Regio- and Stereospecific Prenylation of Flavonoids by *Sophora flavescens* Prenyltransferase

Ridao Chen,[a] Xiao Liu,[a] Jianhua Zou,[a] Yunze Yin,[a] Bin Ou,[a] Jianhua Li,[a] Ruishan Wang,[a] Dan Xie,[a] Peicheng Zhang,[a] and Jungui Dai[a,*]

[a] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College & Chinese Academy of Medical Sciences, 1 Xian Nong Tan Street, Beijing 100050, People's Republic of China
Fax: (+86)-10-6301-7757; phone: (+86)-10-6316-5195; e-mail: jgdai@imm.ac.cn

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Abstract: Prenylflavonoids are valuable natural products that are widely distributed in plants. They often possess divergent biological properties, including phytoestrogenic, anti-bacterial, anti-tumor, and anti-diabetic activities. The reaction catalyzed by prenyltransferases represents a Friedel–Crafts alkylation of the flavonoid skeleton in the biosynthesis of natural prenylflavonoids and often contributes to the structural diversity and biological activity of these compounds. However, only a few plant flavonoid prenyltransferases have been identified thus far, and these prenyltransferases exhibit strict substrate specificity and low catalytic efficiency. In this article, a flavonoid prenyltransferase from *Sophora flavescens*, SfFPT, has been identified that displays high catalytic efficiency with high regiospecificity acting on C-8 of structurally different types of flavonoid (i.e., flavanone, flavone, flavanonol, and dihydrochalcone, etc.). Furthermore, SfPFT exhibits strict stereospecificity for levorotatory flavanones to produce (2*S*)-prenylflavanones. This study is the first to demonstrate the substrate promiscuity and stereospecificity of a plant flavonoid prenyltransferase *in vitro*. Given its substrate promiscuity and high catalytic efficiency, SfFPT can be used as an environmentally friendly and efficient biological catalyst for the regio- and stereospecific prenylation of flavonoids to produce bioactive compounds for potential therapeutic applications.

Keywords: enzyme catalysis; flavonoid prenylation; prenyltransferase; regioselectivity; *Sophora flavescens*; stereoselectivity

Introduction

Prenylflavonoids are valuable natural products that are mainly found within a few families, including the Leguminosae, Moraceae, Cannabaceae, Guttiferae, Umbelliferae, and Euphorbiaceae. They exhibit various biological properties, including phytoestrogenic, anti-bacterial, anti-tumor, and anti-diabetic activities.[1] The prenylation of flavonoids contributes significantly to the structural variation of plant secondary metabolites and enhances the bioactivity and bioavailability of these plant secondary metabolites; this enhancement is likely a result of the presence of the lipophilic prenyl side chain, which increases the membrane permeability and/or enhances the binding affinity of target proteins of these compounds.[2]

The enzymatic reaction catalyzed by prenyltransferase is crucial for the biosynthesis of bioactive prenyl-

flavonoids. However, the study of plant membrane proteins is challenging; thus, only five flavonoid prenyltransferases have been characterized at the molecular level to date.[3–6] Three of these prenyltransferases were identified in *Sophora flavescens* (i.e., SfN8DT-1, SfG6DT, and SfiLDT), while the other two were isolated from *Glycine max* (i.e., G4DT) and *Lupinus albus* (i.e., LaPT1). SfN8DT-1, the first flavonoid-specific prenyltransferase to be identified, was shown to be responsible for the prenylation of a very few select flavanones (i.e., naringenin, liquiritigenin, and hesperetin) at the C-8 position. SfG6DT was found to specifically prenylate the isoflavone genistein at the C-6 position. The chalcone-specific prenyltransferase SfiLDT was shown to prenylate isoliquiritigenin. G4DT was discovered to specifically act on a pterocarpan substrate, glycinol. Finally, LaPT1 was found to prenylate the B-rings of isoflavones, such as

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genistein and 2′-hydroxygenistein. These membrane-bound prenyltransferases exhibit strict substrate specificities, as they only accept dimethylallyl diphosphate (DMAPP) as a prenyl donor and a few similarly structured substrates as prenyl acceptors. Given this substrate specificity, these plant prenyltransferases are quite different from the soluble prenyltransferases found in fungi, bacteria, and actinomycetes, which display remarkable substrate tolerance and notable catalytic promiscuity.[7–10]

Because plants usually do not have biosynthetic gene clusters like microorganisms do and given the transmembrane properties of the prenyltransferases, the detailed function of a plant prenyltransferase is much more difficult to elucidate. In addition, the prenyltransferases identified thus far exhibit strict substrate specificity and low catalytic efficiency, which significantly restricts their use in the structural derivation of flavonoids to produce biologically active compounds for therapeutic applications.

The medicinal plant *S. flavescens* can produce diverse prenylated flavonoids, including different types of flavonoids with prenyl or lavandulyl group substitutions (i.e., flavanones, flavanonols, flavonols, and chalcones, etc.).[11–13] It has previously been demonstrated that enzyme promiscuity plays a role in protein evolution, as well as in the biosynthesis of natural products for plant defense, where multiple functions have been associated with a single enzyme.[14,15] Thus, it can be hypothesized that either diverse flavonoid prenyltransferases with strict substrate specificities solely exist in *S. flavescens* or some flavonoid prenyltransferases in *S. flavescens* with broad substrate spectra have yet to be discovered; both hypotheses would allow *S. flavescens* to biosynthesize various prenylated flavonoids.

In this study, a flavonoid prenyltransferase gene from *S. flavescens*, *SfFPT*, was cloned and successfully expressed heterologously in yeast (*Saccharomyces cerevisiae*). A sequence alignment analysis indicated that SfFPT may be a paralogue of the previously reported flavonoid prenyltransferase SfN8DT-1 (93% identity). Interestingly, SfFPT recognized a diverse array of flavonoid structure types as prenyl acceptors, including flavanone, flavone, flavanonol, and dihydrochalcone; furthermore, the regio- and stereospecificity of the transferred prenyl group into the flavonoid skeleton was performed with high catalytic efficiency when DMAPP was used as a prenyl donor. Meanwhile, SfFPT could also accept geranyl diphosphate (GPP) as a prenyl donor in some cases.

To the best of our knowledge, this study is the first demonstration of a plant flavonoid prenyltransferase that exhibits broad substrate specificity. The heterologous over-expression of SfFPT could provide a convenient approach for the production of diverse, bioactive prenylated flavonoids by enzymatic synthesis and

provide insight into the enzymatic promiscuity and reaction selectivity of plant flavonoid prenyltransferases, which would then explain the structural diversity of the prenylflavonoids found in nature. The prenylation of structurally different flavonoids by the same enzyme illustrates the economical use of a "combinatorial biosynthetic" strategy in nature to create structural diversity. The results indicate that SfFPT may contribute to the biosynthesis of diverse prenylflavonoids in *S. flavescens*. Moreover, SfFPT can be used as an environmentally friendly and efficient biological catalyst for the regio- and stereospecific prenylation of flavonoids, which have potential therapeutic applications in humans.

Results and Discussion

The Molecular Cloning of a Flavonoid Prenyltransferase cDNA

S. flavescens plants, as well as cultured cells from these plants, can produce various types of prenylated flavonoids, such as prenylated flavonol, flavanone, flavanonol, and chalcone. The existence of flavonoid prenyltransferases and the elucidation of their catalytic abilities were first demonstrated by an analysis of the enzymatic prenylation activity of the microsomal fraction prepared from cultured cells of *S. flavescens*. Diverse prenyltransferase activities were observed in the microsomal fraction. Specifically, the results indicated that the microsomal fraction can catalyze the prenylations of pinocembrin (**1**), naringenin (**2**), chrysin (**3**), and taxifolin (**4**) *in vitro*, which further supported the hypothesis that either diverse flavonoid prenyltransferases or some flavonoid prenyltransferases with broad substrate specificity were present in *S. flavescens* (Supporting Information, Figure S1). Based upon these results, we focused on the identification of candidate prenyltransferases that could be responsible for the prenylation of different flavonoids and investigated the use of these candidates in the enzymatic synthesis of prenylflavonoids *in vitro*. As a result, a candidate cDNA, *SfFPT* (GenBank accession KC513505), was cloned from the cultured cells of *S. flavescens* by homology-based RT-PCR. A 1665 bp full-length cDNA containing a 1224 bp open reading frame (ORF) and encoding a 407 amino acid polypeptide was obtained using the rapid amplification of cDNA ends (RACE) method. The deduced protein sequence of SfFPT showed very high sequence similarity to those of *S. flavescens* flavonoid prenyltransferases that were previously reported (Supporting Information, Figure S2); in particular, it shared a sequence identity of 93% with SfN8DT-1. In addition, a molecular evolutionary genetics analysis using Clustal X[16] and MEGA5[17] indicated that SfFPT be-

