Synthesis of acridone derivatives via heterologous expression of a plant type III polyketide synthase in *Escherichia coli*

Gyu-Sik Choi¹, Hye Jeong Choo¹, Bong-Gyu Kim² and Joong-Hoon Ahn¹*

**Abstract**

**Background:** Acridone alkaloids are heterocyclic compounds that exhibit a broad-range of pharmaceutical and chemotherapeutic activities, including anticancer, antiviral, anti-inflammatory, antimalarial, and antimicrobial effects. Certain plant species such as *Citrus microcarpa*, *Ruta graveolens*, and *Toddaliopsis bremekampii* synthesize acridone alkaloids from anthranilate and malonyl-CoA.

**Results:** We synthesized two acridones in *Escherichia coli*. Acridone synthase (ACS) and anthraniloyl-CoA ligase genes were transformed into *E. coli*, and the synthesis of acridone was examined. To increase the levels of endogenous anthranilate, we tested several constructs expressing proteins involved in the shikimate pathway and selected the best construct. To boost the supply of malonyl-CoA, genes coding for acetyl-coenzyme A carboxylase (ACC) from *Photorhabdus luminescens* were overexpressed in *E. coli*. For the synthesis of 1,3-dihydroxy-10-methylacridone, we utilized an N-methyltransferase gene (NMT) to supply N-methylanthranilate and a new N-methylanthraniloyl-CoA ligase. After selecting the best combination of genes, approximately 17.3 mg/L of 1,3-dihydroxy-9(10H)-acridone (DHA) and 26.0 mg/L of 1,3-dihydroxy-10-methylacridone (NMA) were synthesized.

**Conclusions:** Two bioactive acridone derivatives were synthesized by expressing type III plant polyketide synthases and other genes in *E. coli*, which increased the supplement of substrates. This study showed that it is possible to synthesize diverse polyketides in *E. coli* using plant polyketide synthases.

**Keywords:** Acridone, Metabolic engineering, Polyketide synthase

**Background**

Natural compounds are valuable in cosmetics, food, and pharmaceutical industries [1]. Therefore, natural and nature-inspired, chemically synthesized compounds have extensively been developed and exploited for countless industrial purposes. Phytochemicals are typical natural compounds that have additional biological, nutritive, and/or pharmacological value. Among the diverse phytochemicals, secondary metabolites such as alkaloids, phenylpropanoids, and terpenoids have been extensively studied, and some of them have been employed in various fields [2].

Acridones are heterocyclic alkaloids that contain a tricyclic ring with nitrogen at the 10th position and a carbonyl group at the 9th position [3]. Acridone alkaloids are secondary metabolites that are generally found in the plant family, Rutaceae [4]. Various acridone derivatives (glyforine, acronycine, thioacridones, and substituted 9-aminoacridines, etc.) have been reported to exert a wide range of chemotherapeutic effects including anticancer, antimicrobial, antimalarial, antipsoriatic activities [5–8]. The synthesis of acridone alkaloids in plants...
N-methylacridone (1,3-dihydroxy-10-methylacridone) was discovered in Ruta graveolens [10]. Some genes in acridone biosynthesis have been characterized, and the acridone biosynthetic pathway has been elucidated in R. graveolens. The first committed step in acridone alkaloid biosynthesis is the conversion of anthranilate into N-methylanthranilate by anthranilate N-methyltransferase (ANMT). Next is the N-methylanthranilate-catalyzed synthesis of N-methylanthraniloyl-CoA using coenzyme A (CoA). Then, acridone synthase (ACS)—one of the plant polyketide synthases (PKSs)—catalyzes the condensation of N-methylanthraniloyl-CoA and malonyl-CoA. ANMT and ACS were cloned in R. graveolens [11, 12]. ACSs from Huperzia serrata [13] and Citrus microcarpa [14] have also been cloned. Notably, to date, plant anthranilate coenzyme A ligase has not been cloned.

Microorganic biosynthetic platforms have emerged as the leading platforms for the production of natural and synthetic value-added compounds, such as flavonoids, alkaloids, polyketides, and various chemicals. Due to its well-established genetics and physiology, Escherichia coli has become one of the representative microorganisms in biosynthetic platforms [15]. One of the secondary metabolic pathways of E. coli, the shikimate pathway has received considerable attention as it is a major pathway for the production of aromatic compounds [16]. Biosynthetic pathways for aromatic amino acid production (l-tryptophan, l-tyrosine and l-phenylalanine) including the shikimic acid pathway provide the chemical building blocks for the synthesis of various chemicals through specific intermediates, such as chorismate and shikimate [17–21].

