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Identification of SVM-based classification model, synthesis and evaluation of prenylated flavonoids as vasorelaxant agents

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ABSTRACT

Support vector machine (SVM) was applied to predict vasorelaxation effect of different structural molecules. A good classification model had been established, and the accuracy in prediction for the training, test, and overall datasets was 93.0%, 82.6%, and 89.5%, respectively. Furthermore, the model was used to predict the activity of a series of prenylated flavonoids. According to the estimated result, eleven molecules **1**–**11** were selected and synthesized. Their vasodilatory activities were determined experimentally in rat aorta rings that were pretreated with phenylephrine (PE). Structure–activity relationship (SAR) analysis revealed that flavanone derivatives showed the most potent activities, while flavone and chalcone derivatives exhibited medium activities.

1. Introduction

Cardiovascular diseases are the main cause of death in most countries. One of the main reasons in cardiovascular diseases is involvement of vascular tissues through increasing tonicity or losing their capacity to relaxation. Therefore, vascular studies have been paid much attention and the vasodilator agents would be beneficial in treating cardiovascular disease.[1–4] In recent years, flavonoids have been recognized as compounds with potent biological activities that may be active in prevention of cardiovascular disease.[5,6] As far as now, many flavonoids have been found to exhibit vasodilator effects in isolated vascular preparation and animal models.[7–9] However, the mechanism(s) for their action and SAR have not been fully understood until now, because the investigation of flavonoids by the SAR method is hampered by their vast structural diversity.

Quantitative structure–activity relationship (QSAR) analysis is an effective method in rational drug design and the mechanism of research of drug actions. In addition, it is useful in areas like design of combinatorial libraries with appropriate ADMET properties.[10–12] There are many statistical methods applied for QSAR development,[13,14] such as multiple linear regression (MLR), artificial neural network (ANN), genetic algorithm (GA), and support vector machine (SVM). Among these methods, SVM is a popular algorithm developed from the machine learning community. QSAR studies of vasodilators have been performed by Vilara et al. The authors performed LDA method capable of differentiating between active and inactive compounds.[15] However, the models reported were linear in nature, and the compounds used did not comprise flavonoids, which were also important vasodilators. In present work, support vector machine (SVM) was applied to construct classification model which involved a large number of descriptors calculated by Dragon 5.0 software and could classify active and inactive compounds (flavonoids were employed).

Previously, our group reported the synthesis of quercetin derivatives (or analogs) together with their vasorelaxant actions in the isolated rat thoracic aorta rings and found that flavonoids had stronger activity with the augment of log*P values.*[7] As a continuation of our ongoing project related to the discovery and optimization of flavonoids as vasodilators, we introduced prenyl group into flavonoids in this study. It was reported that prenyl groups could increase the lipophilicity and confer to the molecule a strong affinity to biological membranes, and result in significant enhancement of bioactivities.[16,17] Despite intensive studies for prenylated flavonoids, few studies about their vasodilator effect have been reported.

The aim of this study was to develop a relatively accurate classification model to predict vasodilatory activity of prenylated flavonoids and to synthesize compounds which were estimated as active groups by SVM classification model. Eleven prenylated flavonoids **1–11** were synthesized (Fig. 1) considering the effect of different skeletons (chalcone, flavanone, and flavone) and different substituents of hydroxyl, halogen (Br and Cl), or alkoxyl (methoxyl and methlenedioxyl) in prenylated flavonoids. Vasodilatory activity of these compounds was subsequently evaluated in rat thoracic aorta rings model against phenylephrine (PE)-induced contraction.

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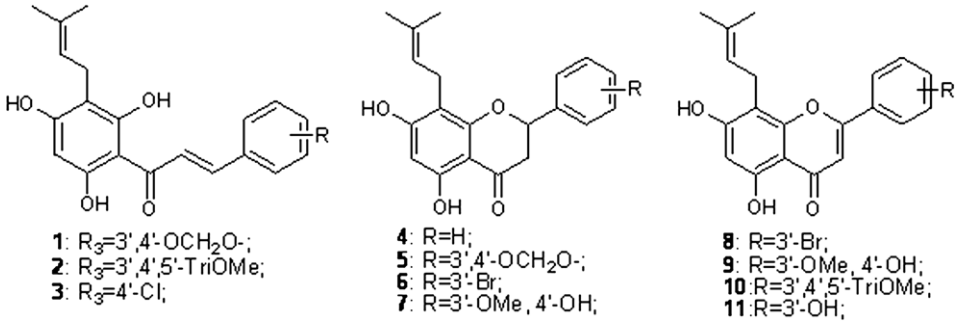


Figure 1. The structure of synthesized prenylated flavnoids.

2. Materials and methods

2.1. Data preparation

The studied compounds and their classification were taken from the literature,[8,18,19] and 343 compounds were employed for QSAR development and divided into train set ($n = 228$) and test set ($n = 115$), as shown in Tables 1 and 2, respectively. In the dataset, thirty flavonoids derivatives (24 active and 6 inactive ones) were introduced in order to identify a reliable model for predicting correct classification of prenylated flavonoids. Active compounds have marked vasodilatory effects according to the literature. In order to ensure the prediction accuracy of potent vasodilators rather than weak ones, flavonoids with weak activities were classified into inactive group (i.e., rutin, epicatechin). All compounds were sketched and optimized in Discovery Studio 2.0 software package.

2.2. Descriptors calculation and selection

The resulted geometry was transferred into software Dragon,[20] which can calculate constitutional descriptors, topological descriptors, walk and path counts, information indices, 2D autocorrelations, edge adjacency indices, Burden eigenvalue descriptors, Burden eigenvalue descriptors, etc. Among them, the descriptors used in previous QSAR model were considered preferential.[15,18] After the calculation of the molecular descriptors, those that stayed constant for all molecules were eliminated, and pairs of variables with a correlation coefficient greater than 0.80 were classified as intercorrelated, and one of them in each correlated pair was deleted. Then stepwise multiple linear regression (Stepwise-MLR) and feature selection tool[21] were used to select the most relevant descriptors. Finally, nine descriptors were selected, as shown in Table 3. The correlation matrix of these descriptors is shown in Table 4.

2.3. SVM model development

Support vector machine (SVM)[22,23] algorithms are mainly developed by Vapnik's and Burges's work. The major advantage of SVM is that it adopts the structure risk minimization (SRM) principle, which has been proved to be superior to the traditional empirical risk minimization (ERM) principle, employed by conventional neural networks. Theories of support vector classification can be found in the tutorials for SVM.[23] SVM classifiers are generated by a two-step procedure: first, the sample data vectors are mapped to a very high-dimensional space. The dimension of this space is significantly larger than that of the original data space. Then, the SVM algorithm finds a hyperplane in this space with the largest margin separating classes of data. The decision function is

$$f(x) = \mathrm{sign}\left(\sum_{i=1}^{l} y_i \alpha_i K(x, x_i) + b\right),$$

where sign is simply a sign function which returns +1 for positive argument and −1 for a negative argument; y_i are input class labels that take a value of −1 or +1, x_i is a set of descriptors, and $K(x,x_i)$ is a kernel function, whose value is equal to the inner product of two vectors x and x_i in the feature space $\Phi(x)$ and $\Phi(x_i)$. That is, $K(x;x_i) = \Phi(x)^{*}\Phi(x_i)$. Any function that satisfies Mercer's condition can be used as the kernel function and α_i is the Lagrangian multiplier.

3. Results and discussion

3.1. Result of SVM

In current study, the radial basis function (RBF) was used in the training of the SVM model, RBF is commonly used in many studies because of its good general performance and facile adjustment involving few parameters.[24] The RBF is formulated as $\exp(-\gamma(x - x_i)^2)$. The width of RBF (γ) and capacity parameter (C) should be optimized. C is a regularization parameter that controls the tradeoff between maximizing the margin and minimizing the training error. The parameter of the kernel (γ) controls the amplitude of the Gaussian function and further controls the generalization ability of SVM. Since two parameters exhibit strong interactions, grid search (GS) was performed in this study. In the grid search, we considered parameter $\lg 2\gamma$ from −10 to 10 with 1 as the increment. Parameter $\lg 2C$ was chosen from −5 to 15 with 1 as the increment. Values of g and C for this dataset were finally fixed to 0.035 and 100, respectively.

