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•Review•

Targeting the biological activity and biosynthesis of hyperforin: a mini-review

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[ABSTRACT] Hyperforin is a representative polycyclic polyprenylated acylphloroglucinols (PPAPs) that exerts a variety of pharmacological activities. The complete biosynthesis pathway of hyperforin has not been elucidated due to its complex structure and unclear genetic background of its source plants. This mini-review focuses on the bioactivity and biosynthesis of hyperforin. These analyses can provide useful insights into the biosynthesis investigations of hyperforin and other PPAPs with complex structures.

[KEY WORDS] Hyperforin; PPAPs; Bioactivity; Biosynthesis; Prenyltransferase
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Introduction

Natural products derived from medicinal plants are a major source of pharmaceutically active compounds for clinical treatment and drug discovery [1]. Due to their complex structures, these bioactive molecules are usually obtained by natural extraction instead of total chemical synthesis. However, their extraction and separation are not easy in many cases, which results from the complex composition of natural plants, high costs of extraction and isolation, and uncertainty in the availability of natural materials largely restricted by cultivated land resources, climate, diseases and insect pests, pesticide residues and other factors.

In recent years, increasing attention has been drawn towards natural product biosynthesis, as it provides an alternative solution to tackle drug shortage and paves a new way for natural product production. In pace with the rapid development of enzymatic catalysis, combinatorial biosynthesis and synthetic biology, breakthroughs have been made in the biosynthesis of many important natural products such as artemisinin [2], morphine [3], tropane and alkaloids [4]. Many of these bioactive molecules and their precursors can be successfully produced by engineered microorganisms [5, 6]. However, there are still some bottlenecks, especially in the biosynthesis pathways of natural products with complex structures.

Hyperforin is a representative polycyclic polyprenylated acylphloroglucinols (PPAPs), which possesses a highly oxygenated double-ring skeleton and multiple prenyl substitutions (Fig. 1). It was first isolated from *Hypericum perforatum* L. (St. John's wort) in the 1970s, and then well-recognized for its significant antidepressant activity [7]. However, due to the complex structure and unclear genetic background of its source plants, the biosynthesis of hyperforin has not been fully elucidated.

Pharmaceutical Activities of Hyperforin

H. perforatum, commonly known as St. John's wort, is a medicinal plant widely used in Europe and America for the treatment of postpartum depression and other mild to moderate depression for many years [8, 9]. In China, the aerial part of *H. perforatum* is used for traditional Chinese medicine (TCM) therapy, and characterized by soothing the liver, relieving depression, clearing heat, draining dampness, eliminating swelling, and increasing milk secretion [10]. Hyperforin, mainly derived from *H. perforatum*, is considered to be most relevant to the antidepressant activity of the plant [11]. Furthermore, an increasing number of studies have been conducted to evaluate its antitumor [12] and neuroprotective [13] activities. Hyperforin also exerts a wide range of pharmacological activities including anti-inflammatory, antioxidant, antibacterial, and antiplasmodial effects. Thus, hyperforin has been consi-

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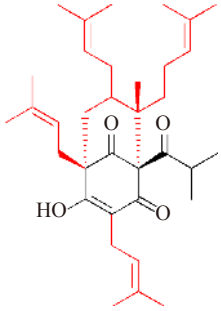
Hypericum perforatum



Hyperforin

Fig. 1 *Hypericum perforatum* and the chemical structure of hyperforin

dered as a powerful and potential lead compound. However, drug interactions still need to be concerned in clinical trials [14].

Anti-depressive activity

There is increasing consensus that hyperforin exhibited its antidepressant effect by inhibiting the re-uptake of multiple neurotransmitters, such as 5-hydroxytryptamine (5-HT), noradrenaline (NA), dopamine (DA), L-glutamine, and γ-aminobutyric acid (GABA) [15, 16]. In detail, hyperforin was capable of triggering transient receptor potential canonical-6 (TRPC6) to interfere the re-uptake. Additionally, a more recent study has confirmed that hyperforin stimulated the activity of the transcription factor AP-1 *via* TRPC6 [17].