We synthesized two acridones (1,3-dihydroxy-9(10H)-acridone [DHA] and 1,3-dihydroxy-10-methylacridone [NMA]) using engineered E. coli and two substrates, namely anthranilate, and malonyl-CoA. To optimize the substrate supply for the synthesis of acridone, we prepared several sets of constructs; the first set for the synthesis of anthranilate using genes coding for proteins involved in the shikimate pathway and the second set for the synthesis of malonyl-CoA by overexpressing acetyl-coenzyme A carboxylases (ACCs). For the synthesis of NMA (1,3-dihydroxy-10-methylacridone), we additionally introduced the N-methyltransferase gene (NMT) to supply N-methylanthranilate by using endogenous anthranilate. The overall scheme of the biosynthesis of these two compounds is shown in Fig. 1. Through a combination of these genes along with ACS, badA, and pqsA, which are involved in CoA utilization or substrate cyclization, we were able to synthesize 17.3 mg/L DHA and 26.0 mg/L NMA.

Results

Screening of constructs to synthesize DHA and NMA

DHA and NMA are synthesized from anthranilate or N-methylanthranilate and malonyl-CoA, respectively. Anthranilate and N-methylanthranilate are activated by coenzyme A. We tested two CoA ligases, badA—encoding benzoate coenzyme A ligase—and pqsA encoding anthranilate coenzyme A ligase. Two ACSs, RgACS, and CmACS were tested. E. coli—harboring each of the four constructs pC-RgACS-badA, pC-CmACS-badA, RgACS-pqsA or pC-CmACS-pqsA—was exposed to 100 μM anthranilate or N-methylanthranilate. A new peak was observed in culture filtrates from E. coli strains harboring RgACS-badA or pC-CmACS-badA when they were supplied with anthranilate (Fig. 2d, e). The molecular mass of the synthesized product was 227.06 Da, which corresponded to the predicted mass of DHA. However, E. coli cells harboring RgACS-pqsA or pC-CmACS-pqsA that were supplied with N-methylanthranilate synthesized a new product whose molecular mass was 240.87 Da, which is the predicted mass of NMA (Fig. 2e, g). Based on the structure—using nuclear magnetic resonance spectroscopy (NMR)—we confirmed that the two compounds were DHA and NMA, respectively, (see “Methods”). These results indicated that badA could potentially convert anthranilate into anthraniloyl-CoA and that pqsA is responsible for the conversion of N-methylanthranilate into N-methylanthraniloyl-CoA.

Escherichia coli strains harboring RgACS synthesized 11.80 mg/L DHA (51.96 μM) when 100 μM anthranilate was supplied, and synthesized 17.52 mg/L (72.62 μM) NMA when 100 μM N-methylanthranilate was provided. This yield exceeded that obtained using E. coli harboring CmACS, which synthesized 1.4 mg/L DHA and 6.0 mg/L NMA. In addition, the amount of byproduct such as 2,3-dihydroxyquinoline (DHQ) were found more in E. coli harboring CmACS and the unreacted N-methylanthranilate was observed in E. coli harboring CmACS. This result indicates that RgACS effectively synthesizes DHA and NMA. We observed the synthesis of 2,4-dihydroxyquinoline (DHQ) in E. coli strains harboring RgACS-badA or CmACS-badA. DHQ also used anthranoyl-CoA and malonyl-CoA. Two molecules of malonyl-CoA instead of three, are used to synthesize DHQ. The amount of the synthesized DHQ was 2.6 mg/L in E. coli harboring CmACS-badA and 3.6 mg/L in E. coli harboring RgACS-badA, while the amount of DHA was 1.3 mg/L in CmACS and 10.5 mg/L in RgACS.

The synthesis of N-methylquinoline (NMQ) was observed in the culture filtrate of E. coli harboring CmACS-pqsA. Nevertheless, we could not observe any detectable NMQ in E. coli harboring RgACS-pqsA. Enzymatic reactions with N-methylanthranilate using
CmANS revealed that the synthesized products resulted from the incorporation of two \((N\text{-}methylquinolone)\) or three molecules \((N\text{-}methylacridone)\) of malonyl-CoA with a preference towards \(N\text{-}methylacridone\) synthesis \([14]\). However, the enzymatic reaction using RgACS with \(N\text{-}methylanthranilate\) produced only NMA (but not \(N\text{-}methylquinolone\)) \([22]\). These results indicate that RgACS is better than CmACS at synthesizing DHA and NMA. Therefore, we selected constructs containing RgACS for further experiments.

