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Synthesis of Unnatural 2-Substituted Quinolones and 1,3-Diketones by a Member of Type III Polyketide Synthases from *Huperzia serrata*

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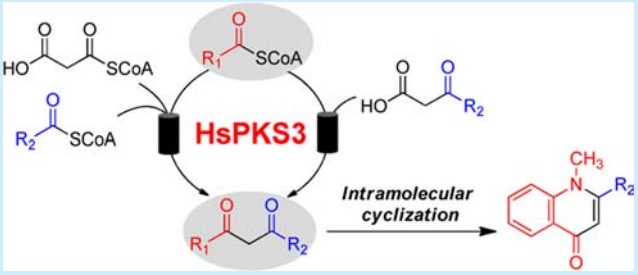
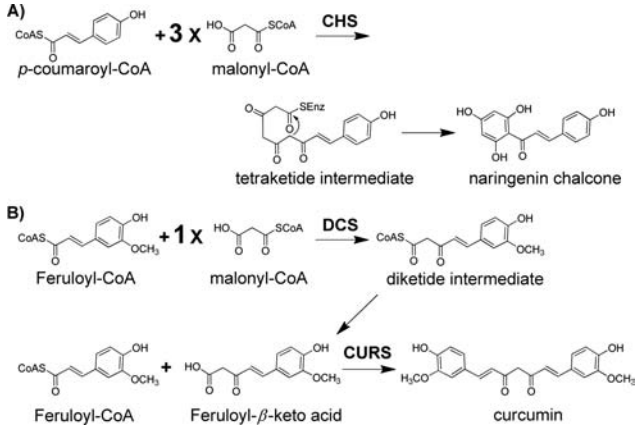
Supporting Information

ABSTRACT: A curcuminoids, benzalacetone-, and quinolone-producing type III polyketide synthase (HsPKS3) from *Huperzia serrata* uniquely catalyzes the formation of unnatural 2-substituted quinolones and 1,3-diketones via head-to-head condensation of two completely different substrates. The broad range of substrate tolerance of HsPKS3 facilitates accessing structurally diverse 2-substituted quinolones and 1,3-diketones.



Type III polyketide synthases (PKSs) are a class of architecturally simple homodimeric proteins that catalyze iteratively decarboxylative condensations of a starter coenzyme A (CoA) thioester and C_2 units derived from malonyl-CoA to produce a dazzling array of secondary metabolites contributing to plant defense and human health.[1] A representative example is that chalcone synthase (CHS) catalyzes sequential condensations of *p*-coumaroyl-CoA with three molecules of malonyl-CoA to produce naringenin chalcone (Scheme 1A).[2] Despite the fact that type III PKSs share a common three-dimensional overall fold and utilize an identical catalytic triad of Cys-His-Asn, the catalytic functions of type III PKSs are extremely divergent, and a subtle variation of the amino acid residues lining the active-site cavity could dramatically change the catalytic functions.[1,3] Therefore, screening novel type III PKSs from plants and probing their catalytic potentials would be not only advantageous to understand the biosynthesis of plant metabolites but also instructive to enzymatically or biomimetically synthesize structurally diverse and chemically complex unnatural natural products.

Scheme 1. Formation of (A) Naringenin Chalcone by CHS and (B) Curcumin by DCS and CURS



2-Substituted quinolones and 1,3-diketones are two classes of natural products with a broad spectrum of biological activities, such as antibacterial, antitumor, and antioxidative activities.[4] In addition, 1,3-diketones are synthetically useful intermediates in heterocyclic chemistry. The high reactivity of the electrophilic carbonyl carbons and the nucleophilic methylene facilitates accessing a variety of pharmaceutically important heterocyclic compounds such as isoxazoles, pyrazoles, and pyrimidines.[5] Although chemical syntheses of 2-substituted quinolones and 1,3-diketones have been extensively reported,[6] enzymatic syntheses of these two kinds of scaffolds are currently confined to a handful of compounds such as curcuminoids. Regarding the enzymatic synthesis of curcuminoids, a two-step reaction mechanism involving two type III PKSs, diketide-CoA synthase (DCS) and curcumin synthase (CURS) from *Curcuma longa* (Scheme 1B),[7] and a one-pot reaction mechanism involving a single type III PKS (CUS) from *Oryza sativa* have been reported.[8]

