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Engineering a promiscuous prenyltransferase for selective biosynthesis of an undescribed bioactive cannabinoid analog



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Cannabinoids are unique meroterpenoids, with cannabigerolic acid (CBGA) serving as a dedicated precursor. This study introduces a fungal aromatic prenyltransferase AscC into the engineered *Escherichia coli* to catalyze the transfer of C₅–C₁₅ terpenoid linear precursors to olivetolic acid. Four CBGA derivatives (compounds **1–4**) with diverse C₅, C₁₀, or C₁₅ prenyl chains are isolated and identified, with compound **4** being an undescribed product featuring a C₁₅ prenyl chain at the C-5 position. Compound **4** demonstrates the highest anti-neuroinflammatory and antibacterial activities, with IC₅₀ values of 3.06 μM for TNF-α and 4.31 μM for IL-6, alongside EC₅₀ values ranging from 0.87 to 3.16 μM against three Gram-positive bacteria. An efficient construct is established by incorporating an additional copy of AscC, resulting in a yield of 14.85 ± 0.91 mg/L of compound **4**. Two mutants, L180Y and L180F, are engineered to selectively produce compound **4**. These findings provide a foundation for enriching the chemical diversity of bioactive cannabinoid analogs with various prenyl moieties through combinatorial biosynthesis.

Natural products play pivotal roles in chemical biology and drug discovery due to their unique chemical structures and specific biological targets¹. The construction of natural product analogs is important for exploring structure-activity relationships, developing drugs with enhanced bioactivity and bioavailability, and uncovering disease mechanisms as chemical biology tools². With the advances in synthetic biology, combinatorial biosynthesis exploiting enzymatic and pathway promiscuity provides an alternative approach to generate novel natural product analogs^{3–5}.

Meroterpenoids represent a class of natural products with conspicuous biological activities derived from the terpenoid biosynthetic pathway coupled with other biogenic pathways. Among these, cannabinoids stand out as unique meroterpenoids initially isolated from the plant *Cannabis sativa*, a herbal medicine with a history spanning over 3500 years⁶. More than 120 phytocannabinoids have been isolated from *Cannabis*, most of which are alkyl resorcinol derivatives with a monoterpene unit⁷. Notably, cannabinoids, such as Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD), exhibit potent pharmacological effects, including analgesic, antiemetic, antispasmodic, antimicrobial, and anti-inflammatory activities^{8–10}, which

renders them of great medicinal importance¹¹. The pharmacological activities of cannabinoids principally occur through interacting with cannabinoid receptors CB1 and CB2, belonging to the superfamily of G-protein coupled receptors which mediate most of physiological responses and so have great potential as therapeutic targets for a broad spectrum of diseases^{12,13}. The important influence of the C-3 side chain of cannabinoids on the pharmacological activities has been identified through chemical synthesis of a series of C-3 alkyl analogs^{14–16}, however, the effect of the prenyl chain on pharmaceutical properties remains unexplored.

The biosynthesis of cannabinoids is derived from a monoterpene unit produced by 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway and an aromatic moiety olivetolic acid (OA) catalyzed by an olivetol synthase and an OA cyclase using malonyl-CoA and hexanoyl-CoA as substrates¹⁷. Cannabigerolic acid (CBGA) is the committed biosynthetic precursor of cannabinoids, formed by a C-C Friedel-Craft alkylation of OA with geranyl diphosphate (C₁₀, GDP)¹⁸. Subsequently, the geranyl moiety of CBGA undergoes cyclization reaction to form cannabinoids such as Δ⁹-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), or

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cannabichromenic acid (CBCA), which can be decarboxylated to their neutral forms (Fig. 1)¹⁹.

Heterologous biosynthesis of cannabinoids has been achieved in engineered yeasts, paving an alternative way for access to cannabinoids^{20–22}. Based on enzyme promiscuity and engineered biosynthetic pathways, a series of unnatural cannabinoid analogs with distinct alkyl moieties at the C-3 position were obtained by combinatorial biosynthesis through feeding different fatty acids^{20,23}. Given the diversity of terpenoids which includes hemiterpenoids, monoterpene, sesquiterpenoids, diterpenoids, and sesquiterpenoids, we are particularly interested in the biosynthesis of CBGA analogs with distinct terpenoid chains. Several aromatic prenyltransferases have been reported, among which AscC, a UbiA-type prenyltransferase from *Fusarium* sp. NBRC 100844, could catalyze the conversion of 2-methyl-4,6-dihydroxy benzaldehyde into the corresponding farnesylated product²⁴. In this work, we synthesized four CBGA analogs 1–4 with a C₅, C₁₀, or C₁₅ prenyl unit using the engineered *Escherichia coli* harboring the AscC gene. Molecular docking and site-directed mutagenesis resulted in two mutants selectively producing the undescribed compound 4. In addition, the anti-neuroinflammatory and antibacterial activities of these compounds were observed.

Results

Heterologous expression of promiscuous aromatic prenyltransferases in engineered *E. coli* strains for cannabinoid analogs production

To biosynthesize cannabinoid analogs featuring diverse prenyl side chains, the engineered *E. coli* strains were recruited, which contain an exogenous MVA pathway along with a farnesyl diphosphate synthase

(FDPS) or geranylgeranyl diphosphate (GGDPS) or geranylgeranyl diphosphate synthase (GGDPS) previously constructed for terpenoid biosynthesis²⁵. The aromatic prenyltransferase (CsPT4) from *C. sativa* and four fungal promiscuous aromatic prenyltransferases (aPTases), including AscC from *Fusarium* sp., Trt2 and AtaPT from *Aspergillus terreus*, and Orf2 from *Streptomyces* sp.^{24,26–29}, were picked out to test their activity for transferring various prenyl groups to the aromatic ring of OA (Supplementary Fig. 1). The full-length cDNA of these aPTases was synthesized after codon optimization and then constructed into the corresponding expression vector.

The four fungal aromatic prenyltransferases could express substantial amounts of recombinant proteins with expected molecular mass in the soluble fraction. These aPTases were subsequently transformed into the engineered *E. coli* strains producing C₅–C₂₅ prenyl diphosphates. By feeding with OA, four specific peaks could be detected in the extract of engineered *E. coli* containing AscC using high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) analysis (Fig. 2). The pseudo-molecular ion peaks ($[M-H]^-$) of specific products were 291.1601, 359.2226, 427.2853, and 427.2851, indicating that AscC could catalyze the introduction of C₅, C₁₀, and C₁₅ prenyl chains into OA (Fig. 2). The conversion rate of OA was 70.48% by AscC. By contrast, no obvious product was observed in the engineered bacteria harboring Trt2, AtaPT, and Orf2. In addition, we tested the catalytic activity of AscC towards a series of OA analogs (S1–S7), but no products were observed (Supplementary Fig. 2). Compared to OA, these analogs lack a hydroxyl group at the C-4 position, except S4 that has an ester group at the C-1 position instead of a carboxyl group. These results suggest that both the C-4 hydroxyl group and the C-1 carboxyl group in the substrate are important for enzyme activity of AscC.