**Synthesis of NMA**

\(N\text{-}methylanthranilate\) is the building block of NMA, but \(E.\ coli\) does not synthesize \(N\text{-}methylanthranilate.\) Anthranilate NMT was employed to synthesize NMA. In order to increase the substrate for NMT, \(\text{trpE}\) was overexpressed. The second substrate of NMA synthesis is malonyl-CoA. The effects the four constructs that reportedly affect intracellular malonyl-CoA were individually tested with respect to NMA synthesis. Three of them (PDHm, acs, and \(\text{ackA-pta}\)) increased the level of acetyl-CoA \([23, 24]\) and one of them (acc) synthesized malonyl-CoA from acetyl-CoA \([24]\). We engineered five \(E.\ coli\) strains \((B\text{-}NMA3} \sim B\text{-}NMA7) and tested the synthesis of NMA. Four strains synthesized NMA. Among them, the strain B-NMA3 produced the highest amount of NMA \((30.6\ mg/L)\) followed by B-NMA4 \((24.2\ mg/L),\) B-NMA5 \((22.2\ mg/L),\) B-NMA6 \((19.3\ mg/L),\) and B-NMA7 \((18.3\ mg/L)\) (Fig. 3).

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**Fig. 1** Biosynthesis scheme for producing two acridone derivatives in \(Escherichia\ coli\). Dotted arrows represent the metabolic pathways in \(E.\ coli\). Bold arrows indicate the pathway engineered for the synthesis of the two acridones \((\text{dihydroxyacridone (DHA) and } N\text{-}methylacridone (NMA)).\) Introduced genes are as follows: ACS, acridone synthase from \(Ruta\ graveolens\); badA, benzoate CoA ligase from \(R.\ palustris\); \(\text{pqsA},\) anthranilate coenzyme A ligase from \(P.\ aeruginosa\); NMT, \(N\text{-}methyltransferase from \(Ruta\ graveolens\);\) acs, acetyl-CoA synthase, PDH, pyruvate dehydrogenase complex, \(\text{ackA} \& \text{pta},\) acetate kinase \(\&\) phosphate acetyltransferase from \(E.\ coli\); and acc, acetyl-coenzyme A carboxylase from \(\text{Photorhabdus luminescens}\).
the overexpression of gene involved in acetyl-CoA or malonyl-CoA increased the synthesis of NMA and the enhancement of malonyl-CoA synthesis by acc is more effective in the synthesis of NMA than the increase of acetyl-CoA by pta-ackA, PDHm, or acs.

We also tried to increase endogenous anthranilate levels by overexpressing aroG and the feedback-inhibition-free version of aroG (aroG'). Two more E. coli strains (B-NMA-8 and B-NMA-9) were tested. However, we could not detect the synthesis of NMA. Only the accumulation of anthranilate and N-methylanthranilate was observed. The unreacted anthranilate and N-methylanthranilate in B-NMA-8 were 16.0 and 35.0 mg/L, respectively; only 7.2 mg/L N-methylanthranilate was observed in B-NMA-3, whereas anthranilate was not observed.

The rapid synthesis of anthranilate or N-methylanthranilate seemingly inhibited the synthesis of NMA. Notably, higher copy number plasmids containing RgACS and pqxA did not further increase NMA synthesis. Likely, the activities of these two downstream proteins got saturated when converting the synthesized N-methylanthranilate into NMA. Fine-tuning of the whole process is critical to increasing the final yield of the product [25, 26].

Using the strain B-NMA3, we monitored the synthesis of NMA and N-methylanthranilate for 27 h. The synthesis of both NMA and N-methylanthranilate showed a similar pattern (Fig. 4). Both reached their maximal synthesis at 24 h, at which time, approximately 26.0 mg/L NMA and 5.4 mg/L N-methylanthranilate were synthesized.