The results obtained in the classification of the compounds that make up the training and prediction series are shown in Table 5. The accuracy in prediction for the training, test, and overall datasets are 93.0%, 82.6%, and 89.5%, respectively, and cross validation accuracy of this model is 80.1%. It was suggested that this SVM classification model derived in this study would be valuable and reliable in classifying structurally diverse compounds for vasodilatory activity.

3.2. Chemistry

Once the classification model had been obtained, eleven prenylated flavonoids **1–11** which were predicted as active compounds were synthesized. The synthetic pathway is outlined in Scheme 1. Compound **13** was obtained from prenylation of 2,4,6-trihydroxyacetophenone **12** with 3,3-dimethylallyl bromide, according to reported methods.[25] Selective methoxymethylation of **13** with chloromethoxymethyl ether and anhydrous K_2CO_3 in dry acetone gave compound **14**.[26] Condensation of **14** with corresponding benzaldehydes was preceded in aqueous alcoholic alkali, affording chalcones **15a–g**. Compounds **15a–f**

Table 1
Compounds in train set for SVM classification model, and their corresponding experimental and theoretical classification

Compounds	Classification		Compounds	Classification	
	Exp.	SVM		Exp.	SVM
Adenosine	1	1	Ibopamine	1	−1
Alprostadile	1	1	Iloprost	1	1
Amotriphene	1	1	Inositol niacinate	1	1
Apigenin	1	1	Isosorbide dinitrate	1	1
Benfurodil	1	1	Isosorbide-5-mononitrate	1	1
Betahistine	1	1	Isoxsuprine	1	1
2-(2-Biphenylyloxy)-triethylamine	1	1	Khellin	1	1
Bosentan	1	1	Lidoflazine	1	1
Buflomedil	1	1	Luteolin	1	1
Tolazoline	1	1	5-Methyoxyflavone	1	1
Carbochromen	1	1	Minoxidil	1	1
Chloracizine	1	−1	Moxisylyte	1	−1
Chlorpromazine	1	1	Naftidrofurile	1	1
Ciclonicate	1	−1	Nicametate	1	1
Clonitrate	1	1	Nicergoline	1	1
Cyclandelate	1	1	Nicofuranose	1	1
3,4-Cyclohexen-7-hydroxy-8-methoxycoumarin	1	−1	Nifedipine	1	1
3,4-Cyclopenten-4′-methyl-8-methoxyfuro[3,2-*g*]coumarin	1	1	Nitroglycerin	1	1
Daizdein	1	1	Oxyfedrine	1	1
Diltiazem	1	1	Papaverine	1	1
Dimoxyline	1	1	pentifylline	1	−1
Dipyridamole			Perhexiline	1	1
Droprenylamine	1	1	Pimefyllin	1	1
Efloxate	1	1	Pentrinitrol	1	1
Fendiline	1	1	Pinocembrin	1	1
Fenoxedil	1	1	Piribedil	1	1
Floranol	1	1	Prazosin	1	1
Genistein	1	1	propatyl nitrate	1	1
Glycetein	1	1	Resveratrol	1	−1
Hesperintin	1	1	Suloctidil	1	1
Hexestrol bis(diethylaminoethyl ether)	1	1	Trapidil	1	−1
Hexobendine	1	1	Trimetazidine	1	1
5-Hydroxyflavone	1	1	Vinburnine	1	1
6-Hydroxyflavanone	1	1	Vincamine	1	1
7-Hydroxy-4-methyl-5-methoxycoumarin	1	−1	Visnadin	1	1
Abacavir	−1	1	Niacinamide	−1	−1
Acediasulfone	−1	−1	Niclofolan	−1	−1
Acrivastine	−1	−1	Niclosamide	−1	−1
Ambucaine	−1	−1	Nifenazone	−1	−1
Ancymidol	−1	−1	Nifuratel	−1	−1
Asulam	−1	−1	Nifurtoinol	−1	−1
Azanidazole	−1	−1	Ninhydrin	−1	−1
3,4-Benzo-4′-methyl-8-methoxyfuro[3,2-*g*]coumarin	−1	−1	Nitramine	−1	−1
Bifenox	−1	−1	Nitrodan	−1	−1
Brodimoprim	−1	−1	Nitrofurantoin	−1	−1
Captan	−1	−1	Octaverine	−1	1
Catalin	−1	−1	Oxacillin	−1	−1
Cicrotoic acid	−1	−1	Oxazepam	−1	−1
Clonazepam	−1	−1	Oxymetazoline	−1	−1
5,7-Dihydroxy-4-methylcoumarin	−1	−1	Phenoxyacetic acid	−1	−1
Dichloroisoproterenol	−1	−1	Pidylon	−1	−1
Dimethisoquin	−1	−1	Pivalizid	−1	−1
Dimethophrine	−1	−1	Pantothenic Acid	−1	−1
Dopamine	−1	−1	Patulin	−1	−1
Doxofylline	−1	−1	Pebulate	−1	−1
(−)-Epicatechin	−1	−1	Pecilocin	−1	−1
Ellagic acid	−1	−1	Perfluidone	−1	−1
Ergonovine	−1	−1	Phenolphthalol	−1	−1
Eritadenine	−1	−1	phenoxyacetic acid	−1	−1
Etonitazene	−1	−1	Phenpentermine	−1	−1
Fenadiazole	−1	−1	Phloroglucinol	−1	−1
Fentichlor	−1	−1	Phthalofyne	−1	−1
Fluazinam	−1	−1	Phytol	−1	−1
Flumetramide	−1	−1	Piposulfan	−1	−1
Fluorosalan	−1	−1	Pirenoxine	−1	−1
Flutamide	−1	−1	Previscan	−1	−1
Frequentin	−1	−1	Probucol	−1	−1
Fropenem	−1	1	Propanil	−1	−1
Galipine	−1	−1	Propiconazole	−1	−1
Genistin	−1	−1	Puerarin	−1	−1
Gepefrine	−1	−1	Pymetrozine	−1	−1
Halazone	−1	−1	Pyrantel	−1	−1
Haloxon	−1	−1	Pyrifenox	−1	−1

(*continued on next page*)

Table 1 (*continued*)

Compounds	Classification		Compounds	Classification	
	Exp.	SVM		Exp.	SVM
Hetacillin	−1	−1	Pyrrolnitrin	−1	−1
Hymecromone	−1	−1	Quazepam	−1	−1
4-Hydroxymethyl-7-hydroxy-8-methoxycoumarin	−1	−1	Rabeprazole	−1	1
7-Hydroxy-4-methyl-8-methoxycoumarin	−1	−1	Rafoxanide	−1	−1
Idrocilamide	−1	−1	Resistomycin	−1	−1
Imazaquin	−1	−1	Rhodamine B	−1	−1
Indomethacin	−1	−1	Rimiterol	−1	−1
Iodamide	−1	−1	Rioprostil	−1	−1
Iodinated glycerol	−1	−1	Rolicyprine	−1	−1
Isoaminile	−1	−1	Salicylsulfuric acid	−1	−1
Isobuzole	−1	−1	Saluzid	−1	−1
Isoetharine	−1	−1	Satigrel	−1	−1
2-Isovaleryl-1,3-indanedione	−1	−1	Simazine	−1	−1
Ketamine	−1	−1	Solan	−1	−1
Lamivudine	−1	−1	Spasmolytol	−1	−1
Lapachol	−1	−1	Stepronin	−1	−1
Maclurin	−1	−1	Suclofenide	−1	−1
Mafenide	−1	−1	Sulfaethoxypyridazine	−1	−1
Maleic hydrazide	−1	−1	Sulfametrole	−1	−1
Maltol	−1	−1	Sulfentrazone	−1	1
Mebendazole	−1	−1	Sulforidazine	−1	−1
Meclofenamic acid	−1	−1	Suxibuzone	−1	−1
Mefenamic acid	−1	−1	Talipexole	−1	−1
Menichlopholan	−1	−1	Taxifolin	−1	−1
Mephenesin	−1	−1	Teflubenzuron	−1	−1
Mepivacaine	−1	−1	Thiophanate	−1	−1
Metahexamide	−1	−1	Thyropropic acid	−1	−1
Metaproterenol	−1	−1	Tiletamine	−1	−1
Methaqualone	−1	−1	Tolycaine	−1	−1
Methestrol	−1	−1	Tranilast	−1	−1
Methidathion	−1	−1	Triadimefon	−1	−1
Methylphenidate	−1	1	Trifluralin	−1	−1
Metobromuron	−1	−1	Trimecaine	−1	−1
Metribuzin	−1	−1	Ujothion	−1	−1
Mexacarbate	−1	−1	Veralipride	−1	−1
Midodrine	−1	−1	Verazide	−1	−1
Mofezolac	−1	−1	Vinclozolin	−1	−1
2-(2-Naphthyloxy)ethanol	−1	−1	Warfarin	−1	−1
Norbutrine	−1	−1	Xylazine	−1	−1
Narcobarbital	−1	−1	Xylopropamine	−1	−1
Neocinchophen	−1	−1	Zopolrestat	−1	−1