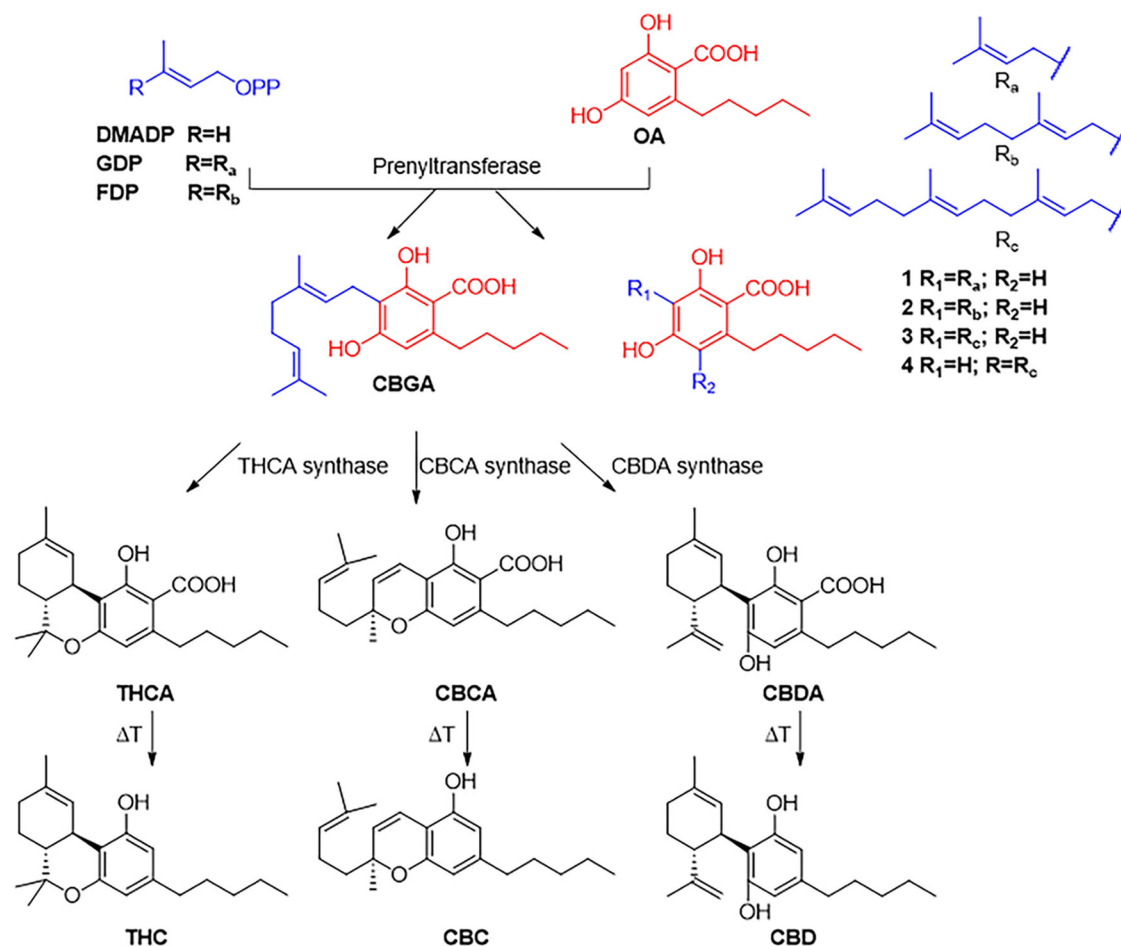
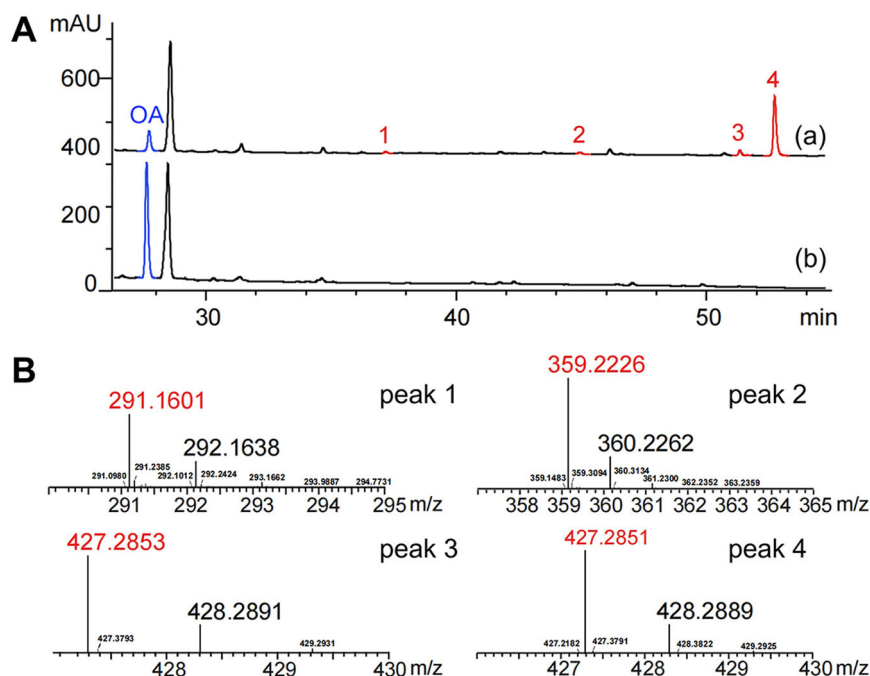


Fig. 1 | The biosynthetic pathway of cannabinoids.

Fig. 2 | The conversion of olivetolic acid (OA) to prenylation products by engineered *E. coli* harboring AscC. A HPLC analysis of the extract of engineered bacteria harboring pET28a/AscC (a) and empty vector pET28a (b). **B** The mass spectrometry of peaks 1–4.



Identification of cannabinoid analogs produced by AscC in engineered *E. coli*

The plasmid pET28a/AscC was transferred into *E. coli* strain BL21 harboring the pBbA5c-MevT-MBIS plasmid, which contains a complete gene set of the mevalonate (MVA) pathway and a FDPS gene³⁰, resulting in an engineered *E. coli* strain BPA1. To elucidate the structure of cannabinoid analogs, a 10 L scale fermentation of the engineered BPA1 strain was performed and extracted with ethyl acetate. The *E. coli* extracts were further purified with a combination of repeated silica-gel and Sephadex LH-20 column chromatography. Afterward, semi-preparative HPLC was carried out using a mixture of acetonitrile and acid water as a mobile phase to afford compounds 1–4. Notably, compound 4 emerged as the predominant component (Fig. 2).

Compound 4 was obtained as a yellowish powder and characterized by a molecular formula of $C_{27}H_{40}O_4$ with eight degrees of unsaturation based on high-resolution electron ionization mass spectroscopy (HR-EI-MS) (m/z 427.2851 [M-H]⁺, calcd. 427.2854) and NMR spectroscopic data (Supplementary Figs. 3–10). Compound 4 showed the same molecular weight as compound 3 but a different retention time, implying that they might be prenylated products of OA at different positions. Compound 3 was determined as a cannabinoid analog 2,4-dihydroxy-3-[(2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl]-6-pentylbenzoic acid through careful inspection of the NMR data (Supplementary Figs. 11, 12) and comparison with those reported in the literature³¹. The 1D and 2D NMR spectra of 4 closely resembled those of 3 with a key difference being the upfield movement of the carbon at C-3 (δ_C 102.2) and C-1'' (δ_C 31.5) in 4 compared to 3 (δ_C 111.1 for C-3 and δ_C 36.7 for C-1''). Additionally, the carbon at C-5 (δ_C 119.9) and C-1' (δ_C 25.1) in 4 moved downfield compared to 3 (δ_C 111.6 for C-5 and δ_C 22.2 for C-1') indicating a C_{15} farnesyl chain attached to C-5. Confirmation of this attachment was provided by the 1H - 1H correlation spectroscopy (COSY) correlations of H-1'/H-2', H-4'/H-5'/H-6' as well as heteronuclear multiple bond correlation (HMBC) correlations of H-1' with C-4/C-5/C-6/C-2'/C-3' (Fig. 3). The 1H - 1H COSY correlations of H-1''/H-2''/H-3''/H-4''/H-5'', combined with the HMBC correlations of H-1'' with C-1/C-5/C-6, provided evidence for the attachment of the *n*-pentyl chain to C-6 and confirmed the planar structure of 4 (Fig. 3). Accordingly, the structure of 4 was determined as 2,4-dihydroxy-5-[(2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl]-6-pentylbenzoic acid (Fig. 3). The structures of

compounds 1 and 2 were identified as 2,4-dihydroxy-3-(3-methylbut-2-en-1-yl)-6-pentylbenzoic acid³² and CBGA³³ (Supplementary Figs. 13–16), respectively, through comparison of their NMR data with those reported in the literature (Fig. 3). Compounds 1–4 are cannabinoid analogs, in which compound 4 is an undescribed compound. The results indicated that engineered *E. coli* harboring AscC could produce four cannabinoid analogs with a C_5 , C_{10} , or C_{15} prenyl chain, likely attributed to the spacious hydrophobic substrate-binding pocket of promiscuous aPTases³⁴.

