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Quantitative Structure–Activity Relationship and Molecular Docking Studies of Imidazolopyrimidine Amides as Potent Dipeptidyl Peptidase-4 (DPP4) Inhibitors

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Type 2 diabetes (T2DM) is a metabolic disorder disease and DPP-4 inhibitors are a class of oral hypoglycemic that blocks the dipeptidyl peptidase-4 (DPP-4) enzyme. DPP-4 inhibitors reduce glucagon and blood glucose levels and don't have side effects such as hypoglycemia or weight gain. In this paper, a series of imidazolopyrimidine amides analogues as DPP4 inhibitors were selected for quantitative structure-activity relationship (QSAR) analysis and docking studies. A collection of chemometric methods such as multiple linear regression (MLR), factor analysis-based multiple linear regression (FA-MLR), principal component regression (PCR), genetic algorithm for

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variable selection-MLR (GA-MLR) and partial least squared combined with genetic algorithm for variable selection (GA-PLS), were conducted to make relations between structural features and DPP4 inhibitory of a variety of imidazolopyrimidine amides derivatives. GA-PLS represented superior results with high statistical quality (R^2 = 0.94 and Q^2 = 0.80) for predicting the activity of the compounds. Docking studies of these compounds reveals and confirms that compounds 15, 18, 25, 26, and 28 are introduced as good candidates for DPP-4 inhibitors were introduced as a good candidate for DPP-4 inhibitory compounds.

Keywords: Imidazopyrimidine derivatives; DPP-4 inhibitors; QSAR; molecular docking.

1. INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder disease that the body doesn't have the ability to produce insulin or is resistant to insulin so it cannot function properly. Dipeptide peptidase 4 (DPP-4) inhibitors are a new therapy Target that does not complicate previous medications such as hypoglycemia, weight gain and cardiovascular risk [1]. DPP-4 is a membrane protease that has a specific selectivity on the secretion of incretins hormones. Therefore, the mechanism of DPP-4 inhibitors is blocking the action of DPP-4 enzyme, so the incretin levels increased. Glucagon release is decreased and in turn the level of insulin secretion is increased, so that the blood glucose level is controlled [2].

The quantitative structure-activity relationship (QSAR) research field provides medicinal chemists with the ability to predict drug activity by mathematical equations which construct a relationship between the biological activity of the molecules and descriptors [1,2]. These mathematical equations are in the form of y = Xb+e that describe a set of predictor variables (X) with a predicted variable (y) by means of a regression vector (b) [3]. The most important step in building QSAR models is the appropriate representation of the structural and physicochemical features of structures [4-10]. These features called molecular descriptors are the ones with higher impact on the biological activity of interest. Nowadays, a wide range of descriptors are being used in QSAR studies which can be classified into different categories according to the Karelson approach including; constitutional, geometrical, topological, quantum, chemical and so on [8].Hyperchem and Dragon are two well-known computational software provide us with more than 1000 of these descriptors [11-12]. There are different variable selection methods available including; stepwise multiple linear regression (MLR), genetic algorithm (GA), principal component or factor analysis (PCA) and so on.

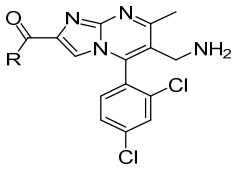
Here, we consider the DPP4 inhibitory activity of a novel series of imidazolopyrimidine amides which have been recently designed and synthesized by W. Meng [13]. Our research shows that these series of compounds don't evaluate for QSAR studies. Different statistical methods were applied to model the relationship between the structural features and the DPP-4 inhibitory activity of the studied compounds. These methods are: (i) multiple linear regression (MLR), (ii) principal component regression (PCR), MLR with factor analysis as the data pre-processing step for variable selection (FA-MLR) (iii), genetic algorithm-multiple linear regression (GA-MLR) (iv), genetic algorithm-partial least squares (GA-PLS) (v). Molecular docking simulation technique was also performed on twenty-nine compounds to reach the details of molecular binding models for these compounds interacting with the key active site DPP-4 inhibitors.

2. MATERIALS AND METHODS

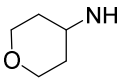
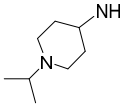
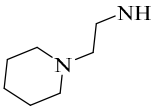
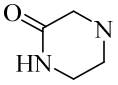
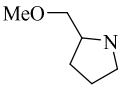
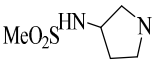
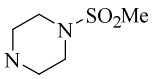
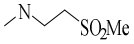
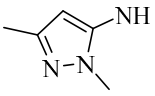
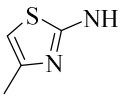
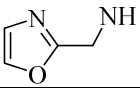
2.1 Data Set

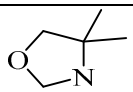
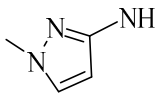
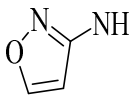
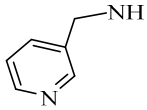
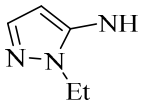
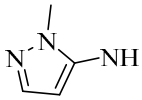
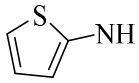
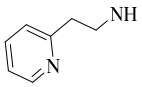
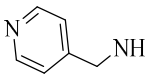
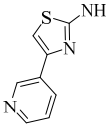
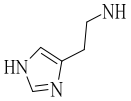
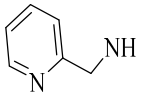
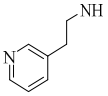
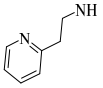
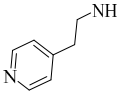
The biological activity was used in this study, were the DPP-4 inhibitory activity of a set of thirty-one imidazolepyrimidine amides derivatives [13], which were designed, synthesized and evaluated for their ability as potential treatments for type II diabetes. The structural features and biological activities of these compounds are listed in Table 1. The biological data were converted to logarithmic scale (pIC_{50}) and then used for subsequent QSAR analysis as dependent variable.