![UV:270 nm](image-url)
Synthesis of DHA

Anthranilate and malonyl-CoA are substrates for DHA. Endogenous levels of these two compounds are probably critical determinants of the final yield. To increase DHA synthesis, we used two strategies. The first strategy was to increase endogenous anthranilate. The shikimate pathway synthesizes anthranilate. Genes in this pathway were overexpressed. The second strategy was to use a plasmid with different copy number to express RgACS and badA. We constructed eight different E. coli strains. The levels of synthesized DHA increased from 2.56 in B-DHA3 to 6.39 mg/L in D-BHA5, and the strain D-BHA6 produced approximately 3.98 mg/L of DHA. Importantly, the levels of unreacted anthranilate continued to increase, and were 0.72 mg/L in B-DHA3 and 593.40 mg/L in B-DHA6. It seemed that higher production of anthranilate inhibited the synthesis of DHA, and that the conversion of the synthesized anthranilate into DHA was critical for increasing the yield of DHA. In order to augment the conversion of anthranilate, greater and better involvement of downstream genes (badA and RgACS) seems necessary. Therefore, we tested the strain B-DHA7-10. The synthesis of DHA increased from 1.12 mg/L in B-DHA7 to 17.3 mg/L in B-DHA10 (Fig. 5). In particular, the strain(s) that were expected to synthesize more anthranilate produced more DHA. Besides, the levels of unreacted anthranilate in these strains were less than those in corresponding strains harboring a lower copy of badA and RgACS. Taken together, the higher copy number of RgACS and badA facilitated the synthesis of DHA.

We also tested the four constructs that were supposed to increase intracellular levels of malonyl-CoA.

![Fig. 3 Effect of the precursor pathway genes on the production of N-methylacridone. B-NMA3 overexpressed acc (acetyl-CoA carboxylase), B-NMA4 overexpressed ackA (acetate kinase), and pta (phosphate acetyltransferase), B-NMA5 overexpressed PDHm (pyruvate dehydrogenase complex variant), and B-NMA6 overexpressed acs (acetyl-coenzyme A synthetase)。

![Fig. 4 Production of N-methylacridone using the strain B-NMA3]
Like for the synthesis of NMA, the placcABCD was also the best in the context of DHA synthesis (data not shown). The synthesis of DHA in the strain B-DHA10 was monitored (13 mL of 1% YM9 broth in 100 mL flask). DHA levels continued to increase until 15 h and remained almost the same until 21 h. The levels of unreacted anthranilate also continued to increase until 40 h. Approximately 15.7 mg/L DHA was synthesized at 24 h (Fig. 6).

**Discussion**

In our present works, we successfully synthesized two acridone derivatives, 1,3-dihydroxy-9(10H)-acridone and 1,3-dihydroxy-10-methylacridone, using engineered *E.*
coli. Genes coding for proteins in the shikimate pathway and TrpE encoding anthranilate synthase were tested and selected for the synthesis of the 1st substrate, anthranilate. Acetyl-CoA-carboxylase from P. luminescens was introduced to increase the available levels of the 2nd substrate, malonyl-CoA (for ACS). We tested ACS from R. graveolens and C. microcarpa to select the one that was better with respect to the synthesis of DHA and NMA. The results of in vitro enzymatic efficacy tests showed that ACS from R. graveolens outperformed that from C. microcarpa [11, 14, 22]. Sometimes, in vitro enzymatic results did not correlate with the in vivo results due to the presence of unknown substrates in vivo, which expectedly inhibit or divert the enzymatic activity [27]. Therefore, we tested the in vivo synthesis of acridone using both genes. In this study, in vivo biosynthesis of acridones by RgACS showed better productivity than CmACS. Based on the in vitro properties of ACS and on the in vivo acridone biosynthesis experiment, we could identify a positive correlation between enzyme properties and acridone biosynthesis.