Compounds 1–4 displayed potent anti-inflammatory and anti-bacterial activities

Cannabinoids are known for their potent anti-inflammatory and immunosuppressive properties, likely through interacting with G protein-coupled receptors that are closely related to immune regulation and neurodegeneration^{12,13,35}. Therefore, four cannabinoid analogs were evaluated for their anti-neuroinflammatory activity by investigating the inhibition of TNF- α and IL-6 secretion in BV-2 microglial cells. As shown in Table 1, compound 4 showed potent inhibitory effects on TNF- α and IL-6 secretion, with IC_{50} values of 3.06 and 4.31 μM , respectively. Compounds 1–3 showed moderate activity against TNF- α and IL-6 secretion with 34.29–48.17% inhibition rates at 20 μM . By comparing the structures and activity of compounds 1–4, it can be concluded that the C_{15} farnesyl unit at the C-5 position of OA played an important role in anti-inflammatory activity.

Considering the reported broad-spectrum antimicrobial activity of many cannabinoids, we aimed to assess the antimicrobial potential of compounds 1–4. Three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and *Micrococcus luteus*), one Gram-negative bacteria (*E. coli*), and one fungus (*Trichophyton rubrum*) were recruited. As shown in Table 2, compounds 1–4 exhibited specific inhibitory activities against Gram-positive bacteria but were inactive against *E. coli* and *T. rubrum*. The EC_{50} values of compounds 1–4 against *S. aureus*, *B. subtilis*, and *M. luteus* ranged from 0.87 to 35.13 μM . The substrate OA showed no activity, emphasizing the importance of prenylation for antibacterial activity. Notably, compound 4 exhibited the most potent antibacterial activity among these cannabinoid analogs, with EC_{50} values of 0.87–3.16 μM , suggesting that the C_{15} farnesyl group at the C-5 position of OA also contributed to the antibacterial property against Gram-positive bacteria.

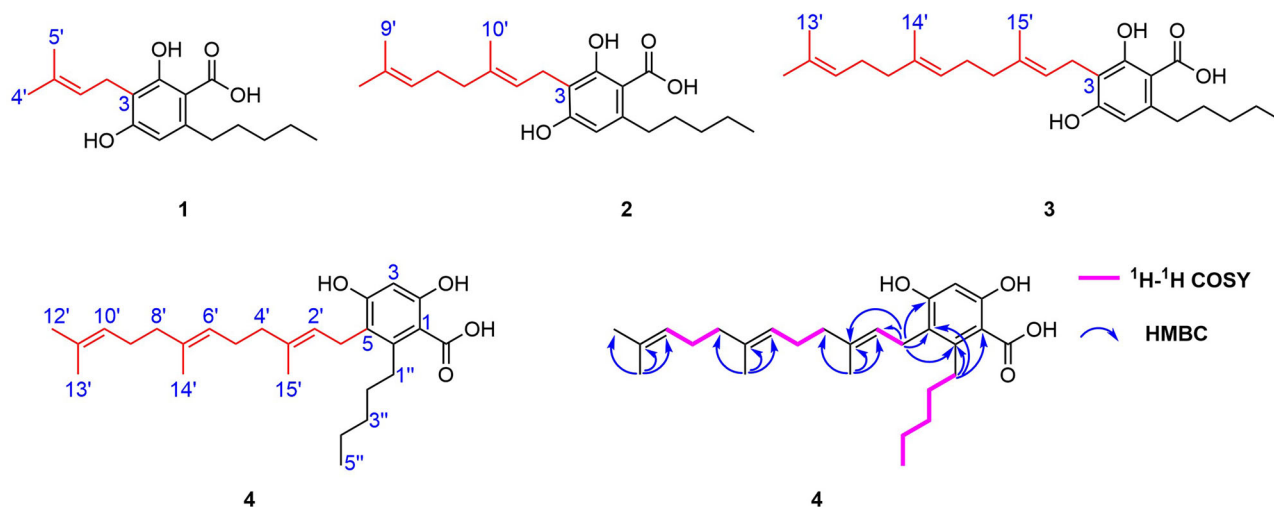


Fig. 3 | Chemical structures of the products produced by engineered *E. coli* harboring AscC and key ¹H-¹H COSY and HMBC correlations of compound 4.

Improved production of compounds 1–4 in *E. coli*

We analyzed the production of four compounds in *E. coli* strains carrying pBbA5c-MevT-MBIS and pET28a/AscC using an external standard method. HPLC analysis showed that the yields of compounds 1–4 were 0.12 ± 0.02 mg/L, 0.08 ± 0.01 mg/L, 0.38 ± 0.05 mg/L, and 7.31 ± 0.60 mg/L, respectively. Notably, a significant amount of unconsumed prenyl substrate FDP was observed in the engineered *E. coli*, prompting an attempt to enhance the yield of compounds 1–4 by increasing the copy numbers of the AscC gene. For this purpose, an additional plasmid pCold-TF/AscC was transformed into the *E. coli* harboring pBbA5c-MevT-MBIS and pET28a/AscC while the pCold-TF empty vector was co-transformed into the same strain as a negative control. HPLC analysis showed that the yields of compounds 1–4 significantly improved to 2.06 ± 0.10 mg/L, 1.63 ± 0.12 mg/L, 0.91 ± 0.09 mg/L and 14.85 ± 0.91 mg/L respectively in the strain containing two AscC plasmids, which were 17.85, 20.80, 2.42 and 2.03-fold of those in the strain containing plasmid pET28a/AscC, respectively (Fig. 4). The results indicated that increasing AscC copy numbers led to a substantial increase in the yields of compounds 1–4. Different yields of compounds 1–4 may be attributed to the availability of various prenyl diphosphates, as FDP was the predominant product in the engineered *E. coli* containing the pBbA5c-MevT-MBIS plasmid. However, the production of compounds 1 and 2 showed a significantly higher increase compared to compounds 3 and 4 after introducing an additional copy of AscC, suggesting that the enzymatic efficiency of AscC has a greater impact on the product profiles than the availability of prenyl diphosphates. Although prenylation at either the C-3 or C-5 position of OA occurs when using FDP as a prenyl donor, the yield of compound 4 was significantly higher than that of compound 3. These results suggest that prenylation reactions catalyzed by AscC exhibit a regioselective preference for the *ortho* position of the *n*-pentyl group over the two electron-donating hydroxyl groups on the aromatic ring.

Table 1 | Inhibitory effects of compounds 1–4 on TNF- α and IL-6 secretion of BV-2 microglial cells induced by LPS

Compound	TNF- α secretion		IL-6 secretion	
	Inhibition rate (%) ^a	IC ₅₀ (μ M)	Inhibition rate (%) ^a	IC ₅₀ (μ M)
1	34.29 $\pm$ 6.84	–	35.01 $\pm$ 5.94	–
2	40.62 $\pm$ 7.93	–	48.17 $\pm$ 5.57	–
3	41.20 $\pm$ 6.36	–	46.21 $\pm$ 5.84	–
4	85.35 $\pm$ 7.32	3.06	81.08 $\pm$ 6.76	4.31

^aInhibition rate was determined at the concentration of 20 μ M.