were cyclized by refluxing in a solution of NaOAc in ethanol, affording flavanone **16a–f**, respectively. Demethoxymethylation of **15b, 15e, 15g**, and **16a**–**f** was carried out in 2% HCl in MeOH/THF obtaining products **1–7** and **17e**–**f**, respectively. Finally, products **8–11** were obtained by dehydrogenating the corresponding prenylflavanones **6–7** and **17e–f** with iodine and pyridine. Among the eleven prenylated flavanoids, the galabranin **4**, (±)exiguaflavanone K **7** and 5,7,4′-trihydroxy-3′-methoxy-8-(3,3-dimethylallyl)-flavone **9** were natural products, and others were novel compounds. The structures of compounds prepared were elucidated by ^{1}H NMR and ESI-MS.

3.3. Biological activity

Vasodilatory activity was investigated in aortic rings with endothelium pre-contracted with 1 μM phenylephrine. Among all the tested compounds **1–11**, promoted relaxation was in a dose-dependent manner with the maximal effect observed at 300 μM. According to their ability to induce vasorelaxation, these flavonoids are classified into three categories based on their potency (EC_{50}) and efficacy (E_{max}). Flavonoids **1**, **4**, **7**, and **10** inducing 50% relaxation at small concentration (less than 100 μM) with good efficacy (from 90% to 100%) are considered to be good relaxing agents. Flavonoids **2**, **5**, **6**, **8**, and **9** inducing moderate E_{max} (70–90%) with small EC_{50} (less than 100 μM) are moderate relaxing agents. Compounds **3** and **11** are weak vasodilators with EC_{50} more than 100 μM and induce limited relaxation (less than 70%) at 300 μM. Concentration–relaxation curves of these flavonoids in three categories are shown in Figure 2. The theoretical results of them were in good agreement with the experimental values, as shown in Table 6. Among the eleven compounds **1–11**, which were predicted as active compounds by the SVM classification model, nine compounds **1, 2**, **4–10** showed medium to good vasodilatory activity, while only two compounds **3** and **11** showed weak activity.

Insight into the observed effects of different skeletons and substituents revealed that flavanones show the most potent activities, while flavones and chalcones exhibit medium activities. Compounds **6** and **7** (flavanones) exhibit much more potent vasodilatory activity than **8** and **9** (flavones). Compound **1**, a chalcone with similar substituent to **5**, showed slight less potency than **5**. Both compounds **2** and **9** have trimethoxyl substituent in B-ring, while flavone **9** exhibited more potency. In the flavanone class, compound **4**, without any substituent in B-ring, showed the highest E_{max}. 3′-Br or 3′,4′-methylenedioxyl substituent of in B-ring could enhance the potency (less EC_{50}) but reduce the efficacy (less E_{max}). In the flavone class, hydrophobic substituents in B-ring of flavone exhibit medium activities (Br, trimethoxyl), while the 3-hydroxylation substituent attenuated the relaxation activity. In the chalcone class, only 3′,4′-methylenedioxyl in B-ring of chalcone exhibits good activity, while other substituents (4′-chloride and 3′,4′,5′-trimethoxyl) reduced the activity.

Table 2
Compounds in test set for SVM classification model, and their corresponding experimental and theoretical classification

Compounds	Classification		Compounds	Classification	
	Exp.	SVM		Exp.	SVM
Acacetin	1	1	Flunarizine	1	1
Acetabutone	1	1	Galangin	1	1
Amiodarone	1	−1	Glabridin	1	1
Bufenine	1	1	Hepronicate	1	1
Benziodarone	1	−1	Ibudilast	1	−1
Butalamine	1	1	Ifenprodil	1	1
Cetiedil	1	1	Kaempferol	1	1
Cinepazet Maleate	1	1	Naringenin	1	1
Cinepazide	1	1	Pelargonidin	1	1
Hesperetin	1	1	Pentoxifylline	1	−1
7-Hydroxy-4,8-dimethylcoumarin	1	−1	Pentaerythritol tetranitrate	1	1
4′-Hydroxyflavanone	1	1	Phloretin	1	1
7-Hydroxyflavone	1	1	Prenylamine	1	1
Chrysin	1	1	Propentofylline	1	−1
Cloricromen	1	1	Quercetin	1	1
Dihydroergochristine	1	1	Verapamil	1	1
Dilazep	1	1	Vinpocetine	1	1
EGCG	1	−1	Viquidil	1	−1
Etafenone	1	1	Wogonin	1	1
Etofylline nicotinate	1	−1	Zolertine	1	1
Floredil	1	1			
Acenocoumarin	−1	−1	4-Hexylresorcinol	−1	−1
Acetanilide	−1	−1	2-(2-Hydroxy-1-naphthyl)-cyclohexanone	−1	−1
Acetophenazine	−1	1	Iloperidone	−1	1
Acetylpheneturide	−1	−1	Isosafrole	−1	−1
Acroteben	−1	−1	Ketoprofen	−1	−1
Alizapride	−1	−1	Medmain	−1	−1
Benorilate	−1	−1	Meparfynol	−1	−1
Benzyl salicylate	−1	−1	Methetoin	−1	−1
Bromindione	−1	−1	Metizoline	−1	−1
Broxuridine	−1	−1	Naphazoline	−1	−1
Buclizine	−1	1	Nemotin	−1	−1
Capravirine	−1	−1	Nicoclonate	−1	−1
Catechin	−1	1	Nikethamide	−1	−1
Carindacillin	−1	−1	Nitrophenide	−1	−1
Centalun	−1	−1	Normolaxol	−1	1
Clorophene	−1	−1	Osalmid	−1	−1
Cyanofenphos	−1	−1	Pelletierine	−1	−1
3,4-Cyclopenten-7-hydroxy-8-methoxycoumarin	−1	1	Pentylenetetrazole	−1	1
Cymiazole	−1	−1	Phenetamine	−1	−1
Diallate	−1	−1	Pholedrine	−1	−1
Dihydrotachysterol	−1	1	Propylparaben	−1	−1
Edifenphos	−1	−1	Reposal	−1	−1
Ephedrine	−1	−1	Rutin	−1	−1
Estazolam	−1	−1	Salicil	−1	−1
Etaqualone	−1	−1	Sobrerol	−1	−1
Ethylmethylthiambutene	−1	−1	4-Stilbazole	−1	−1
1-Ethynylcyclohexanol	−1	−1	Sulfenone	−1	−1
Eugenol	−1	−1	Tebufenozide	−1	1
Equipax	−1	−1	Terbacil	−1	−1
Farnesol	−1	−1	Tetrahydrozoline	−1	1
Fenbendazole	−1	−1	Tetrantoin	−1	−1
Fenpentadiol	−1	−1	Thiofuradene	−1	−1
Flavoteben	−1	−1	Thionazin	−1	−1
Furfural	−1	−1	Tranylcypromine	−1	1
Glaphenin	−1	−1	Versalide	−1	−1
Guaiacol	−1	−1	Yellow OB	−1	−1
Hexobarbital	−1	−1	Zaleplon	−1	−1