In order to increase the final yield of the two acridones, we tested the genes coding for proteins involved in the shikimate pathway. We observed a dramatic increase in the levels of intermediates, such as anthranilate, instead of an increase in DHA levels during DHA synthesis. Importantly, during the synthesis of DHA, the rate-limiting step was likely the conversion of anthraniloyl-CoA into DHA by PKS. However, the conversion of anthranilate into N-methylantranilate and/or the conversion of N-methylantraniloyl-CoA into NMA were limiting steps during the synthesis of NMA. Exposure of E. coli harboring CoA ligase and PKS to either anthranilate or N-methylantranilate resulted in no further synthesis of DHA and NMA, (~500 μM of anthranilate and 300 μM of N-methylantranilate). The endogenous levels of anthranilate upon expressing the genes coding for proteins in the shikimate pathway increased more than 500 μM (Fig. 5b), a concentration at which the synthesis of DHA is likely inhibited. These findings indicated that PKS was probably a rate-limiting step. In case of NMA synthesis, we found that the conversion of anthranilate into N-methylantranilate was a limiting step [21]. In vitro enzymatic study using the purified ACS from R. graveolens also showed that ACS was inhibited by 250 μM N-methylantraniloyl-CoA [28]. The construct that minimizes the accumulation of the anthranilate appeared to be the best for the synthesis of NMA and DHA. Fine-tuning of the overall pathway is critical to enhancing the final yield of the product.

Aerobic growth of E. coli produces ATP, ubiquinol-8, CO₂ and a considerable amount of acetic acid as a byproduct through the acetate producing pathways [29]. The production of acetic acid could be a negative influence on the synthesis of acridone derivatives. The synthesized acetic acid is neutralized upon converting acetic acid into acetyl-CoA by acetyl-CoA synthase (acs) [30]. Acetyl-CoA is then converted into malonyl-CoA by ACC. Overexpression of acs or acc enhanced the production of DHA and NMA because it resulted not only in the supply of the second substrate (malonyl-CoA), but also in the reduction of the byproduct, acetic acid. These results agreed with the previous reports that overexpression of acs, acc, or PDH enhanced the synthesis of flavonoids and triacetic acid lactone [23, 24, 31].

**Conclusions**

We synthesized two acridones (DHA and NMA) in *Escherichia coli* using two substrates, namely anthranilate and malonyl-CoA. Towards this, plant acridone synthase (ACS) and anthraniloyl-CoA ligase genes were transformed into E. coli. To optimize the substrate supply for acridone synthesis, we prepared several sets of constructs; the first set for the synthesis of anthranilate using genes coding for proteins involved in the shikimate pathway—major pathway for the production of aromatic compounds—and the second set for the synthesis of malonyl-CoA by overexpressing acetyl-coenzyme A carboxylases (ACCs). For the synthesis of NMA, we additionally introduced the N-methyltransferase gene (NMT) to supply N-methylantranilate by using endogenous anthranilate. Through a combination of these genes along with ACS, *badA* and *pqxA*, which are involved in CoA utilization or substrate cyclization, we were able to synthesize 17.3 mg/L DHA and 26.0 mg/L NMA.

**Methods**

**Constructs**

ACS from *R. graveolens* (RgACS; GenBank: AJ297786.2) was cloned using reverse transcription and polymerase chain reaction (RT-PCR). Two primers 5′-ATGGATCC GATGGAATCCCTGAAGGAGATG-3′ and 5′-ATGCG GCCGCTCATGCTTTCAACGGGACAC-3′ were used (restriction sites for EcoRI and NotI have been underlined). ACS from *Citrus microcarpa* (GenBank: AB823699) was also cloned by RT-PCR using two primers 5′-aagattcaATGGTAAACCATGGAGGAGATATG-3′ and 5′-aaggcgccgTCATGCTTTCAACGGGACACGTG-3′ (restriction sites for EcoRI and NotI have been underlined).

*badA* from *Rhodopseudomonas palustris* (GenBank: L42322.1) and *pqxA* from *Pseudomonas aeruginosa* (Ndel/Xhol) had been previously cloned. badA was first cloned into pCDF-duet1 (Ndel/KpnI) and then ACS
was subcloned into pCDF-duet1 containing bada (pC-RgACS-bada) (BamHI/NotI). Subsequently, RgACS-bada was subcloned into pET-duet1 and pRSF-duet1 (BamHI/EcoRV) using polymerase chain reaction (PCR). Two primers 5'-ATGGATCCGATGGATCCCTGAA GGAGATG-3' and 5'-GCGGCGGCTACGCCCACAC ACCCTTG-3' were used (restriction sites for BamHI and NotI have been underlined). pqsa was first cloned into pCDF-duet1 (Ndel/Xhol) and ACS was subcloned into pCDF-duet1 containing pqsa (pC-RgACS-pqsa) (BamHI/NotI) and then RgACS-pqsa was subcloned into pET-duet1 (pE-RgACS-pqsa) (BamHI/Xhol).