Engineering AscC for selective production of compound 4

Given that four different cannabinoid analogs are produced by AscC, we are motivated to engineer the enzyme to improve its product specificity. The AscC protein model contained a sizable central cavity capable of accommodating diverse prenyl substrates (Fig. 5A). In the model of AscC docked with structurally geometry-optimized FDP and OA, we found 14 amino acid residues interacting with the bound substrates (Fig. 5B). The residues Y162, K166, and D226 located in the conserved motifs (Y₁₆₂XXXXK₁₆₆ and D₂₂₆XXXXD₂₃₀) and R110 interacted with the pyrophosphate group of FDP were excluded because of their crucial roles for enzyme activity³⁶. Then, computational alanine scanning on the residues within 3 Å of OA or FDP was performed, and the amino acids with binding energy exceeding 0.4 were selected as candidates for further investigation (Supplementary Table 1). The in silico prediction showed that mutating V215 to alanine would result in the destabilization of the protein when FDP served as the lignan. Mutating D219 and L176 to alanine was predicted to increase protein stability and lead to substantial energy alterations, but mutation of D219A would disrupt the essential hydrogen bond between D219 and FDP. Mutating Y309 to alanine would lead to a significant change in binding energy and enhance protein stability, but the π -alkyl interaction between Y309 and FDP would be disrupted. Mutations L180A and Y222A exhibited a neutral effect and moderate energy changes. Therefore, we obtained six variants by site-directed mutagenesis, including L176F, L176Y, L180F, L180Y, Y222L, and Y222F, based on $\Delta\Delta G$ changes to improve the thermal stability of the protein. These mutants were individually transformed into *E. coli* harboring the pBbA5c-MevT-MBIS plasmid.

Interestingly, the variants L180Y and L180F selectively utilized FDP as a prenyl donor and specifically catalyzed the isopentenyl reaction of OA at the C-5 position to produce compound 4 (Fig. 5C). We constructed the protein model of L180F using AlphaFold 2 and docked the substrates OA and FDP into the binding pocket. OA was observed to exhibit different conformations in the wild-type and mutant enzymes (Fig. 5E). In L180F, there were more interactions between the residues R41, G47, and R90 and the carboxyl or 2-hydroxyl group of OA (Fig. 5F, G), which likely reduced the binding flexibility of OA within the pocket. Moreover, a π -alkyl interaction between Y222 and the alkyl side chain of OA in L180F replaced the alkyl-alkyl interaction between M50 and the alkyl side chain of OA in AscC. Additional π -alkyl interactions between OA and FDP were also observed. Accordingly, the positioning of OA was likely stabilized within the enzyme pocket, facilitating regioselective Friedel-Craft alkylation at C-5 (Fig. 5D). In addition, the yields of compound 4 in variants L180Y and L180F reached up to 11.71 and 12.95 mg/L, respectively. The switch from an alkyl-alkyl to a π -alkyl interaction between the amino acid at position 180 and FDP, coupled with the additional π -alkyl interaction between Y218 and FDP, may

Table 2 | Antimicrobial effects of compounds 1–4

Microorganism	EC ₅₀ (μM)					
	1	2	3	4	OA	CK ^a
<i>S. aureus</i>	24.21 ± 2.95	4.93 ± 0.70	9.62 ± 1.45	0.87 ± 0.14	NA	0.48 ± 0.05
<i>B. subtilis</i>	12.50 ± 1.17	2.62 ± 0.14	5.34 ± 0.56	2.08 ± 0.12	NA	0.21 ± 0.03
<i>M. luteus</i>	35.13 ± 3.78	8.85 ± 0.50	17.62 ± 1.36	3.16 ± 0.42	NA	0.27 ± 0.03
<i>E. coli</i>	NA	NA	NA	NA	NA	0.15 ± 0.02
<i>T. rubrum</i>	NA	NA	NA	NA	NA	3.38 ± 0.76

^a The positive control for *S. aureus*, *B. subtilis* and *M. luteus* was ampicillin sodium. The positive control for *E. coli* was cefotaxime sodium, and the positive control for *T. rubrum* was terbinafine hydrochloride.

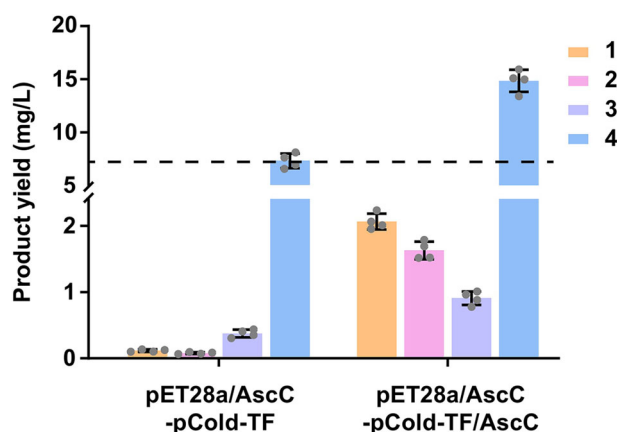


Fig. 4 | The yields of compounds 1–4 in engineered *E. coli* harboring AscC. Co-transformation pET28a/AscC and pCold-TF empty vector in engineered *E. coli* containing the pBbA5c-MevT of MBIS plasmid served as a control for determining the effect of AscC gene copy on product yields. Four biologically independent experiments were performed. The error bars represent the standard deviation (S.D.) of the mean.

provide enhanced cation stabilization. Besides, the reduced distance between C-5 and C-1 of FDP (from 6.7 to 6.2 Å) might also contribute to the improved conversion efficiency in variants L180Y and L180F. Furthermore, the natural substrates 2-methyl-4,6-dihydroxy benzaldehyde and FDP were also docked into the wild-type and mutant enzymes, respectively. The results suggested that L180F might still catalyze Friedel-Craft alkylation at the C-3 position, probably attributed to the methyl group at the C-1 position facilitating substrate flexibility within the enzyme pocket (Supplementary Fig. 17). However, the experiment was not conducted due to the unavailability of the substrate.

The variants L176Y and L176F resulted in a substantial loss of enzymatic activity (Fig. 5C). Y222 interacted with FDP via π -alkyl interactions, and the mutants Y222L and Y222F exhibited a dramatic reduction in enzymatic activity, with 21% and 0.4% production of wild-type AscC, respectively. The residues R41, G47, and R90 were predicted to form hydrogen bonds or carbon interaction with OA, suggesting they play an important role in the binding and stabilizing of OA. This was confirmed by the substantial loss of enzymatic activity upon mutation R90E (Fig. 5C). The results highlighted the importance of these residues in enzyme catalysis.

Discussion

The development of natural product analogs holds significant promise in chemical biology and drug discovery. Cannabinoids represent a unique class of natural products with substantial pharmaceutical values. Notable efforts have focused on the generation of cannabinoid analogs with distinct alkyl moieties, such as combinatorial biosynthesis of unnatural cannabinoid analogs through feeding different fatty acids^{20,23}. In this study, cannabinoid analogs with varied terpenoid chains were created by introducing a promiscuous fungal aromatic prenyltransferase AscC into engineered bacteria

strains capable of terpenoid production. The most abundant product, compound 4, is an undescribed compound featuring a C₁₅ prenyl chain at the C-5 position of olivetolic acid.