Table 3
The involved molecular descriptors and their corresponding definition

Symbol	Class	Definition
D/D	Topological descriptors	Distance/detour index
Jhetp	Topological descriptors	Balaban-type index from polarizability weighted distance matrix
TIC1	Information indices	Total information content index (neighborhood symmetry of 1-order)
SEigp	Eigenvalue-based indices	Eigenvalue sum from polarizability weighted distance matrix
SP20	Randic molecular profiles	Shape profile No. 20
RDF030m	RDF descriptors	Radial distribution function-3.0
Mor06u	3D-MoRSE descriptors	3D-MoRSE-signal 06
ATS8v	2D autocorrelations	Broto-Moreau autocorrelation of a topological structure
JGI1	Topological charge indices	Mean topological charge index of order1

Table 4
Correlation matrix of the nine descriptors

	D/D	Jhetp	TIC1	SEigp	SP20	RDF030m	Mor06u	ATS8v	JGI1
D/D	1.000								
Jhetp	−0.326	1.000							
TIC1	0.782	−0.489	1.000						
SEigp	−0.502	0.377	−0.438	1.000					
SP20	0.568	−0.293	0.605	−0.337	1.000				
RDF030m	0.569	−0.098	0.407	−0.207	−0.060	1.000			
Mor06u	−0.334	0.109	−0.371	0.104	−0.290	−0.145	1.000		
ATS8v	0.712	−0.498	0.791	−0.389	0.581	0.381	−0.280	1.000	
JGI1	−0.077	0.452	−0.164	−0.162	−0.095	−0.032	−0.068	−0.198	1.000

Table 5
Results of SVM classification model

	SVM		Accuracy (%)	
	Active	Inactive		
Train set active	61	9	87.1	93.0
Train set inactive	151	7	95.5	
Test set active	32	9	78.0	82.6
Test set inactive	63	11	85.1	
Total accuracy	—	—	89.5	
Cross validation	—	—	80.1	

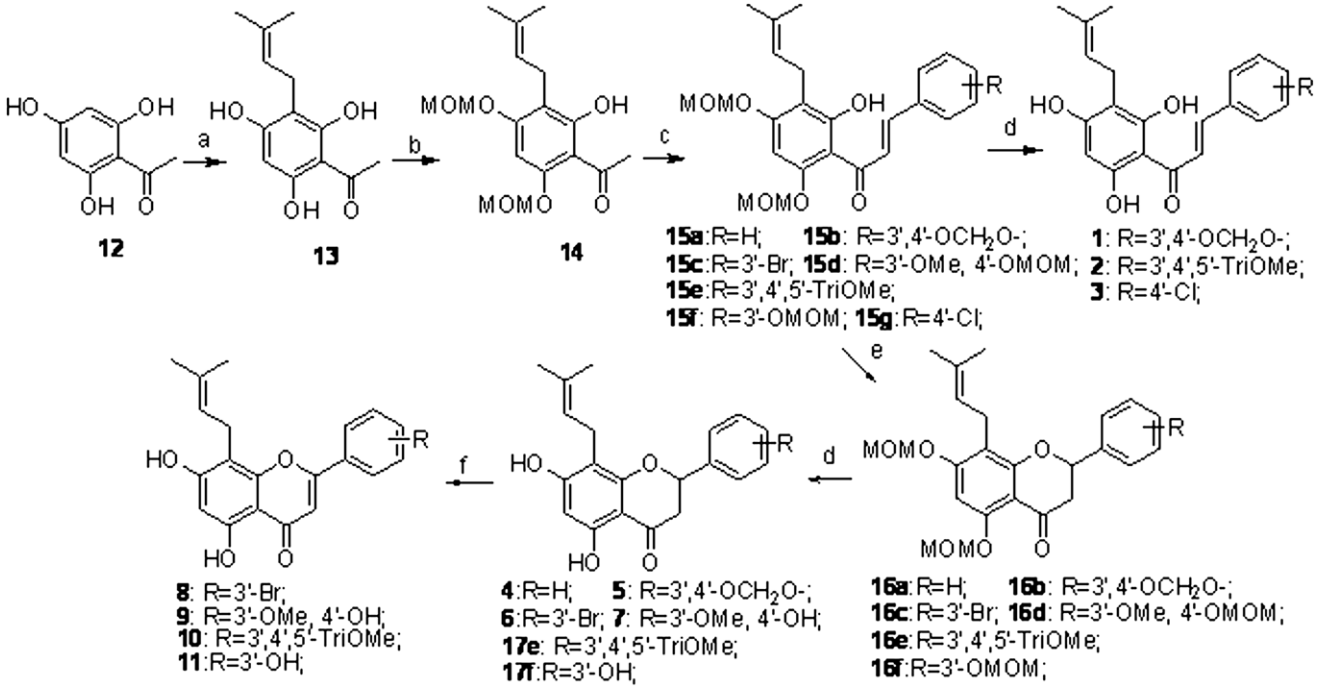
4. Conclusions

In this work, we applied nonlinear models (support vector machine) to classify 343 compounds with or without vasodilator activity using descriptors calculated from the molecular structure. SVM method produced good classification results with good predictive ability (total accuracy 89.5%) with cross validation of 80.1%. The SVM classification model developed here was used to predict the activity of prenylated flavonoids derivatives. Eleven prenylated flavonoids **1–11**, estimated as active compounds, with consideration the effect of different skeletons (chalcone, flavanone, and flavone) and different substituents of hydroxyl, halogen (Br and Cl), or alkoxyl (methoxyl and methylenedioxyl) in prenylated flavonoids were synthesized. The theoretical results of them were in good agreement with the experimental values. Among the eleven compounds, nine compounds **1**, **2**, **4–10**, showed medium to good vasodilatory activity, while two other compounds **3** and **11** showed weak activity. Some compounds showed more potent vasorelaxation than the positive control quercetin based on the result of either E_{max} or EC_{50}. SAR studies of prenylated flavonoids revealed that flavanones showed the most potent activities, while flavones and chalcones exhibited medium activities.

5. Experimental

5.1. Chemistry

Melting points were obtained on a B-540 Buchi melting-point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM 400 instrument at 400 MHz (chemical shifts are expressed as δ values relative to TMS as internal standard). Mass spectra (MS) and ESI (positive) were recorded on an Esquire-LC-00075 spectrometer. Elemental analyses were performed on a Flash EA 1112 elemental analyzer.



Scheme 1. Synthesis of prenylated flavonoids **1**–**11**. Reagents and conditions: (a) 3,3-dimethylallyl bromide, 10% KOH; (b) MOMCl, K_2CO_3, acetone 0 °C–rt; (c) corresponding benzaldehyde, 10% KOH, ethanol/H_2O, rt; (d): 5% HCl, MeOH/THF, reflux; (e) NaOAc, EtOH, reflux; (f) I_2, pyridine, 90 °C.