Table 1. Chemical structure of imidazolopyrimidine amides analogues used and their experimental and cross validated-predicted activity by (GA-PLS) for DPP4 inhibitory and their docking bonding energies



1-31

NO	R	Exp.pIC$_{50}$	Pred. pIC$_{50}$ by GA-PLS	Binding Energ (kcal/mol)
1*	OEt	9.39	------	------
2		8.6	8.38	-8.1
3		8.5	8.7	-8.7
4		8.6	8.3	-7.9
5**		8.5	8.65	-8.6
6		8.3	8.3	-8.1
7		8.06	8.1	-8.2
8		9	8.7	-8.1
9		8.5	8.5	-8
10		9.69	9.8	-8.2
11		9.3	9.3	-8.2
12		9.5	9.6	-7.9
13		9.04	8.9	-8.9
14		8.18	8.5	-8.4
15		8.69	8.7	-9

16*		------	------	------
17**		8.7	8.37	-8.5
18		8.40	8.60	-9.3
19**		8.58	8.3	-8.9
20		8.95	8.9	-8.6
21**		8.69	8.68	-8.6
22		8.49	8.48	-8.7
23**		9.15	9.1	-8.9
24		8.58	8.4	-8.4
25**		8.39	8.4	-9.4
26		8.3	8.35	-9.2
27		8.32	8.4	-8.3
28**		8.26	8.28	-9.4
29**		8.8	8.72	-8.8
30		8.8	8.6	-8.9
31**		8.8	8.73	-8.3

**: outlier data*
***: molecules as test set*

2.2 Molecular Descriptors

All structures were generated with HyperChem program (Hyper-cube Inc., Version 8.0.3) [11] and optimized by MM+ method and then semi-empirical AM1 method in hyperchem software. The molecular structures were optimized using the Polak-Ribiere algorithm until the root mean square gradient was 0.01 kcal mol^{-1}. Some chemical parameters including molar volume (V), molecular surface area (SA), hydrophobicity (logP), hydration energy (HE) and molecular polarizability were calculated by using Hyperchem software. The resulted geometry was transferred into Dragon program package, which was developed by Milano Chemometrics and QSAR Group. Dragon software (version 5.5) [12] calculated the different topological, geometrical, charge, empirical and constitutional descriptors for each molecule. 2D autocorrelations aromaticity indices, atom-centred fragments and functional groups were also calculated by dragon software.

In the case of docking procedure, each optimized structures in HyperChem 8.0.3 program were thereafter converted to PDBQT using MGLtools 1.5.6 [14]. The three-dimensional crystal structure of dipeptidyl peptidase iv human (PDB ID:5j3j) were retrieved from protein data bank [15]. Co-crystal ligand molecules were excluded from the structures and the PDBs were corrected in terms of missing atom types by modeller9.12 [16]. An in house application (MODELFACE) was used for generation of python script and running modeler software [17]. Subsequently, the enzymes were converted to PDBQT and gasteiger partial charges were added using MGLtools1.5.6.

2.3 Data Screening and Model Building

The calculated descriptors were collected in a data matrix, D whose number of rows and columns were the number of molecules and descriptors, respectively. First, the descriptors were checked for constant or near constant values and those detected were removed from the original data matrix. The correlated descriptors with each other's and with the activity data were determined and removed from the pool of descriptors.

Five different methods were used: (1) stepwise-multiple linear regression (2) MLR with factor analysis as the data pre-processing step for variable selection (FA-MLR), (3) principal component regression analysis and (4) genetic algorithm- multiple linear regression (GA-MLR) (5) genetic algorithm- partial least squares (GA-PLS).

MLR with stepwise selection and elimination of variables was applied for developing QSAR models by using SPSS software (SPSS Inc., version 21). The resulted models were validated by leave-one out cross-validation procedure by using MATLAB software version 2014. However, this procedure did not produce good results and therefore we used a genetic algorithm (GA-PLS) to select the best variables. FA-MLR was performed on the dataset. Factor analysis was used to reduce the number of variables. Principal component regression analysis was also tried for the dataset along with FA-MLR. With PCRA collinearities among X variables are not a disturbing factor and the number of variables included in the analysis may exceed the number of observations [18]. In this method, factor scores, as obtained from FA, are used as the predictor variables [19]. In PCRA, all descriptors are assumed to be important while the aim of factor analysis is to identify relevant descriptors. Partial least squares (PLS) linear regression is a recent technique that generalizes and combines features from principal component analysis and multiple regressions. PLS is the best method for overcoming the problems in MLR related to multicollinear or over-abundant descriptors [20]. Application of PLS method thus allows the construction of larger QSAR equations while still avoiding over-fitting and eliminating most variables. This method is normally used in combination with cross-validation to obtain the optimum number of components [21]. The PLS regression method used was the NIPALS-based algorithm existed in the chemometrics toolbox of MATLAB software (version 8.0.3.532 Math Work Inc.).

2.4 Docking Procedures

An in house batch script (DOCK-FACE) for automatic running of AutoDock 4.2 was used to carry out the docking simulations [22] in a parallel mode [23]. To prepare the receptor structure, the three-dimensional crystal structure of Dipeptidyl Peptidase-4 (PDB ID: 5j3j) was acquired from Protein Data Bank (PDB database; http://www.rcsb.org) [24] and water molecules and co-crystal ligand were removed from the structure. The PDB was then checked for missing atom types with the python script as implemented in MODELLER 9.17 [25]. The

ligand structures were made by Hyper Chem software package (Version 7, Hypercube Inc). For geometry optimization, Molecular Mechanic (MM^+), followed by semi-empirical AM1 method was performed. The prepared Ligands were given to 100 independent genetic algorithm (GA) runs. 150 population size, a maximum number of 2,500,000 energy evaluations and 27,000 maximum generations were used for Lamarckian GA method. The grid points of 30, 30, and 30 in x-, y-, and z directions 20.3, 3.7 and 51.3 were used. Number of points in x, y and z respectively. All visualization of protein ligand interaction was evaluated using VMD software [26]. Cluster analysis was performed on the docked results using a root mean square deviation (RMSD) tolerance of 2.4 Å.