Herein, we report a new type III PKS (HsPKS3) from *Huperzia serrata* and enzymatic synthesis of a variety of unnatural 2-substituted quinolones and 1,3-diketones by HsPKS3 (Figure 1A).

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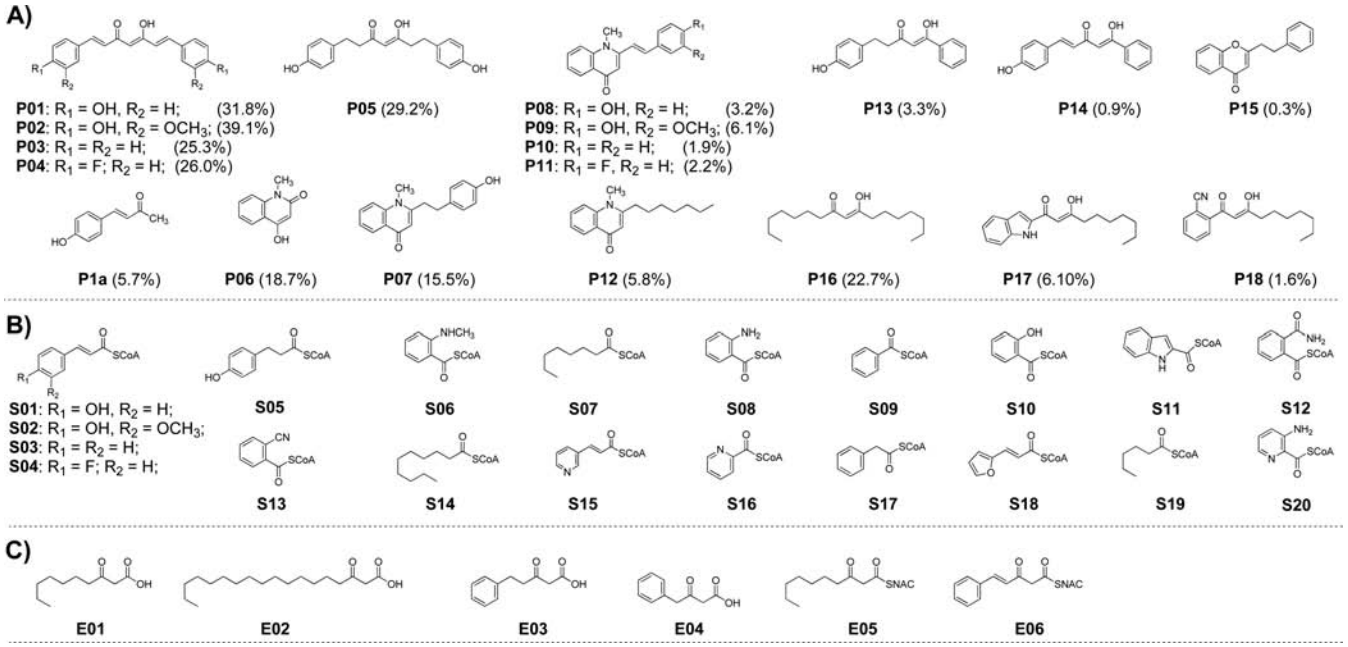

Figure 1. Products and substrates. (A) Representative products enzymatically synthesized by HsPKS3. Compounds **P01**−**P06**, **P1a**, **P12**, and **P15** were identified using authentic compounds (Supporting Information, Figures S3−S9); compounds **P07**−**P11**, **P13**, **P14**, and **P16**−**P18** were elucidated on the basis of their HRESIMS and NMR data (Supporting Information, Tables S1−S3, Figures S10−S50). (B) Synthesized starters. (C) Synthesized extenders. The numbers in parentheses represent the yield of each enzymatic product. Note: 1,3-Diketones produced by HsPKS3 are presented as a relatively stable enol form.

