Determination of FSH-receptor antagonistic property of 6-amino-4-phenyltetrahydroquinoline derivatives by employing QSDAR method

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ABSTRACT

This work presents prediction of observed antagonist activity of 6-amino-4-phenyltetrahydroquinoline derivatives, using chemical environment data. Based on general interaction properties function a quantum mechanical *ab initio* calculations were employed to optimize their molecular electronic structures. A series of carbon chemical shifts descriptors were computed using the Gaussian98 software. Relevance descriptors were explored and a reliable QSDAR model was achieved using multiple linear regression method (MLR). The developed model was confirmed by applying some trustworthy tests. The results reveal that the carbon NMR chemical shift of C4, δ_{C4} which directly affects by the 4-phenyl ring and its corresponding substitutions, shows a major contribution to govern the observed property of pIC$_{50}$ of the 6-amino-4-phenyltetrahydroquinoline derivatives. The model proposes that the low electronic charge symmetry around the C$_4$ is more favorable for the FSH receptor antagonistic activity of 6-amino-4-phenyltetrahydroquinoline derivatives, implying positive effect of hydrophobicity on their FSH receptor antagonistic activity.
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INTRODUCTION

Substituted 6-amino-4-phenyl-tetrahydroquinoline derivatives are described that are antagonists for the G$_s$-protein-coupled human follicle-stimulating hormone (FSH) receptor. These compounds show high antagonistic efficacy *in vitro* using a CHO cell line expressing the human FSH receptor. This antagonist also showed a submicromolar IC$_{50}$ in a more physiologically relevant rat granulosa cell assay and was found to significantly inhibit follicle growth and ovulation in an *ex vivo* mouse model. This compound class may open the way toward a novel, non-steroidal approach for contraception[1].

In a SAR study done by E. Manivannan et al., among the 6-amino-4-phenyltetrahydroquinoline derivatives as antagonists for the Gs-protein-coupled human follicle-stimulating hormone (FSH) receptor activity of ligands derivatives it have demonstrated that, the correlation between three descriptors of ClogP, amid linkage of Y=NH-(CO), indicator I$_{sub[R1]}$ and observed FSH receptor antagonistic activity is excellent in terms of its statistical parameters provided 84.3 percent variance in FSH receptor antagonistic activity among the 19 compounds[2]. They have revealed that unsubstituted

4-phenyl ring of tetrahydroquinolines as well as hydrophobicity is conductive to FSH -receptor antagonistic activity among the all congeners. In order to explain the results, they have claimed that a hydrophobic type of interactions as dominant intermolecular interaction between these ligands and FSH receptor governs its activity as antagonists. Furthermore, unsubstituted 4-phenyl ring of tetrahydroquinoline is favorable for FSH receptor antagonistic activity.

Concerning the role of hydrophobicity 6-amino-4-phenyl-tetrahydroquinoline derivatives, in our previous work[3], we have conducted a quantum mechanical detail studies and shown that the inter- and intra-molecular proton transfer govern the hydrophobicity. While the former tends to decrease hydrophobicity, the latter show an increasing effect as involving the lone pairs inside the 6-amino-4-phenyltetrahydroquinoline derivatives.

Herein, we will perform a quantum mechanical insight study to construct a quantitative relationship between FSH -receptor antagonistic activity and electronic structure of all congeners. Results will help one to have better understanding on interactions of 6-amino-4-phenyltetrahydroquinoline derivatives and the target molecule leading to synthesis of more efficient antagonists for Gs-protein-coupled human FSH-receptor.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most important techniques, which has long been frequently used not only for the structural elucidation of simple and complicated molecules but also for the detailed explanation of some important processes, including structural configuration, reaction mechanisms, molecular dynamics, chemical equilibrium, structural genomics, and even three-dimensional structures[4–7]. In addition, NMR is a non-destructive technique; that is, compounds may always be recovered. Since the chemical information in NMR spectroscopy is encoded in the form of chemical shift, intensity, and multiplicity[8]. In NMR spectroscopy data, the tensor of chemical shift is mainly composed of two terms, such as diamagnetic and paramagnetic, for which, the diamagnetic term is directly related to the electrostatic potential at the nucleus, whereas the paramagnetic term is mainly dependent upon the orbital configuration[9]. In the case of carbon NMR data, there are sufficiently large differences between these two diamagnetic and paramagnetic terms. This suggests that the spectral regions of

the orbital configurations for different carbon atoms can be separated very easily from each other.