pA-accABC encoding acetyl-CoA carboxylase had been cloned previously [32]. TrpE was cloned as described in Lee et al. [20] and subcloned into pCDF-duet1 (pC-trpE) (BamHI/BglII/Xhol). aroG and aroGI—both of which had been previously cloned [18]—were subcloned into pC-trpE (EcoRI/NotI). pC-aroL-aroE-aroD-aroB-aroGII-pqsa-tpkA-trpE had been constructed previously [21]. The E. coli pyruvate dehydrogenase complex variant (PDHm) was cloned as described in Bocanegra et al. [33]. ackA encoding acetate kinase (AAC75356) and pta encoding phosphate acetyltransferase (AAC75357) were cloned from E. coli using PCR. pta was subcloned into pACYC-duet1 (Ndel/Xhol), and ackA was subcloned into the resulting plasmid (EcoRI/HindIII) to give rise to pA-ack-pta. acs—encoding E. coli acetyl-coenzyme A synthetase (BAE78071)—was subcloned into pACYC-duet1 (EcoRI/HindIII) (pA-acs). ACC encoding acetyl-CoA carboxylase from Photobacterium luminescens (accABC) had been cloned previously [21].

NMT from Ruta graveolens had been cloned previously [20]. In order to prepare the pC-aroG-NMT-trpE construct, trpE was amplified using a forward primer containing a BamHI site and a reverse primer containing a Xhol site, and was subcloned into pCDF-duet1 (BglII/BamHI) (pC-trpE). NMT was amplified with a forward primer containing a BamHI site, and a reverse primer containing an AflII site, following which it was subcloned into pC-trpE (BamHI/AflII). The resulting construct was digested with BamHI/Xhol, and was then subcloned into pCDF-duet1 (BglII/Xhol) (pC-NMT-trpE). aroG or aroGI were amplified using primers containing EcoRI (forward primer) and NotI sites (reverse primer) and were subcloned into pC-NMT-trpE (EcoRI/NotI). The constructs and the strains used in this study were listed in Table 1.

Production and analysis of DHA and NMA in E. coli

The overnight cultures of E. coli transformants were inoculated into a fresh LB containing appropriate antibiotics and growth at 37 °C until OD600=1. Cells were harvested and resuspended in M9 medium containing 2% glucose, 1% yeast extract, antibiotics, 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) in a test tube except that the synthesis of NMA and DHA was monitored for 27 h in a flask. The cells were grown at 30 °C with shaking for 24 h. The culture supernatant was extracted with three volumes of ethyl acetate (EA). The upper layer—after centrifugation—was collected and dried. The dried sample was dissolved in 60 μL dimethyl sulfoxide (DMSO).

To analyze the formation of DHA and NMA, Thermo Ultimate 3000 HPLC (high performance liquid chromatography) equipped with a photodiode array (PDA) detector and a Varian C18 reversed-phase column (Varian, 4.60 × 250 mm, 3.5 μm particle size) was used [21].