Anti-tumor activity

Hyperforin is a potential anticancer agent, especially in the treatment of chronic lymphoid leukemia (CLL) and acute myeloid leukemia (AML) [18, 19]. Hyperforin stimulated the expression of the pro-apoptotic Noxa in primary CLL cells, while in AML cell lines and primary AML cells, it directly inhibited the kinase activity of the serine/threonine protein kinase B/AKT1, leading to the activation of the pro-apoptotic protein Bad [20, 21]. A previous review highlighted their remarkable potential in cancer prevention through modulating inflammatory signaling cascades, reactive oxygen species (ROS) generation, and proton dynamics [22].

Neuroprotective activity

Studies have shown that hyperforin may serve as a potent neuroprotectant, promising to be a potential therapeutic candidate in the treatment of Alzheimer's disease [13]. Experiments indicated that hyperforin protected PC12 cells and SH-SY5Y cells against damage and apoptosis induced by aluminum maltolate [23]. According to a recent review, hyperforin targeted TGF-β1 signaling and increased TGF-β1 production in the central nervous system to improve cognition [24]. However, more clinical evidence is needed to confirm the effect of hyperforin on neurodegenerative disorders [25].

Anti-inflammatory and antioxidant effect

H. perforatum extracts have been traditionally used as an anti-inflammatory agent, though the exact mechanisms remain unclear [26]. It is demonstrated that hyperforin suppressed the activities of 5-lipoxygenase (5-LO) and cyclooxygenase-1 (COX-1) and exerted a potential therapeutic effect on inflammatory disorders [27]. Hyperforin also suppressed prostaglandin E_2 biosynthesis by inhibiting microsomal prostaglandin E_2 synthase-1 (mPGES-1) which plays a key role in inflammation and tumorigenesis [28]. Moreover, hyperforin exerted significant antioxidant effects. Topical treatment of mild to moderate atopic dermatitis with a hyperforin rich hypericum-cream was significantly superior to the placebo [29]. It should be noted that hyperforin reduced ultraviolet-induced oxidative stress without *in vitro* phototoxic effects [30]. It has been demonstrated that hyperforin acts as an outstanding free radical scavenger, partially due to its anti-inflammatory and UV-protective effects [30]. Furthermore, hyperforin even exhibited DNA-protective function based on the free radical scavenger ability [31]. All the evidence makes hyperforin an ideal anti-inflammatory and antioxidant agent.

Antibacterial and antiplasmodial activity

Hyperforin is recognized as an antibacterial constituent of St. John's Wort [32]. It exhibited antimicrobial activity against multiresistant *Staphylococcus aureus* and other Gram-positive bacteria, without effects on gram-negative bacteria or *Candida albicans* [33]. A hydrogenated hyperforin analog with higher stability was found to be effective against microorganisms in their planktonic and biofilm forms [34]. Furthermore, hyperforin inhibited the growth of *Plasmodium falciparum* at micromolar concentrations, where its activity was not dependent on either its phenol-like sensitivity to autooxidation or the presence of double bonds on the prenyl residues according to a structure-activity study [35].

Herb-drug interaction

Limitations still exist on the application of hyperforin in clinical trials. Since the first case reported in 1999 [36], co-administration of St. John's wort containing hyperforin has been known to be associated with clinical interactions for a set of medicines (mainly CYP3A4 and p-glycoprotein substrates) [37]. For instance, it was reported that *H. perforatum* displayed influence on the pharmacokinetics and pharmacodynamics of rivaroxaban in humans [38]. It has been demonstrated that these herb-drug interactions induced drug-metabolizing enzymes and transporters, resulting in less systematic exposure and even therapeutic failure. Therefore, a daily dose of no more than 1 mg hyperforin is recommended to minimize the risk of interactions [14].

