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Novel applications of plant polyketide synthases

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The structurally and mechanistically simple type III polyketide synthases (PKSs) catalyze iterative condensations of CoA thioesters to produce a variety of polyketide scaffolds with remarkably diverse structures and biological activities. By exploiting the enzymes, we combined precursor-directed biosynthesis with nitrogen-containing substrates and structure-based enzyme engineering and generated unnatural, novel polyketide–alkaloid scaffolds with promising biological activities. The nucleophilic nitrogen atom and the engineered enzymes thus facilitated the formation of additional C–C and C–N bonds during the enzymatic transformations. The methodology will contribute to the further production of chemically and structurally divergent, unnatural natural products, as well as the rational design of novel biocatalysts with unprecedented catalytic functions.

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Introduction

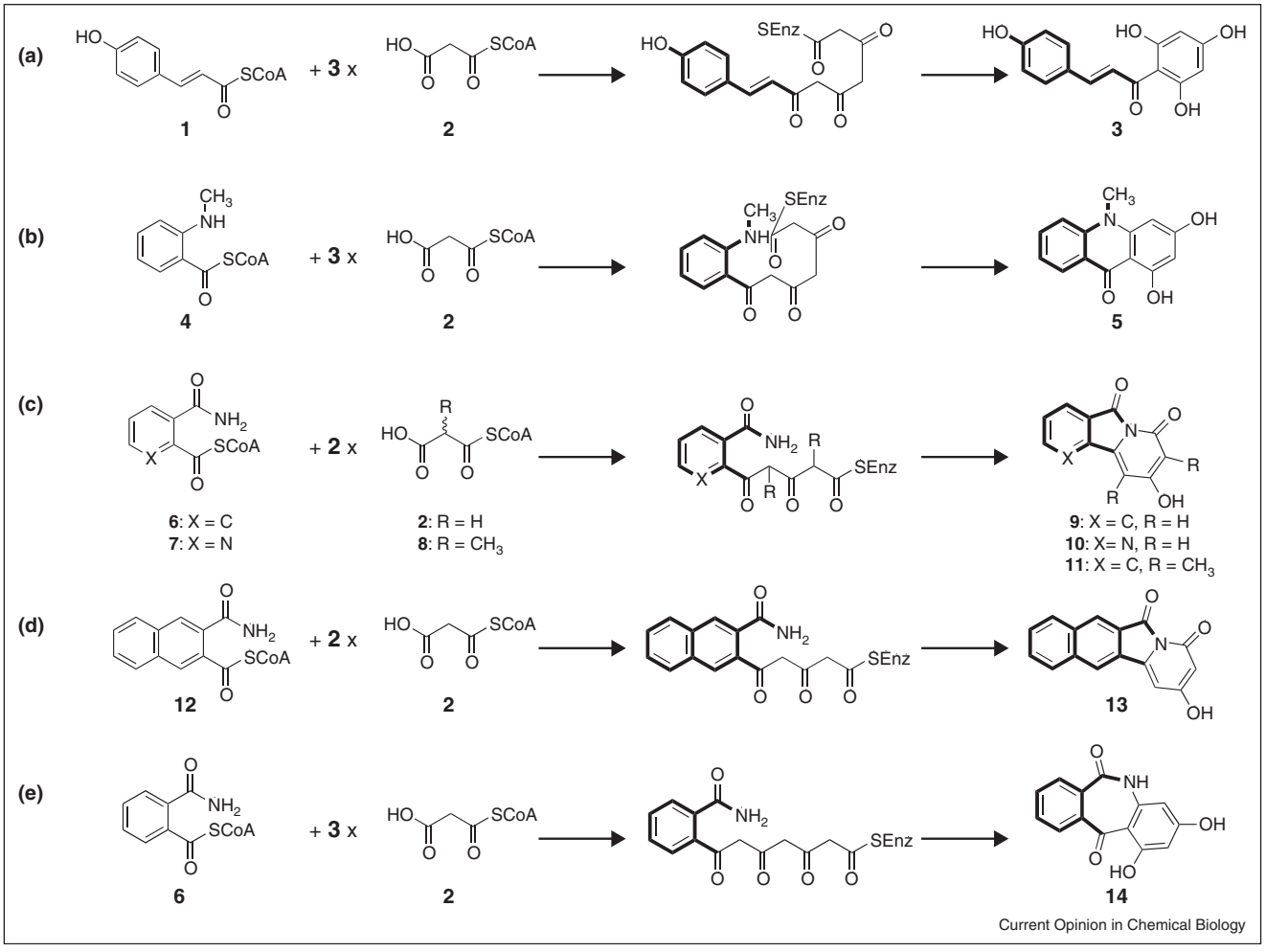
The chalcone synthase (CHS) superfamily of type III polyketide synthases (PKSs) comprises structurally simple, homodimeric proteins [1,2•,3•,4]. The enzyme reaction is initiated by the loading of the starter substrate at the active-site Cys, and continues by iterative, decarboxylative Claisen-type condensations of the extender substrate, malonyl-CoA. Subsequent cyclization of the enzyme-bound poly-β-keto intermediates leads to the formation of a variety of polyketide scaffolds with remarkably diverse structures and biological activities, including chalcone, stilbene, phloroglucinol, resorcinol, benzophenone, biphenyl, naphthalene, chromone, pyrone, acridone, quinolinone, and curcumin [1,2•,3•,4]. The catalytic diversity of the type III PKSs is derived from the differences in the selection of the starter molecules, the number of malonyl-CoA condensations, and the mechanisms of the cyclization reactions [1,2•,3•,4]. Recent crystallographic and site-directed mutagenesis studies have begun to reveal intimate structural details of the enzyme-catalyzed processes [5–16]. It is noteworthy that only a slight modification of the active site dramatically expands the catalytic repertoire of the enzymes [17,18,19•,20–25]. In addition, the type III PKSs exhibit unusually broad substrate specificities and accept a series of nonphysiological substrate analogs to produce chemically and structurally divergent, novel unnatural polyketides [26–37]. The catalytic promiscuity and versatility of the enzymes thus make type III PKSs an excellent platform for the further production of unnatural products and ideal candidates for rational design of novel biocatalysts with unprecedented catalytic functions [38••].