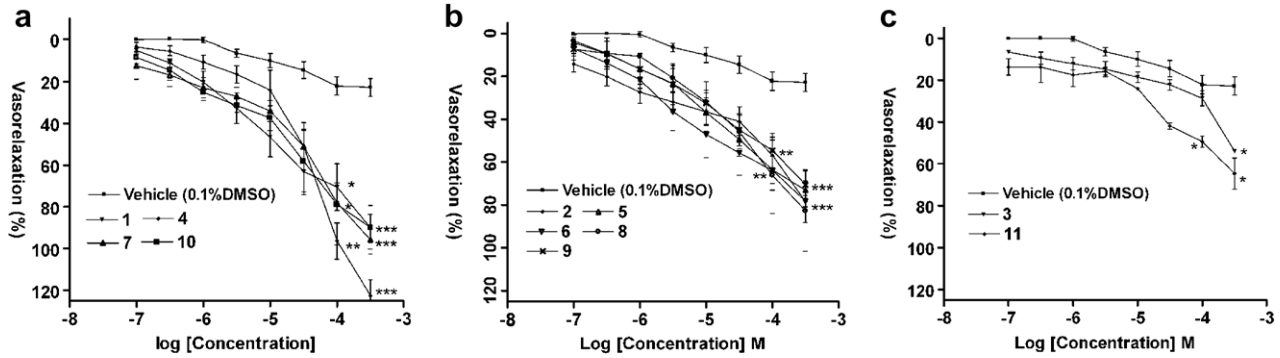


Figure 2. Effects of flavonoids on relaxation in aortic rings with endothelium pre-contracted with 1 μM phenylephrine. Flavonoids were added cumulatively to achieve the appropriate concentrations. Results are expressed as means ± standard error of mean in terms of percentage relaxation of the contraction to PE ($n = 4$). (a) Flavonoids with good relaxation effect; (b) flavonoids with moderate relaxation effect; and (c) flavonoids with weak relaxation effect. Level of statistical significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ versus vehicle group, as determined by ANOVA tests.

Table 6
The vasorelaxant activity induced by compounds **1**–**11**, in rat aorta rings pre-contracted with PE

Entry	EC_{50} (10 μM)[a]	E_{max} (%)	Classification[b]	Entry	EC_{50} (10 μM)[a]	E_{max} (%)	Classification[b]
Quercetin	24.4 ± 2.2	91.3 ± 13.2	Active	**6**	1.56 ± 0.31	78.2 ± 4.7	Active
1	4.21 ± 0.51	90.0 ± 12.9	Active	**7**	10.1 ± 1.22	95.8 ± 13.7	Active
2	N.D.[d]	78.5 ± 19.5	Active	**8**	95.9 ± 10.80	82.8 ± 20.7	Active
3	679 ± 150	64.4 ± 10.8	Active[c]	**9**	49.3 ± 4.52	70.6 ± 14.0	Active
4	5.79 ± 0.82	122.5 ± 15.4	Active	**10**	64.3 ± 12.6	90.1 ± 23.0	Active
5	1.32 ± 0.23	77.3 ± 6.1	Active	**11**	N.D.[d]	64.6 ± 18.3	Active[c]

[a] Each value is the mean ± SD from four experiments.
[b] The classification of compounds was provided by SVM model.
[c] Misclassified compounds.
[d] N.D. means not determined.

The preparation of compound **13** was achieved by treating 2,4,6-trihydroxyacetophenone **12** with 3,3-dimethylallyl bromide, according to the approach in reference.[25]

4,6-Dimethoxymethoxy-3-(3,3-dimethylallyl)-2-hydroxy-acetophenone **14** was obtained by selective methoxymethylation of **13** with chloromethoxymethyl ether according to our previous literature.[26]

5.2. General method for synthesis of compounds 15a–g

To a cold solution of the acetophenone **14** and corresponding benzaldehyde in 3 mL H_2O/EtOH (1:4v/v), 20% KOH in 3 mL H_2O/EtOH (1:4 v/v) was added. The resulting mixture was stirred under nitrogen at room temperature for 36 h. The whole mixture was poured into ice-water, acidified to pH 5 with 1 N HCl, and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified on silica gel column using petroleum ether/ethyl acetate (20:1) to give chalcone **15a**–**g**.

5.2.1. 2-Hydroxy-3-(3,3-dimethylallyl)-4,6-dimethoxymethoxy-chalcone (15a)

Reagents: compound **14** (500 mg, 1.54 mmol) and benzaldehyde (172 mg, 1.62 mmol). Yellow oil (445 mg, 70%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.79 (s, 3H), 3.33 (d, 2H, $J = 6.8$ Hz), 3.48 (s, 3H), 3.51 (s, 3H), 5.23 (m, 1H), 5.25 (s, 2H), 5.28 (s, 2H), 6.40 (s, 1H), 7.40 (m, 3H), 7.60 (m, 2H), 7.77 (d, 1H, $J = 15.6$ Hz), 7.91 (d, 1H, $J = 15.6$ Hz), 13.76 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 413.

5.2.2. 2-Hydroxy-3-(3,3-dimethylallyl)-4,6-dimethoxymethoxy-3′,4′-methylenedioxy-chalcone (15b)

Reagents: compound **14** (500 mg, 1.54 mmol) and 3,4-methylenedioxy-benzaldehyde (243 mg, 1.62 mmol). Yellow oil (528 mg, 75%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.79 (s, 3H), 3.33 (d, 2H, $J = 6.8$ Hz), 3.49 (s, 3H), 3.52 (s, 3H), 5.21 (m, 1H), 5.24 (s, 2H), 5.27 (s, 2H), 6.02 (s, 2H), 6.39 (s, 1H), 6.83 (d, 1H, $J = 7.6$ Hz), 7.08 (dd, 1H, $J = 7.6$, 1.2 Hz), 7.10 (d, 1H, $J = 1.2$ Hz), 7.71 (d, 1H, $J = 15.6$ Hz), 7.77 (d, 1H, $J = 15.6$ Hz), 13.81 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 457.

5.2.3. 2-Hydroxy-3-(3,3-dimethylallyl)-4,6-dimethoxymethoxy-3′-bromo-chalcone (15c)

Reagents: compound **14** (500 mg, 1.54 mmol) and 3-bromobenzaldehyde (300 mg, 1.62 mmol). Yellow oil (546 mg, 72%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.79 (s, 3H), 3.33 (d, 2H, $J = 7.2$ Hz), 3.49 (s, 3H), 3.53 (s, 3H), 5.22 (m, 1H), 5.24 (s, 2H), 5.28 (s, 2H), 6.38 (s, 1H), 7.28 (t, 1H, $J = 8.0$ Hz), 7.49 (dd, 1H, $J = 2.0$, 8.0 Hz), 7.51 (d, 1H, $J = 8.0$ Hz), 7.65 (d, 1H, $J = 15.2$ Hz), 7.75 (t, 1H, $J = 2.0$ Hz), 7.89 (d, 1H, $J = 15.2$ Hz), 13.68 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 491.

5.2.4. 2-Hydroxy-3-(3,3-dimethylallyl)-4,6,4′-trimethoxyme thoxy-3′-methoxy-chalcone (15d)

Reagents: compound **14** (500 mg, 1.54 mmol) and 3-methoxy-4-methoxymethoxy-benzaldehyde (318 mg, 1.62 mmol). Yellow oil (465 mg, 60%);^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.80 (s, 3H), 3.33 (2H, d, $J = 6.8$ Hz), 3.51 (s, 3H), 3.54 (s, 6H), 3.95 (s, 3H), 5.22 (m, 1H), 5.23 (s, 2H), 5.28 (s, 4H), 6.40 (s, 1H), 6.85 (d, 1H, $J = 7.6$ Hz), 7.09 (dd, 1H, $J = 7.6$, 1.2 Hz), 7.12 (d, 1H, $J = 1.2$ Hz), 7.73 (d, 1H, $J = 15.6$ Hz), 7.79 (d, 1H, $J = 15.6$ Hz), 13.90 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 503.

5.2.5. 2-Hydroxy-3-(3,3-dimethylallyl)-4,6-dimethoxymethoxy-3′,4′,5′-trimethoxy-chalcone (15e)

Reagents: compound **14** (500 mg, 1.54 mmol) and 3′,4′,5′-trimethoxy-benzaldehyde (318 mg, 1.62 mmol). Yellow oil (519 mg, 67%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.79 (s, 3H), 3.33

(d, 2H, J = 6.8 Hz), 3.48 (s, 3H), 3.52 (s, 3H), 3.89 (s, 9H), 5.22 (m, 1H), 5.24 (s, 2H), 5.26 (s, 2H), 6.85 (s, 2H), 7.69 (d, 1H, J = 15.6 Hz), 7.84 (d, 1H, J = 15.6 Hz), 13.78 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 503.