3. RESULTS AND DISCUSSION

The structural feature and the experimental DPP-4 inhibitory activity (represented as pIC_{50}) of the molecules used in this study are shown in Table 1. To obtain the effects of the structural parameters of the investigated derivatives on their DPP-4 activity, QSAR analysis was performed with various molecular descriptors. Among the different chemometrics tools available for modeling the relationship between the biological activity and molecular descriptors, five methods (i.e., stepwise MLR, PCR, FA-MLR, GA-MLR and GA-PLS) were applied and compared here. The calculated descriptors from whole molecular structures are briefly described in Table 2.

3.1 MLR Models for a Subset of Molecules

Firstly, separate stepwise selection-based MLR analyses were performed using different types of descriptors, and then, a MLR equation was obtained utilizing the pool of all calculated descriptors. First principal component analysis was done to detect outlier data and was drawn PC1 on PC2 (Fig. 1), as it can show the molecule number of 1 and 16 are outlier data so omitted. Then Kennard stone algorithm was used to divide data set to calibration and prediction set. MLR models with a maximum number of variables of 5 were selected. Statistical parameters such as correlation coefficient (R^2), the correlation coefficient for the test set (R^2test set or R^2predic), standard error of the regression (SE), and Fisher ratio (F) at specified degrees of freedom, leave-one-out cross-validation correlation coefficient (Q^2) was shown in Table 3. Equation 1 was selected as the best equation in the MLR model because of its greatest statistical parameters. The selected variables demonstrate that 2D-autocorrelation (MATS1m), constitutional (Ms), topological charge indices (GGI5), topological (DELS), 3D-MORSE descriptors (Mor25m) effect on the inhibitory activity of the studied compounds.

Table 2. Brief name of molecular descriptors was used in the models

Descriptor type	Descriptors	Brief description
Constitutional	Ms.	Mean electropological state
Topological	Jhetm	Balaban-type index from mass weighted distance matrix
	DELS	Molecular electropological variation
Connectivity indices	X0A	Average connectivity index chi-0
2D-autocorrelation	MATS1m	Moran autocorrelation – lag1/weighted by atomic Masses
Edge adjacency indices	EEig09d	Eigen values 09 from edge adj. matrix weighted by dipole moment
	EEig13d	Eigen values 13 from edge adj. matrix weighted by dipole moment
Burden Eigenvalues	BELm6	Lowest eigenvalue n.6 of burden matrix/weighted by atomic masses
Topological charge indices	GGI5	Topological charge index of order 5
	GGI7	Topological charge index of order 7
3-D Morse Descriptors	Mor27u	3D-MoRSE-signal 27/unweighted
	Mor25m	3D-MoRSE-signal 25/weighted by atomic masses
WHIM Descriptors	E2m	2nd component accessibility directional WHIM index/weighted by atomic masses

Table 3. The results of different QSAR model analysis

Models	Equation	N	R^2	Q^2	F	SE	R^2p
MLR	PIC_{50}=9.508 MATS1m (±2.252) +4.286 Ms (±0.78) +4.319 GGI5 (±0.816)-0.105 DELS (±0.028) +0.538 MOR25m (±0.182)-3.903(±1.868)	29	0.91	0.84	31.7	0.14	0.92
PCR	PIC_{50}= 0.245 PC1(±0.243) +0.13 PC3 (±0.043) +0.129 PC2(±0.043)-0.121 PC7(±0.043) + 8.695(±0.042)	29	0.77	0.75	14. 6	0.22	0.83
FA-MLR	PIC_{50}=11.953 MATS1m(±2.8) +2.65 Ms (±0.83) +2.61(±1.96)	29	0.67	0.53	13.2	0.12	0. 63
GA-MLR	PIC_{50}=2.072GGI7((±0.676)+4.427Ms((±0.678)+8.047BELm6(±1.753)-0.453Mor27u(±0.187)-14.411(±3.275)	29	0.94	0.88	28.5	0.16	0.91
GA-PLS	----------	29	0.94	0.80	-------	0.49	0.95

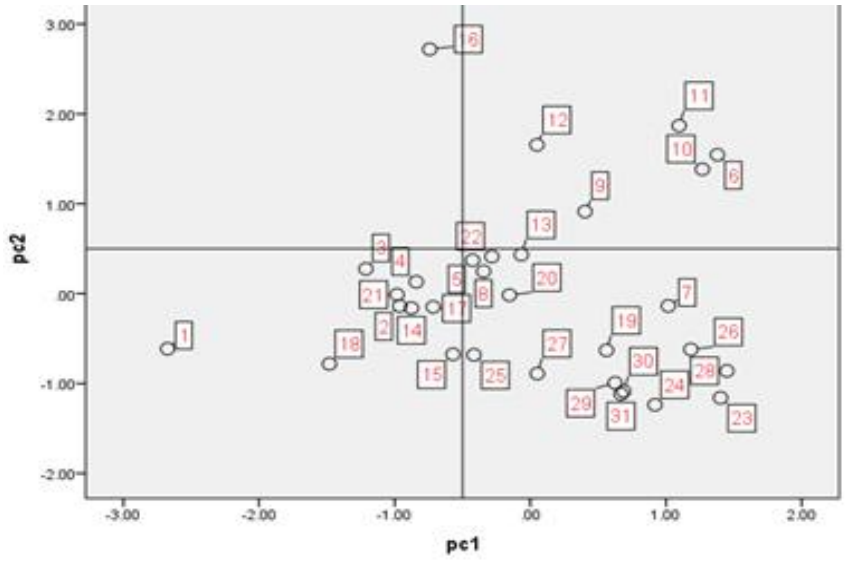


Fig. 1. Principal component analysis diagram for detection of outlier data

A small difference between the conventional and cross-validate correlation coefficients of the different MLR equations (Table 4) reveals that none of the models is over fitted, which can be partially attributed to the absence of collinearity between the variables in one hand and use of no extra variables on the other hand. Equation 1 (as the best equation in this series) could explain 91% of the variance and predict 84% of the variance in ($-\log IC_{50}$) data. All of the descriptors that used in this equation have positive effect on DPP-4 inhibitory expect DELS as topological descriptors. Fig. 2 shows the plots of linear regression predicted versus the experimental value of the DPP4 inhibitory activity of ligand. The plots for this model show to be more convenient with R^2cv= 0.84.

