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QSAR Study of Flavonoids as Platelet-Activating Factor (PAF) Receptor Binding Antagonists

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ABSTRACT

Flavonoids are natural inhibitors against platelet activating factor (PAF) receptor. PAF is involved in several patho-physiological conditions such as inflammation, atherosclerosis, asthma and thrombosis. The PAF actions can be inhibited by blocking its receptor with an antagonist. The aim of this study was to find the structure activity relationship between the PAF receptor binding antagonist activity and the structure based properties of flavonoids **1-11** by using quantum chemical quantitative structure activity relationship (QSAR) approach. The semi-empirical quantum chemical method was used to optimize the molecular structures of the flavanoids and various quantum chemical descriptors were calculated from the optimized structures. Heuristic and multi-linear regression methods were used to determine the correlation between the quantum chemical properties and the experimental PAF receptor binding inhibitory activity. The best correlation equation was obtained using multi-linear correlation method with three descriptors and correlation coefficient, R^2= 0.9632.

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INTRODUCTION

Platelet activating factor (PAF) (1-*O*-hexadecyl/octadecyl-2-acetyl-sn-glycero-3-phosphocholine) [1] is a potent phospholipid mediator. This protein is formed by several different cells such as eosinophils, macrophages, platelets, neutrophils and vascular endothelial cells. Its function was to transmit signals between neighboring cells and acts as a hormone, cytokines, and other signaling molecules [2]. The PAF unregulated signaling system however, can trigger inflammatory and thrombotic cascades, amplify these cascades when acting with other mediators, and mediate molecular and cellular interactions (cross talk) between inflammation and thrombosis.

This biologically active phospholipid involved in several pathophysiological condition such as inflammation, atherosclerosis [3] asthma [4] and thrombosis [5]. PAF is also involved in tissue injury during endotoxemia[1] and also participate in several physiological responses including attraction and activation of leucocytes [6], increased in vascular permeability [7], cardiovascular actions, [8], effects in liver [9] and the central nervous system. Platelets could be activated by stimuli such as thrombin to synthesis and secrete PAF[3]. The attachment of PAF to it receptor form a series of intracellular signals to finally cause pathophysiological and physiological processes. The PAF actions could be prevented by blocking its receptor with an antagonist. Thus, it is important to find a compound(s) that could act as the PAF receptor binding antagonist to prevent a variety of inflammation, respiratory, immunological and cardiovascular disorder [10].

One of the natural PAF antagonistsisginkgolides from *Ginkobiloba*[11]. Three quantitative structure activity relationship (QSAR) approaches*i.e*CoMFA, CoMSIA, and HQSAR, were used to investigate the relationship between 117 ginkgolide analogues and their bioactivities againstPAF receptor. The models revealed that steric, electrostatic, hydrophobicity, and individual atoms affect molecular bioactivity as antagonists of PAF.

However, ginkgolides has a cage skeleton consisting of six five-membered rings, therefore, are very tough to be synthesized and they have their specific mode of action against selected pathophysiological condition cause by PAF.

Flavonoids, another natural inhibitor against PAF receptor [12] could be found in many plant species and have simpler skeleton, which is easy to synthesize and modify. Hence an investigation of the relationship

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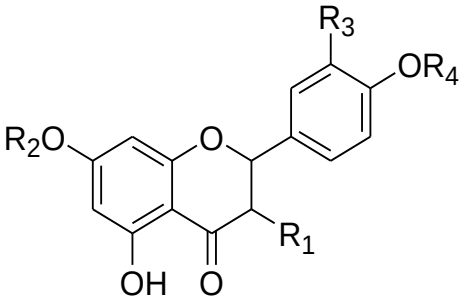
between the flavonoids analogues structures and their activity as the PAF receptor binding antagonist, embarked in this research, so that the understanding derived from this study can be used to predict and synthesize highly active flavonoids.

MATERIALS AND METHODS

The structure of flavonoids **1-11** and their PAF receptor binding inhibitory activity were obtained from literature [12]. The data were expressed as IC_{50}(μM)and converted into log IC_{50} in order to improve the normal distribution of the experimental data points.