5.2.6. 2-Hydroxy-3-(3,3-dimethylallyl)-4,6,3′-trimethoxymethoxy-chalcone (15f)

Reagents: compound **14** (500 mg, 1.54 mmol) and 3-methoxymethoxy-benzaldehyde (269 mg, 1.62 mmol). Yellow oil (473 mg, 65%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.65 (s, 3H), 1.76 (s, 3H), 3.31 (d, 2H, J = 6.8 Hz), 3.47 (s, 3H), 3.49 (s, 3H), 3.50 (s, 3H), 5.18 (s, 2H), 5.20 (m, 1H), 5.23 (s, 2H), 5.28 (s, 2H), 6.39 (s, 1H), 7.04 (dd, 1H, J = 2.0, 8.0 Hz), 7.21 (d, 1H, J = 8.0 Hz), 7.29 (t, 2H, J = 8.0 Hz), 7.71 (d, 1H, J = 16.0 Hz), 7.88 (d, 1H, J = 16.0 Hz), 13.75 (s, 1H). ESI-MS: m/z $[M+H]^+$ 473.

5.2.7. 2-hydroxy-3-(3,3-dimethylallyl)-4,6-dimethoxymethoxy-4′-chloro-chalcone (15g)

Reagents: compound **14** (500 mg, 1.54 mmol) and 4′-chlorobenzaldehyde (228 mg, 1.62 mmol). Yellow oil (496 mg, 72%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.69 (s, 3H), 1.80 (s, 3H), 3.35 (d, 2H, J = 7.2 Hz), 3.50 (s, 3H), 3.52 (s, 3H), 5.23 (m, 1H), 5.26 (s, 2H), 5.28 (s, 2H), 6.40 (s, 1H), 7.38 (d, 2H, J = 8.4 Hz), 7.53 (d, 2H, J = 8.4 Hz), 7.72 (d, 1H, J = 16.0 Hz), 7.88 (d, 1H, J = 16.0 Hz), 13.76 (s, 1H). ESI-MS: m/z $[M+H]^+$ 447.

5.3. General method for synthesis of compounds 16a–f

A solution of **15** and 500 mg sodium acetate in 5 mL ethanol containing three drops of water was refluxed for 24 h. The mixture was poured into cold water and extracted with ethyl acetate. The organic phase was washed with brine and dried over anhydrous Na_2SO_4. After removing the solvent, the residue was purified on silica gel column using petroleum ether/ethyl acetate (15:1) to afford flavanones **16a–f**.

5.3.1. 5,7-Dimethoxymethoxy-8-(3,3-dimethylallyl)-flavanone (16a)

Reagents: compound **15a** (400 mg, 0.97 mmol) and sodium acetate (500 mg). Pale yellow syrup (272 mg, 68%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.64 (s, 3H), 1.65 (s, 3H), 2.82 (dd, 1H, J = 16.4 Hz, 2.8 Hz), 2.99 (dd, 1H, J = 12.8, 16.4 Hz), 3.32 (d, 2H, J = 6.8 Hz), 3.48 (s, 3H), 3.53 (s, 3H), 5.18 (m, 1H), 5.24 (s, 2H), 5.26 (s, 2H), 5.41 (dd, 1H, J = 2.8, 12.8 Hz), 6.57 (s, 1H), 7.34–7.47 (m, 5H). ESI-MS: m/z $[M+H]^+$ 413.

5.3.2. 5,7-Dimethoxymethoxy-3′,4′-methylenedioxy-8-(3,3-dimethylallyl)-flavanone (16b)

Reagents: compound **15b** (400 mg, 0.88 mmol) and sodium acetate (500 mg). Pale yellow syrup (252 mg, 63%);^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.65 (s, 6H), 2.77 (dd, 1H, J = 3.2, 16.4 Hz), 2.95 (dd, 1H, J = 12.8, 16.4 Hz), 3.30 (d, 2H, J = 6.8 Hz), 3.48 (s, 3H), 3.53 (s, 3H), 5.16 (m, 1H), 5.23 (s, 2H), 5.25 (s, 2H), 5.31 (dd, 1H, J = 3.2, 12.8 Hz), 5.99 (s, 2H), 6.56 (s, 1H), 6.82 (d, 1H, J = 8.0 Hz), 6.88 (dd, 1H, J = 1.2, 8.0 Hz), 6.96 (d, 1H, J = 1.2 Hz). ESI-MS: m/z $[M+H]^+$ 457.

5.3.3. 5,7-Dimethoxymethoxy-3′-bromo-8-(3,3-dimethylallyl)-flavanone (16c)

Reagents: compound **15c** (400 mg, 0.81 mmol) and sodium acetate (500 mg). Pale yellow syrup (260 mg, 65%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.65 (s, 3H), 1.66 (s, 3H), 2.80 (dd, 1H, J = 2.8, 16.4 Hz), 2.96 (dd, 1H, J = 12.8, 16.4 Hz), 3.30 (d, 2H, J = 6.8 Hz), 3.47 (s, 3H), 3.52 (s, 3H), 5.16 (m, 1H), 5.23 (s, 2H), 5.25 (s, 2H), 5.49 (dd, 1H, J = 2.8, 12.8 Hz), 6.57 (s, 1H), 7.33 (t, 1H, J = 7.6 Hz), 7.39 (d, 2H, J = 7.6 Hz), 7.81 (s, 1H). ESI-MS: m/z $[M+H]^+$ 491.

5.3.4. 5,7,4′-Trimethoxymethoxy-3′-methoxy-8-(3,3-dimethylallyl)-flavanone (16d)

Reagents: compound **15e** (400 mg, 0.80 mmol) and sodium acetate (500 mg). Pale yellow syrup (228 mg, 57%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.68 (s, 3H), 2.80 (dd, 1H, J = 16.4, 2.8 Hz), 2.95 (dd, 1H, J = 13.4, 16.4 Hz), 3.34 (d, 2H, J = 6.8 Hz), 3.49 (s, 6H), 3.55 (s, 3H), 3.93 (s, 3H), 5.17 (m, 1H), 5.20 (s, 2H), 5.24 (s, 2H), 5.26 (s, 2H), 5.48 (dd, 1H, J = 2.8, 13.4 Hz), 6.59 (s, 1H), 6.99 (d, 1H, J = 8.0 Hz), 7.05 (s, 1H), 7.22 (s, 1H, J = 8.0 Hz). ESI-MS: m/z $[M+H]^+$ 503.

5.3.5. 5,7-Dimethoxymethoxy-3′,4′,5′-trimethoxy-8-(3,3-dimethylallyl)-flavanone (16e)

Reagents: compound **15d** (400 mg, 0.80 mmol) and sodium acetate (500 mg). Pale yellow syrup (240 mg, 60%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.66 (s, 3H), 1.67 (s, 3H), 2.79 (dd, 1H, J = 16.4, 2.8 Hz), 2.95 (dd, 1H, J = 13.4, 16.4 Hz), 3.32 (d, 2H, J = 6.8 Hz), 3.49 (s, 3H), 3.55 (s, 3H), 3.95 (s, 9H), 5.16 (m, 1H), 5.21 (s, 2H), 5.24 (s, 2H), 5.35 (dd, 1H, J = 2.8, 13.4 Hz), 6.58 (s, 1H), 6.92 (s, 2H). ESI-MS: m/z $[M+H]^+$ 503.