Hyperforin is a Representative PPAP

Hyperforin is a typical member of PPAPs [39, 40]. The complexity and diversity of PPAPs are attributable to the types of acryl group, the number and positions of prenyl group, the oxidation degree of prenyl residue, the position of ether ring and secondary cyclization. According to their biosynthetic pathways, PPAPs can be divided into three groups: bicyclic polyprenylated acylphloroglucinols (BPAPs), cage-like polycyclic polyprenylated acylphloroglucinols (caged PPAPs) derived from further cyclization of BPAPs, and other polycyclic polyprenylated acylphloroglucinols derived from direct cyclization of monocyclic polyprenylated acyl-

phloroglucinols (MPAPs) rather than being derived from BPAPs (other PPAPs) (Fig. 2). Generally, PPAPs can also be divided into type A and type B depending on the position of the acyl group on the phloroglucinol core, where type A and type B have the acyl group located at the C-1 position and at the C-3 position, respectively [41]. Apparently, hyperforin belongs to type A PPAPs. From the biogenetic point of view, introduction of multi-prenyl residues at the acylphloroglucinol core (yielding MPAPs) represents the requisite starting point of PPAP biosynthesis. On the basis of MPAPs, the prenyl groups are cyclized with benzene rings followed by a set of tailoring reactions to convert into such analogs with distinct structures.

PPAP analogs usually exist in groups within the same plant species and share similar physicochemical properties. However, the complicated and unstable architecture makes isolation, purification, and structure identification a real challenge. In recent years, there has been an increased interest in the discovery and activity study of PPAPs. For example, garsubelone A was the first dimeric PPAP derivative isolated from *Garcinia subelliptica*, featuring complex 6/6/6/6/6/6/6 heptacyclic architecture containing 10 stereogenic centers [42]. Hyperberins A and B, type B PPAPs with a bicyclo[5.3.1] hendecane core, were isolated from *H. beanii* and exhibited moderate cytotoxicity and potent anti-inflammatory activities [43]. Hypatone A bearing an unprecedented cagelike skeleton was isolated from *H. patulum* by Xu's group and found to be a natural Cav3.1 agonist with the most potent activity reported so far (EC_{50} = 3.80 μmol·L^{-1}). Furthermore, it normalized the pathological gating of a mutant Cav3.1 channel found in spinocerebellar ataxia 42 (SCA42, an incurable hereditary neurodegenerative disorder), which paves a way for developing new drugs against relative diseases [44].

Due to their particularly complex structures, PPAPs attract the attention from synthetic chemists and a large number of chemical synthesis studies have been carried out. The first catalytic asymmetric total synthesis of *ent*-hyperforin was accomplished by Shibasaki's group in 2010 [45]. Although the synthesis of PPAPs has raised continuous attention in recent years and more than 20 natural PPAPs' total syntheses have been accomplished, the synthesis of these compounds is still challenging due to the complicated and unstable structure and multiple chiral centers [39, 46].

The Biosynthesis Pathway of Hyperforin

Hyperforin is a hybrid of prenyl units and acylphloroglucinol core structure. Labeling experiments revealed that the acylphloroglucinol moiety of hyperforin was generated *via* the polyketide biosynthesis pathway, and the remaining parts consisted of five prenyl units mainly derived from the 1-deoxy-D-xylulose 5-phosphate pathway (DXP pathway) [47]. That is, the biosynthesis of acylphloroglucinol moiety in hyperforin is formed by polyketide synthase (PKS, *e.g.* isobutyrophenone synthase) from isobutyryl-CoA and three molecules of malonyl-CoA, and the prenyl units are plausible integrated into the skeleton by prenyltransferases from prenyl pyrophosphates (Fig. 3).

Hyperforin is characterized by its unique multi-prenylated bicyclic structure. It was hypothesized that different prenyltransferases contribute to the formation of multi-prenylated bicyclic structure during the biosynthesis of hyperforin [46-49]. To begin with, three prenyl moieties (including one geranyl from GPP and two dimethylallyl units from DMAPP) are introduced to the acylphloroglucinol skeleton through electrophilic substitution of the aromatic nucleus by prenyltransferases. However, the sequence of the necessary steps is not fully understood. Next, the 2′/3′ double bond of preimplanted geranyl chain attacks a third DMAPP to trigger ring-