Aromadendrin 4'-methyl ether (**1**): R_1=OH; R_2,R_3=H; R_4=CH_3
Eriodictyol 7,4'-dimethyl ether (**2**); R_1=H; R_2,R_4=CH_3; R_3=OH
Quercetin 7,4'-dimethyl ether (**3**): R_1=OH, R_3=H; R_2, R_4=CH_3
Naringenin 4'-methyl ether (**4**): R_1, R_2, R_3=H; R_4=CH_3
Kaempferol 4'-methyl ether (**5**): R_1=OH, R_2,R_3=H; R_4=CH_3
Quercetin 3-*O*-rutinoside (**6**): R_1=*O*-glu(6→1) rham; R_2, R_4=H, R_3=OH
Kaempferol 3-*O*-rutinoside (**7**): R_1=*O*-glu(6→1) rham; R_2,R_3,R_4=H
Taxifolin 4'-methyl ether (**8**): R_1, R_3=OH; R_2=H; R_4=CH_3
Taxifolin 7-methyl ether (**9**): R_1, R_3=OH; R_2=CH_3; R_4=H
Quercetin 4'-methyl ether (**10**): R_1, R_2=OH; R_3=OH; R_4=CH_3
Quercetine 7-methyl ether (**11**): R_1=OH, R_4=H; R_2=CH_3; R_3=OH

Fig. 1: Structure of Flavonoids **1-11.**

The structures (Figure 1) were drawn using Chem3D ultra 7 software. Their initial geometry were optimized using molecular mechanics methods and the resultant structures were subjected to quantum mechanical self-consistent field (SCF) calculations usingMOPACsoftware [13] and RM1 Semi empirical quantum chemical method [14]. Gradient norm limit of 0.0 kcal/Åwas used as stopping criteria for optimized structures. The optimized geometries along with the molecular orbital and vibrational frequency informationwere be used to calculate over 400 molecular descriptors containing constitutional, topological, geometrical, charge-related, semi empirical and thermo-dynamical descriptors usingCODESSA software [16]. The CODESSA software v2.6 were used to calculate descriptors from the produced electronic structural data and finally to get linear correlations between the calculated and experimental PAF receptor binding inhibitory activity values using either best multi linear (BMLR) or Heuristic method.

Among the several possible QSAR models, the best QSARcould be determined from several statistical criteria such as R^2 and the F value. Cross validation is one of the model validation techniques to internally check the on the derived model. It gives the estimation of the predictive power of the model to predict the values of the untested compounds. It was performed in this study using the leave-one-out method where one compound is removed from the data set and its activity is predicted using the model derived from the rest of the data set [18]. The corresponding squared cross-validated correlation coefficient (R^2_{cv}) for the selected model is calculated automatically by the validation module implemented in CODESSA software v 2.6 [16].

RESULTS AND DISCUSSION

QSA Rmodeling:

From the Heuristic method, thirteen linear calculations were obtained (Table 1), where as the best multi linear calculation gave five linear correlations (Table 2). Over fitting phenomenon occurs when exceedingly large number of descriptors was used in the generation of the best multi linear model for a given property [15]. The over fitting was avoided by the procedure called break point technique as illustrated in Fig. 2 that shows the change in slope in the plot of R^2 versus the number of descriptors added [16]. If the addition of a new descriptor leads to only a small (<0.02) increase in the correlation coefficient, then the added descriptor is adding very little information to the QSAR model. The procedure was stopped when the difference between R^2 of the two consequent regression equations was less than or equal 0.02 [16]. This method managed to avoid over fitting of the regression equations by monitoring the increase of R^2 in the equations with successive number of descriptors involved. Hence, the three descriptors correlation obtained using best multi linear method was selected as the best QSAR model and the parameters for this equation (Eq. 1) is given in Table 3.