3.2 PCR Analysis

When factor scores were used as the predictor parameters in a multiple regression equation (Table 5), a predictive QSAR model with factor scores of 1, 2, 3 and 7 as input variables, was obtained (Table 3, Equation 2). This equation shows statistical quantities similar to those obtained by the FA-MLR method.

Considering this information in modelling, it may apparently increase the model variances (i.e., R^2) but they are useful for prediction. Fig. 2 shows the plots of linear regression predicted versus the experimental value of the DPP-4 inhibitory activity of ligand. The plots for this model show to be more convenient with R^2cv= 0.75.

3.3 FA-MLR Analysis

FA-MLR was performed on the dataset. Factor analysis (FA) was used to reduce the number of variables and to detect structure in the relationships between them. This data-processing step is applied to identify the important predictor variables and to avoid collinearities among them. Principal component regression analysis, PCRA, was tried for the dataset along with FA-MLR. With PCRA collinearities among **X** variables are not a disturbing factor and the number of variables included in the analysis may exceed the number of observations [27]. In this method, factor scores, as obtained from FA, are used as the predictor variables [28]. In PCRA, all descriptors are assumed to be important while the aim of factor analysis is to identify relevant descriptors. Table 5 shows the two-factor loadings of the variables (after VARIMAX rotation) for the compounds tested against dipeptidyl peptidase 4 inhibitors'. As it is observed, about 77% of variances in DPP4 inhibitors' could be explained by the selected two factors. It is observed; about 0.67 of variances in the original data matrix can be explained by selected 2 factors, MATS1m as 2D-autocorrelation descriptors and Ms as Constitutional descriptors. And also have weakly predicted variance in DPP4 inhibitory. Fig. 2 shows the plots of linear regression predicted versus the experimental value of the DPP4 inhibitory activity of ligand. The plots for this model show to be more convenient with R^2cv= 0.53.

3.4 GA-MLR Analysis

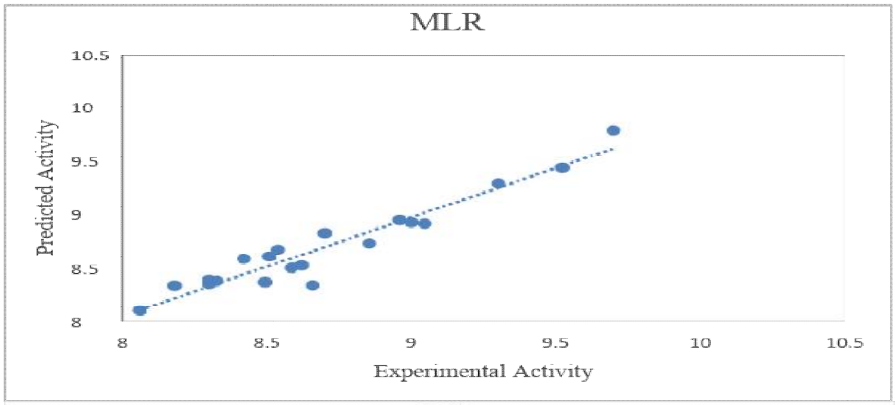
Genetic algorithm technique was employed as a selection tool to select the most relevant descriptors with respect to an objective function. The genetic algorithm (GA) starts with the creation of a population of randomly generated parameter sets. the parameters set used for the GA includes population size (160), initial terms 18%, max generation (250) and %convergences (90%), These selected subsets of variables are further evaluated by their fitness to predict inhibitory activity values. multiple linear regression analysis was performed on the training set and then, evaluated by the test set. Using genetic algorithm-multiple linear regression (GA-MLR) analysis resulted in the development of a predictive QSAR model with four descriptors with the following equation:

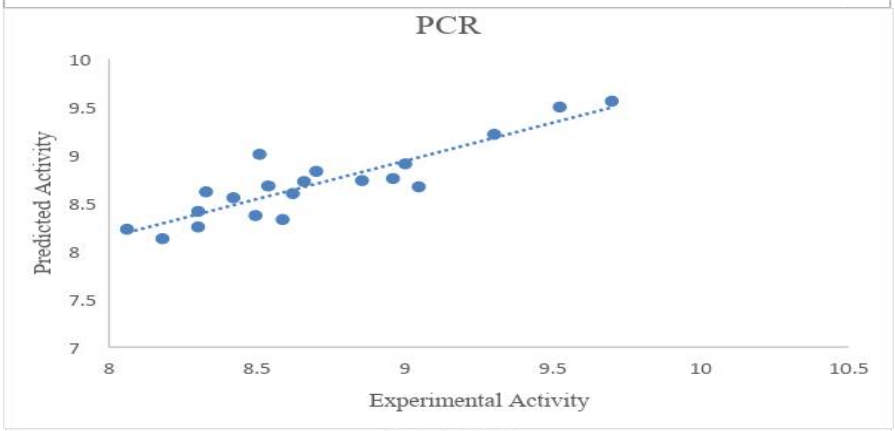
PIC50=2.072GGI7(±0.676)+4.427Ms((±0.678)+8
.047BELm6(±1.753)-0.453MOr27u(±0.187)-
14.411(±3.275)