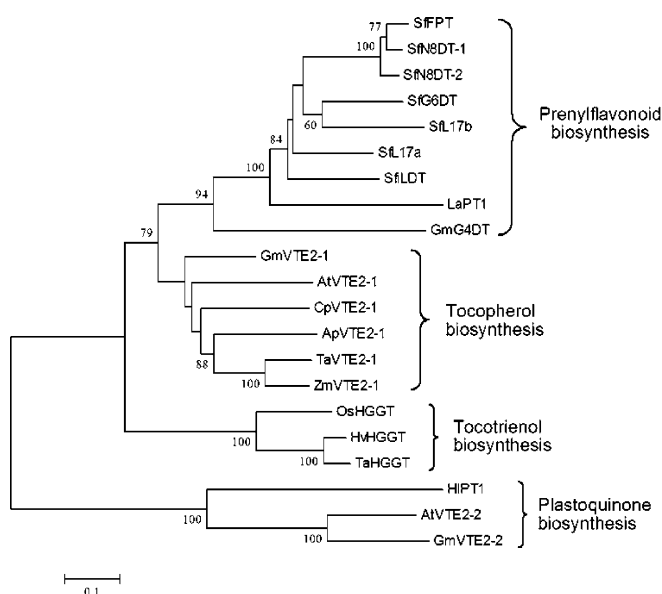↖↖ **These are not the final page numbers!**

Figure 1. The phylogenetic relationships between SfFPT and related prenyltransferases from plants. The results were calculated using the MEGA version 5 software. Method: Neighbor-joining, Bootstrap: 1000. The branch lengths represent relative genetic distances. The protein sequences and corresponding accession numbers that were used for this comparison are as follows: SfFPT, KC513505; SfN8DT-1, AB325579; SfN8DT-2, AB370330; SfL17a, AB371287; SfL17b, AB370329; SfG6DT, BAK52291; SfiLDT, AB604223; GmG4DT, AB434690; LaPT1, JN228254; HIPT1, AB543053; AtVTE2-2, DQ231060; GmVTE2-2, DQ231061; OsHGGT, AY222862; HvHGGT, AY222860; TaHGGT, AY222861; CpVTE2-1, DQ231058; AtVTE2-1, AY089963; ApVTE2-1, DQ231057; GmVTE2-1, DQ231059; TaVTE2-1, DQ231056; ZmVTE2-1, DQ231055.

longed to the *S. flavescens* flavonoid prenyltransferase clade and exhibited close evolutionary relationships with the other prenyltransferases (Figure 1).

As with previously characterized flavonoid prenyltransferases (i.e., SfN8DT-1, G4DT, SfG6DT, and LaPT1), the SfFPT polypeptide was predicted to contain eight transmembrane α-helices using TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). In addition, SfFPT was predicted to localize to plastids using WoLF PSORT (http://wolfpsort.org/), which is similar to the other known flavonoid prenyltransferases. However, the predicted polypeptide sequence did not contain a transit peptide at the N-terminal end, as assessed by ChloroP 1.1 (http://www.cbs.dtu.dk/services/ChloroP/), TargetP 1.1 (http://www.cbs.dtu.dk/services/TargetP/), iPSORT (http://ipsort.hgc.jp/), and the SignalP 4.0 Server (http://www.cbs.dtu.dk/services/SignalP/). Two characteristically conserved motifs, NQ×× D×××D and KD(I/L)×D×(E/D)GD, of flavonoids and homogentisate prenyltransferase were also present in the polypeptide sequence of SfFPT. In addition, SfFPT might be a basic protein with a

theoretical pI of 9.2, as predicted by ExPASy (http://web.expasy.org/cgi-bin/protparam/protparam). It was also indicated that the protein would exhibit increased stability and activity under basic conditions.

The Functional Characterization of Recombinant SfFPT

The coding region of *SfFPT* was cloned into a yeast expression vector, pESC, under the control of the GAL10 yeast promoter to yield pESC-*SfFPT*. After verification of the sequence, the plasmid was introduced into YPH499 yeast for expression analysis. The microsomal fraction of the recombinant yeast was prepared for an enzymatic activity analysis using 400 μM DMAPP as a prenyl donor and 400 μM pinocembrin, naringenin, chrysin, or taxifolin as prenyl acceptors, which were chosen because the prenylated derivatives were found in both the primary plant and cultured cells of *S. flavescens*. Recombinant SfFPT exhibited clear prenyltransferase activity in the enzymatic reaction analyses (Figure 2). It can regioselectively and efficiently catalyze the prenylation of pinocembrin (in 58.8% yield), naringenin (in 62.6% yield), chrysin (in 16.4% yield), and taxifolin (in 33.5% yield) at the C-8 position. In contrast, no prenyltransferase activity was observed when microsomes isolated from yeast cells transformed with an empty vector or boiled recombinant protein was used; furthermore, no reactivity was observed when DMAPP, a prenyl acceptor or Mg^{2+} was absent from the enzymatic reaction (Supporting Information, Figure S3).

Previous studies reported that the catalytic activity of plant flavonoid prenyltransferases is dependent on divalent cations.[3,4] Further investigations in this study revealed that a variety of divalent cations can contribute to the activity of SfFPT; specifically, while Mg^{2+} (100%) is the most effective, Ba^{2+} (84.3%), Ca^{2+} (83.6%), Fe^{2+} (55.1%), Co^{2+} (45.9%), Cu^{2+} (40.6%), Zn^{2+} (36.8%), and Mn^{2+} (36.6%) also contribute to SfFPT activity. The optimal pH for enzyme activity was approximately 9.0 and is likely related to the basic protein characteristics of SfFPT, which has a theoretical pI of 9.2. The optimal reaction temperature was approximately 40°C, and the catalytic activity of SfFPT decreased rapidly when the reaction temperature increased above 60°C, which is likely due to the denaturation of the enzyme (Supporting Information, Figure S4).

The Prenylations of Flavonoids by Recombinant SfFPT

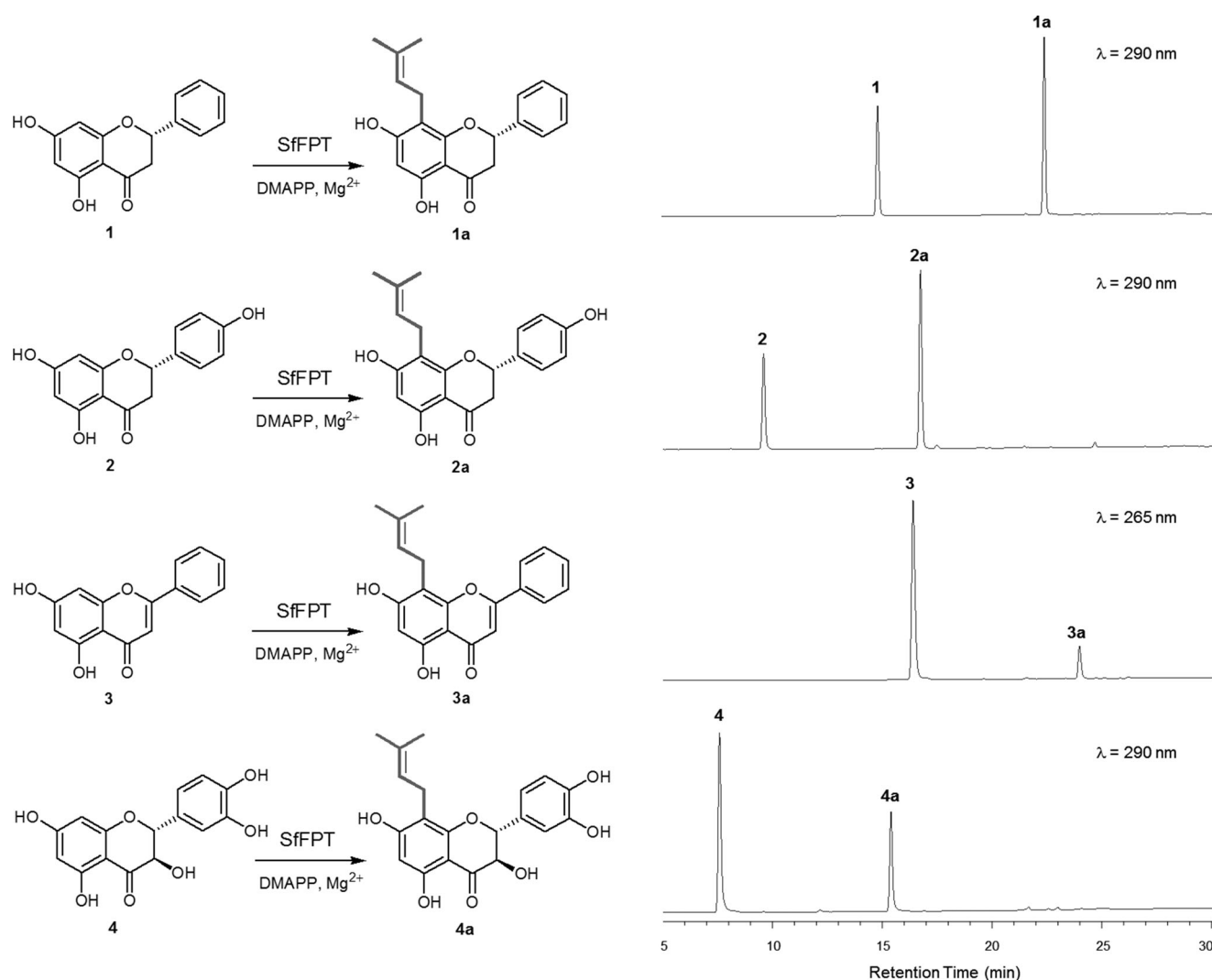To analyze the prenyl acceptor specificity of recombinant SfFPT, various putative, aromatic substrates

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Figure 2. The functional characterization of recombinant SfFPT with DMAPP as a prenyl donor and pinocembrin (**1**), naringenin (**2**), chrysin (**3**), or taxifolin (**4**) as prenyl acceptors. The existence of flavonoid prenyltransferases and the elucidation of their catalytic abilities in *S. flavescens* were demonstrated by analyzing the microsomal fraction enzyme reactivity of cultured cells (Supporting Information, Figure S1).