Considering the steric hindrance of the *n*-pentyl group and the electron-donating effect of the hydroxyl groups in the aromatic ring, prenylation of OA would theoretically favor the C-3 position over the C-5 position. However, when using farnesyl diphosphate (FDP) as the prenyl donor, the amount of compound 4 prenylated at the C-5 position was significantly higher than that of compound 3 at the C-3 position. Notably, C-5 substituted cannabinoids are rare, with the exception of 5-geranyl olivetolate isolated from *Helichrysum umbraculigerum*³⁷. A number of aPTases responsible for catalyzing the attachment of prenyl groups to aromatic substrates have been reported. For instance, UbiA-type PTases CsPT1 and CsPT4 reported from *Cannabis* catalyze the attachment of a geranyl group to OA²⁰, and NphB (formerly Orf2) identified from *Streptomyces* sp. strain CL190 could catalyze the attachment of a geranyl group to aromatic substrates²⁸. Recently, the core cannabinoid biosynthetic enzymes have also been identified from *H. umbraculigerum*, including four aPTases HuPT1–4 among that HuPT4 (HuCBGAS4) geranylated the aromatic dihydrostilbenic acid³⁸. Notably, these identified aPTases predominantly catalyze prenylation at the C-3 position of aromatic substrates^{20,31,39}. By contrast, AscC can catalyze OA to produce farnesylated products at both the C-3 and C-5 positions, suggesting a distinct catalytic mechanism. The unusual regioselectivity of AscC warrants further investigation, and the rise of artificial intelligence and language modes will help enzyme engineering in future⁴⁰. The unique structure of compound 4 presents significant challenges for regioselective chemical synthesis. Guided by structure-based enzyme engineering, we successfully improved the selectivity of AscC, and developed two variants, L180Y and L180F, which exclusively produce compound 4. These findings provide promising catalysts for manipulating the production of compound 4 using synthetic biology.

We found that compounds 1–4 exerted significant anti-neuroinflammatory and antimicrobial properties, with compound 4 being the most effective. This indicates that the prenyl side chains at the C-5 position of OA play crucial roles in these activities. Additionally, novel cannabinoids with remarkable bioactivities could potentially be generated via prenyl cyclization. For instance, CBD demonstrates enhanced anti-inflammatory and antimicrobial activity compared to CBGA^{41,42}. Further efforts should focus on exploring more cyclases and expression systems to expand the chemical space of cannabinoids.

Methods

General experimental procedures

Column chromatography was performed on silica gel (200 – 300 mesh, Qingdao Dingkan Silicone Co., Ltd., P. R. China) and Sephadex LH-20 (20 – 100 μm, Amersham Pharmacia Biotech, Sweden). HPLC-DAD analysis was carried out on an Agilent 1260 Infinity II equipped with a Zorbax SB-C₁₈ (5 μm, 4.6 × 250 mm, 1 mL/min). Semi-preparative HPLC analysis was performed on a HITACHI Primaide series instrument with a Shimadzu Shim-pack GIST-C₁₈ column (5 μm, 10 × 250 mm, 3 mL/min). Mass spectra were obtained on a Waters Synapt XS spectrometer equipped

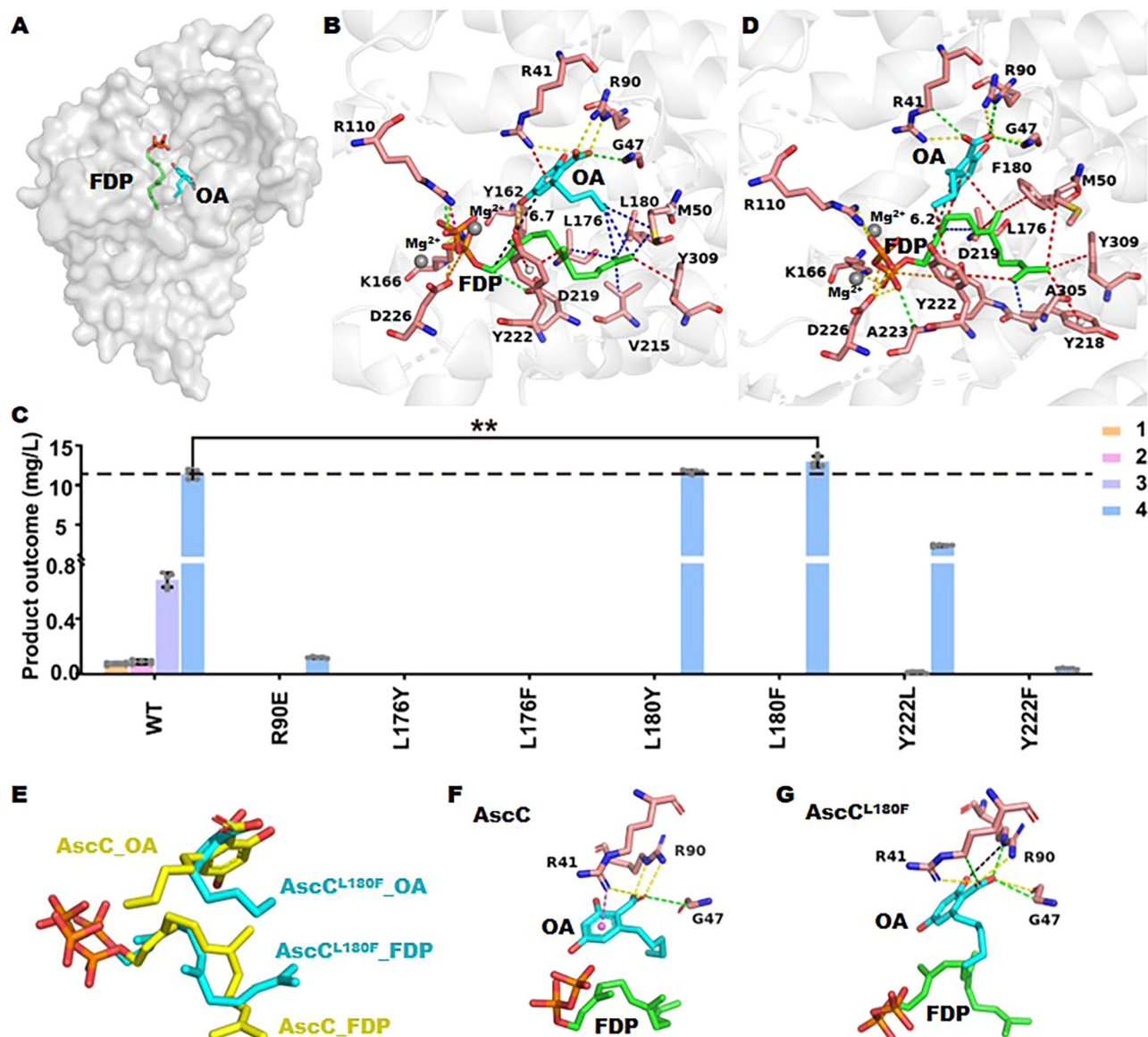


Fig. 5 | Analysis of crucial residues in AscC. **A** Overview of AscC model docked with FDP and OA. **B** Interactions between the substrates and individual residues in AscC. Yellow, blue, red, green, orange, violet, and black dash lines represent hydrogen bonds, alkyl interactions, π -alkyl interactions, carbon interactions, charge interactions, π -cation interactions, and donor-donor interactions, respectively. The visualization was generated using Pymol. **C** Compound yields of wild-type AscC and

its mutants expressed in the pET28a vector in engineered *E. coli* containing the pBbA5c-MevT-MBIS plasmid. $**P < 0.01$ vs WT. Five biologically independent experiments were performed. The error bars represent the standard deviation (S.D.) of the mean. **D–G** Interactions between the substrates and residues in AscC and its mutant L180F.

with a Zorbax Extend-C₁₈ column (1.8 μ m, 4.6 $\times$ 50 mm, 0.3 mL/min). NMR spectra were recorded on a Bruker AV-700 (700 MHz) spectrometer, with TMS as an internal standard. IR spectra were recorded on a Bruker Tensor-27 spectrometer with KBr pellets. Genes were synthesized by Tsingke Biotechnology Co., Ltd.. OA was purchased from J&K Scientific Co., Ltd.. The ethyl acetate used for extraction was analytical grade and acetonitrile used for HPLC was chromatographic grade. Other reagents were analytical grade.