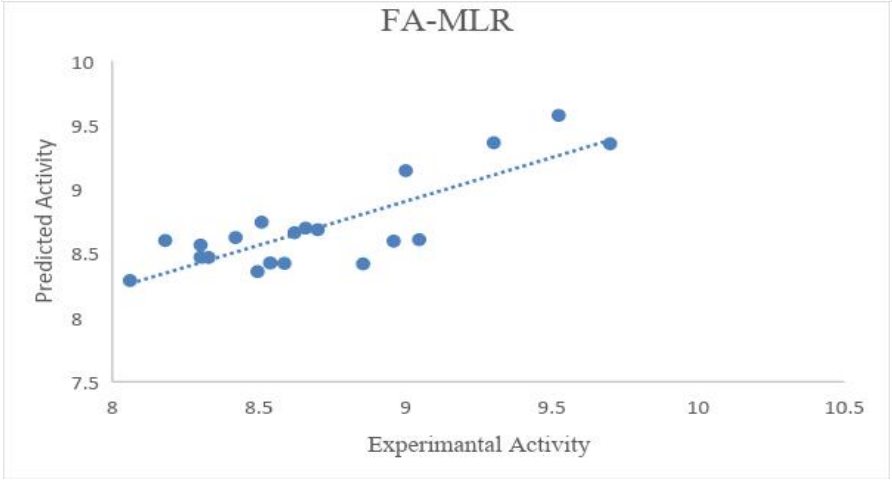
The statistical parameters of GA-MLR model are shown in Table 3.and could explain 94% of the variance and predict 88% of the variance in ($-\log IC_{50}$) data. This equation describes the effect of GGI7 (Topological charge indices), Ms (Constitutional), BELm6(Burden Eigenvalues) and MOr27U (3-D Morse Descriptors) in dpp4 inhibitory. All the descriptors have a positive coefficient except MOR27u and indicated that increase this descriptor (MOR27u) could result in decreasing PIC_{50}. Fig. 2 shows the plots of linear regression predicted versus the experimental value of the dpp4 inhibitory activity of ligand. The plots for this model show to be more convenient with R^2cv= 0.88.

Table 4. Correlation coefficient (R2) matrix for descriptors represented in multiple linear regression

	MATS1m	Ms	GGI5	DELS	Mor25m
MATS1M	1	0.413	0.649	0.712	0.077
MS		1	0.345	0.668	0.250
GGI5			1	0.859	-0.125
DELS				1	0.079
Mor25M					1







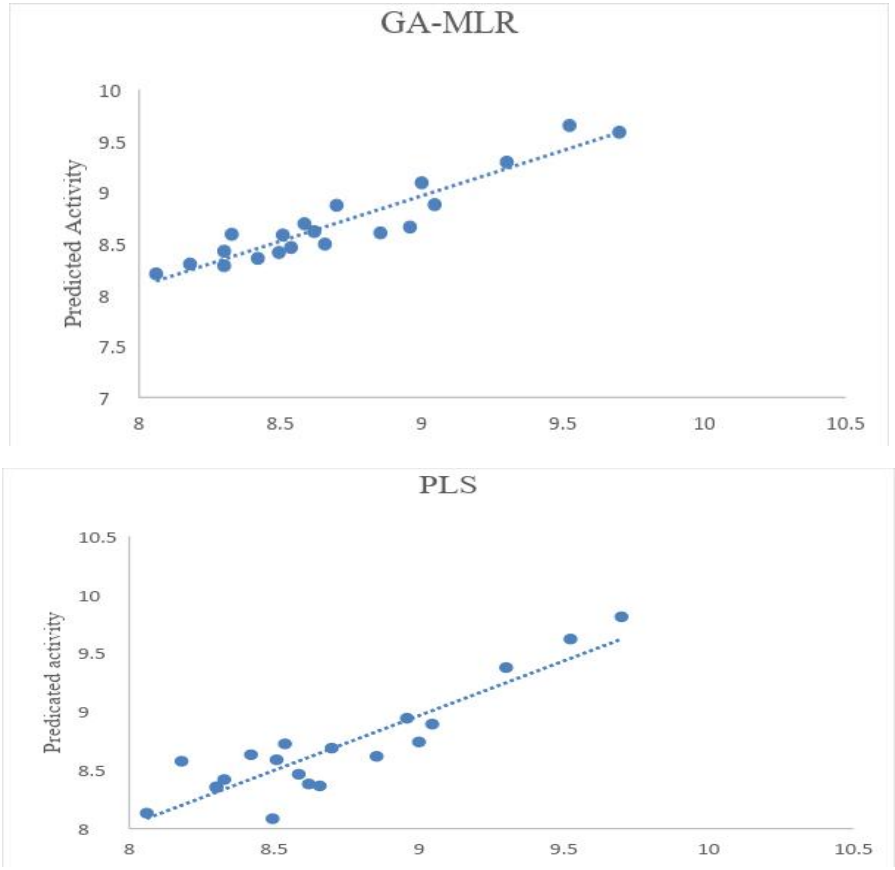


Fig. 2. Plots of the cross-validated predicted activity against the experimental activity for the QSAR models obtained by different chemometrics methods

3.5 GA-PLS Analysis

In PLS analysis, the descriptors data matrix is decomposed to orthogonal matrices with an inner relationship between the dependent and independent variables. Therefore, unlike MLR analysis, the multi collinearity problem in the descriptors is omitted by PLS analysis. Because a minimal number of latent variables are used for modelling in PLS; this modelling method coincides with noisy data better than MLR. In order to find the more convenient set of descriptors in PLS modeling, genetic algorithm was used. To do so, many different GA-PLS runs were conducted using the different initial set of populations.

The data set (n = 29) was divided into two group: calibration set (n = 20) and prediction set (n = 9). Given 20 calibration samples; the leave-one out cross-validation procedure was used to find the optimum number of latent variables for each PLS model.

The most convenient GA-PLS model that resulted in the best fitness contained 9 indices, five of them being those obtained by MLR. The PLS estimate of coefficients for these descriptors are given in Fig. 3. As it observed, a combination of Constitutional, Topological, Connectivity indices, 2D-autocorrelation, Edge adjacency indices, Topological charge indices, 3-D Morse Descriptors, WHIM Descriptors has been selected by GA-PLS to account the Dipeptidyl Peptidase-4 (DPP4) inhibitory activity of imidazolopyrimidine amides derivatives. The resulted GA-PLS model possessed a high statistical quality R^2 = 0.94 and Q^2 = 0.80. The predictive ability of the model was measured by applying to 10 external tests set molecules. The squared correlation coefficient for prediction was 0.95 and the standard error of prediction was 0.49. The values of pIC_{50} using GA-PLS model (refined from cross-validation or external prediction set) are shown in Table 1**.** This Fig. 3 describes the effect of Ms (Constitutional), X0A (Connectivity indices), MATSIM(2D-

autocorrelation), EEig09d, EEig13d (Edge adjacency indices), GGi5(Topological charge indices), Mor25m (3-D Morse Descriptors), E2M (WHIM Descriptors) and DELS (Topological) on inhibitory DPP4 activity. And also describe that X0A, EEig13d and DELS have negative coefficient on DPP4 inhibitory but the other descriptors have a positive effect on DPP4 activity. Fig. 2 shows the plots of linear regression predicted versus the experimental value of the DPP4 inhibitory activity of ligand. The plots for this model show to be more convenient with R^2cv= 0.80.

