a thermodynamic descriptor that describes the sum of the electrostatic energy terms resulting from interaction of two dipoles, shows positive contribution to the inhibitory activity. Positive coefficient of this term in the model indicates that substitution with the functional moieties those are capable of Dipole-Dipole interaction with the binding site of enzyme will be conducive for activity which reaffirms the significance of hydrogen bonding interaction between the para hydroxyl group of the 6-aryl moiety and amino acid residues (Glu97 and Asp200) of the enzyme.

Calculated values of all the descriptors in the selected models are shown in Table 5. Predicted activity values were calculated for each compound using the correlations developed and a comparison was made with the observed values (Table 6, Fig. 2 and 3).

The QSAR analysis resulted in statistically significant quantitative models with good predictive ability. It was observed from generated QSAR models that GSK-3α inhibitory activity of 6-aryl-

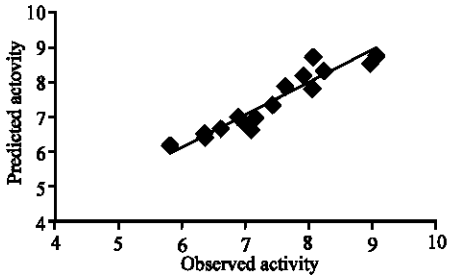

Fig. 2: Scatter plot between observed and predicted gsk-3α inhibitory activity (Model 1)

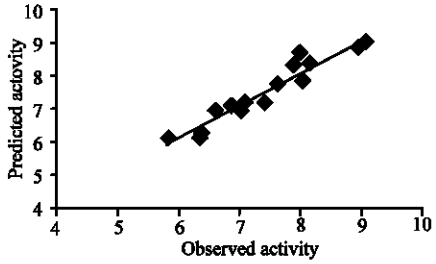

Fig. 3: Scatter plot between observed and predicted gsk-3α inhibitory activity (Model 2)

pyrazolo (3,4-*b*) pyridines is governed by thermodynamic and steric features of the molecules. The results of the study suggests that introduction of bulky groups at C-5 position of the pyrazolopyridine ring will increase the GSK-3α inhibitory potency as it may involve in hydrophobic interaction with the ATP binding site of the enzyme. Moreover, the study also illustrates the significance of the para hydroxyl group of the 6-aryl moiety for the drug-enzyme interaction. The findings of the QSAR analysis are consistent with molecular modeling studies performed on these analogs. The results obtained from the QSAR study emphasize the combined utility of the QSAR analysis and the molecular modeling studies to optimize the design of potent GSK-3α inhibitory molecules.

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