(400 μM concentration) were utilized, including various types of flavonoids and structurally simple phenols that have flavonoid-like structures; DMAPP (400 μM) was used as a prenyl donor. Analyses of the enzymatic products revealed the significant substrate promiscuity of SfFPT. In addition to the four previously tested flavonoids (i.e., pinocembrin, naringenin, chrysin, and taxifolin), SfFPT could also catalyze the prenylation of eight other flavonoids, including seven flavanones, specifically liquiritigenin (**5**, in 45.8% yield), steppogenin (**6**, in 29.9% yield), eriodictyol (**7**, in 63.4% yield), hesperetin (**8**, in 45.1% yield), sakuranetin (**9**, in 3.0% yield), isosakuranetin (**10**, in 37.9% yield), and tsugafolin (**11**, in 18.3% yield), and one dihydrochalcone, phloretin (**12**, in 22.1% yield) (Figure 3; Supporting Information, Figures S5a and S5b). The prenylated products for most of these mole-

cules were not discovered in the plant *S. flavescens*. The apparent Michaelis–Menten constants (K_m) were determined for eleven flavonoids using Lineweaver–Burk plots (Table 1); the Michaelis–Menten constant was not determined for sakuranetin because of its low conversion rate. The results indicate that SfFPT exhibited substantially stronger affinities and higher catalytic efficiencies with various flavonoids than other previously reported plant flavonoid prenyltransferases. For example, the apparent K_m values of naringenin and DMAPP (naringenin was used as a prenyl acceptor) for SfFPT were calculated to be 0.236 μM and 0.105 μM compared with 55 μM and 106 μM for SfN8DT-1, respectively.

Given the high efficiency and conversion rate of flavonoid prenyltransferase SfFPT, most of the enzymatic products could be efficiently prepared for struc-
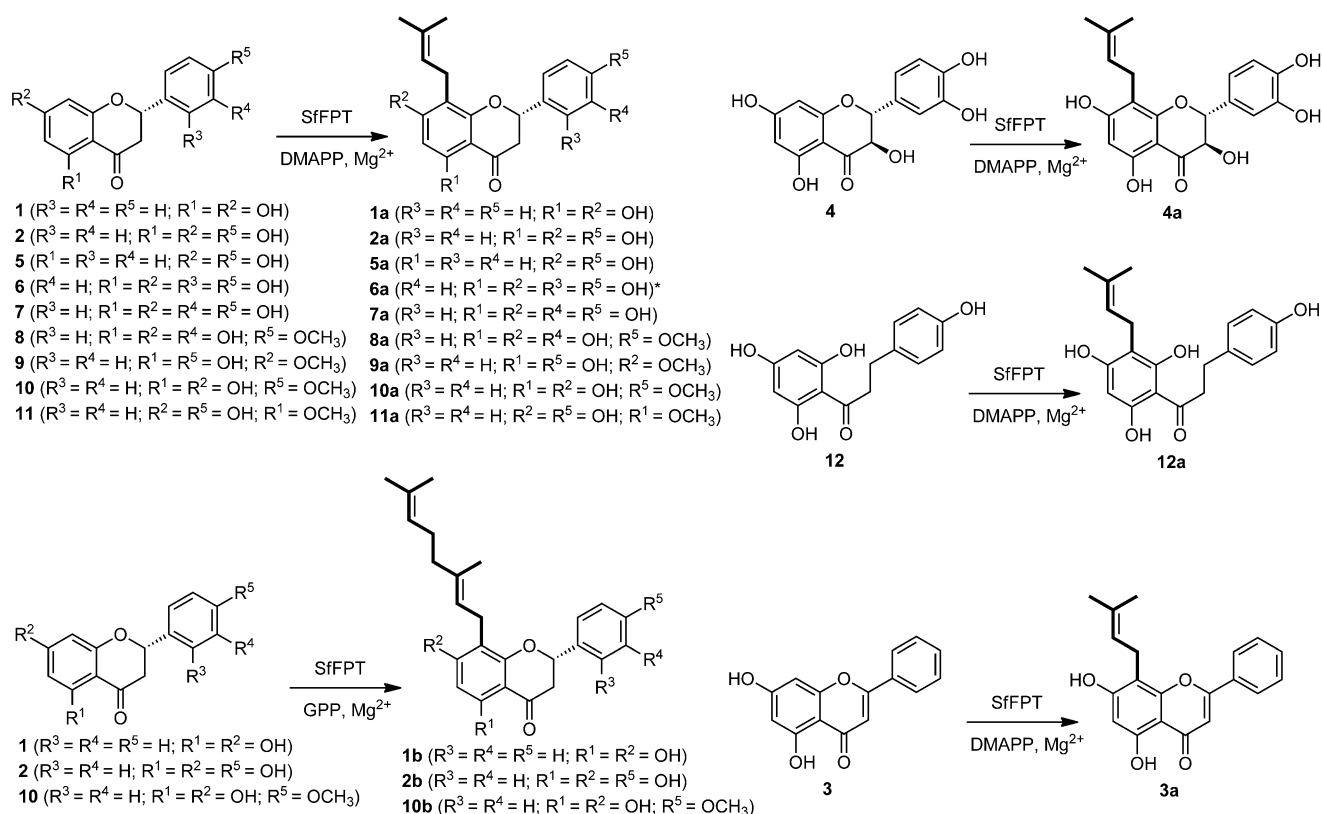
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Figure 3. The prenylation of flavonoids by recombinant SfFPT.

tural elucidation. Eleven flavonoids were used in larger scale reactions, and the prenylated products were purified by semi-preparative RP-HPLC. Identification of the enzymatic products was carried out by comparison of their NMR and MS data with those of know compounds in the published literature (see Experimental Section). In addition, the prenylated product of sakuranetin was identified using an HPLC-

DAD/ESI-MS^n analysis (Supporting Information, Figure S6).

The substrate promiscuity of SfFPT was further demonstrated by prenyl donor specificity analyses. The prenyl donor specificity of SfFPT was tested using DMAPP, GPP, and FPP as prenyl donors and pinocembrin, naringenin, liquiritigenin, steppogenin, eriodictyol, hesperetin, sakuranetin, isosakuranetin, tsugafolin, taxifolin, phloretin, and chrysin were used as prenyl acceptors. The results indicated that SfFPT could also recognize GPP as a prenyl donor and catalyze the geranylation of pinocembrin, isosakuranetin, and naringenin (Figure 3). The prenylation products were identified using HPLC-DAD/ESI-MS^n analyses. (Supporting Information, Figures S7–S9). This study is the first to report that a plant flavonoid prenyltransferase can accept both DMAPP and GPP as prenyl donors.

The Substrate Structure–Enzyme Selectivity Relationship of SfFPT

The prenylation of natural prenylflavonoids always occurs with the substitution of hydroxy group(s) or methoxy group(s) at the flavanone skeleton, which

Table 1. The apparent K_m values of recombinant SfFPT for various flavonoids.