In order to investigate the relative importance of the variable appeared in the final model obtained by GA-PLS method, variable important in projection (VIP) was employed [29]. VIP values reflect the importance of terms in PLS model. According to Erikson et al. X-variables (predictor variables) could be classified according to their relevance in explaining y (predicted variable), so that VIP > 1.0 and VIP < 0.8 mean highly or less influential, respectively, and 0.8 < VIP< 1.0 means moderately influential [30].

The VIP analysis of PLS equation is shown in Fig. 4. VIP analysis shows that Ms which is constitutional descriptors, X0A as Connectivity indices descriptors, MATS1m which is 2D-autocorrelation and GGI5 which is topological charge indices parameter, are the most important indices in the QSAR equation derived by PLS analysis. In addition, the other descriptors have been found to be low influential parameters.

Table 5. Numerical values of factor loading numbers 1–7for some descriptors after VARIMAX rotation (against DPP4 inhibitory activity)

	Component						
	1	**2**	**3**	**4**	**5**	**6**	**7**
volume	.524	.262	.073	.125	.214	-.110	-.325
Ms	.252	.792	.340	.019	-.208	.198	-.119
nH	.472	-.700	.088	.075	.369	-.295	.113
STN	-.163	.087	-.754	-.030	.464	.225	.058
DELS	.753	.448	.131	.132	.283	.005	.031
X0A	.335	.038	.881	.115	.084	-.127	.040
IVDE	.122	.190	.905	.185	.145	-.012	.150
IC0	.202	.925	.173	.205	.007	.043	.079
MATS1m	.849	.054	.267	-.049	.046	.041	-.155
GATS6m	-.179	-.646	-.430	-.251	-.120	.299	-.231
EEig09d	.670	.296	.117	.081	.372	-.295	.163
EEig13d	.252	-.104	-.570	-.063	.548	-.358	.176
GGI5	.598	.295	.270	.278	.508	-.093	.119
GGI4	-.054	.093	.319	.058	.785	.052	.239
JGT	.008	.319	.842	.136	.283	.035	.135
RDF015m	.874	-.157	-.142	-.121	.021	.311	.123
Mor04m	-.029	-.482	-.330	.096	-.442	.190	.180
Mor25m	.053	-.006	-.130	-.055	-.079	-.003	-.885
Mor19m	.243	.052	-.009	-.043	-.176	.905	.179
Mor18p	.383	-.212	.282	.034	-.226	-.699	.321
E2m	.822	.165	.140	.097	-.144	-.040	-.068
HATS3e	-.304	.081	.133	.023	-.752	.080	.171
R4e	.559	-.465	.027	.139	.355	-.399	.210
ALOGP2	-.075	-.110	-.117	-.975	.006	.043	-.062
TE1	.321	.162	.195	.900	.056	-.012	-.012
TPSA(Tot)	.206	.817	-.099	.148	.346	.065	-.014
F03[C-O]	.175	-.031	-.064	-.976	-.001	.027	-.011

Table 6. Leverage (*h*) of the external test set molecules for different models. The last row (*h) is the warning leverage**

Molecular no	MLR	PCR	FA-MLR	GA-MLR	GA-PLS
5	0.13	0.07	0.049	0.37	1.09
17	0.26	0.10	0.052	0.26	0.3
19	0.13	0.20	0.048	0.20	0.65
21	0.23	0.15	0.052	0.19	0.30
23	0.089	0.25	0.047	0.32	1.0
25	0.076	0.065	0.052	0.137	0.37
28	0.129	0.23	0.047	0.114	0.52
29	0.24	0.095	0.048	0.100	0.34
31	0.3	0.105	0.048	0.10	0.48
h*	0.71	0.6	0.3	0.6	1.35

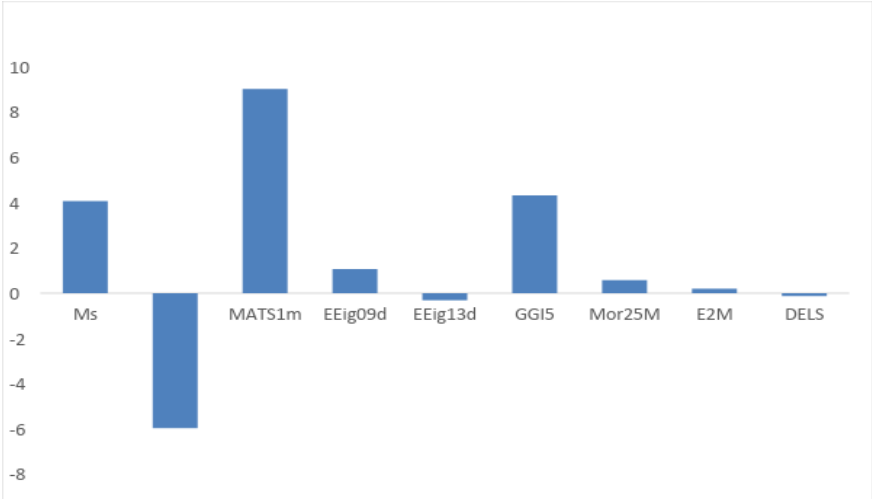


Fig. 3. Plots of the cross-validated predicted activity against the experimental activity for the QSAR models obtained by GA-PLS methods