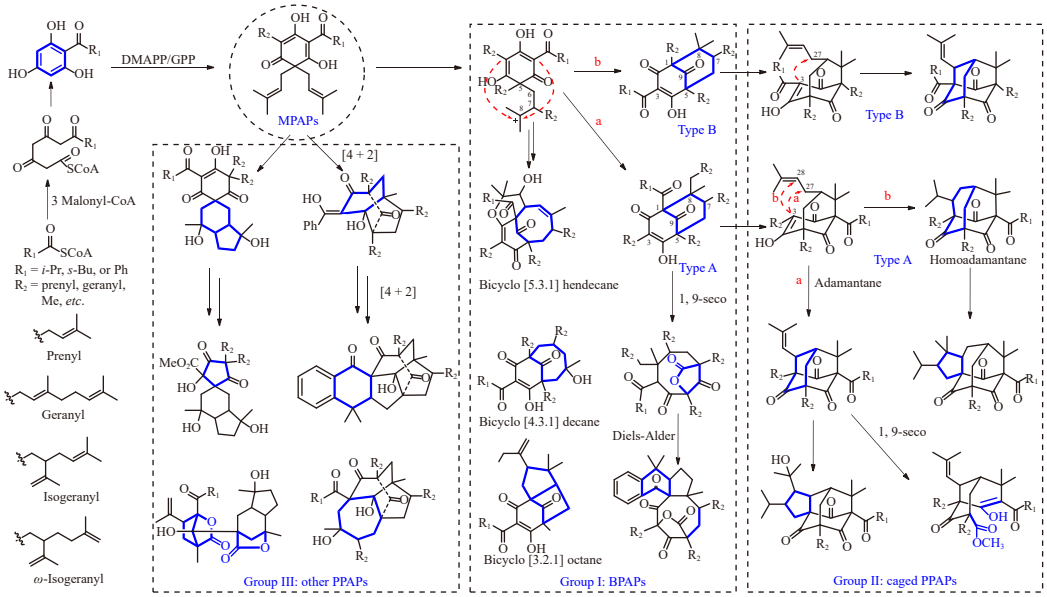


Fig. 2 The supposed biogenic pathway of PPAP analogs [39]

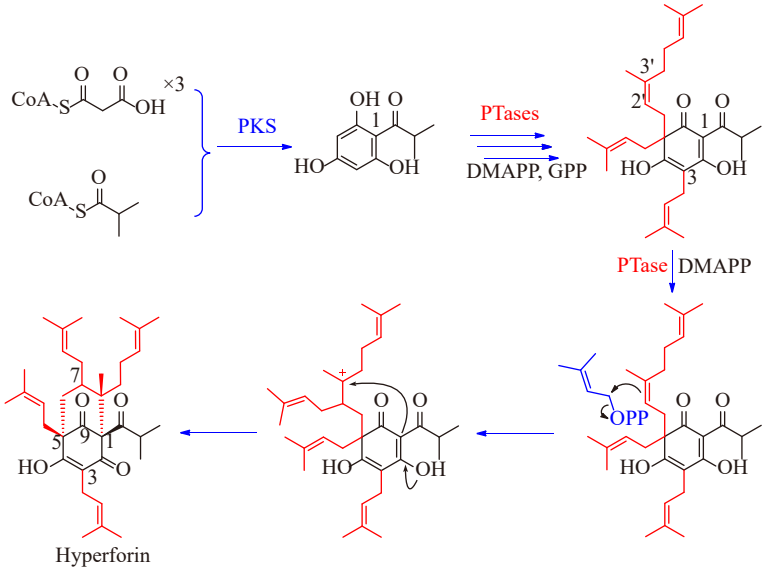


Fig. 3 The proposed hyperforin biosynthetic pathway

closure reaction, and ultimately leads to the formation of a unique hyperforin bicyclic scaffold (Fig. 3). Up till now, prenyltransferases that participate in the prenylation process of hyperforin biosynthesis remain unknown. Cloning and characterization of the key prenyltransferases in hyperforin biosynthesis is expected to be a breakthrough point to reveal the biosynthesis process of this compound.