The carbon NMR data of a compound provides a specific pattern of frequencies that corresponds directly to the quantum mechanical properties of the nucleus of each carbon atom present in that molecule. Thus, the attached and adjacent carbon atoms must have to show a significant effect on the carbon NMR chemical shift. It is important to note that the carbon NMR chemical shifts have been successfully used to predict the chemical structure of compounds and vice versa[9-11].

A number of computational programs are also commercially available, which can easily and accurately predict the carbon NMR spectra of the chemicals. By considering the features of carbon NMR spectroscopy as well as the recent advancement in this area, its chemical shifts can be used successfully as a molecular descriptor for QSAR/QSPR modeling. The development of QSAR/QSPR models using carbon NMR chemical shifts as descriptor is usually referred to as the quantitative spectrometric data-activity relationship (QSDAR). Thus, models developed between the carbon NMR spectral data of a set of molecules and their biological activity/chemical reactivity or physical characteristics are considered as QSDAR models. In the past two decades, the potential use of carbon NMR chemical shifts as input parameters to develop models for biological activities, chemical reactivity, and physical properties has successfully been demonstrated by extensive publications. It has been observed from the previous results that the QSDAR model using chemical shifts of carbon NMR works very well when attempted on a set of compounds with a large proportion of carbon nuclei or on similar structural motifs[12].

One of the major advantages of using the carbon NMR chemical shifts as input parameters in the development of QSDAR models is that, it is a very sensitive technique, such that a very small change in the electronic structure can be clearly observed as major changes in the spectra. The importance of the QSDAR methodology has further been promoted by some patents[13-15].

In present work, attempts were made to build a proper QSDAR model to predict the observed antagonist activity of 6-amino-4-phenyltetrahydroquinoline derivatives, using their parent molecules' carbon NMR

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chemical shifts. Multiple linear regression (MLR) analysis was employed to detect the relevant carbon NMR chemical shifts governing the observed antagonist activity of 6-amino-4-phenyltetrahydroquinoline derivatives.

METHOD

Computer hardware and software

All calculations were run on a 2.5 GHz Intel® Core™2 Quad Q 8300 CPU with 2 GB of RAM using all four available cores under Windows XP operating system. Modeling and geometry optimization of all molecular structures (Z-matrix) were employed by HyperChem (version 7.1, HyperCube, Inc.)[16]. For full optimization of molecular structures and calculation of quantum chemical descriptors, Gaussian 98 software was used. Variables selection was performed by using SPSS software version 11.5.0 (SPSS Inc., 2001) by employing the multiple linear regression method.

Quantum chemical computations

Selection of appropriate electronic features that are significantly related to the property of interest is highly important for predictive QSDAR model. The descriptors can be chosen using domain knowledge about the examined property, or mathematical methods for the selection of descriptors can be applied. The experimental values of observed activity of nineteen 6-amino-4-phenyltetrahydroquinoline derivatives (TABLE 1) were taken from ref.[2]. We draw molecular structures in HyperChem Software and pre-optimized each molecule in it using semiemperical method of AM1 as prior step[16]. Then, we calculated carbon NMR chemical shifts (δ_C), using HF/3-21G nmr=giao keyword. The descriptor can be obtained as:

$$\delta_C = ICS_{reference} - ICS \qquad (1)$$

Where $ICS_{reference}$ and ICS are referred to isotropic chemical shielding of tetra methyl silan as reference and the isotropic chemical shielding of any carbon on the parent molecule (Figure 1). The values of all carbon NMR chemical shifts descriptors, we could obtain using *ab initio* calculation are provided in supporting information.
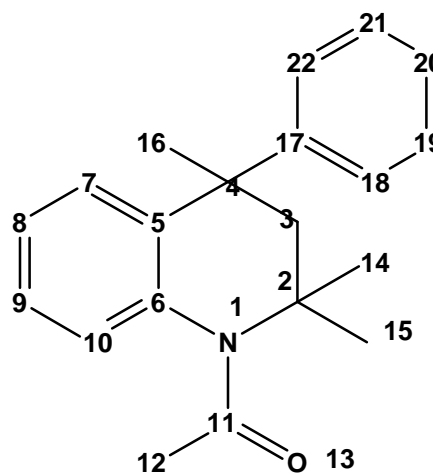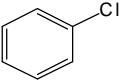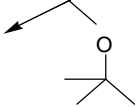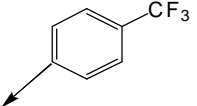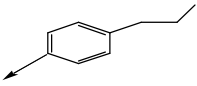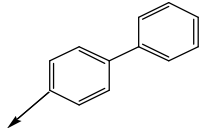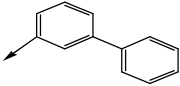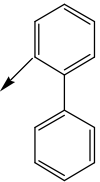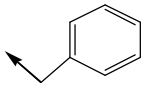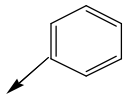