5.3.6. 5,7,3′-Trimethoxymethoxy-8-(3,3-dimethylallyl)-flavanone (16f)

Reagents: compound **15f** (400 mg, 0.85 mmol) and sodium acetate (500 mg). Pale yellow syrup (220 mg, 55%); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.68 (s, 3H), 2.86 (dd, 1H, J = 16.4 Hz, 2.8 Hz), 3.01 (dd, 1H, J = 13.4, 16.4 Hz), 3.30 (d, 2H, J = 6.8 Hz), 3.45 (s, 3H), 3.48 (s, 3H), 3.51 (s, 3H), 5.17 (m, 1H), 5.21 (s, 2H), 5.24 (s, 2H), 5.26 (s, 2H), 5.38 (dd, 1H, J = 2.8 Hz, 13.4 Hz), 6.59 (s, 1H), 7.07 (d, 1H, J = 8.0 Hz), 7.10 (d, 1H, J = 8.0 Hz), 7.17 (s, 1H), 7.35 (1H, t, J = 8.0 Hz). ESI-MS: m/z $[M+H]^+$ 473.

5.4. General method for synthesis of compounds 1–7 and 17e–f

To a solution of **16a–f** in 10 mL methanol, 2 mL of 3 N HCl was added. The resulting mixture was refluxed for 45 min, then poured into cold water and extracted with ethyl acetate. The organic phase was washed with brine and then dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified over silicon gel column. Elution with petroleum ether/ethyl acetate 5:1 gave **1–7 and 17e–f**.

5.4.1. 2,4,6-Trihydroxy-3′,4′-methylenedioxy-3-(3,3-dimethylallyl) -chalcone (1)

Reagent: compound **15b** (100 mg, 0.27 mmol). Yellow amorphous powder (49.2 mg, 61%), mp: 174–175 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ): 1.60 (s, 3H), 1.69 (s, 3H), 3.11 (d, 2H, J = 7.2 Hz), 5.13 (m, 1H), 6.03 (s, 1H), 6.10 (s, 2H), 6.99 (d, 1H, J = 8.0 Hz), 7.20 (d, 1H, J = 8.0 Hz), 7.22 (s, 1H), 7.63 (d, 1H, J = 16.0 Hz), 7.99 (d, 1H, J = 16.0 Hz), 10.37 (s, 1H, OH), 10.67 (s, 1H, OH), 14.41 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 369. Anal. Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.10; H, 5.69.

5.4.2. 2,4,6-Trihydroxy-3′,4′,5′-trimethoxyl-3-(3,3-dimethylallyl) -chalcone (2)

Reagent: compound **15d** (100 mg, 0.24 mmol). Yellow amorphous powder (45.3 mg, 55%), mp: 180–181 °C. ^{1}H NMR (acetone-d_6, 400 MHz, δ): 1.68 (s, 3H), 1.70 (s, 3H), 3.21 (d, 2H, J = 7.2 Hz), 3.72 (s, 3H), 3.83 (s, 6H), 5.19 (m, 1H), 6.09 (s, 1H), 6.96 (s, 2H), 7.66 (d, 1H, J = 16.0 Hz), 7.82 (d, 1H, J = 16.0 Hz), 9.12 (s, 1H, OH), 9.56 (s, 1H, OH), 14.30 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 415. Anal. Calcd for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32. Found: C, 66.38; H, 6.15.

5.4.3. 2,4,6-Trihydroxy-4′-chloro-3-(3,3-dimethylallyl)-chalcone (3)

Reagent: compound **15g** (100 mg, 0.28 mmol). Yellow amorphous powder (46.6 mg, 58%), mp: 150–151 °C. ^{1}H NMR (ace-

tone-d_6, 400 MHz, δ): 1.61 (s, 3H), 1.69 (s, 3H), 3.13 (d, 2H, J = 7.2 Hz), 5.15 (m, 1H), 6.02 (s, 1H), 7.42 (d, 2H, J = 8.0 Hz), 7.67 (d, 2H, J = 8.0 Hz), 7.69 (d, 1H, J = 16.0 Hz), 8.19 (d, 1H, J = 16.0 Hz), 9.16 (s, 1H, OH), 9.74 (s, 1H, OH), 14.16 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 359. Anal. Calcd for $C_{20}H_{19}ClO_4$: C, 66.95; H, 5.34. Found: C, 66.61; H, 5.68.

5.4.4. (±)Galabranin (4)

Reagent: compound **16a** (200 mg, 0.49 mmol). White amorphous powder (102.2 mg, 65%), mp: 147–148 °C. ^{1}H NMR (acetone-d_6, 400 MHz, δ): 1.56 (s, 6H), 2.78 (dd, 1H, J = 3.6, 16.8 Hz), 3.09 (dd, 1H, J = 12.4, 16.8 Hz), 3.19 (d, 2H, J = 6.8 Hz), 5.15 (m, 1H), 5.53 (dd, 1H, J = 3.6, 12.4 Hz), 5.99 (s, 1H), 7.35 (m, 1H), 7.41 (t, 2H, J = 7.6 Hz), 7.54 (d, 1H, J = 7.6 Hz), 9.57 (s, 1H), 12.08 (s, 1H). ESI-MS: m/z $[M+H]^+$ 325. Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21. Found: C, 74.36; H, 6.43.

5.4.5. (±)5,7-Dihydroxy-3′,4′-methylenedioxy-8-(3,3-dimethylallyl)-flavanone (5)

Reagent: compound **16b** (200 mg, 0.44 mmol). White amorphous powder (101.7 mg, 63%), mp: 164–165 °C. ^{1}H NMR (acetone-d_6, 400 MHz, δ): 1.55 (s, 3H), 1.56 (s, 3H), 2.75 (dd, 1H, J = 2.8, 17.2 Hz), 3.10 (dd, 1H, J = 12.8, 17.2 Hz), 3.18 (d, 2H, J = 7.2 Hz), 5.16 (m, 1H), 5.39 (dd, 1H, J = 2.8, 12.8 Hz), 6.00 (s, 1H), 6.03 (s, 2H), 6.84 (d, 1H, J = 8.0 Hz), 6.91 (dd, 1H, J = 2.0, 8.0 Hz), 7.00 (d, 1H, J = 2.0 Hz), 9.56 (s, 1H, OH), 12.08 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 369. Anal. Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.31; H, 5.65.

5.4.6. (±)5,7-Dihydroxy-3′-bromo-8-(3,3-dimethylallyl)-flavanone (6)

Reagent: compound **16c** (200 mg, 0.41 mmol). White amorphous powder (98.5 mg, 60%), mp: 148–149 °C. ^{1}H NMR (acetone-d_6, 400 MHz, δ): 1.59 (s, 6H), 2.84 (dd, 1H, J = 3.6, 15.6 Hz), 3.08 (dd, 1H, J = 12.4, 15.6 Hz), 3.21 (d, 2H, J = 6.8 Hz), 5.16 (m, 1H), 5.56 (dd, 1H, J = 3.6, 12.4 Hz), 6.01 (s, 1H), 7.37 (t, 1H, J = 7.6 Hz), 7.41 (d, 2H, J = 7.6 Hz), 7.75 (s, 1H), 9.60 (s, 1H, OH), 12.04 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 403. Anal. Calcd for $C_{20}H_{19}BrO_4$: C, 59.57; H, 4.75. Found: C, 59.88; H, 4.65.

5.4.7. (±)Exiguaflavanone K (7)

Reagent: compound **16d** (200 mg, 0.40 mmol); White amorphous powder (66.3 mg, 45%), mp: 138–139 °C. ^{1}H NMR (acetone-d_6, 400 MHz, δ): 1.56 (s, 6H), 2.72 (dd, 1H, J = 3.6, 17.2 Hz), 3.11 (dd, 1H, J = 12.8, 17.2 Hz), 3.18 (d, 2H, J = 7.2 Hz), 3.84 (s, 3H), 5.16 (m, 1H), 5.39 (dd, 1H, J = 3.6, 12.8 Hz), 5.98 (s, 1H), 6.83 (d, 1H, J = 8.0 Hz), 6.96 (dd, 1H, J = 1.6, 8.0 Hz), 7.16 (d, 1H, J = 1.6 Hz), 7.69 (s, 1H, OH), 9.50 (s, 1H, OH), 12.10 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 371. Anal. Calcd for $C_{21}H_{22}O_6$: C, 68.10; H, 5.99. Found: C, 68.18; H, 5.88.