The C–C bond forming reactions catalyzed by the type III PKSs are primarily governed by the carbonyl chemistry of aldol-type and Claisen-type condensation reactions. The highly reactive poly-β-keto intermediates readily react with the nucleophilic nitrogen atoms to form Schiff bases, which should facilitate additional C–C or C–N bond forming chemistry, such as in a Mannich-type reaction [38••]. It was thus anticipated that if a promiscuous type III PKS could accept a rationally designed nitrogen-containing analog as a starter substrate and subsequently catalyze the polyketide chain elongation by the condensation with malonyl-CoA, then it would be possible to generate more complex and biologically active alkaloid molecules. This review summarizes our recent advances in the enzymatic synthesis of unnatural, novel polyketide–alkaloid scaffolds by exploiting plant type III PKSs, using an approach combining precursor-directed biosynthesis and structure-based enzyme engineering.

Synthesis of novel pyridoisoindole and dibenzoazepine derivatives

The PKS1 from the primitive club moss *Huperzia serrata* is a type III PKS with remarkable substrate tolerance and catalytic potential [39,40]. For example, *H. serrata* PKS1, which normally catalyzes the formation of naringenin chalcone (**3**) by sequential condensations of 4-coumaroyl-CoA (**1**) with three molecules of malonyl-CoA (**2**) (Figure 1a), also accepts the bulky *N*-methylanthraniloyl-CoA (**4**) as a starter substrate to produce 1,3-dihydroxy-*N*-methylacridone (**5**), after three condensations with malonyl-CoA (Figure 1b). Therefore, we first designed and synthesized 2-carbamoylbenzoyl-CoA (**6**), which has a similar molecular size as *N*-methylanthraniloyl-CoA and a nitrogen atom that can react with the carbonyl group of the elongating poly-β-keto intermediates during the enzyme reaction [41••]. In this case, the

Figure 1



Enzymatic formation of naringenin chalcone and alkaloids by type III PKSs. **(a)** Chalcone by CHS or *H. serrata* PKS1, **(b)** acridone by *H. serrata* PKS1, **(c)** the 6.5.6-fused pyridoisoindoles by *H. serrata* PKS1, **(d)** the 6.6.5.6-fused benzopyridoisoindole by *H. serrata* PKS1, and **(e)** the ring-expanded 6.7.6-fused dibenzoazepine by the *H. serrata* PKS1 S348G mutant.

less reactive carbamoyl group, instead of the primary amine, did not undergo the spontaneous intramolecular cyclization of the substrate with the concomitant removal of CoASH.

When incubated with 2-carbamoylbenzoyl-CoA as a substrate, the *H. serrata* PKS1 afforded an unnatural novel alkaloid, 2-hydroxypyrido[2,1-*a*]isoindole-4,6-dione (**9**), as a single product in 6.4% yield [41••]. Thus, the enzyme accepted the nitrogen-containing analog as a starter substrate and catalyzed sequential condensations with two molecules of malonyl-CoA to produce the pyridoisoindole scaffold, with the 6.5.6-fused tricyclic ring system (Figure 1c). A steady-state kinetics analysis revealed a $K_M = 7.6\ \mu M$ and a $k_{cat} = 4.5 \times 10^{-2}\ min^{-1}$ for 2-carbamoylbenzoyl-CoA, with respect to the pyridoisoindole-forming activity, representing 7-fold and 453-fold increases in the k_{cat}/K_M values of the chalcone-forming and acridone-forming activities of HsPKS1, respectively [41••]. In addition, interestingly, *H. serrata* PKS1 also accepted pyridine-containing 3-carbamoylpicolinoyl-CoA (**7**) as a starter substrate, and (2*RS*)-methylmalonyl-CoA (**8**) as an extender, to produce an unnatural novel pyrido[2,3-*a*]indolizine (**10**) and a dimethylated pyridoisoindole (**11**), respectively, but with lower efficiency [41••] (Figure 1c).

Furthermore, to our surprise, *H. serrata* PKS1 even accepted a bulky naphthalene-containing 3-carbamoyl-2-naphthoyl-CoA (**12**) as a starter substrate to produce 2-hydroxybenzo[*f*]pyrido[2,1-*a*]isoindole-4,6-dione (**13**), another unnatural novel alkaloid scaffold with the 6.6.5.6-fused tetracyclic ring system, as a single product in 13% yield [41••] (Figure 1d). A steady-state kinetics analysis revealed a $K_M = 6.8\ \mu M$ and a $k_{cat} = 3.2 \times 10^{-1}\ min^{-1}$ for 3-carbamoyl-2-naphthoyl-CoA (**12**), which