Strains, plasmids and media

All primers used in this study were listed in Supplementary Table 2. *E. coli* DH5 α was used for plasmid maintenance and *E. coli* BL21 (DE3) was used for gene heterologous expression. Plasmid pBbA5c-MevT-MBIS was kindly provided by Prof. Tao Liu at Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. Codon-optimized CsPT4 from *C. sativa*, AscC from the fungus *Fusarium* sp., Trt2 and AtaPT from *Aspergillus*

terreus, and Orf2 from *Streptomyces* sp. were subcloned into *Nde*I and *Xho*I sites of expression vector pET28a using ClonExpress® Ultra One Step Cloning Kit (Vazyme Bio.), generating corresponding plasmids. Similarly, the cDNA of AscC was inserted into *Sall* and *Xho*I sites of expression vector pCold-TF, resulting in the plasmid pCold-TF/AscC. The recombinant plasmids were individually transformed into *E. coli* expression strain BL21(DE3) that harbored the pBbA5c-MevT-MBIS vector to generate the corresponding strains. pET28a empty vector was transformed into the same strain as a negative control. The plasmid pCold-TF/AscC was co-transformed with pET28a/AscC into *E. coli* strain BL21 harboring pBbA5c-MevT-MBIS to generate the strain BPA3. pCold-TF empty vector was transformed into the same strain as a negative control (BPA4). All strains constructed in this study were listed in Supplementary Table 3. The culture media used for *E. coli* strains were Luria-Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) or Terrific Broth (TB) medium (12 g/L tryptone, 24 g/L yeast extract, 4 mL/L glycerol, 2.31 g/L KH₂PO₄,

12.54 g/L K_2HPO_4). Ampicillin sodium (100 μ g/mL), chloramphenicol (34 μ g/mL), and kanamycin (50 μ g/mL) were supplemented to media for screening positive recombinant strains.

Product assay of engineered *E. coli*

The colonies of strains constructed in this study were screened using LB solid plates containing corresponding antibiotics and further confirmed by colony-PCR. Seed cultures (10 mL) of confirmed transformants were grown overnight at 37 °C in TB medium supplemented with corresponding antibiotics. 1 mL of seed culture was inoculated into 50 mL fresh TB broth supplemented with the same antibiotics and cultured at 37 °C until the cultures reached the optical density (OD_{600}) of 0.6–0.8. Subsequently, 50 μ L of 0.3 mM isopropyl- β -D-thiogalactopyranoside (IPTG) was added to induce the expression of the target proteins at 16 °C for 16 h. Afterward, 0.25 mg substrates (OA, compound **S1–S7**: salicylic acid, acetylsalicylic acid, ginkgoneolic acid, 2,4-dihydroxy-6-pentylbenzoate, 2-hydroxy-6-methylbenzoic acid, 2-methoxy-4-methylbenzoic acid or 4-ethyl-3-hydroxybenzoic acid) dissolved in anhydrous ethanol (10 mg/mL) was added to the cultures which were incubated at 25 °C for 72 h. Subsequently, the cultures were extracted with ethyl acetate by ultrasound (100 mL $\times$ 3, 30 mins per time) and the solvents were then removed by rotary evaporators. The residues were dissolved in 1 mL methanol and filtered for HPLC and LC-MS analysis. The mobile phase was composed of acetonitrile (solvent A) and 0.2% formic acid in water (solvent B), at a flow rate of 1.0 mL/min and a column temperature of 30 °C. The elution gradient was as follows: 0–40 min, 5–75% A; 40.01–50 min, 75–95% A; 50.01–55 min, 95% A (for OA); while 0–5 min, 5% A; 5.01–30 min, 5–100% A; 30.01–35 min, 100% A (for **S1–S7**). The injection volume was 20 μ L. LC-MS was performed under the same condition of HPLC, while electrospray ionization mass spectrometry was used in the negative ion mode with a capillary voltage of 3500 V.

Isolation and structural elucidation of compounds produced by engineered *E. coli*

A total of 10 L engineered *E. coli* cells harboring pET28a-AscC and pBbA5c-MevT-MBIS were cultivated. The cultures were extracted three times with ethyl acetate and the organic phase was combined and concentrated in vacuo. The crude extract (7 g) was subjected to silica gel column chromatography and eluted with petroleum ether-EtOAc (100:0, 10:1, 5:1, 2:1 and 0:1, v/v) to obtain five fractions (Fr.1–Fr.5). Fr.3 (1.2 g) was subjected to Sephadex LH-20 and eluted with CH_2Cl_2 /MeOH (1:1, v/v), and then purified by semipreparative HPLC using MeCN/ H_2O (1:1, v/v) as a mobile phase to yield compound **1** (6 mg, t_R = 37.0 min), **2** (3 mg, t_R = 44.8 min), **3** (2 mg, t_R = 51.1 min) and **4** (32 mg, t_R = 52.5 min). Afterward, the compounds were subjected to extensive NMR analysis.

Analytical data of compounds 1–4

Compound **1**: Yellowish powder; 1H NMR (700 MHz, CD_3OD): δ 6.18 (s, 1H), 5.21 (t, J = 6.9 Hz, 1H), 3.26 (d, J = 7.2 Hz, 2H), 2.88 (d, J = 7.7 Hz, 2H), 1.76 (s, 3H), 1.65 (s, 3H), 1.55 (m, 2H), 1.33 (m, 4H), 0.90 (t, J = 6.7 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD): δ 175.8, 164.2, 160.5, 146.7, 131.6, 124.3, 113.8, 110.6, 104.5, 37.4, 33.3, 32.9, 26.0, 23.6, 22.9, 17.9, 14.4.

Compound **2**: Yellowish powder; 1H NMR (700 MHz, $CDCl_3$): δ 6.27 (s, 1H), 5.28 (t, J = 6.6 Hz, 1H), 5.05 (t, J = 6.4 Hz, 1H), 3.43 (d, J = 7.1 Hz, 2H), 2.88 (t, J = 7.9 Hz, 2H), 2.11 (m, 2H), 2.06 (m, 2H), 1.81 (s, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 1.56 (m, 2H), 1.33 (m, 4H), 0.89 (t, J = 6.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 175.4, 163.7, 160.6, 147.4, 139.4, 132.2, 123.9, 121.5, 111.6, 111.3, 103.4, 39.9, 36.7, 32.2, 31.6, 26.5, 25.8, 22.7, 22.2, 17.9, 16.4, 14.2.

Compound **3**: Yellowish powder; 1H NMR (700 MHz, $CDCl_3$): δ 6.24 (s, 1H), 5.27 (t, J = 7.2 Hz, 1H), 5.08 (m, 2H), 3.39 (m, 2H), 2.87 (m, 2H), 2.11 (m, 2H), 2.05 (m, 4H), 1.96 (m, 2H), 1.81 (s, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 1.58 (s, 3H), 1.55 (m, 2H), 1.29 (m, 4H), 0.88 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 174.9, 163.6, 160.3, 147.2, 139.2, 135.7, 131.4, 124.5,

123.8, 121.5, 111.6, 111.1, 103.6, 39.9, 39.8, 36.7, 32.2, 31.6, 26.8, 26.5, 25.8, 22.7, 22.2, 17.8, 16.4, 16.2, 14.2.