Table 1: The statistical parameters for the QSAR models selected using Heuristic method for flavonoids **1-11.**

No.	R^2	F	S^2	R^2_{cv}	No. of descriptor
1	0.9777	43.8808	0.0013	0.9340	5
2	0.9776	43.6726	0.0013	0.9236	5
3	0.9771	42.6824	0.0014	0.9068	5
4	0.9769	42.2275	0.0014	0.9300	5
5	0.9763	41.1531	0.0014	0.9173	5
6	0.9762	41.0771	0.0014	0.9398	5
7	0.9760	40.6914	0.0014	0.9070	5
8	0.9759	40.4520	0.0014	0.8982	5
9	0.9756	40.0457	0.0015	0.8912	5
10	0.9748	38.6960	0.0015	0.9078	5
11	0.9671	44.0566	0.0016	0.9102	4
12	0.9482	42.7007	0.0022	0.8853	3
13	0.9644	40.6386	0.0018	0.9018	4

Table 2: The statistical parameters for the QSAR models selected using BML method for flavonoids**1-11**.

No.	R^2	F	S^2	R^2_{cv}	No. of descriptor
1	0.8233	18.6362	0.0015	0.6650	2
2	0.9632	61.0291	0.0012	0.9279	3
3	0.9761	61.3906	0.0012	0.9416	4
4	0.9792	47.0306	0.0016	0.9356	5
5	0.9800	32.7146	0.0066	0.4544	6

Table 3: The coefficients, statistical errors and the t-test values for thebest nonlinear three descriptorQSAR models selected using BML method for flavonoids**1-11.**

S/N	X	±ΔX	t-test	Descriptor
0	-6.1354e+01	7.3171e+00	-8.3850	Intercept
1	1.8592e-01	1.5696e-02	11.8450	Total dipole of the molecule (td)
2	-1.5194e-01	3.6146e-02	-4.2035	Total molecular 2-center resonance energy/no of atoms (tm)
3	6.9251e+00	8.6434e-01	8.0120	Minimum e-n attraction for a H atom (me)

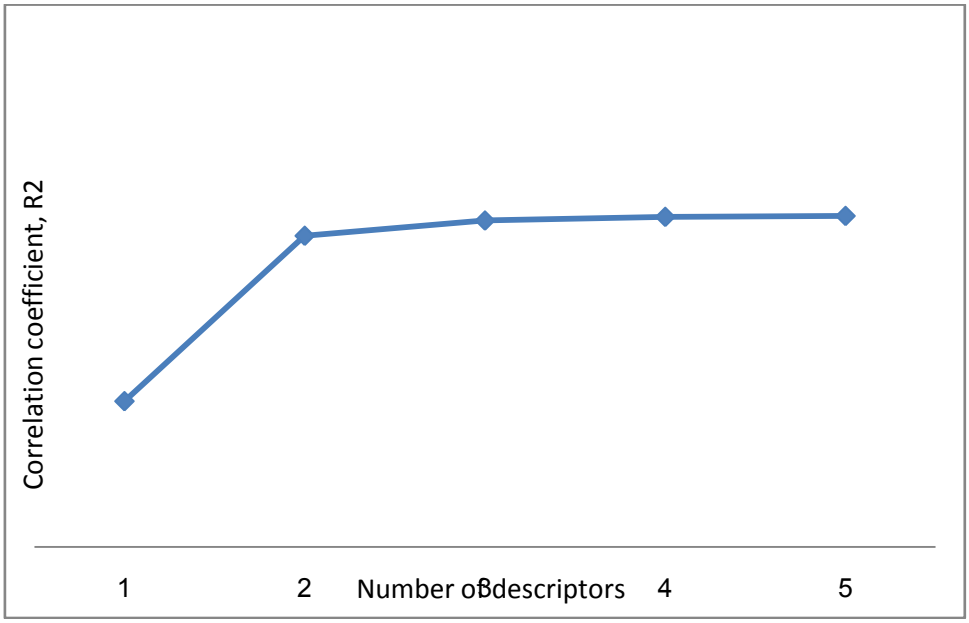


Fig. 2: Correlation coefficient R^2, versus number of descriptors in the QSAR models using BMLR method.

The best QSAR model with the parameter could be expressed by the equation:

$$\text{Log } IC_{50} = 0.18592\ (td) - 0.15194\ (tm) + 6.9251(me) - 61.354 \quad (1)$$

The correlations between the calculated and experimental activity according to the above equation is shown in Fig. 3. The experimental and predicted log IC_{50} of the flavonoids **1-11** is presented in table 4. The result shows small differences between the experimental and predicted log IC_{50} values (less than 5%) implying good predictive power of the QSAR model.