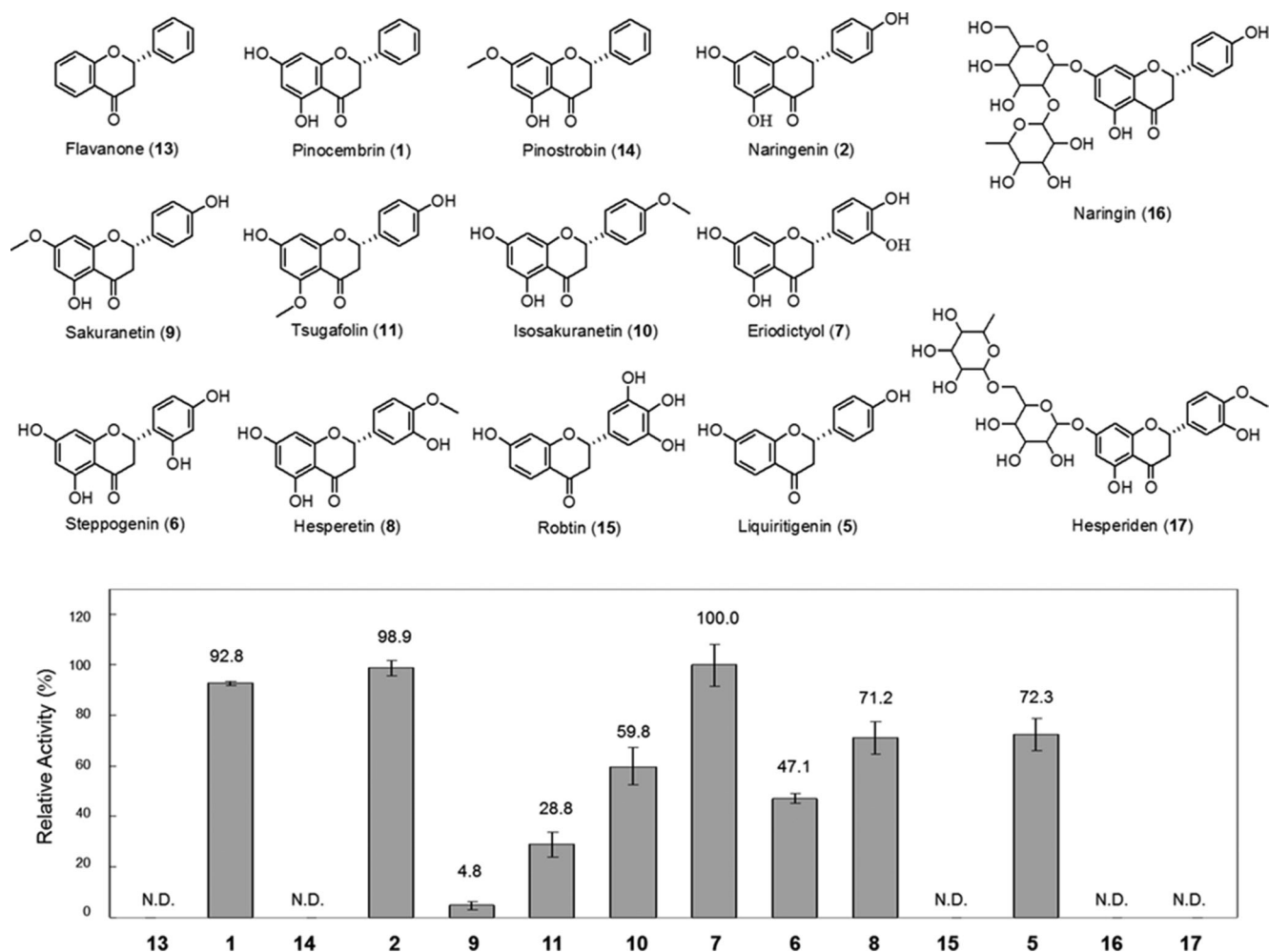| Substrate	K_m [μM]	Substrate[a]	K_m [μM]	Conversion yields [%]
pinocembrin	0.586	pinocembrin	0.120	58.8
naringenin	0.236	naringenin	0.105	62.6
liquiritigenin	0.092	liquiritigenin	0.128	45.8
steppogenin	0.013	steppogenin	0.031	29.9
eriodictyol	0.092	eriodictyol	0.125	63.4
hesperetin	0.228	hesperetin	0.140	45.1
isosakuranetin	0.207	isosakuranetin	0.047	37.9
tsugafolin	0.032	tsugafolin	0.446	18.3
taxifolin	0.114	taxifolin	0.869	33.5
phloretin	0.045	phloretin	0.102	22.1
chrysin	0.020	chrysin	2.030	16.4

[a] Apparent K_m value for DMAPP.

Figure 4. The relative enzyme activities of recombinant SfFPT on various flavanones. N.D.: not detected.

may affect the biological activities of these compounds.[18,19] On the other hand, different substitution patterns and the substitution of different groups at the flavanone skeleton may affect the substrate selectivity of a prenyltransferase. Through substrate selectivity analyses, it was revealed that SfFPT mainly prenylates flavanones at the C-8 position. To provide insight into the "substrate structure–enzyme selectivity relationship" and to provide valuable strategies for the further chemo-enzymatic synthesis of active prenylflavonoids, the substrate selectivity of SfFPT for fourteen flavanones, including flavanone (**13**), pinocembrin, pinostrobin (**14**), naringenin, sakuranetin, tsugafolin, isosakuranetin, eriodictyol, steppogenin, hesperetin, robtin (**15**), liquiritigenin, naringin (**16**), and hesperiden (**17**), was compared (Figure 4). The following conclusions were preliminarily made about the effects of different substitution groups on the prenylation and substrate selectivity of SfFPT.

Firstly, hydroxy groups at the C-7/5 positions on the A-ring enhanced the electron density around the C-8 position, which was crucial for the prenylation of the

C-8 position by SfFPT, and improved the reactivity and regioselectivity of the prenyl group substitution at the C-8 position of the flavanone skeleton, which was in accordance with previous reports.[20,21] In contrast to hydroxy groups, methoxy groups at the C-7/5 positions reduced the prenylation efficiency of SfFPT. Because the prenylation rate of naringenin (**2**) and the yield of its prenylated product by SfFPT were higher than those of tsugafolin (**11**) and sakuranetin (**9**), respectively, and because the prenylation rate of tsugafolin (**11**) was higher than the rate of sakuranetin (**9**), it can be concluded that a hydroxy group substituted at the C-7 position is crucial for a prenylation at the C-8 position by SfFPT. This conclusion is also confirmed by the fact that SfFPT could not prenylate pinostrobin (**14**) (a methoxy group is substituted at the C-7 position of pinocembrin), naringin (**16**, an *O*-glycosylation group is at the C-7 position of naringenin), and hesperiden (**17**, an *O*-glycosylation group is at the C-7 position of hesperetin).

Secondly, hydroxy groups at the C-3′/4′ positions on the B-ring increased the prenylation efficiency of

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SfFPT. For example, the conversion rate of eriodictyol (**7**) by SfFPT was higher than that of naringenin (**2**), and the conversion rate of naringenin was higher than that of pinocembrin (**1**). In addition, flavanones with hydroxy groups at the C-3′,4′,5′ positions on the B-ring cannot be accepted as substrates given that liquiritigenin (**5**), and not robtin (**15**), can be prenylated by SfFPT. Similar results were observed in the enzymatic reaction of taxifolin (dihydroxy groups at the C-3′,4′ positions) and dihydromyricetin (trihydroxy groups at the C-3′,4′,5′ positions).

Thirdly, a flavanone with a methoxy group on the B-ring was less effectively converted by SfFPT than flavanones with a hydroxy group. For example, the conversion rate of eriodictyol (**7**) was higher than that of hesperetin (**8**), and the conversion rate of naringenin (**2**) was higher than that of isosakuranetin (**10**).

Fourthly, overall, the conversion rate of flavanones by SfFPT was influenced by the hydroxy or methoxy group substitution as follows: hydroxy groups in the C-7/5 positions on the A-ring and the C-3′/4′ positions on the B-ring had a beneficial effect on the prenylation of flavanones by SfFPT, while methoxy groups in these positions obviously decreased the conversion rate.

The Regiospecific and Stereospecific Prenylation of Flavonoids by SfFPT

While various flavonoids, including flavanone, flavone, dihydrochalcone, and flavonol, were accepted as substrates by SfFPT, a prenyl side chain was regioselectively introduced into the skeleton of the flavonoids only at the C-8 position. In contrast, when steppogenin was incubated with SfFPT, DMAPP, and MgCl$_2$ in Tris-HCl buffer (pH 9.0), both C-6 and C-8 prenylated products were simultaneously detected. These findings are consistent with previously reported results that steppogenin was prenylated by the microsomal fraction of *S. flavescens* cultured cells.[13] However, further investigation of this reaction revealed that 6-dimethylallylsteppogenin and 8-dimethylallylsteppogenin can easily and automatically transform into each other in the reaction buffer without enzyme. Thus, 6-prenylsteppogenin is unlikely to be a direct enzymatic product of steppogenin when catalyzed by SfFPT. These results strongly suggest that SfFPT is strictly regiospecific for the prenylation of flavonoids at the C-8 position.

Interestingly, regardless of whether optically active or optically inactive flavanones were incubated with recombinant SfFPT, the enzyme products were always determined to be in the levorotatory form (2*S*). These findings prompted us to investigate the stereoselectivity of recombinant SfFPT. (+)-Naringenin and (−)-naringenin, a pair of optical isomers, were used as prenyl acceptors, and the products were analyzed by chiral chromatography. The results indicated that the pair of optical isomers can transform into each other in the reaction buffer without recombinant SfFPT. On the other hand, a dextrorotatory product [i.e., (+)-8-dimethylallylnaringenin] was not detected when (+)-naringenin was incubated with SfFPT and DMAPP (Figure 5). These results demonstrate that SfFPT is stereospecific for levorotatory flavanone. Similar results were also observed when optical iso-
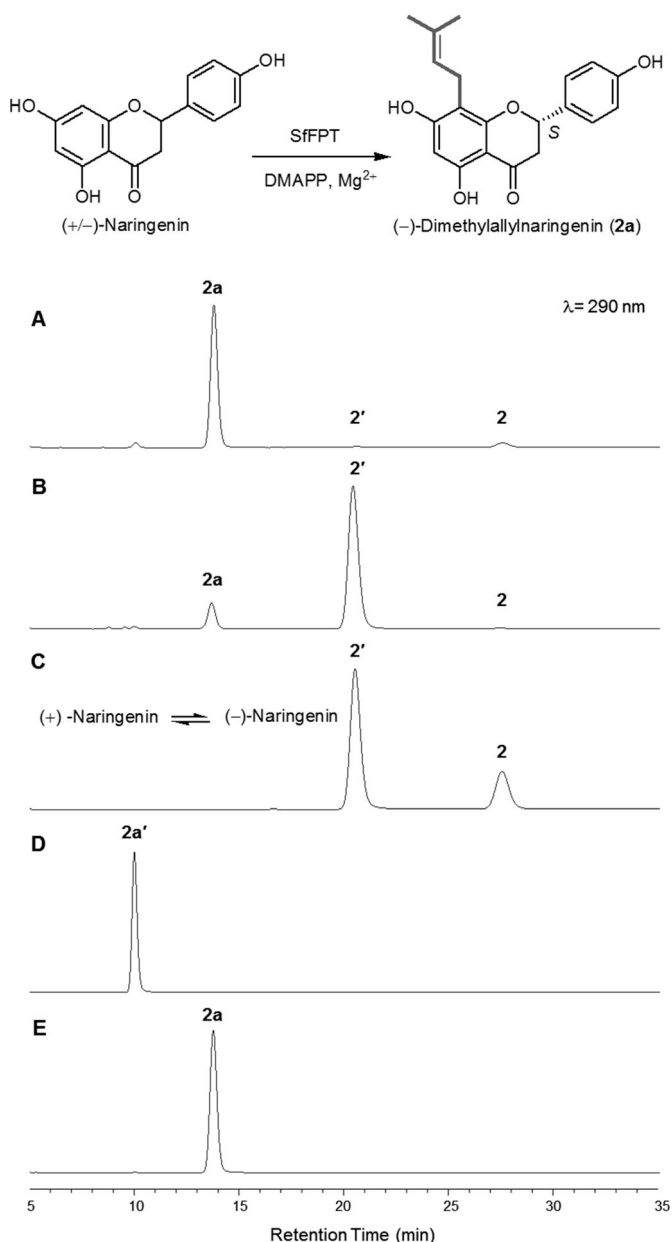