5.4.8. (±)5,7-Dihydroxy-3′,4′,5′-trimethoxy-8-(3,3-dimethylallyl)-flavanone (17e)

Reagent: compound **16e** (200 mg, 0.40 mmol); White amorphous powder (85.8 mg, 52%), mp: 215–216 °C. ^{1}H NMR (acetone-d_6, 400 MHz, δ): 1.57 (s, 3H), 1.59 (s, 3H), 2.77 (dd, 1H, J = 2.8, 17.2 Hz), 3.10 (dd, 1H, J = 12.8, 17.2 Hz), 3.20 (d, 2H, J = 7.2 Hz), 3.70 (s, 3H), 3.82 (s, 6H), 5.21 (m, 1H), 5.44 (dd, 1H, J = 2.8, 12.8 Hz), 6.00 (s, 1H), 6.87 (s, 2H), 9.54 (s, 1H, OH), 12.07 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 415. Anal. Calcd for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32. Found: C, 66.81; H, 6.23.

5.4.9. (±)5,7-Dihydroxy-3′-hydroxy-8-(3,3-dimethylallyl)-flavanone (17f)

Reagent: compound **16f** (200 mg, 0.43 mmol). White amorphous powder (69.2 mg, 48%), mp: 175–176 °C. ^{1}H NMR (acetone-d_6, 400 MHz, δ): 1.55 (s, 6H), 2.72 (dd, 1H, J = 3.2, 16.8 Hz), 3.12 (dd, 1H, J = 12.8, 16.8 Hz), 3.18 (d, 2H, J = 7.2 Hz), 5.16 (m, 1H), 5.45 (dd, 1H, J = 3.2, 12.8 Hz), 6.02 (s, 1H), 6.82 (dd, J = 2.0, 8.0 Hz), 6.98 (d, 1H, J = 8.0 Hz), 7.03 (d, 1H, J = 2.0 Hz), 7.22 (t, 1H, J = 8.0 Hz), 8.46 (s, 1H, OH), 9.54 (s, 1H, OH), 12.10 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 341. Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.57; H, 5.92. Found: C, 70.35; H, 5.79.

5.5. General method for synthesis of compounds 8–11

A stirred solution of corresponding prenylflavanone and iodine in dry pyridine (4 mL) was heated to 90 °C for 6 h. The mixture was cooled and poured into cold water. The precipitate was separated and the mixture was extracted with ethyl acetate. The combined organic phase was washed with saturated sodium thiosulfate and water, successively. Then the organic layer was dried with sodium sulfate and concentrated. The residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (2:1) to afford **8**–**11**.

5.5.1. 5,7-Dihydroxy-3′-bromo-8-(3,3-dimethylallyl)-flavone (8)

Reagents: compound **6** (40 mg, 0.10 mmol) and iodine (25.2 mg, 0.10 mmol). Light yellow solid (26.3 mg, 66%), mp: 202–203 °C. ^{1}H NMR (acetone-d_6, 400 MHz, δ): 1.61 (s, 3H), 1.76 (s, 3H), 3.50 (d, 2H, J = 7.2 Hz), 5.23 (m, 1H), 6.31 (s, 1H), 6.79 (s, 1H), 7.51 (t, 1H, J = 8.0 Hz), 7.72 (d, 1H, J = 8.0 Hz), 7.82 (d, 1H, J = 8.0 Hz), 8.16 (s, 1H), 9.68 (s, 1H, OH), 13.05 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 401. Anal. Calcd for $C_{20}H_{17}BrO_4$: C, 59.87; H, 4.27. Found: C, 59.68; H, 4.39.

5.5.2. 5,7,4′-Trihydroxy-3′-methoxy -8-(3,3-dimethylallyl)-flavone (9)

Reagents: compound **7** (40 mg, 0.11 mmol) and iodine (27.5 mg, 0.11 mmol). Light yellow solid (22.3 mg, 56%), mp: 196–198 °C. ^{1}H NMR (acetone-d_6, 400 MHz, d): 1.61 (s, 3H), 1.76 (s, 3H), 3.52 (d, 2H, J = 6.8 Hz), 3.94 (s, 3H), 5.28 (m, 1H), 6.31 (s, 1H), 6.64 (s, 1H), 6.98 (dd, 1H, J = 1.6, 7.6 Hz), 7.55 (dd, 1H, J = 1.6, 7.6 Hz), 7.58 (d, 1H, J = 1.6 Hz), 8.41 (s, 1H, OH), 9.53 (s, 1H, OH), 12.92 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 369. Anal. Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.18; H, 5.29.

5.5.3. 5,7-Dihydroxy-3′,4′,5′-trimethoxy-8-(3,3-dimethylallyl)-flavone (10)

Reagents: compound **17e** (40 mg, 0.10 mmol) and iodine (25.2 mg, 0.10 mmol). Light yellow solid (27.9 mg, 70%), mp: 229–230 °C. ^{1}H NMR (acetone-d_6, 400 MHz, d): 1.61 (s, 3H), 1.75 (s, 3H), 3.54 (d, 2H, J = 7.2 Hz), 3.79 (s, 3H), 3.92 (s, 6H), 5.32 (m, 1H), 6.31 (s, 1H), 6.76 (s, 1H), 7.34 (s, 2H), 9.56 (s, 1H, OH), 12.81 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 413. Anal. Calcd for $C_{23}H_{24}O_7$: C, 66.98; H, 5.87. Found: C, 67.09; H, 5.69.

5.5.4. 5,7-Dihydroxy-3′-hydroxy-8-(3,3-dimethylallyl)-flavone (11)

Reagents: compound **17f** (40 mg, 0.12 mmol), iodine (29.9 mg, 0.12 mmol). Light yellow solid (25.4 mg, 64%), mp: 228–229 °C. ^{1}H NMR (acetone-d_6, 400 MHz, d): ^{1}H NMR (acetone-d_6, 400 MHz, d): 1.61 (s, 3H), 1.77 (s, 3H), 3.51 (d, 2H, J = 6.8 Hz), 5.25 (m, 1H), 6.30 (s, 1H), 6.66 (s, 1H), 7.04 (dd, 1H, J = 1.6, 8.0 Hz), 7.38 (t, 1H, J = 8.0 Hz), 7.48 (d, 1H, J = 1.6 Hz), 7.49 (d, 1H, J = 8.0 Hz), 8.82 (s, 1H, OH), 9.60 (s, 1H, OH), 12.80 (s, 1H, OH). ESI-MS: m/z $[M+H]^+$ 339. Anal. Calcd for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36. Found: C, 70.18; H, 5.49.

5.6. Vasodilator effect assay

Vascular rings were prepared from the aorta of male Sprague–Dawley rats (4 to 6 months old and weighing on average 250 g),

and contraction studies were performed following the general procedure detailed in the literature.[27] After an equilibration period of at least 1 h, isometric contractions induced by PE (1 mM) were obtained. When contraction of the tissue in response to this vasoconstrictor agent had stabilized (after about 20 min), cumulatively increasing concentrations of the tested compounds were added to the bath at 15–20 min intervals (the time needed to obtain steady-state relaxation). Control tissues were simultaneously subjected to the same procedures, but omitting the compounds and adding the vehicle. All data were expressed as mean ± SD. The prenylated flavonoids-induced maximal relaxation (E_{max}) in aortic rings was calculated as a percentage of the contraction in response to PE (1 μM). The half maximum effective concentration (EC_{50}) was defined as the concentration of the flavonoids that induced 50% of maximum relaxation from the contraction elicited by PE (1 μM) and was calculated from the concentration–response curve by nonlinear regression (curve fit) using GraphPad Prism (Version 4.0). Statistical comparisons were made using one-way ANOVA followed by Newman–Keuls test. $P < 0.05$ was considered to be statistically significant.

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