Using a homologous cloning strategy, a new type III PKS (HsPKS3) was successfully cloned from the leaves of *H. serrata*. The full-length cDNA of HsPKS3 contained a 1239 bp ORF encoding a protein with 413 amino acids including the Cys-His-Asn catalytic triad conserved in all known plant type III PKSs. Sequence alignment revealed that HsPKS3 shared 60.53% amino acid identity with HsPKS1 from *H. serrata*,[9] 44.36% with CUS from *O. sativa*,[8] 47.94% with BAS from *R. palmatum*,[10] 47.46% with CURS from *C. longa*,[7] 50.61% with QNS from *A. marmelos*,[11] and 56.42% with CHS from *M. sativa*,[2] respectively. Phylogenetic analysis revealed that HsPKS3 was grouped into nonchalcone synthases.

Incubation of *p*-coumaroyl-CoA (**S01**), malonyl-CoA, and the recombinant enzyme HsPKS3 resulted in the production of a diarylheptanoid scaffold of bisdemethoxycurcumin (**P01**), accompanying the formation of a C_6−C_3 scaffold of *p*-hydroxybenzalacetone (**P1a**). Further investigations revealed that HsPKS3 could also catalyze the formation of various diarylheptanoids (**P02**−**P05**) from cinnamoyl-CoA analogues (**S02**−**S05**). Interestingly, HsPKS3 accepts nitrogen-containing, bulky *N*-methylanthraniloyl-CoA (**S06**) as a starter to produce quinolone alkaloid **P06**. To the best of our knowledge, HsPKS3 is the first type III PKS with the multifunctions producing benzalacetone, curcuminoids, and a quinolone alkaloid.

When *p*-hydroxyphenylpropinoyl-CoA (**S05**) was incubated with *N*-methylanthraniloyl-CoA (**S06**) and malonyl-CoA, the enzyme reaction generated an unknown product (**P07**) (Figure 2A). The HRESIMS spectrum of **P07** showed the presence of a protonated ion at *m*/*z* 280.1336 ($[M + H]^+$), consistent with an empirical molecular formula of $C_{18}H_{17}NO_2$. Analysis of the 1D- and 2D-NMR spectra allowed establishing the structure of **P07** as 2-(4-hydroxyphenethyl)-1-methylquinolin-4(1*H*)-one. Considering that HsPKS3 could not produce a head-to-head condensation product from two molecules of *N*-methylanthraniloyl-CoA (**S06**) and malonyl-CoA, the mechanism of the quinolone alkaloid (**P07**) formation was proposed as follows: HsPKS3 first catalyzed the condensation of *p*-hydroxyphenylpropinoyl-CoA and malonyl-CoA to produce a *β*-keto acid intermediate (Scheme 2A), and then, the *β*-keto acid intermediate serves as the second extender, performing the ongoing head-to-head condensation with *N*-methylanthraniloyl-CoA to form a 1,3-diketone intermediate, followed by nucleophilic attack of a carbonyl carbon by a nitrogen atom to produce the final product (Scheme 2B). Further co-incubation of cinnamoyl-CoA analogues (**S01**−**S04**) with *N*-methylanthraniloyl-CoA and malonyl-CoA yielded quinolone alkaloids (**P08**−**P11**). However, when octanoyl-CoA (**S07**) was incubated with *N*-methylanthraniloyl-CoA (**S06**) and malonyl-