Figure 5. Chiral HPLC analyses of enzymatic reaction products of recombinant SfFPT. (A) (−)-Naringenin (**2**) was used as a prenyl acceptor. (B) (+)-Naringenin (**2′**) was used as a prenyl acceptor. (C) Control reaction without recombinant enzyme. (D) Authentic (+)-8-dimethylallylnaringenin (**2a′**). (E) Authentic (−)-8-dimethylallylnaringenin (**2a**).

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mers of other flavanones (e.g., pinocembrin and hesperetin, data not shown) were employed as prenyl acceptors. It is well-known that the efficient synthesis of optically pure flavanones by chemical asymmetric catalysis methods is highly attractive to chemists. However, this type of synthesis has faced many difficulties and challenges in this field.[22] The chemical synthesis of optically pure prenylflavanones has not been reported thus far. Interestingly, SfFPT exhibits a desired reaction specificity and high catalytic efficiency, which makes it a useful tool for the production of bioactive chiral prenylflavanones. These results suggest a powerful method for the preparation of optical prenylflavanones by dynamic resolution and SfFPT prenylation.

Conclusions

The prenyl group substitution at the flavonoid skeleton contributes to the structural diversity and biological activity of natural flavonoids in the plant kingdom. The enzymatic reaction catalyzed by a prenyltransferase is crucial for the biosynthesis of these diverse prenylated flavonoids. However, only a few plant flavonoid prenyltransferases have been identified, and they exhibit strict substrate specificity and low catalytic efficiency. The present work describes the identification of a flavonoid prenyltransferase SfFPT from *S. flavescens*. We discovered that SfFPT exhibits significant substrate promiscuity *in vitro* and has an unprecedented ability to recognize diverse flavonoids as prenyl acceptors when DMAPP is used as a prenyl donor; SfFPT could also accept GPP as a prenyl donor in some cases, which has not yet been found in the reported plant flavonoid prenyltransferases. Intriguingly, SfPFT exhibits strict stereospecificity for levorotatory flavanones. The results indicate that SfFPT may not only contribute to the biosynthesis of diverse prenylflavonoids in *S. flavescens*, but also can be used as an efficient biological catalyst for the regio- and stereospecific prenylation of flavonoids, which have potential therapeutic applications in humans.

Experimental Section

Plant Material and Culture Method

S. flavescens cultured cells were maintained in Murashige and Skoog's basal medium[23] supplemented with 0.5 mg L^{-1} α-naphthaleneacetic acid (NAA), 0.5 mg L^{-1} 6-benzylaminopurine (6-BA), 0.2 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 30 g L^{-1} sucrose, and 5 g L^{-1} agar. Cell suspension cultures were performed in 500-mL flasks containing 125 mL of the same medium without agar. Methyl jasmonate (MJ) solution in EtOH (100 mM) was added to the medium (at a final concentration of 100 μM) on the 12th day of subculture to induce prenyltransferase gene expression.

Chemicals

The prenyl donors dimethylallyl diphosphate (DMAPP), geranyl diphosphate (GPP), and farnesyl diphosphate (FPP) were chemically synthesized as previously reported.[24] Most of the tested aromatic substrates were purchased from Sigma–Aldrich (St. Louis, MO, USA) and BioBioPha (Kunming, Yunnan, China). We sincerely thank Prof. Min Ye from Peking University for the gift of liquiritigenin for enzymatic activity analyses. Optically active flavanones [i.e., (−)-naringenin, (+)-naringenin, (−)-8-dimethylallylnaringenin, and (+)-8-dimethylallylnaringenin] were isolated from their optically inactive enantiomers by chiral HPLC, and their enantiomeric excess values were over 99%.

HPLC grade methanol was purchased from Merck (Darmstadt, Germany); water was purified and deionized by a water purification system from Tautobiotech (Shanghai, China). The formic acid used in this study was of HPLC grade (Mreda, Columbia, USA). All the other chemicals used in this study were of analytical grade for laboratory use.

Analytical Methods

The analyses of substrate specificity and the determinations of the conversion rates were performed on an Agilent 1200 series HPLC system (Agilent Technologies, Böblingen, Germany) coupled with an LCQ Fleet ion trap mass spectrometer (Thermo Electron Corp., San Jose, CA, USA) equipped with an electrospray ionization (ESI) source.

The LC chromatograph consisted of a quaternary pump equipped with an on-line solvent degasser unit, an auto sampler, a column temperature controller and a diode-array detector coupled with an analytical workstation. HPLC analyses were performed on a Shiseido capcell pak C18 MG II column (250 mm×4.6 mm i.d., 5 μm, Shiseido Co., Ltd., Tokyo, Japan) at a flow rate of 1 mL min^{-1}, and the column temperature was maintained at 30 °C. The injection volume was 10 μL. The mobile phase was a gradient elution of solvents A (i.e., 0.1% formic acid aqueous solution) and B (i.e., methanol). The gradient program was as follows: 0–25 min, a linear change from A:B (50:50, v/v) to A:B (0:100, v/v), and then an isocratic elution for 10 min. The re-equilibration duration was 10 min between individual runs.

For HPLC-MS analyses, ultra-high purity helium (He) was used as the collision gas, and high purity nitrogen (N$_2$) was used as the nebulizing gas. The optimized ESI source parameters were as follows: sheath gas flow rate, 20 (arbitrary units); auxiliary gas flow rate, 5 (arbitrary units); spray voltage, 5.0 kV; capillary temperature, 350 °C; source collision-induced decomposition (CID), 35 V; tube lens offset voltage, −75 V. The spectra were recorded in the 100–1000 *m/z* range for a full scan MS analysis. The split ratio of effluent from the LC to ion source was 2:1. The data were analyzed using XCalibur software.

The resolution of flavanone enantiomers and the determination of enantiomeric excess values were carried out utilizing a Chiralpak AD-H column (250 mm×4.6 mm i.d., 5 μm, Daicel Chemical Industries Ltd., Tokyo, Japan). The mobile phase consisted of *n*-hexane and 2-propanol (87:13, v/v). Resolution analyses were carried out at 30 °C at a flow rate of 1.1 mL min^{-1} with detection at 290 nm (UV).

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cDNA Cloning

Twelve-day-old cell cultures of *S. flavescens* treated with MJ for 24 h were used for total RNA preparations; the RNA was isolated using the RNA prep pure Plant Kit (TianGen Biotech, China). Total RNA was reverse-transcribed using SMARTScribe reverse transcriptase (Clontech, USA). A core cDNA fragment encoding a flavonoid prenyltransferase was cloned by homology-based RT-PCR using TransStart FastPfu DNA Polymerase (TransGen Biotech, China) and a degenerate primer pair, MC-GP-F/MC-GP-R, that was designed using the conserved sequences of plant flavonoid prenyltransferases (MC-GP-F: 5′-THGARATAGACAAGRTWAA CAAGCCIKATCTTCC-3′; MC-GP-R: 5′-CYCCTTCMATRT CAGGTATATCCTTRAAYAATGC-3′). The 3′-end and 5′-end amplifications were carried out using the Smart RACE cDNA Amplification Kit (Clontech, USA) according to the manufacturer's protocol. The full-length cDNA of SfFPT was isolated by RT-PCR using gene-specific primer pairs.

The Expression of the cDNA

The coding region of SfFPT was amplified using N-terminal and C-terminal primers: 5′-GTCGAATTCATGGGTTC TATGCTTCTTGCATCTTTTCC-3′ (the *EcoR* I site is underlined) and 5′-ATTTGCGGCCGCTCATCTAAACAAAGGTATGAG GAAG-3′ (the *Not* I site is underlined). The amplified cDNA was cloned into the *EcoR* I/*Not* I site of the expression vector pESC (Stratagene, La Jolla, CA, USA) *via* the cloning vector pEASY-B (TransGen Biotech, China). After the verification of the sequence, the plasmid was transformed into YPH499 yeast for heterologous expression.