Compound **4**: Yellowish powder; $[\alpha]_D^{20}$ = - 5.13 (c 0.04 M in MeOH); 1H NMR (700 MHz, $CDCl_3$): δ 6.33 (s, 1H), 5.08 (m, 3H), 3.35 (d, J = 6.6 Hz, 2H), 2.96 (t, J = 8.3 Hz, 2H), 2.11 (m, 2H), 2.04 (m, 4H), 1.96 (m, 2H), 1.81 (s, 3H), 1.67 (s, 3H), 1.59 (s, 6H), 1.54 (m, 2H), 1.37 (m, 4H), 0.92 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 176.1, 163.9, 161.1, 147.4, 137.9, 135.6, 131.5, 124.5, 123.8, 122.4, 119.9, 104.7, 102.2, 39.8, 39.8, 32.6, 31.5, 31.0, 26.8, 26.5, 25.8, 25.1, 22.6, 17.8, 16.5, 16.2, 14.2 (see Supplementary Table 4); IR (KBr) ν_{max} : 3389, 2927, 1631, 1608, 1449, 1252, 1178 cm^{-1} ; UV (MeOH): λ_{max} 200 nm; HR-ESI-MS (m/z): $[M-H]^-$ calcd. for $C_{27}H_{39}O_4$, 427.2854; found, 427.2851.

Modeling and molecular docking simulations

The alphafold model of AscC and AscC^{L180F} was generated using web service (<https://wemol.wecomput.com/>). Hydrogen atoms and atom charges were subsequently added. The substrates FDP and OA undergoing structural optimization were docked into the binding pocket of AscC and AscC^{L180F} using the AutoDockTools-1.5.6. The docking results were then visualized and analyzed by PyMOL.

Site-directed mutagenesis of AscC

Alanine scanning was used to identify candidate residues with a Radius within 3 Å, while affinity maturation was employed to design mutants with a focus on those exhibiting a binding energy change exceeding 0.4. Mutants were constructed using overlap extension PCR and then subcloned into the pET28a vector. The primers were listed in Supplementary Table 2. Each mutant was confirmed by DNA sequencing. Afterward, the resultant variants were transformed into *E. coli* BL21 (DE3) harboring pBbA5c-MevT-MBIS.

Qualitative analysis of the products in engineered *E. coli* strains

Calibration curves of compounds **1**, **2**, and **4** were generated using HPLC following the method described above. Five concentration gradients of the isolated compound **1** (80, 40, 20, 10, 5 μ g/mL), compound **2** (60, 30, 15, 7.5, 3.75 μ g/mL) and compound **4** (500, 250, 125, 62.5, 31.25 μ g/mL) were prepared. Each concentration was injected in triplicate, and the linearity of the standard curve was determined by plotting the peak area versus concentration. The resulting equation and correlation coefficient obtained from the linearity study were $y = 19252x + 55.984$ ($R^2 = 0.9981$) for compound **1**, $y = 13688x + 43.024$ ($R^2 = 0.9973$) for compound **2**, and $y = 15153x + 192.93$ ($R^2 = 0.9993$) for compound **4**. The yields of compound **3** were calculated according to the calibration curve of compound **4**.

Anti-inflammatory activity assay of compounds 1–4

The assay was performed according to the literature with certain modifications⁴³. Briefly, BV-2 microglia cells were cultured in DMEM high-glucose medium containing 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μ g/mL) at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air. BV-2 cells (2×10^5 cells/well) were seeded in 96-well plates and cultured overnight until reaching 70–90% confluence. Subsequently, the cells were pretreated with different concentrations of test compounds (80, 40, 20, 10, and 5 μ M) for 1 h, followed by exposure to 1 μ g/mL of LPS for 24 h. The levels of TNF- α and IL-6 in the culture supernatants were assessed using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's protocol (BD Biosciences). The absorbance value (A) of each well at 450 nm (570 nm calibration) was measured with a microplate reader (Molecular Devices). Inhibitory rates of TNF- α and IL-6 production were calculated according to the following formula: Inhibitory rate = $(1 - (A_{sample} - A_{blank}) / (A_{solvent} - A_{blank})) \times 100\%$. IC_{50} values were analyzed by log (inhibitor) vs. normalized response - Variable slope using Prism 9.0 software. Each experiment was conducted with three independent biological replicates.

Antimicrobial activity assay of compounds 1–4

T. rubrum ATCC4438 was bought from Hospital for Skin Diseases, Institute of Dermatology Chinese of Medical Sciences, Peking Union Medical College. *E. coli* ATCC25922 was bought from HuanKai Microbial Co., Ltd. *S. aureus*, *B. subtilis*, and *M. luteus* were bought from Institute of Resource Insects, Chinese Academy of Forestry Sciences. The antimicrobial assays of compounds 1–4 were conducted using a micro-broth dilution method in 96-well culture plates according to the Clinical and Laboratory Standards Institute (CLSI). Compounds 1–4 were prepared as a 20 mM stock solution in dimethyl sulfoxide (DMSO). Ampicillin sodium and cefotaxime sodium were used as the positive agents for the antibacterial assay of Gram-positive bacteria and Gram-negative bacteria, respectively, while terbinafine hydrochloride was used as the positive agent for antifungal activity. The four bacterial strains were cultured on LB agar plates, whereas the fungal strain was cultured on the potato-dextrose agar (PDA) plates. The colonies were taken from the agar plates and incubated in the corresponding medium. The microbe suspension was deliquated with fresh LB or potato-dextrose broth (PDB) to 10^5 cfu/mL. The diluted suspensions were then transferred to a 96-well plate (200 μ L per well) containing different concentrations of the test compound. Serial dilutions of the stock solution were performed with final concentrations ranging from 50 to 0.39 μ g/mL (including 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625 μ g/mL). The microplates were incubated at 37 °C for 24 h for the bacteria or at 28 °C for 96 h for the fungi. All assays were performed in quadruplicate. EC_{50} was defined as the minimum concentration inhibiting 50% of microbial growth and was calculated using GraphPad Prism 9.0 software.

Statistics and reproducibility

We calculated the compound yields synthesized by the engineered strains using peak areas and the calibration curves derived from purified compounds. The compound yields for each strain were measured in triplicate biological replicates, with each replicate originating from different positive clones.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data in this study are available in the main text and the supplementary information. The source data behind the graphs in the paper can be found in Supplementary Data 1. The plasmids have been deposited in Addgene under Deposit ID 85347.