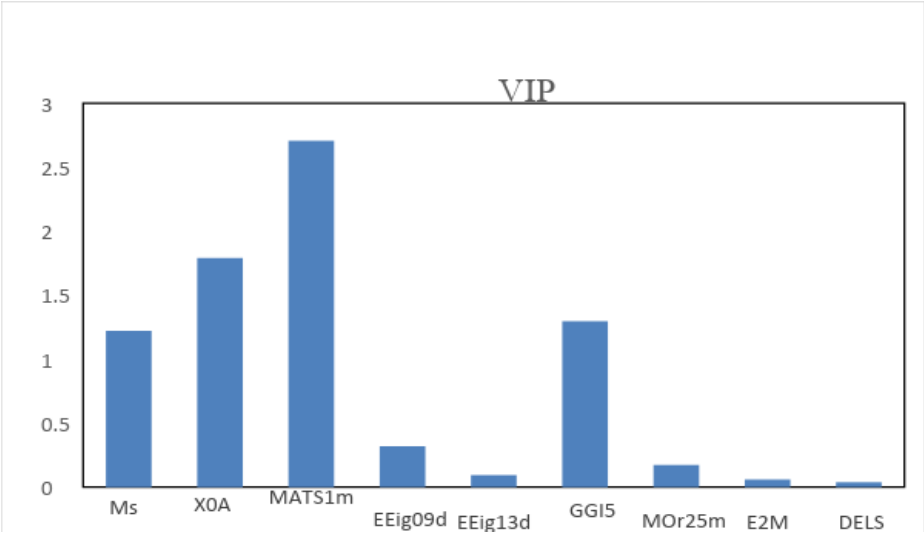


Fig. 4. Plot of variables important in projection (VIP) for the descriptors used in GAPLS model

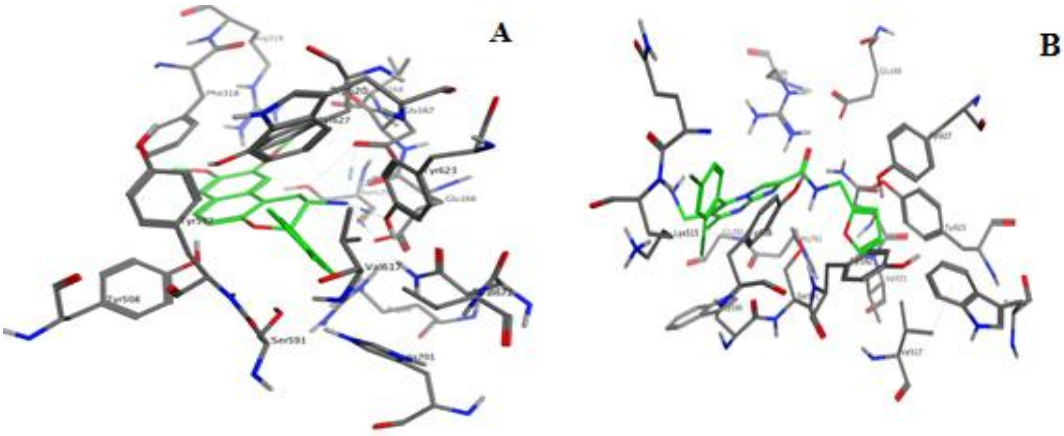


Fig. 5. Interactions of A) HL1 and B) compound 25 with the residues in the binding site of DPP4 (5j3j) receptor

3.6 Robustness and Applicability Domain of the Models

Leverage is one of the standard methods for this purpose. Warning leverage (h^*) is another criterion for interpretation of the results. The warning leverage is, generally, fixed at $3k/n$, where n is the number of training compounds and k is the number of model parameters. Leverage greater than warning leverage h^* means that the predicted response is the result of substantial extrapolation of the model and therefore may not be reliable [31]. The calculated leverage values of the test set samples for different models and the warning leverage, as the threshold value for accepted prediction, are listed in Table 6. As seen, the leverages of all test samples are lower than h^* for all models. This means that all predicted values are acceptable.

3.7 Docking Study

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays a great role in the rational design of drugs. Here, docking studies were carried out on our compounds to find their binding site, binding modes and the best direction on the base of their binding energy. Having completed the docking process, the protein– ligand complex was analyzed to investigate the type of interactions. The conformation with the lowest binding energy was considered as the best docking result in each case resource.

As it was shown in Table 1, Compounds 15, 18, 25, 26 and 28 based on their highest docking binding energy can be a good candidate for DPP-4 inhibitors.

On the other hand, promising results such as the ligand-receptor binding site and binding modes were obtained from docking analyses. The results for each ligand were compared to its corresponding co-crystal ligand.

The NH_2 group of co-crystal ligand formed H-bond interaction with amino acid, Glu 166, Glu 167 and Tyr 623 of the receptor (salt bridge) and Trifluoro phenyl group of co-crystal ligand was occupying S1 hydrophobic pocket of DPP-4 inhibitors with val 627, His701, Val617, Tyr 592, Tyr 627 and Tyr506 residue of receptor [32] and also Dimethoxy phenyl group of co-crystal ligand formed pi-pi interaction with Phe 318 of the receptor (figure 5A). in compound 25, dichloro phenyl group of ligand formed pi-pi interaction with Tyr 508 and were occupying S1 pocket of enzyme on the other side, the methyl group of imidazole pyrimidine formed arene- hydrogen interaction with Trp 590 of receptor.

4. CONCLUSION

In this study, five different QSAR modelling methods, MLR, FA-MLR, PCR, GA-PLS and GA-MLR were used in the construction of a QSAR model for DPP4 inhibitory of imidazolopyrimidine amides and the resulting models were compared. The reliability, accuracy and predictability of the proposed models were evaluated by root mean square error of cross-validation (RMSECV) and