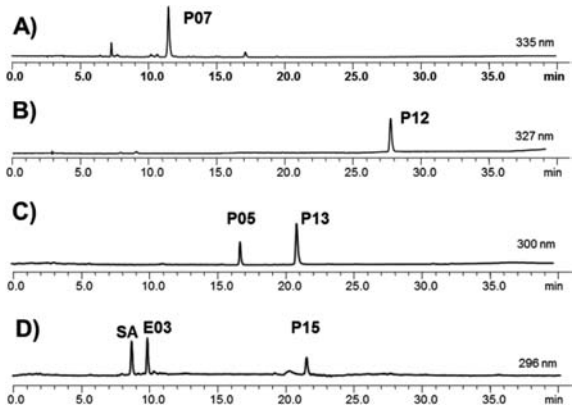

Figure 2. HPLC chromatograms of the reaction products produced by HsPKS3 from (A) *p*-hydroxyphenylpropinoyl-CoA, *N*-methylanthraniloyl-CoA, and malonyl-CoA; (B) *N*-methylanthraniloyl-CoA and 3-oxodecanoic acid; (C) *p*-hydroxyphenylpropinoyl-CoA, benzoyl-CoA, and malonyl-CoA; (D) salicyloyl-CoA and 3-oxo-5-phenylpentanoic acid. SA represents the abbreviation of salicylic acid.

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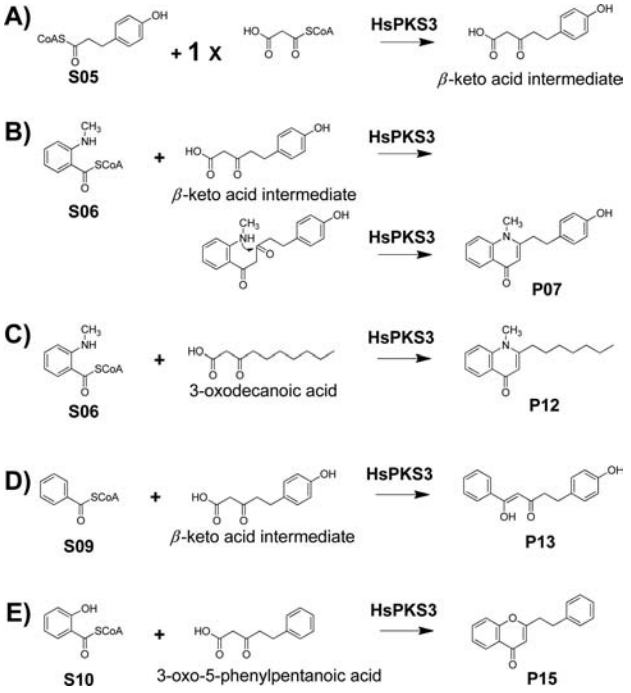
Scheme 2. HsPKS3 Catalyzed the Formation of β-Keto Acid Intermediate (A), 2-Substituted Quinolones (B and C), Diarylpentanoid (D), and 2-(2-Phenylethyl)chromone (E)



CoA, only an LC-MS-detectable quinolone alkaloid was produced, which was presumably caused by the poor capability of forming a β-keto acid intermediate from the condensation of octanoyl-CoA and malonyl-CoA. Accordingly, 3-oxodecanoic acid (**E01**) was chemically synthesized and incubated with *N*-methylanthraniloyl-CoA (**S06**). Expectedly, a single product, 2-heptyl-*N*-methylquinolin-4(1*H*)-one (**P12**), was obtained with respectable yield (Figure 2B, Scheme 2C). By replacement of *N*-methylanthraniloyl-CoA (**S06**) with anthraniloyl-CoA (**S08**), 2-heptyl-quinolin-4(1*H*)-one could be synthesized by HsPKS3. Moreover, HsPKS3 could also catalyze a head-to-head condensation of very long chain 3-oxooctadecanoic acid (**E02**) and *N*-methylanthraniloyl-CoA to produce quinolone alkaloids (Supporting Information, Figure S51), suggesting the great potential of HsPKS3 in enzymatic synthesis of 2-substituted quinolones with flexible variation of the alkyl chain length.