Advances in Prenyltransferase

The reported prenyltransferases included aromatic prenyltransferase (aPTase), isoprenyl diphosphate synthases and protein prenyltransferase. More specifically, aPTases catalyzed the electrophilic alkylation between prenyl diphosphates and aromatic acceptors. Isoprenyl diphosphate synthases facilitated the extension of the prenyl chain by catalyzing the consecutive condensations of two prenyl diphosphates (commonly head-to-head or head-to-tail). Protein prenyltransferases were reported to be responsible for transferring the prenyl cation to the C-terminal cysteine side chain, leading to the formation of C-S bond [50].

In the recent decade, great progress has been achieved in the aspect of aPTase involved in the biosynthesis of natural products, which mainly focuses on the cloning and characterization of aPTase genes from bacteria and plants, structural biology studies and the clarification of catalytic mechanisms. To the best of our knowledge, four kinds of aPTases have been reported, namely membrane-bound UbiA family from bacteria [51], membrane-bound flavonoid/phenolic aPTase from plants [52], soluble ABBA family aPTase from bacteria [53] and soluble DMATS superfamily (dimethylallyltryptophan synthase superfamily) aPTase [54]. The aPTases involved in hyperforin biosynthesis probably belong to the membrane-bound aPTase family, but it is not excluded that they may be soluble aPTases derived from plants.

The biochemical study of plant-derived aPTase

aPTase plays a pivotal role in the biosynthesis of diverse prenylated aromatic metabolites in plants. It has drawn increasing attention from all over the world since the 1970s. Most of the aPTases from plants are insoluble membrane-bound proteins, such as prenyltransferases from soybean that catalyze glyceollins biosynthesis [55]; prenyltransferases from *Ammi majus* that catalyze coumarin prenylation [56]; and prenyltransferases from *Glycyrrhiza glabra* that catalyze the formation of glabrol [57]. According to a previous study, they are all membrane-bound proteins present in the plastid.

Soluble aPTases from plants are also reported. For example, prenyltransferase from *Cannabis sativa*, that participated in the tetrahydrocannabinol biosynthesis and yielded cannabinoids with hallucinogenic effect, is soluble [58]. Additionally, the prenyltransferase from *Humulus lupulus* that involved in the biosynthesis of bitter acid is also a soluble protein [59].

Molecular biological study of plant-derived aPTase

Among the established plant-derived aPTases, flavonoid prenyltransferases are investigated most thoroughly. In 2008, Yazaki's group identified the first flavonoid prenyltransferase gene (designated as *SfN8DT-1)* from *Sophora flavescens*. The protein encoded by this gene catalyzed the prenylation of naringenin at the C-8 position and preferred DMAPP as its specific donor [60]. It was found that *SfN8DT-1* belongs to the same family as homogentisate prenyltransferases possessing aspartate-riched conserved amino acid sequences NQ × × D × × × D and KD × × D × (E/D) GD [61]. The same group was successfully characterized by isoflavone prenyltransferase *SfG6DT* and chalcone prenyltransferase *SfiLDT* from *S. flavescens* [62], as well as *GmG4DT* [63] from soybean, which plays an essential role in phytoalexin glyceollin production.

Like some DMATS aPTase from microorganisms with catalytic promiscuity, aPTase can also be found in plants, with substrate promiscuity. SfFPT, an aPTase that

catalyzes the broadest substrate scope in plants was characterized from *S. flavescens* by our research team [64]. Using the sequence of SfFPT as a probe, flavonoid C-6 prenyltransferase GuA6DT and chalcone prenyltransferase GuILDT were successfully identified from *Glycyrrhiza uralensis* [65]. In addition, structurally diverse prenylflavonoids were conveniently synthesized through enzymatic catalysis by combining GuILDT and a stereospecific chalcone isomerase GuCHI [66]. Two non-Leguminosae aPTases MaIDT and CtIDT were characterized from *Morus alba* and *Cudrania tricuspidate*, respectively. Phylogenetic analysis revealed that MaIDT and CtIDT may be independently evolved from their homologs in Leguminosae [67, 68]. The study provides valuable gene information for the investigation of prenyltransferase from other plant species.