cross-validation, the root mean square error of prediction (RMSEP). Results confirm that among the applied models, the GA-PLS is superior for the prediction of the pIC_{50} of imidazolopyrimidine amides analogues. All models represent high goodness of fit (measured by R^2), whereas that obtained from GA-PLS is significantly better than that of the other models. The cross-validation statistics reported suggested that the higher prediction ability of the GA-PLS model. This study suggests the importance of constitutional, topological, connectivity indices, 2D-autocorrelation, edge adjacency indices, topological charge indices, 3D Morse descriptors, WHIM Descriptors of molecules for imidazolopyrimidine amides derivatives. Docking study reveals and confirms that compounds 15, 18, 25, 26, and 28 are introduced as good candidates for DPP-4 inhibitors.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hansch C, Hoekman D, Gao H. Comparative QSAR: Toward a deeper understanding of chemicobiological interactions. Chem. Rev. 1996;96:1045-1076.
2. Hansch C, Maloney PP, Fujita T, Muir RM. Correlation of biological activity of phenoxyacetic acids with hammett substituent constants and partition coefficients. Nature. 1962;194:178-180.
3. Fassihi A, Sabet R. QSAR study of $p56^{lck}$ protein tyrosine kinase inhibitory activity of flavonoid derivatives using MLR and GA-PLS, Int. J. Mol. Sci. 2008;9:1876-1892.
4. Hansch T. Fujita. ρ-σ-π Analysis. A method for the correlation of biological activity and chemical structure. J. Am. Chem. Soc. 1964;86:1616-1626.
5. Sabet R, Fassihi A, Moeinifard B. QSAR study of PETT derivatives as potent HIV-reverse transcriptase inhibitors. J. Mol. Graph & Model. 2009;28:146.
6. Hansch C, Hoekman D, Gao H. Comparative QSAR: Toward a deeper understanding of chemicobiological interactions. Chem. Rev. 1996;96:1045-1075.
7. Todeschini R, Consonni V. Handbook of molecular descriptors. Wiley-VCH, Weinheim; 2000.
8. Sabet R, Fassihi A. QSAR study of Isatin analogues as *in vitro* anti-cancer agents. Eur. J. Med. Chem. 2010;45:1113.
9. Sabet R, Fassihi A, Hemmateenejad B, Saghaie L, Miri R, Gholami M. Computer-aided drug design of novel antibacterial 3-hydroxypyridine-4-ones: Application of QSAR methods based on the MOLMAP approch. Journal of Computer-Aided Molecular Design. 2012;26:349.
10. Karbakhsh R, Sabet R. Application of different chemometrics tools in QSAR Study of Azolo-adamantanes against influenza a virus. 2011;6:23.
11. Visit the Hyperchem official. Available:http://www.hyper.com
12. Todeschini R. Milano chemometrics and QSPR group. Available:http://michem.disat.unimib.it/
13. Wei Meng, Robert P. Brigance, Hannguang J. Chao, Aberra Fura. Discovery of 6-(Aminome thyl) -5-(2,4-dichlorophe nyl) -7-methy limidazo[1,2-a] pyrimidine-2-carbox amides as Potent, Selective Dipeptidyl Peptidase-4 (DPP4) Inhibitor s. J. Med. Chem. 2010;53:5620–5628.
14. Morris GM, Huey R, Olson AJ. Using auto dock for ligand-receptor docking. Curr Protoc Bioinformatics. 2008;(Chapter 8): Unit 8.14.
15. Hikisz P, Szczupak Ł, Koceva-Chyła A, Oehninger L, Ott I, Therrien B, et al. Anticancer and antibacterial activity studies of gold (I)-alkynyl chromones. Molecules. 2015;20:19699-718.
16. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen MY, et al. Comparative protein structure modeling using modeller. Curr Protoc Bioinformatics. 2006;(Chapter 5):Unit 5.6.
17. Sakhteman A. PreAupos SOM. Available:https://www.biomedicale.univ-paris5.fr/aupossom/
18. Leardi R. Application of genetic algorithm-PLS for feature selection in spectral data sets. J. Chemomtr. 2000;14:643-655.
19. Siedlecki W, Sklansky J. On automatic feature selection. Int. J. Pattern Recog. Artif. Intell. 1988;2:197-220.
20. Schmidi H. Multivariate prediction for QSAR. Chemom. Intell. Lab. Sys. 1997;37: 125-134

21. Hansch C, Kurup A, Garg R, Gao H. Chem-bioinformatics and QSAR. A Review of QSAR Lacking Positive Hydrophobic Terms. Chem. Rev. 2001;101:619-672.
22. Humphrey W, Dalke A, Schulten K. VMD: Visual molecular dynamics. Journal of Molecular Graphics. 1996;14(1):33-8.
23. Fereidoonnezhad M, Faghih Z, Mojaddami A, Sakhteman A, Rezaei Z. A comparative docking studies of dichloroacetate analogues on four isozymes of pyruvate dehydrogenase kinase in humans. Indian J Pharm Educ. 2016;50(2):S32-S8.
24. Mirjalili BF, Zamani L, Zomorodian K, Khabnadideh S, Haghighijoo Z, Malakotikhah Z, et al. Synthesis, antifungal activity and docking study of 2-amino-4H-benzochromene-3-carbonitrile derivatives. Journal of Molecular Structure. 2016; 1116:102-8.
25. Li Z, Gu J, Zhuang H, Kang L, Zhao X, Guo Q. Adaptive molecular docking method based on information entropy genetic algorithm. Applied Soft Computing. 2015;26:299-302.
26. Feng J, Ablajan K, Sali A. 4-Dimethy-laminopyridine-catalyzed multi-component one-pot reactions for the convenient synthesis of spiro[indoline-3,4′-pyrano[2,3-c]pyrazole] derivatives. Tetrahedron. 2014; 70(2):484-9.
27. Fassihi A, Abedi D, Saghaie L, Sabet R, Fazeli H, Bostaki G, Deilami O, Sadinpour H. Eur. J. Med. Chem; 2008. DOI: 10.1016/j.ejmech.2008.10.022
28. Sharaf MA, Illman DL, Kowalski BR. Chemometrics. New York: Wiley. 1986; 332.
29. Olah M, Bologa C, Oprea TI. An automated PLS search for biologically relevant QSAR descriptors. J. Comput. Aided Mol. Des. 2004;18:437-449.
30. Mohajeri A, Hemmateenejad B, Mehdipour A, Miri R. Modeling calcium channel antagonistic activity of dihydropyridine derivatives using QTMS indices analyzed by GA-PLS and PC-GA-PLS. J. Mol. Graph. Model. 2008;26:1057-1065.
31. Brereton R. Chemometrics data analysis for the laboratory and chemical plant. Wiley. 2004;47–54.
32. Liu Y, Liu T. Recent in non-peptidomimetic dipeptidyl peptidase 4 inhibitors: Medicinal chemistry and preclinical aspects. Current Medicinal Chemistry. 2012;19:3982-3999.

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