Figure 1 : The relative position of carbon atoms in the parent molecule of 6-amino-4-phenyltetrahydroquinoline which are subjected in carbon chemical shift calculations
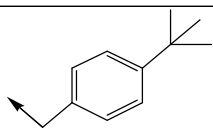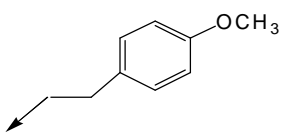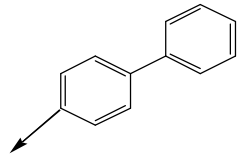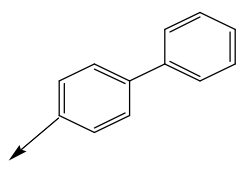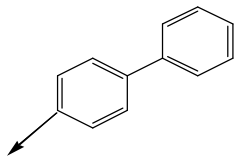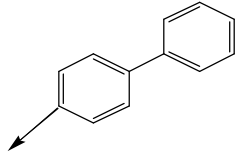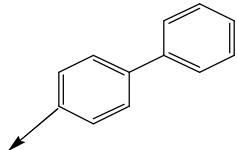
RESULTS AND DISCUSSION

A series of nineteen structures of 6-amino-4-phenyltetrahydroquinoline derivatives (TABLE 1) were optimized and their corresponding carbon NMR chemical shift descriptors were calculated. In order to select the most relevant variables, a data matrix of twenty calculated carbon NMR chemical shift data was subjected to stepwise selection procedure, which combines the forward selection and backward elimination approaches. This procedure considers first the descriptive variable most highly correlated with the response. If the inclusion of this variable results in a significant improvement of the regression model, evaluated with an overall *F*-test, it is retained and the selection continues. In a next step the variable that gives the largest significant decrease of the regression sum of squares, evaluated with a partial F-test, is added. After each forward selection step a backward elimination step is performed. In this step a partial *F*-test for the variables, already in the equation, is carried out. If a variable is no longer contributing significantly to the regression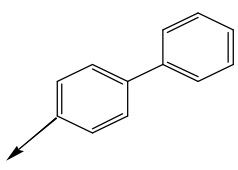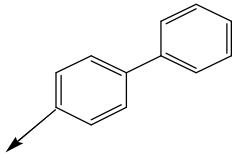 model, it is removed. The procedure stops at the moment that no variables fulfill the requirements anymore to be removed or entered. After this selection procedure classical MLR can be applied on the retained variables to build a predictive model.

The stepwise variable selection-based MLR analysis led to development of following tetra-carbon NMR shifts equation:

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TABLE 1 : Observed (ref.14) and predicted activity values of 6-amino-4-phenyltetrahydroquinoline derivatives

NO.	X	Y	R	R$_1$	R$_2$	pIC50-Expt.	pIC50-Calc.	Abs(Res.)
1	NH	CO		H	H	7.51	7.40	0.11
2	NH	CO		H	H	7.12	7.08	0.04
3	NH	CO		H	H	7.57	7.48	0.09
4	NH	CO		H	H	8.16	8.04	0.12
5	NH	CO		H	H	8.30	8.08	0.22
6	NH	CO		H	H	7.55	7.02	0.53
7	NH	CO		H	H	8.05	7.76	0.29
8	NH	CO		H	H	7.60	7.17	0.43
9	NH	SO2		H	H	5.55	6.37	0.82
10	NH	CONH		H	H	6.24	5.85	0.39