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This model has the correlation coefficient (R^2) value of 0.9632, cross-validated correlation coefficient (R^2_{CV}) value of 0.9279, squared standard deviation (S^2) value of 0.0012 and Fisher criterion (F) value of 61.0291. The close agreement between R^2 and R^2_{CV}shows the good predictive power of the QSAR model.

The type of descriptors involved in the equation 1 are total dipole of the molecule (td) (electronic descriptor), total molecular 2-center resonance energy divided by the no of atoms (tm) (quantum chemical descriptor) and min e-n attraction for H atom(me) (electronic descriptor) (Table 3). The equation showed that the increasing in total dipole of the molecule and min e-n attraction for a H atom as well as the decreasing in total molecular 2-center resonance energy/no of atoms would increase the IC_{50} value of PAF receptor antagonist activity which showed the reducing in the activity.

The total dipole of the molecule (td) is given by the following formula [16]:

$$\mu = -\sum_{i=1}^{occ} \int_{(V)} \emptyset_i \check{r} \emptyset_i dv + \sum_{a=1}^{M} Z_a \vec{R}_a \quad (2)$$

Where $\emptyset$are the molecular orbitals, $\check{r}$ are the electron position operator; Z_a are the a-th atomic nuclear charge and $\vec{R}_a$ is the position vector of a-th atomic nucleus. Based on the QSAR model (Eq. 1), the log IC_{50} value is found to be directly proportion to the dipole moment and thus implying that the inhibition activity will drop if the polar functional groups, such as hydroxyl groups, are introduced. This explains why compounds**4**and **7** whichcontains no hydroxyl groups are more active than other compounds containing hydroxyl groups.
The resonance energy between given two atomic species can be expressed as [16]:

$$E_R(AB) = \sum_{\mu \in} \sum_{A\nu \in B} P_{\mu\nu}\, \beta_{\mu\nu} \quad (3)$$

Where A s the given atomic species; B another atoms; $P_{\mu\nu}$ density matrix elements over atomic basis $\{\mu\nu\}$; $\beta_{\mu\nu}$ is resonance integrals on atomic basis$\{\mu\nu\}$.

This resonance energy is much related to the benzene ring in the flavonoids structure, thus indicates the importance of resonance in the activity as the antagonist of PAF receptor binding.

The min nuclear-electron attraction energy for hydrogen atom is defined as [16]:

$$E_{ne}(AB) = \sum_B \sum_{\mu,\nu \in A} P_{\mu\nu} \left\langle \mu \left| \frac{Z_B}{R_{iB}} \right| \nu \right\rangle \quad (4)$$

The first summation is performed over all atomic nuclei in the flavonoid molecule (B) whereas the second summation is carried out over all atomic orbitals at a given atom, i.e. hydrogen (A) [16]. The term,$\left\langle \mu \left| \frac{Z_B}{R_{iB}} \right| \nu \right\rangle$, represent the nuclear-electron attraction integrals on the given atomic basis. This energy may be related to the conformational such as rotational inversional changes in the molecule. The presence of this term in the QSAR equation emphasizes the importance of conformation in predicting the activity of the molecule.