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References

- Atanasov, A. G., Zotchev, S. B., Dirsch, V. M. & Supuran, C. T. Natural products in drug discovery: advances and opportunities. *Nat. Rev. Drug Discovery* **20**, 200–216 (2021).
- Goss, R. J. M., Shankar, S. & Abou Fayad, A. The generation of “unnatural” products: synthetic biology meets synthetic chemistry. *Nat. Prod. Rep.* **29**, 870–889 (2012).
- Winter, J. M. & Tang, Y. Synthetic biological approaches to natural product biosynthesis. *Curr. Opin. Biotechnol.* **23**, 736–743 (2012).
- Sun, H. H., Liu, Z. H., Zhao, H. M. & Ang, E. L. Recent advances in combinatorial biosynthesis for drug discovery. *Drug Des., Dev. Ther.* **9**, 823–833 (2015).
- Tang, Y. L., Xiang, L., Zhang, F. Y., Tang, K. X. & Liao, Z. H. Metabolic regulation and engineering of artemisinin biosynthesis in *A. annua*. *Med. Plant Biol.* **2**, 4 (2023).
- Bonini, S. A. et al. *Cannabis sativa*: a comprehensive ethnopharmacological review of a medicinal plant with a long history. *J. Ethnopharmacol.* **227**, 300–315 (2018).
- Lu, D., Nikas, S. P., Han, X. W., Parrish, D. A. & Makriyannis, A. Synthesis and characterization of a compact tricyclic resorcinol from (+)- and (-)-3-pinanol. *Tetrahedron Lett.* **53**, 4636–4638 (2012).
- Pellati, F. et al. *Cannabis sativa* L. and nonpsychoactive cannabinoids: Their chemistry and role against oxidative stress, inflammation, and cancer. *BioMed Res. Int.* **2018**, 1691428 (2018).
- Liu, Y. et al. *Cannabis sativa* bioactive compounds and their extraction, separation, purification, and identification technologies: an updated review. *TrAC, Trends Anal. Chem.* **149**, 116554 (2022).
- Burstein, S. Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. *Bioorg. Med. Chem.* **23**, 1377–1385 (2015).
- Whiting, P. F. et al. Cannabinoids for medical use: a systematic review and meta-analysis. *JAMA, J. Am. Med. Assoc.* **313**, 2456–2473 (2015).
- Keimpema, E., Di Marzo, V. & Harkany, T. Biological basis of cannabinoid medicines. *Science* **374**, 1449–1450 (2021).
- Rosenbaum, D. M., Rasmussen, S. G. F. & Kobilka, B. K. The structure and function of G-protein-coupled receptors. *Nature* **459**, 356–363 (2009).
- Thakur, G. A., Duclos, R. I. & Makriyannis, A. Natural cannabinoids: templates for drug discovery. *Life Sci.* **78**, 454–466 (2005).
- Karas, J. A. et al. The antimicrobial activity of cannabinoids. *Antibiotics (Basel)* **9**, 406 (2020).
- Bow, E. W. & Rimoldi, J. M. The structure-function relationships of classical cannabinoids: CB1/CB2 modulation. *Perspect. Medicin. Chem.* **8**, PMC.S32171 (2016).
- Bloemendal, V., Spierenburg, B., Boltje, T. J., van Hest, J. C. M. & Rutjes, F. One-flow synthesis of tetrahydrocannabinol and cannabidiol using homo- and heterogeneous Lewis acids. *J. Flow Chem.* **11**, 99–105 (2021).
- Fellermeier, M. & Zenk, M. H. Prenylation of olivetolate by a hemp transferase yields cannabigerolic acid, the precursor of tetrahydrocannabinol. *FEBS Lett.* **427**, 283–285 (1998).
- Appendino, G., Chianese, G. & Tagliatela-Scafati, O. Cannabinoids: occurrence and medicinal chemistry. *Curr. Med. Chem.* **18**, 1085–1099 (2011).
- Luo, X. et al. Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. *Nature* **567**, 123–126 (2019).
- Zirpel, B., Degenhardt, F., Martin, C., Kayser, O. & Stehle, F. Engineering yeasts as platform organisms for cannabinoid biosynthesis. *J. Biotechnol.* **259**, 204–212 (2017).
- Zhang, Y. et al. Development of an efficient yeast platform for cannabigerolic acid biosynthesis. *Metab. Eng.* **80**, 232–240 (2023).
- Lee, Y. E., Nakashima, Y., Kodama, T., Chen, X. R. & Morita, H. Dual engineering of olivetolic acid cyclase and tetraketide synthase to generate longer alkyl-chain olivetolic acid analogs. *Org. Lett.* **24**, 410–414 (2022).
- Quan, Z. Y., Awakawa, T., Wang, D. M., Hu, Y. & Abe, I. Multidomain P450 epoxidase and a terpene cyclase from the ascochlorin biosynthetic pathway in *Fusarium* sp. *Org. Lett.* **21**, 2330–2334 (2019).
- Li, D. S. et al. An extremely promiscuous terpenoid synthase from the Lamiaceae plant *Colquhounia coccinea* var. *mollis* catalyzes the formation of sester-/di-/sesqui-/mono-terpenoids. *Plant Commun.* **2**, 100233 (2021).
- Itoh, T. et al. Identification of a key prenyltransferase involved in biosynthesis of the most abundant fungal meroterpenoids derived from 3,5-dimethylorsellinic acid. *ChemBioChem* **13**, 1132–1135 (2012).
- Zhou, K., Wunsch, C., Dai, J. G. & Li, S. M. Gem-diprenylation of acylphloroglucinols by a fungal prenyltransferase of the dimethylallyltryptophan synthase superfamily. *Org. Lett.* **19**, 388–391 (2017).
- Kuzuyama, T., Noel, J. P. & Richard, S. B. Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products. *Nature* **435**, 983–987 (2005).

29. Sands, L. B., Haiden, S. R., Ma, Y. & Berkowitz, G. A. Hormonal control of promoter activities of *Cannabis sativa* prenyltransferase 1 and 4 and salicylic acid mediated regulation of cannabinoid biosynthesis. *Sci. Rep.* **13**, 8620 (2023).
30. Redding-Johanson, A. M. et al. Targeted proteomics for metabolic pathway optimization: Application to terpene production. *Metab. Eng.* **13**, 194–203 (2011).
31. Tanaya, R. et al. Catalytic potential of *Cannabis* prenyltransferase to expand cannabinoid scaffold diversity. *Org. Lett.* **25** (2023).
32. Darby, C. M. et al. Whole cell screen for inhibitors of pH homeostasis in *Mycobacterium tuberculosis*. *PLoS One* **8**, 124–128 (2013).
33. Taura, F., Morimoto, S. & Shoyama, Y. Cannabis .23. Cannabinerolic acid, a cannabinoid from *Cannabis sativa*. *Phytochemistry* **39**, 457–458 (1995).
34. Chen, R. D. et al. Molecular insights into the enzyme promiscuity of an aromatic prenyltransferase. *Nat. Chem. Biol.* **13**, 226–234 (2017).
35. Giuffrida, A. & McMahon, L. R. In vivo pharmacology of endocannabinoids and their metabolic inhibitors: therapeutic implications in Parkinson's disease and abuse liability. *Prostaglandins Other Lipid Mediat.* **91**, 90–103 (2010).
36. Cheng, W. & Li, W. K. Structural insights into ubiquinone biosynthesis in membranes. *Science* **343**, 878–881 (2014).
37. Bohlmann, F. & Hoffmann, E. Naturally occurring terpene derivatives. Part 208. Cannabigerol-like compounds from *Helichrysum umbraculigerum*. *Phytochemistry* **18**, 1371–1374 (1979).
38. Berman, P. et al. Parallel evolution of cannabinoid biosynthesis. *Nat. Plants* **9**, 817–831 (2023).
39. Valliere, M. A. et al. A cell-free platform for the prenylation of natural products and application to cannabinoid production. *Nat. Commun.* **10**, 565 (2019).
40. Huang, T. & Li, Y. Current progress, challenges, and future perspectives of language models for protein representation and protein design. *Innovation* **4**, 100446 (2023).
41. Atalay, S., Jarocka-Karpowicz, I. & Skrzydlewska, E. Antioxidative and anti-inflammatory properties of cannabidiol. *Antioxidants* **9**, 21 (2020).
42. Karas, J. A. et al. The antimicrobial activity of cannabinoids. *Antibiotics (Basel)* **9**, 590–603 (2020).
43. Zeng, Z. R. et al. Sinunanolobaton A, an anti-inflammatory diterpenoid with bicyclo[13.1.0]pentadecane carbon scaffold, and related casbanes from the sanya soft coral *Sinularia nanolobata*. *Org. Lett.* **23**, 7575–7579 (2021).

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Author contributions

Y.L. and S.L. conceived the overall project, designed the experiments, and wrote the manuscript. Q.Y. generated the majority of the presented data. Y.C. performed the molecular docking and result analysis. X.Y. performed the site-mutagenesis. A.W. screened the function of aromatic prenyltransferases. H.H. performed the anti-inflammatory experiments. X.H., X.T., K.G., and Z.X. help with enzyme assay or NMR analysis.

Competing interests

The authors declare no competing interests.

Additional information

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