HsPKS3 catalyzing the formation of 2-substituted quinolone from two completely different starters motivated us to explore the potential of HsPKS3 in the synthesis of unnatural 1,3-diketone scaffolds. Considering that curcumin-like diarylpentanoids possess diverse biological activities such as anti-inflammatory, antioxidant, and antityrosinase activities,[12] synthesis of a diarylpentanoid scaffold using HsPKS3 was tested. Expectedly, a diarylpentanoid (**P13**) were produced from the condensation of benzoyl-CoA (**S09**), *p*-hydroxyphenylpropinoyl-CoA (**S05**), and malonyl-CoA (Figure 2C, Scheme 2A and D). Likewise, (*E*)-5-(4-hydroxyphenyl)-1-phenylpent-4-ene-1,3-dione (**P14**) could also be synthesized from *p*-coumaroyl-CoA. Interestingly, co-incubation of HsPKS3 with salicyloyl-CoA (**S10**) and 3-oxo-5-phenylpentanoic acid (**E03**) led to the formation of a small amount of 2-(2-phenylethyl)-chromone (**P15**) (Figure 2D, Scheme 2E).

In order to further probe the extremely promiscuous catalytic potential of HsPKS3, structurally varied starters (**S01**−**S20**) (Figure 1B), including aromatic-CoAs (**S01**−**S05**, **S09**, **S10**, and **S17**), aliphatic-CoAs (**S07**, **S14**, and **S19**), heteroaromatic-CoAs (**S11**, **S15**, **S16**, and **S18**), and bulky anthraniloyl-CoA analogues (**S06**, **S08**, **S12**, **S13**, and **S20**), were prepared and co-incubated with 3-oxodecanoic acid (**E01**), and the reaction mixtures were analyzed by LC-MS (Supporting Information, Figures S52−S71). The results revealed that HsPKS3 could efficiently catalyze head-to-head condensations of 3-oxodecanoic acid with all the synthesized starters to produce structurally diverse 1,3-diketones. However, when **S10** and **S20** were used as starters, only trace amounts of 2-heptylchromone- and 2-heptylquinolone-type products could be detected by LC-MS. Unexpectedly, incubation of 3-oxodecanoic acid (**E01**) with 2-carbamoylbenzoyl-CoA (**S12**) produced (*Z*)-2-(3-hydroxydec-2-enoyl)benzonitrile (**P18**), the structure of which was confirmed by co-incubation of **E01** with designedly synthesized 2-cyanobenzoyl-CoA (**S13**). Considering that **S12** was poorly accepted by HsPKS3 and slowly changed into 2-cyanobenzoic acid in the reaction medium, it was tentatively proposed that the dehydration might spontaneously occur in the reaction buffer. For the purpose of further confirmation of the enzymatic products, **P18** from 2-carbamoylbenzoyl-CoA (**S12**), the long-chain alphatic 1,3-diketone (**P16**) from octanoyl-CoA, and nitrogen-containing 1-(1*H*-indol-2-yl) decane-1,3-dione (**P17**) from indole-2-carbonyl-CoA (**S11**) were prepared by large-scale reactions, and their structures were unambiguously elucidated on the basis of their HRESIMS and NMR data. In order to probe the extender tolerance of HsPKS3, β-keto acids (**E01**−**E04**) and β-keto acid thioesters of *N*-acetyl cysteamine (NAC) (**E05** and **E06**) were synthesized and incubated with feruloyl-CoA (**S02**) (Supporting Information, Figures S72−S76). HsPKS3 could accept various extenders including 3-oxo-4-phenylbutanoic acid (**E04**), very long chain 3-oxooctadecanoic acid (**E02**), and structurally stable β-keto acid thioesters of NAC (**E05** and **E06**) to produce 1,3-diketones. For example, head-to-head condensation of 3-oxo-4-phenylbutanoic acid and feruloyl-CoA (**S02**) could produce a diarylhexanoid scaffold.