In terms of molecular characterization of plant-derived aPTase, remarkably rapid progress has been witnessed in the past decade. An increasing number of aPTases sequences derived from plants have been identified, and they are surprisingly capable of catalyzing various types of prenylation reactions, such as phenylpropanoids prenyltransferase AcPT1 from *Artemisia capillaris* [69]; meroterpenoid farnesyltransferase RdPT1 from *Rhododendron dauricum* that is involved in the biosynthesis of anti-HIV agent daurichromenic acid [70]; DMAPP-specific coumarin prenyltransferase PcPT from *Petroselinum crispum* [71]; GPP-specific coumarin prenyltransferase ClPT1 from *Citrus limon* [72]; PcM4DT from *Psoralea corylifolia* that catalyzes the prenylation of pterocarpans [73]; LjG6DT from *Lotus japonicus* that is responsible for isoflavone (wighteone) biosynthesis [74]; and Stilbenoid prenyltransferase MaOGT from *M. alba* that is geranyl diphosphate-specific [75].

Hints for Hyperforin Biosynthesis Investigation

The enzymes responsible for the formation of acylphloroglucinol moiety in hyperforin biosynthesis have not been reported yet. However, several homologous PKSs involved in the formation of phlorisovalerophenone (PIVP) during the biosynthesis of humulone from *H. lupulus* have been characterized [76, 77]. Furthermore, the complete biosynthesis of PIVP was achieved in an engineered *Escherichia coli* [78]. All these studies can provide useful insights for exploring acylphloroglucinol moiety biosynthesis in hyperforin.

Currently, there is no report about molecular characterization of prenyltransferases involved in hyperforin biosynthesis. Beerhues's group found that the cell-free extracts of cell cultures of *H. calycinum* contained a soluble prenyltransferase, which is likely to catalyze the initial prenylation in hyperforin biosynthesis [79]. However, it cannot be fully excluded that the enzyme may be related to the formation of some other prenylated secondary metabolites in the cultures. In a further study, a prenyltransferase HcPT, which catalyzes regiospecific C-8 prenylation of 1,3,6,7-tetrahydroxyxanthone, is cloned and characterized from *H. calycinum* cell cultures (Fig. 4) [80]. Additionally, the same group reported the discovery of four prenyltransferase genes from *H. sampsonii* (*HsPT8px* and *HsPTpat*) and *H. calycinum* cell cultures (*HcPT8px* and *HcPTpat*) [81]. They are all engaged in the biosynthesis of polyprenylated xanthones patulone. Actually, the protein that is encoded by the homologous sequences *HsPT8px* or *HcPT8px* is mainly responsible for the fusion of a single prenyl unit to C-8 in 1,3,6,7-tetrahydroxyxanthone while *HsPTpat* or *HcPTpat* determines the introduction of a second prenyl moiety at the same position to generate *gem*-diprenylation product. Kinetic study shows that HsPT8px and HsPTpat catalyzes the sequential addition of the two prenyl groups to the xanthone skeleton. (Fig. 4). Moreover, they found that HsPT8 preferred Mg^{2+} while HsPTpat favored Mn^{2+} as its cofactor [81].

Prenyltransferases recognized biosynthetic precursors of hyperforin are found in some species. For example, two prenyltransferases named HlPT-1 and HlPT1L from *H. lupulus* catalyzes the prenylation of phlorisobutyrophenone (PlBP), while HlPT2 catalyzes the subsequent prenylation step to introduce a second prenyl residue (Fig. 4). Co-expression of HlPT1L and HlPT2 in engineered yeast demonstrates that they can catalyze three consecutive prenylations in the *β*-bitter acid biosynthesis pathway. Interestingly, HlPT2 only functioned when co-expressed with HlPT1L, implying that HlPT1L and HlPT2 may form a metabolon during the catalyzation [82, 83]. It should be noted that, prenyltransferase with efficient catalyst activity and broad substrate scope can be applied in the precursor biosynthesis and construction of hyperforin biosynthetic pathway. For example, AtaPT, a prenyltransferase with promiscuous catalytic properties from *Aspergillus terreus* characterized by our group, is capable of catalyzing *gem*-diprenylation in PlBP core, make it possible to produce the precursors of hyperforin and other PPAPs (Fig. 4) [84, 85].