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NO.	X	Y	R	R_1	R_2	pIC50-Expt.	pIC50-Calc.	Abs(Res.)
11	NH	Bond		H	H	6.03	6.15	0.12
12	CO	NH		H	H	5.73	5.73	0.00
13	NH	CO		4-Me	H	7.30	8.03	0.73
14	NH	CO		2-OMe	H	6.77	7.36	0.59
15	NH	CO		4-OH	H	6.62	6.61	0.01
16	NH	CO		H	7-Me	8.30	7.86	0.44
17	NH	CO		H	8-OMe	7.52	7.79	0.27
18	O	CO		H	H	7.27	7.01	0.26
19	O	CH2		H	H	6.78	6.86	0.08

$$pIC_{50} = -0.826\delta_{c4}(\pm0.168) + 0.022\delta_{c8}(\pm0.006) + 0.007\delta_{c12}$$
$$(\pm0.002) - 0.039\delta_{c5}(\pm0.014) + 38.42(\pm6.522) \qquad (2)$$
$$N=19, R^2=0.787, SE=0.437, F=12.93, Q^2_{Loo}=0.641,$$
$$Q^2_{LMo}=0.631$$

The values in the parenthesis represent the standard deviation of the coefficients. The symbols N, R^2, SE and F are number of components, correlation coef-

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ficient, standard error of regression and Fisher's F-ratio, respectively.

In order to rely on the obtained model it should be noted that, an excellent model should provide excellent statistics, excellent predictive power as well as stability. Moreover the model should not suffer from co-linearity problem. Concerning the equation (2), the correlation
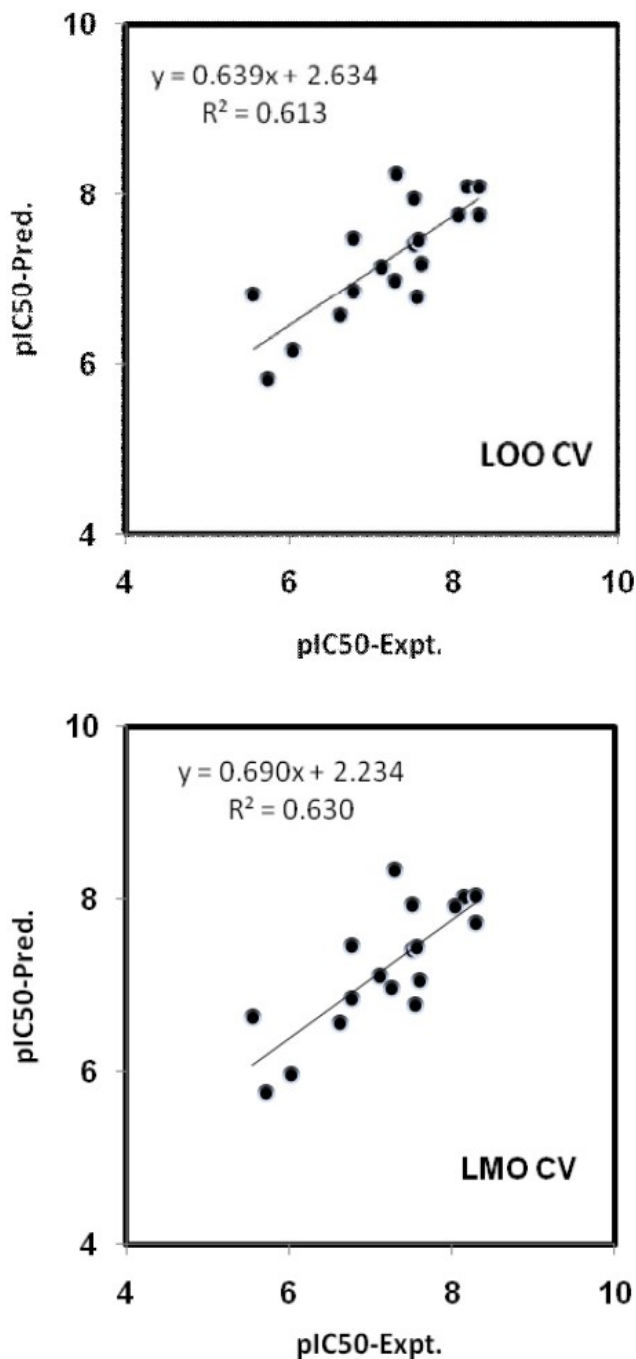