The synthesized DHA was purified using HPLC. The mobile phase consisted of water and acetonitrile (7:3, v/v), and no gradient was applied. The structure of the purified compounds was determined using proton nuclear resonance spectroscopy (NMR). DHA (1,3-dihydroxy-9(10H)-acridone), 1H NMR (500 MHz, DMSO-d6): δ 6.00 (d, J=2.0 Hz, H-2), 6.30 (d, J=2.0 Hz, H-4), 7.25 (ddd, J=8.3, 7.0, 1.0 Hz, H-7), 7.47 (dd, J=8.3, 1.4 Hz, H-8), 7.72 (ddd, J=8.2, 7.0, 1.4, H-6), 8.15 (dd, J=8.2, 1.0 Hz, H-5). To determine the structure of NMA (1,3-dihydroxy-10-methylacridone), thin layer chromatography (TLC, silica gel 60 F254, Millipore) was used to purify the putative NMA. Ethyl acetate and hexane (2:1 (v/v)) were used as developing solvents. The purified sample was dissolved in acetone-d6. The chemical shifts for 1H and 13C NMR data were referenced to that of tetramethylsilane (TMS). In order to verify the structure, COSY, TOCSY, NOE5Y, 1H-13C HMQC, and 1H-13C HMBC were used. The mixing time for TOCSY and NOE5Y was 60 ms and 1 s, respectively. The delay in the evolution of long-ranged couplings was 70 ms in HMBC. 1H NMR (500 MHz, acetone-d6): δ 6.19 (d, J=1.9 Hz, H-2), 6.50 (d, J=1.9 Hz, H-4), 7.78 (dd, J=8.8, 1.2 Hz, H-5), 7.79 (m, H-6), 7.34 (m, H-7), 8.40 (dd, J=8.0, 1.4 Hz, H-8), 3.90 (s, N-CH3). 13C NMR(500 MHz, acetone-d6): δ 95.26(C-2), 90.23(C-4), 114.7(C-5), 133.4(C-6), 120.6(C-7), 125.4(C-8), 179.9(C-9), 33.03(N-CH3). In
| Strains or E. coli strain | Relevant properties or genetic marker | Source or reference |
|--------------------------|--------------------------------------|---------------------|
| Bla (DE3)                |                                      | Novagen             |
| B-DHA1                   |                                      | This study          |
| B-DHA2                   |                                      | This study          |
| B-DHA3                   |                                      | This study          |
| B-DHA4                   |                                      | This study          |
| B-DHA5                   |                                      | This study          |
| B-DHA6                   |                                      | This study          |
| B-DHA7                   |                                      | This study          |
| B-DHA8                   |                                      | This study          |
| B-DHA9                   |                                      | This study          |
| B-DHA10                  |                                      | This study          |
| B-NMA1                   |                                      | This study          |
| B-NMA2                   |                                      | This study          |
| B-NMA3                   |                                      | This study          |
| B-NMA4                   |                                      | This study          |
| B-NMA5                   |                                      | This study          |
| B-NMA6                   |                                      | This study          |
| B-NMA7                   |                                      | This study          |
| B-NMA8                   |                                      | This study          |
| B-NMA9                   |                                      | This study          |

| Strains                  |                                      |                     |
|--------------------------|--------------------------------------|---------------------|
| DH5α                     |                                      | Novagen             |
| BL21 (DE3)               |                                      | Novagen             |
| B-DHA1                   |                                      | This study          |
| B-DHA2                   |                                      | This study          |
| B-DHA3                   |                                      | This study          |
| B-DHA4                   |                                      | This study          |
| B-DHA5                   |                                      | This study          |
| B-DHA6                   |                                      | This study          |
| B-DHA7                   |                                      | This study          |
| B-DHA8                   |                                      | This study          |
| B-DHA9                   |                                      | This study          |
| B-DHA10                  |                                      | This study          |
| B-NMA1                   |                                      | This study          |
| B-NMA2                   |                                      | This study          |
| B-NMA3                   |                                      | This study          |
| B-NMA4                   |                                      | This study          |
| B-NMA5                   |                                      | This study          |
| B-NMA6                   |                                      | This study          |
| B-NMA7                   |                                      | This study          |
| B-NMA8                   |                                      | This study          |
| B-NMA9                   |                                      | This study          |
the $^1$H spectrum, six peaks were observed in the aromatic region, while a single peak was observed at 3.90 ppm. All peaks in the aromatic region were assigned using COSY and TOCSY. The N-attached methyl group was thought to be responsible for the peak at 3.899 ppm as it showed two cross-peaks with H-5 and H-4 in NOESY.

Abbreviations

ACC: Acetyl-coenzyme A carboxylase; ackA: Acetate kinase; ANMT: Anthranilate N-methyltransferase; ASC: Acidine synthase; CoA: Coenzyme A; DHA: 1,3-Dihydroxy-9(10H)-acridone; DMSO: Dimethyl sulfoxide; HPLC: High performance liquid chromatography; IPTG: Isopropyl β-D-thiogalactopyranoside; NMA: 1,3-Dihydroxy-10-methylacridone; NMR: Nuclear magnetic resonance spectroscopy; NMT: N-methyltransferase gene; PDA: Photodiode array; PDM: Pyruvate dehydrogenase complex variant; PKS: Polyketide synthases; pta: Phosphate acetyltransferase.

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Authors’ contributions

GSC, BGK and JHA designed experiments. GSC, HJC, BGK, and JHA performed the experiments and analyzed the data. GSC, HJC and JHA wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated during this study are included in this published article.

Ethics approval and consent to participate

This manuscript does not report data collected from humans or animals.

Consent for publication

This manuscript does not contain any individual persons’ data.

Competing interests

The authors declare that they have no competing interests.

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