The aPTases involved in hyperforin biosynthesis most likely belong to the membrane-bound aPTase family, although they may also be derived from soluble aPTases from plants. An in-depth analysis about the prenyltransferase gene sequences from the related species may help to identify the mono-prenylation or *gem*-diprenylation prenyltransferases involved in the hyperforin biosynthesis.

Perspectives

Hyperforin is a representative PPAPs and possesses extensive pharmacological activities. It is fascinating and rewarding to learn more about its biosynthesis process. With the development in natural product biosynthesis and the comprehensive application of molecular biology, chemical biology and bioinformatics, more biosynthetic genes of hyperforin are expected to be identified. As mentioned, hyperforin is characterized by its unique multi-prenylated bicyclic structure. Given the important role of the prenylation process in hyperforin biosynthesis, cloning and characterization of the responsible prenyltransferases will be a reasonable breakthrough point to reveal the biosynthesis process of this com-

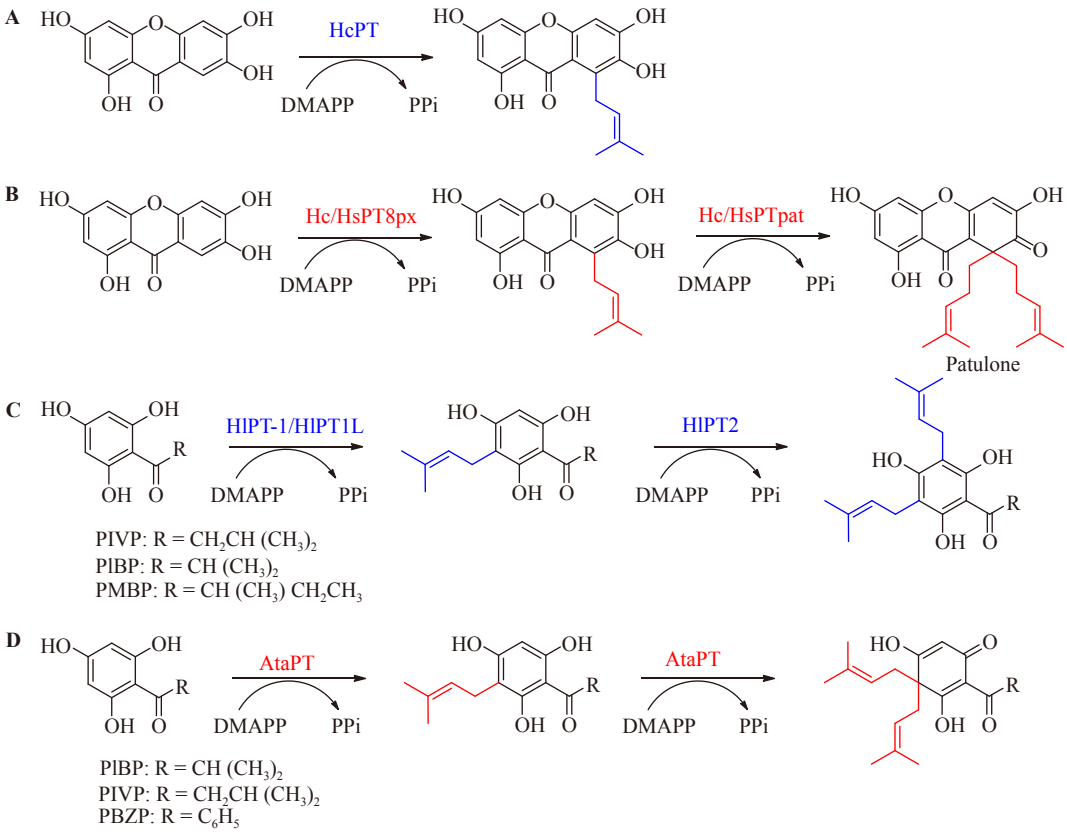


Fig. 4 Enzymatic prenylation of acylphloroglucinols

pound. In-depth elucidation of hyperforin biosynthesis will also provide useful insights to better understanding the biosynthesis of various naturally-occurring PPAPs with complex structures and lay a solid foundation for the efficient biosynthetic production of these bioactive compounds.

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