Figure 2 : Plot of internal tests of leave-one-out and leave-many-out cross-validation observed activity of 6-amino-4-phenyltetrahydroquinoline derivatives

coefficients of leave-one-out and leave-many-out cross-validations are denoted by Q^2_{LOO} and Q^2_{LMO}, respectively. The R^2 value of 0.787 describes that the resultant equation can explain about 78.7 percent of variance in the pIC_{50} data of the 6-amino-4-phenyltetrahydroquinoline derivatives whereas the high value of cross-validated correlation coefficients $Q_{LOO} = 0.614$, $Q_{LMO} = 0.631$, and also their closeness to each other explain the predictive power and stability of the proposed model (Figure 2). The widely used approach to establish the robustness of the resulting models is called Y- randomization. The values of pIC_{50} were randomly attributed to the molecules and the MLR modeling was repeated with the randomized data. The randomization was repeated one hundred times and the maximum values obtained for the R^2 and RMS were 0.49 and 0.43 respectively. The statistical qualities of these models are much lower than the original one. Therefore, it can be considered that our model is reasonable and had not been obtained by chance.

The variance inflation factor (VIF) is another test indicating that the model reaches the statistical requirements. The VIF is defined as below:

VIF=1/(1-R_i^2) (3)

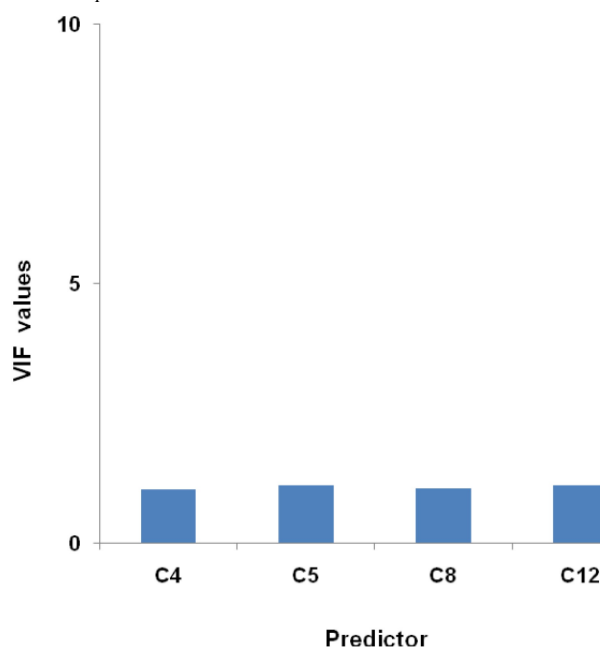Where R_i is the multiple correlation coefficients of the

Figure 3 : The relative VIF values of any of predictor contributed in the obtained model in this work

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*i*th independent variables on all other independent variables. Thus, a VIF is defined for each variable in the equation, not for the equation as a whole, and all the VIF values should be less than 10. The maximum values for this model is around 1.11, and thus much less than 10, confirming that this model achieves the statistical requirements and that there is no co-linearity problem (see Figure 3).

This model was used to reproduce the pIC$_{50}$ of 6-amino-4-phenyltetrahydroquinoline derivatives. Results are shown in TABLE 1.

In order to inspection of the relative importance and contribution of each descriptors in the constructed model, the value of mean effect (MF) was calculated for each descriptors by the following equation and it is shown in Figure 4.

$$MF_j = \frac{\beta_j \sum_{i=1}^{n} d_{ij}}{\sum_{i=1}^{m} \beta_j \sum_{i=1}^{m} \sum_{i=1}^{n} d_{ij}} \qquad (4)$$

Where MF$_j$ is the mean effect for considered descriptor j, β_j is the coefficient of descriptor j, d$_{ij}$ denotes the value of descriptor j of molecule i, m is the number of descriptors in the model and n is the number of molecules in the data sets. The value of mean effect shows the relative contribution of each descriptor on the predicted response.