Enzyme Preparation

Recombinant yeast transformed with foreign plasmid was stored in SD-His dropout medium prior to incubation at 30 °C in a shaking incubator (200 rpm) overnight. Cells were collected by centrifugation at 1,500×g for 5 min at room temperature, and the cell pellet was resuspended in SG-His dropout medium to achieve an OD_{600} of 1.0 to induce gene expression. After another incubation at 28 °C for 24 h, the cells were harvested by centrifugation at 1,500×g for 5 min at 4 °C, and the cell pellet was resuspended in 100 mM Tris-HCl buffer (pH 9.0) containing 1 mM PMSF. The cell pellet was homogenized with acid-washed glass beads (425–600 μm size, Sigma–Aldrich, St. Louis, MO, USA). The mixture was vortexed for 30 s followed by 30 s on ice, and this cycle was repeated eight times for a total of 8 min to lyse the cells. The mixture was centrifuged at 10,000×g for 15 min. Then, the supernatant was further ultracentrifuged at 160,000×g for 90 min at 4 °C to pellet the microsome fraction. After two washes with 100 mM Tris-HCl buffer (pH 9.0), the membrane fraction was resuspended in the same buffer. The protein content was determined using the Bradford method.[25] The microsomal enzyme preparation and reaction procedure of cultured cells is provided in the Supporting Information (Supplemental Experimental Procedures).

Enzyme Reactions

Standard enzyme assays were performed in 100 mM Tris-HCl buffer (pH 9.0). The reaction mixtures contained 10 mM $MgCl_2$, 400 μM prenyl donors, 400 μM prenyl acceptors, and 0.1–0.2 mg of recombinant yeast microsomal protein in a total volume of 200 μL. After incubation for 30 min at 40 °C, the reaction was terminated by the addition of 400 μL of EtOAc and a 2 min vortex step. After the removal of the protein by centrifugation at 12,000×g for 15 min, the organic layer was evaporated until dry, and the product was dissolved in 200 μL MeOH for HPLC-MS analysis. For quantification, three parallel assays were routinely carried out.

To test the necessity of divalent cations for SfFPT activity, $MgCl_2$, $BaCl_2$, $CaCl_2$, $FeCl_2$, $CoCl_2$, $CuCl_2$, $ZnCl_2$, and $MnCl_2$ were used individually. To study the optimal pH, the enzymatic reaction was performed in various reaction buffers (pH 6.0–12.0). To assay for the optimal reaction temperature, the reaction mixtures were incubated at different temperatures (40–80 °C).

Apparent K_m Value Assays

To determine the apparent K_m values, 160 μg of recombinant yeast microsomes in a total volume of 200 μL at 40 °C for 30 min, were incubated with either various concentrations of prenyl acceptors (20–400 μM) and a fixed concentration of DMAPP (800 μM) or various concentrations of DMAPP (20–400 μM) and a fixed concentration of prenyl acceptors (800 μM). All experiments were performed in triplicate, and enzyme activity was evaluated by the production of prenyl-flavonoids (nmol) per mg of microsomal protein per minute. Apparent K_m values were calculated from Lineweaver–Burk plots using the Hyper32 software (http://homepage.ntl-world.com/john.easterby/hyper32.html).

Substrate Specificity Assays

To study the substrate specificity of SfFPT, seventy-two aromatic substrates were used as prenyl acceptors, including flavone, chrysin, apigenin, luteolin, diosmetin, baicalein, hispidulin, acacetin-7-*O*-β-D-rutinoside, fisetin, kaempferol, morin hydrate, quercetin, myricetin, kumatakenin A, (−)-flavanone, (±)-pinocembrin, (−)-pinocembrin, (+)-pinocembrin, (±)-naringenin, (−)-naringenin, (+)-naringenin, (−)-pinostrobin, (−)-sakuranetin, (−)-tsugafolin, (−)-isosakuranetin, (−)-eriodictyol, (−)-steppogenin, (−)-hesperetin, (−)-robtin, (−)-liquiritigenin, (−)-5,6,7-trimethoxyflavanone, (−)-hesperiden, (−)-naringin, (+)-taxifolin, dihydromyricetin, dihydrokaempferol-7-*O*-β-D-glucoside, daidzein, formononetin, genistein, orobol, 2′-hydroxygenistein, sophoricoside, genistin, 2-hydroxychalcone, 4-hydroxychalcone, 2′-hydroxychalcone, 4′-hydroxychalcone, 2′,4′,6′-trihydroxychalcone, isoliquiritigenin, phloretin, 4,4′-dihydroxybenzophenone, 2,4-dihydroxybenzophenone, (−)-maackiain, norathyriol, (+)-catechin, L-epicatechin, resveratrol, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, 2,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 2,6-dihydroxybenzoic acid, 2,3-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 2,4,6-trihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, 2,5-dihydroxyphenylacetic acid/homogentisic acid, 4-hydroxyphenylpyruvic acid, caffeic acid, 4-chromanol, and 4-chromanone.

The prenyl donor specificity of SfFPT was tested with DMAPP, GPP, and FPP when pinocembrin, naringenin, liquiritigenin, steppogenin, eriodictyol, hesperetin, sakuranetin,

isosakuranetin, tsugafolin, taxifolin, phloretin, and chrysin were used as prenyl acceptors in individual reactions.

The Preparation and Identification of the Reaction Products

To prepare the enzymatic products for structural elucidation, each reaction mixture containing 10 mM MgCl$_2$, 2 mM prenyl donor, 4 mM DMAPP, and 5 mg of recombinant yeast microsomal protein in a total volume of 5 mL was incubated for 2 h at 40 °C. The enzymatic product was extracted by EtOAc (3×10 mL). The organic layers were combined and concentrated under vacuum. The residue was further dissolved in MeOH, and the enzymatic products were purified by reversed-phase HPLC. The structures of the enzymatic products were characterized by MS and NMR spectral and polarimetric analyses.

Positive ESI mass spectra were obtained from an LCQ Fleet ion trap mass spectrometer. Optical rotations were determined using a Perkin–Elmer Model 341 LC polarimeter (Perkin–Elmer, Waltham, MA, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian NMR System 600, 500, and 400 spectrometers (Varian Inc., Palo Alto, CA, USA).

8-Dimethylallylpinocembrin (1a): ESI-MS: $m/z=325$ [M+H]$^+$; $[\alpha]_D^{20}$: −68.4 (c 0.19, MeOH); ^{1}H NMR (acetone-d_6, 500 MHz): $\delta=5.56$ (1 H, dd, $J=12.5$, 3.0 Hz, H-2), 3.12 (1 H, dd, $J=17.0$, 12.5 Hz, H-3a), 2.84 (1 H, dd, $J=17.0$, 3.0 Hz, H-3b), 6.04 (1 H, s, H-6), 7.58 (1 H, d, $J=7.0$ Hz, H-2′), 7.46 (1 H, dd, $J=7.0$, 1.5 Hz, H-3′), 7.38 (1 H, m, H-4′), 7.46 (1 H, dd, $J=7.0$, 1.5 Hz, H-5′), 7.58 (1 H, d, $J=7.0$ Hz, H-6′), 3.27 (2 H, d, $J=7.0$ Hz, H-1″), 5.20 (1 H, m, H-2″), 1.70 (3 H, s, H-4″), 1.68 (3 H, s, H-5″), 12.11 (1 H, s, 5-OH); ^{13}C NMR (acetone-d_6, 125 MHz): $\delta=79.75$ (C-2), 43.60 (C-3), 197.13 (C-4), 103.36 (C-4a), 160.89 (C-5), 96.48 (C-6), 164.95 (C-7), 108.38 (C-8), 163.01 (C-8a), 140.38 (C-1′), 129.44 (C-2′), 127.13 (C-3′), 129.44 (C-4′), 127.13 (C-5′), 129.26 (C-6′), 22.27 (C-1″), 123.67 (C-2″), 131.26 (C-3″), 17.84 (C-4″), 25.85 (C-5″).[26]

8-Dimethylallylnaringenin (2a): ESI-MS: $m/z=341$ [M+H]$^+$; $[\alpha]_D^{20}$: −33.8 (c 0.08, MeOH); ^{1}H NMR (acetone-d_6, 600 MHz): $\delta=5.44$ (1 H, dd, $J=12.5$, 3.0 Hz, H-2), 3.13 (1 H, dd, $J=17.0$, 12.5 Hz, H-3a), 2.75 (1 H, dd, $J=17.0$, 3.0 Hz, H-3b), 6.01 (1 H, s, H-6), 7.40 (1 H, d, $J=8.5$ Hz, H-2′), 6.90 (1 H, d, $J=8.5$ Hz, H-3′), 6.90 (1 H, d, $J=8.5$ Hz, H-5′), 7.40 (1 H, d, $J=8.5$ Hz, H-6′), 3.21 (2 H, d, $J=7.0$ Hz, H-1″), 5.19 (1 H, m, H-2″), 1.70 (3 H, s, H-4″), 1.68 (3 H, s, H-5″), 8.47 (1 H, s, -OH), 9.50 (1 H, s, -OH), 12.13 (1 H, s, 5-OH); ^{13}C NMR (acetone-d_6, 150 MHz): $\delta=79.73$ (C-2), 43.44 (C-3), 197.56 (C-4), 103.34 (C-4a), 161.10 (C-5), 96.39 (C-6), 164.88 (C-7), 108.30 (C-8), 162.98 (C-8a), 131.11 (C-1′), 128.82 (C-2′), 116.15 (C-3′), 158.57 (C-4′), 116.15 (C-5′), 128.82 (C-6′), 22.25 (C-1″), 123.18 (C-2″), 131.19 (C-3″), 17.84 (C-4″), 25.86 (C-5″).[27]