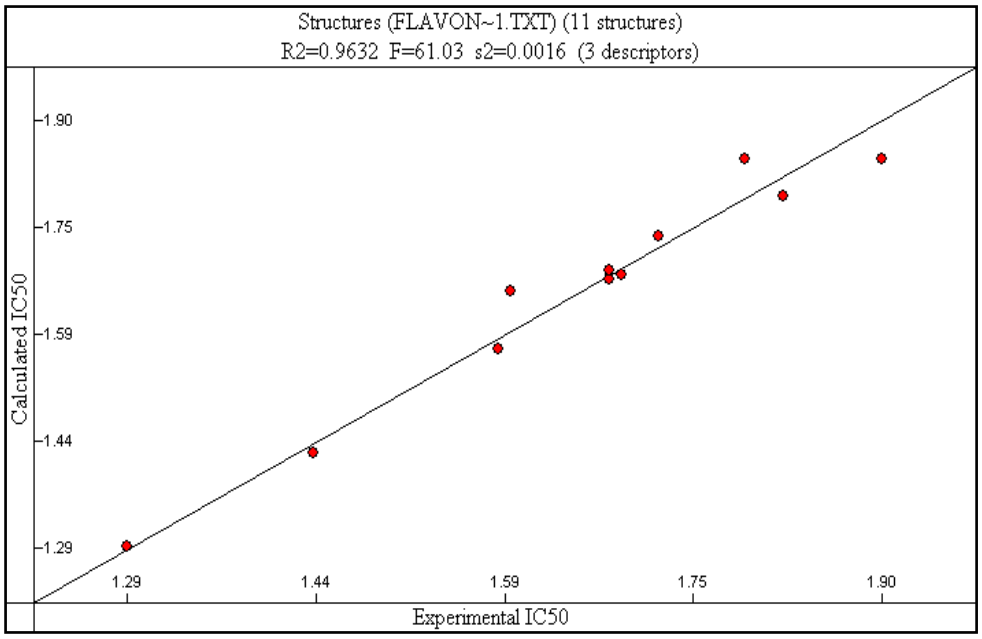
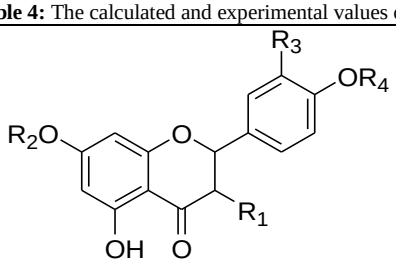
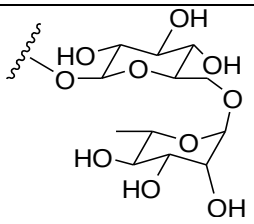


Fig. 3: Calculated versus experimental activity according to BML for flavonoids **1-11.**
(R^2=0.9632, F=61.03, s^2=0.0016).

Table 4: The calculated and experimental values of the best correlation for flavonoids **1-11.**





O-glu(6→1)rham

Compound	R1	R2	R3	R4	Experimental IC_{50}	Predicted IC_{50}	Difference
1	OH	H	H	CH_3	1.9000	1.8434	0.0566
2	H	CH_3	OH	CH_3	1.6900	1.6796	0.0104
3	OH	CH_3	H	CH_3	1.6800	1.6724	0.0076
4	H	H	H	CH_3	1.4400	1.4253	0.0147
5	OH	H	H	CH_3	1.7900	1.8434	0.0534
6	O-glu (6→1)rham	H	OH	H	1.6800	1.6854	0.0054
7	O-glu (6→1)rham	H	H	H	1.2900	1.2937	0.0037
8	OH	H	OH	CH_3	1.6000	1.6571	0.0571
9	OH	CH_3	OH	H	1.5900	1.5733	0.0167
10	OH	H	OH	CH_3	1.7200	1.7345	0.0145
11	OH	CH_3	OH	H	1.8200	1.7920	0.0280

Method validation:

It is important to determine the stability and predictive power of a predictive model such as QSAR model. If the data set is very large, then the data set can be divided into training set which will be used for obtaining the QSAR and tested against the external (test) set. However when the data set is smaller, the internal validation set such as leave-one-out method could be used to evaluate the predictive power of the developed QSAR.

The leave-one-out validation method implemented in the CODESSA software gave the corresponding cross-validated correlation coefficients (R^2_{cv}) to all models. The cross-validated correlation coefficient (R^2_{cv}) for the best three descriptor QSAR equation (Eq. 1) was 0.9279, which is very close to the R^2 (0.9632). The small difference between the R^2 and R^2_{cv} suggest the good predictive ability of the model.

Conclusions:

The quantum chemical quantitative structure activity relationship approach was applied to study the correlation between the PAF receptor binding antagonist activity and the chemical structure of flavonoids **1-11**. This approach is very powerful, and can be applied without the necessity of measuring any additional physic-chemical properties of the active molecules. The polarity of the substituent functional groups, conformation of the active molecules and the resonance energies are found to play significant roles in determining the activity of the molecules. The obtained QSAR was found to have good predictive power, and hence could be used to determine or construct new structure with better inhibition activity.

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