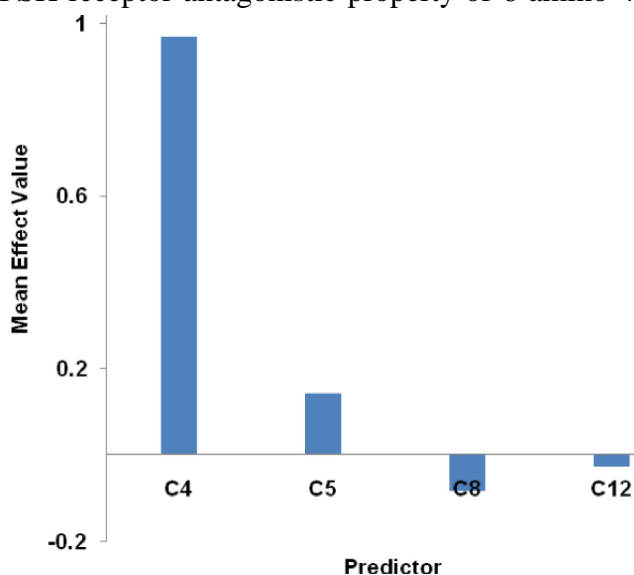The resulted Eq.(2) specifies that the observed FSH-receptor antagonistic property of 6-amino-4-

Figure 4 : Relative contribution of descriptors in observed activity of 6-amino-4-phenyltetrahydroquinoline derivatives

phenyltetrahydroquinoline derivatives pIC$_{50}$, is mainly controlled by carbon NMR chemical shifts of four carbons of C$_4$, C$_5$, C$_8$ and C$_{12}$ (see the Figure 1). While the δ_{c4} and δ_{c5} show decreasing effect, the δ_{c4} and δ_{c5} have increasing contribution, (see the Eq.(2)). In the other word, since the value of carbon NMR chemical shifts of all four carbons are positive, it seems that the charge shift around the C$_8$ and C$_{12}$ and electron charge symmetry around the C$_4$ as well as C$_5$ decrease the observed property of pIC$_{50}$ leading to more effective 6-amino-4-phenyltetrahydroquinoline derivatives. Our results also reveal that among the all four carbon NMR chemical shift descriptors, δ_{c4} which directly affects by the 4-phenyl ring and its corresponding substitutions, shows a major contribution to govern the observed property of pIC$_{50}$ of the 6-amino-4-phenyltetrahydroquinoline derivatives (see the Figure 4). Therefore, the model proposes that the electronic charge symmetry around the C$_4$ (low δ_{c4} value) is more favorable for the FSH receptor antagonistic activity of 6-amino-4-phenyltetrahydroquinoline derivatives. This finding is along with the result reported by E. Manivannan et al.[2] where they proposed that an unsubstituted 4-phenyl ring of the tetrahydroquinolines scaffold is favorable for their FSH receptor antagonistic activity. Their result supports our finding, since the unsubstituted 4-phenyl ring intact the electron charge symmetry around the C$_4$. This subsequently, decreases the δ_{c4} value which is favorable for the FSH receptor antagonistic activity pIC$_{50}$, as our model concerns.

CONCLUSION

Quantum mechanical *ab initio* calculations were employed to optimize nineteen structures of 6-amino-4-phenyltetrahydroquinoline derivatives as antagonists for the Gs-protein-coupled human follicle-stimulating hormone (FSH) receptor. Twenty carbon NMR shifts data on any of parent molecule were calculated. Carbon NMR descriptors affect the observed property of 6-amino-4-phenyltetrahydroquinoline derivatives pIC$_{50}$, were explored using multiple linear regression method. A proper model, verified with many reliable tests, was achieved. It was find that the C4 adjacent with 4-phenyl ring play a major role on governing of FSH receptor antagonistic activity of the under consideration de-

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rivatives. Although C4 might be little far from the active site of amid linkage NH-(CO), it seems that a kind of strong allosteric effect (indirect interaction) from hydrophobia head of 6-amino-4-phenyltetrahydroquinoline derivatives around the C4 position might be happening.

LIST OF SYMBOLS

δ_C	:	Carbon chemical shift
N	:	Number of components
R^2	:	Correlation coefficient
F	:	Fisher's F-ratio
Q^2_{LOO}	:	Correlation coefficients of leave-one-out cross-validations
Q^2_{LMO}	:	Correlation coefficients of leave-many-out cross-validations
pIC_{50}	:	Amount of observed property of 6-amino-4-phenyltetrahydroquinoline derivatives
MF_j	:	Mean effect value for considered descriptor of j
β_j	:	Coefficient of descriptor j
d_{ij}	:	The value of descriptor j of molecule i

Subscripts

LOO	:	Leave one out
LMO	:	Leave many out
i	:	Stand for *i*-th descriptor
j	:	Stand for *j*-th descriptor
c	:	carbon atom

Superscripts

m	:	Number of descriptors in the model
n	:	Number of molecules in the data sets

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