8-Dimethylallylliquiritigenin (5a): ESI-MS: $m/z=325$ [M+H]$^+$; $[\alpha]_D^{20}$: −90.0 (c 0.05, MeOH); ^{1}H NMR (acetone-d_6, 600 MHz): $\delta=5.43$ (1 H, dd, $J=13.2$, 3.0 Hz, H-2), 2.99 (1 H, dd, $J=16.8$, 13.2 Hz, H-3a), 2.68 (1 H, dd, $J=16.8$, 3.0 Hz, H-3b), 7.57 (1 H, d, $J=8.4$ Hz, H-5), 6.61 (1 H, d, $J=8.4$ Hz, H-6), 7.41 (1 H, d, $J=8.4$ Hz, H-2′), 6.89 (1 H, d, $J=8.4$ Hz, H-3′), 6.89 (1 H, d, $J=8.4$ Hz, H-5′), 7.41 (1 H, d, $J=8.4$ Hz, H-6′), 3.32 (2 H, br d, $J=7.2$ Hz, H-1″), 5.22 (1 H, t,

$J=7.2$ Hz, H-2″), 1.62 (3 H, s, H-4″), 1.61 (3 H, s, H-5″); ^{13}C NMR (acetone-d_6, 150 MHz): $\delta=80.29$ (C-2), 44.55 (C-3), 190.88 (C-4), 116.39 (C-4a), 126.25 (C-5), 110.43 (C-6), 162.47 (C-7), 115.23 (C-8), 161.94 (C-8a), 131.561 (C-1′), 128.75 (C-2′), 115.99 (C-3′), 158.36 (C-4′), 115.99 (C-5′), 128.75 (C-6′), 22.76 (C-1″), 123.18 (C-2″), 131.56 (C-3″), 17.90 (C-4″), 25.86 (C-5″).[28]

8-Dimethylallylsteppogenin (6a): ESI-MS: $m/z=357$ [M+H]$^+$; $[\alpha]_D^{20}$: −16.7 (c 0.03, MeOH); ^{1}H NMR (acetone,-d_6 600 MHz): $\delta=5.67$ (1 H, dd, $J=12.6$, 3.0 Hz, H-2), 3.12 (1 H, dd, $J=17.0$, 13.2 Hz, H-3a), 2.83 (1 H, dd, $J=17.0$, 3.0 Hz, H-3b), 6.03 (1 H, s, H-6), 6.47 (1 H, d, $J=2.4$ Hz, H-3′), 6.42 (1 H, dd, $J=8.4$, 2.4 Hz, H-5′), 7.33 (1 H, d, $J=8.4$ Hz, H-6′), 3.24 (2 H, d, $J=6.6$ Hz, H-1″), 5.20 (1 H, m, H-2″), 1.59 (3 H, s, H-4″), 1.61 (3 H, s, H-5″), 12.16 (1 H, br s, 5-OH).[29]

6-Dimethylallylsteppogenin (6a′): ESI-MS; $m/z=357$ [M+H]$^+$; $[\alpha]_D^{20}$: +16.7 (c 0.03, MeOH); ^{1}H NMR (acetone-d_6, 600 MHz): $\delta=5.67$ (1 H, dd, $J=13.2$, 3.0 Hz, H-2), 3.14 (1 H, dd, $J=17.4$, 13.2 Hz, H-3a), 2.70 (1 H, dd, $J=17.4$, 3.0 Hz, H-3b), 6.04 (1 H, s, H-8), 6.47 (1 H, d, $J=2.4$ Hz, H-3′), 6.42 (1 H, dd, $J=8.4$, 2.4 Hz, H-5′), 7.30 (1 H, d, $J=8.4$ Hz, H-6′), 3.25 (2 H, d, $J=6.6$ Hz, H-1″), 5.25 (1 H, t, $J=6.6$ Hz, H-2″), 1.75 (3 H, s, H-4″), 1.64 (3 H, s, H-5″), 12.51 (1 H, br s, 5-OH); ^{13}C NMR (acetone-d_6, 150 MHz): $\delta=75.22$ (C-2), 42.75 (C-3), 197.58 (C-4), 103.42 (C-4a), 162.27 (C-5), 108.91 (C-6), 162.44 (C-7), 95.39 (C-8), 159.46 (C-8a), 117.61 (C-1′), 156.28 (C-2′), 103.42 (C-3′), 159.46 (C-4′), 107.80 (C-5′), 128.94 (C-6′), 21.65 (C-1″), 123.77 (C-2″), 131.01 (C-3″), 17.81 (C-4″), 25.84 (C-5″).[30]

8-Dimethylallyleriodictyol (7a): ESI-MS: $m/z=357$ [M+H]$^+$; $[\alpha]_D^{20}$: −32.9 (c 0.09, MeOH); ^{1}H NMR (acetone-d_6, 600 MHz): $\delta=5.38$ (1 H, dd, $J=12.6$, 3.0 Hz, H-2), 3.09 (1 H, dd, $J=17.4$, 12.6 Hz, H-3a), 2.73 (1 H, dd, $J=17.4$, 3.0 Hz, H-3b), 6.02 (1 H, s, H-6), 7.05 (1 H, d, $J=1.2$ Hz, H-2′), 6.89 (1 H, d, $J=8.4$ Hz, H-5′), 6.87 (1 H, dd, $J=8.4$, 1.2 Hz, H-6′), 3.22 (2 H, d, $J=7.2$ Hz, H-1″), 5.20 (1 H, t, $J=7.2$ Hz, H-2″), 1.61 (3 H, s, H-4″), 1.62 (3 H, s, H-5″), 12.15 (1 H, br s, 5-OH); ^{13}C NMR (acetone-d_6, 150 MHz): $\delta=79.76$ (C-2), 43.58 (C-3), 197.46 (C-4), 103.19 (C-4a), 165.31 (C-5), 96.37 (C-6), 163.01 (C-7), 108.36 (C-8), 161.05 (C-8a), 131.89 (C-1′), 116.03 (C-2′), 146.06 (C-3′), 146.29 (C-4′), 114.67 (C-5′), 118.99 (C-6′), 22.30 (C-1″), 123.75 (C-2″), 131.18 (C-3″), 17.89 (C-4″), 25.92 (C-5″).[31]

8-Dimethylallylhesperetin (8a): ESI-MS: $m/z=371$ [M+H]$^+$; $[\alpha]_D^{20}$: −46.9 (c 0.13, MeOH); ^{1}H NMR (MeOH-d_4, 500 MHz): $\delta=5.24$ (1 H, dd, $J=12.5$, 3.0 Hz, H-2), 2.98 (1 H, dd, $J=17.0$, 12.5 Hz, H-3a), 2.66 (1 H, dd, $J=17.0$, 3.0 Hz, H-3b), 5.86 (1 H, s, H-6), 6.91 (1 H, d, $J=1.5$ Hz, H-2′), 6.88 (1 H, d, $J=8.5$ Hz, H-5′), 6.86 (1 H, dd, $J=8.5$, 1.5 Hz, H-6′), 3.15 (1 H, dd, $J=14.0$, 7.5 Hz, H-1″a), 3.10 (1 H, dd, $J=14.0$, 7.5 Hz, H-1″b), 5.09 (1 H, t, $J=7.5$ Hz, H-2″), 1.57 (3 H, s, H-4″), 1.53 (3 H, s, H-5″), 3.81 (3 H, s, 4′-OCH_3); ^{13}C NMR (MeOH-d_4, 125 MHz): $\delta=80.08$ (C-2), 44.05 (C-3), 197.86 (C-4), 109.18 (C-4a), 166.56 (C-5), 96.54 (C-6), 163.18 (C-7), 112.54 (C-8), 161.44 (C-8a), 133.51 (C-1′), 114.57 (C-2′), 147.79 (C-3′), 149.27 (C-4′), 114.57 (C-5′), 118.87 (C-6′), 22.50 (C-1″), 123.98 (C-2″), 131.61 (C-3″), 17.89 (C-4″), 25.95 (C-5″), 56.46 (4′-OCH_3).[32]

8-Dimethylallylisosakuranetin (10a): ESI-MS:($m/z=355$ [M+H]$^+$; $[\alpha]_D^{20}$: −31.6 (c 0.10, MeOH); ^{1}H NMR (acetone-d_6, 600 MHz): $\delta=5.50$ (1 H, dd, $J=12.6$, 3.0 Hz, H-2), 3.15 (1 H, dd, $J=17.4$, 12.6 Hz, H-3a), 2.78 (1 H, dd, $J=17.4$, 3.0 Hz,

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H-3b), 6.04 (1H, s, H-6), 7.51 (1H, d, $J = 8.4$ Hz, H-2′), 7.00 (1H, d, $J = 8.4$ Hz, H-3′), 7.00 (1H, d, $J = 8.4$ Hz, H-5′), 7.51 (1H, d, $J = 8.4$ Hz, H-6′), 3.23 (2H, d, $J = 7.2$ Hz, H-1″), 5.20 (1H, d, $J = 7.2$ Hz, H-2″), 1.62 (3H, s, H-4″), 1.61 (3H, s, H-5″), 3.83 (3H, q, -OCH₃), 9.58 (1H, s, -OH), 12.14 (1H, s, 5-OH); ¹³C NMR (acetone-d_6, 150 MHz): $\delta = 79.55$ (C-2), 43.44 (C-3), 197.45 (C-4), 103.30 (C-4a), 160.99 (C-5), 96.35 (C-6), 164.92 (C-7), 108.29 (C-8), 162.96 (C-8a), 131.20 (C-1′), 128.69 (C-2′), 114.72 (C-3′), 160.83 (C-4′), 114.72 (C-5′), 128.69 (C-6′), 22.23 (C-1″), 123.65 (C-2″), 132.20 (C-3″), 17.84 (C-4″), 25.86 (C-5″), 55.57 (4′-OCH₃).[33]

8-Dimethylallyltsugafolin (11a): ESI-MS: $m/z = 355$ [M+H]⁺; $[\alpha]_D^{20}$: −28.9 (c 0.05, MeOH); ¹H NMR (acetone-d_6, 600 MHz): $\delta = 5.34$ (1H, dd, $J = 12.6, 3.0$ Hz, H-2), 2.90 (1H, dd, $J = 16.8, 12.6$ Hz, H-3a), 2.60 (1H, dd, $J = 16.8, 3.0$ Hz, H-3b), 6.23 (1H, s, H-6), 7.39 (1H, d, $J = 8.4$ Hz, H-2′), 6.89 (1H, d, $J = 8.4$ Hz, H-3′), 6.89 (1H, d, $J = 8.4$ Hz, H-5′), 7.39 (1H, d, $J = 8.4$ Hz, H-6′), 3.26 (2H, d, $J = 6.6$ Hz, H-1″), 5.21 (1H, d, $J = 6.6$ Hz, H-2″), 1.61 (3H, s, H-4″), 1.60 (3H, s, H-5″), 3.73 (3H, q, -OCH₃); ¹³C NMR (acetone-d_6, 150 MHz): $\delta = 76.47$ (C-2), 46.25 (C-3), 188.54 (C-4), 106.07 (C-4a), 161.16 (C-5), 93.79 (C-6), 162.80 (C-7), 108.97 (C-8), 162.50 (C-8a), 130.92 (C-1′), 128.64 (C-2′), 115.98 (C-3′), 158.29 (C-4′), 115.98 (C-5′), 128.64 (C-6′), 22.54 (C-1″), 123.99 (C-2″), 131.62 (C-3″), 18.87 (C-4″), 25.87 (C-5″), 55.81 (5-OCH3).[34]

8-Dimethylallyltaxifolin (4a): ESI-MS: $m/z = 373$ [M+H]⁺; ¹H NMR (DMSO-d_6, 600 MHz): $\delta = 4.90$ (1H, d, $J = 10.8$ Hz, H-2), 4.40 (1H, d, $J = 10.8$ Hz, H-3), 5.94 (1H, s, H-6), 6.87 (1H, d, $J = 1.2$ Hz, H-2′), 6.74 (1H, d, $J = 8.4$ Hz, H-5′), 6.70 (1H, dd, $J = 8.4, 1.2$ Hz, H-6′), 3.01 (2H, d, $J = 7.2$ Hz, H-1″), 5.07 (1H, m, H-2″), 1.56 (3H, s, H-4″), 1.54 (3H, s, H-5″); ¹³C NMR (DMSO, 150 MHz): $\delta = 82.89$ (C-2), 71.20 (C-3), 196.70 (C-4), 100.04 (C-4a), 160.99 (C-5), 95.63 (C-6), 159.17 (C-7), 107.04 (C-8), 158.00 (C-8a), 130.10 (C-1′), 115.22 (C-2′), 144.83 (C-3′), 145.53 (C-4′), 115.01 (C-5′), 119.06 (C-6′), 21.24 (C-1″), 122.59 (C-2″), 128.39 (C-3″), 17.50 (C-4″), 25.48 (C-5″).[35]

3′-Dimethylallylphloretin (12a): ESI-MS: $m/z = 343$ [M+H]⁺; ¹H NMR (acetone-d_6, 600 MHz): $\delta = 2.88$ (1H, t, $J = 7.8$ Hz, H-β), 3.33 (2H, t, $J = 7.8$ Hz, H-α), 6.07 (1H, s, H-5′), 7.09 (1H, d, $J = 8.4$ Hz, H-2), 6.74 (1H, d, $J = 8.4$ Hz, H-3), 6.74 (1H, d, $J = 8.4$ Hz, H-5), 7.09 (1H, d, $J = 8.4$ Hz, H-6), 3.24 (2H, d, $J = 7.8$ Hz, H-1″), 5.22 (1H, m, H-2″), 1.74 (3H, s, H-4″), 1.63 (3H, s, H-5″); ¹³C NMR (acetone-d_6, 150 MHz): $\delta = 46.88$ (C-β), 73.77 (C-α), 192.00 (C=O), 130.70 (C-1), 130.15 (C-2), 115.95 (C-3), 156.39 (C-4), 115.95 (C-5), 130.15 (C-6), 103.00 (C-1′), 162.00 (C-2′), 108.00 (C-3′), 165.04 (C-4′), 94.94 (C-5′), 161.00 (C-6′), 21.92 (C-1″), 124.18 (C-2″), 133.51 (C-3″), 18.87 (C-4″), 25.85 (C-5″).[7]

8-Dimethylallylchrysin (3a): ESI-MS: $m/z = 323$ [M+H]⁺; ¹H NMR (DMSO-d_6, 400 MHz): $\delta = 6.95$ (1H, s, H-3), 6.30 (1H, s, H-6), 8.04 (1H, d, $J = 8.0$ Hz, H-2′), 7.61 (1H, m, H-3′), 7.61 (1H, m, H-4′), 7.61 (1H, m, H-5′), 8.03 (1H, m, H-6′), 3.45 (2H, d, $J = 6.8$ Hz, H-1″), 5.21 (1H, t, $J = 6.8$ Hz, H-2″), 1.62 (3H, s, H-4″), 1.75 (3H, s, H-5″), 10.85 (1H, s, -OH), 12.75 (1H, s, -OH); ¹³C NMR (DMSO-d_6, 100 MHz): $\delta = 163.1$ (C-2), 104.9 (C-3), 182.1 (C-4), 103.9 (C-4a), 159.1 (C-5), 98.5 (C-6), 161.9 (C-7), 106.2 (C-8), 154.6 (C-8a), 131.0 (C-1′), 126.3 (C-2′), 129.2 (C-3′), 132.0 (C-4′), 129.2 (C-5′), 126.3 (C-6′), 21.3 (C-1″), 122.4 (C-2″), 131.0 (C-3″), 17.8 (C-4″), 25.4 (C-5″).[36]

Accession Numbers

Acknowledgements

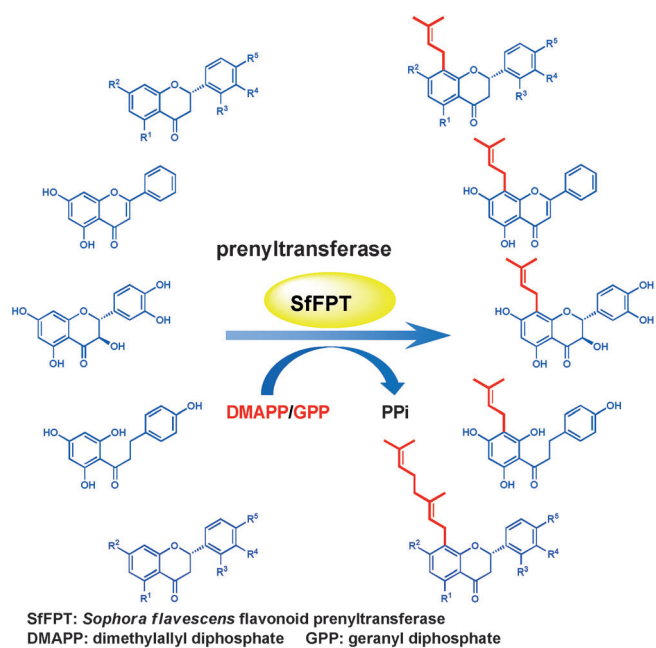## References

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Regio- and Stereospecific Prenylation of Flavonoids by *Sophora flavescens* Prenyltransferase

Ridao Chen, Xiao Liu, Jianhua Zou, Yunze Yin, Bin Ou, Jianhua Li, Ruishan Wang, Dan Xie, Peicheng Zhang, Jungui Dai*

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SfFPT: *Sophora flavescens* flavonoid prenyltransferase
DMAPP: dimethylallyl diphosphate   GPP: geranyl diphosphate

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