Compounds **P07**−**P11**, **P13**, **P14**, and **P16**−**P18** were evaluated for their inhibitory activities against LPS-activated NO production in BV-2 microglial cells. Compounds **P08**, **P13**, **P17**, and **P18** showed inhibitory activity against NO production with IC_{50} values in the range 15.7−29.8 μM (Supporting Information, Table S4).

Despite the fact that both CURS from Curcuma and CUS from *O. sativa* could catalyze the formation of 1,3-diketone scaffolds via head-to-head condensation, these two enzymes reportedly utilize cinnamoyl-CoA analogues as starters, and the products are, accordingly, confined to curcuminoids and gingerol analogues.[7,8] Distinguished from the stepwise synthesis of curcumin by DCS and CURS,[7] HsPKS3 catalyzing the one-pot formation of curcuminoids seems much like rice CUS.[8] However, production of *p*-hydroxybenzalacetone and quinolone by CUS has never been reported. Irrespective of the divergent functions producing benzalacetone, curcuminoids, and quinolone, HsPKS3 reported here has a broad range of substrate tolerance, which facilitates synthesizing a vast array of structurally diverse 1,3-diketones. Remarkably, HsPKS3 unprecedentedly catalyzes the formation of 2-substituted quinolones from a 1,3-diketone intermediate via intramolecular cyclization, greatly expanding the structural diversity and chemical complexity of the enzymatic products. On the other hand, 2-substituted quinolones extensively occurred in

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Rutaceae plants and *Pseudomonas* bacteria.[13] In *Pseudomonas* bacteria, the biosynthesis of 2-substituted quinolones requires the PqsABCD enzymes coded by the *pqsABCD* operon.[14] Enzymes in plants responsible for the biosynthesis of 2-substituted quinolones are currently little understood. Despite that 1,3-diketones and quinolones synthesized here have not been reported from *H. serrata*, and HsPKS3 catalyzing the formation of 2-substituted quinolones and 1,3-diketones would be instructive to understand the biosynthesis of these two kinds of natural products and irradiative to biomimetic synthesis of structurally diverse unnatural 2-substituted quinolones and 1,3-diketones.

In summary, we have identified a unique type III PKS with multifunctions producing curcuminoids, benzalacetone, and quinolone. Probing the catalytic promiscuity of HsPKS3 with synthesized starters led to the synthesis of various 2-substituted quinolones and 1,3-diketones. The broad range of substrate tolerance of HsPKS3 facilitates accessing structurally diverse 2-substituted quinolones and 1,3-diketones.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01501.

Experimental section (general experimental procedures, cDNA cloning, expression of cDNA, enzyme purification, enzyme reaction, and anti-inflammatory assay), phylogenetic analysis, alignment of amino acid sequence, full spectroscopic data (NMR, HRESIMS) for **P07**−**P11**, **P13**, **P14**, and **P16**−**P18**, and LC-MS data for enzymatic reactions (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Abe, I.; Morita, H. *Nat. Prod. Rep.* **2010**, *27*, 809. (b) Austin, M. B.; Noel, J. P. *Nat. Prod. Rep.* **2003**, *20*, 79. (c) Abe, I. *Curr. Opin. Chem. Biol.* **2012**, *16*, 179. (d) Schröder, J. In *Comprehensive Natural Products Chemistry*; Sankawa, U., Ed.; Elsevier: Oxford, 1999; Vol. *1*, p 749.

(2) Ferrer, J.-L.; Jez, J.; Bowman, M. M. E.; Dixon, R. A.; Noel, J. P. *Nat. Struct. Biol.* **1999**, *6*, 775.

(3) (a) Morita, H.; Kondo, S.; Oguro, S.; Noguchi, H.; Sugio, S.; Abe, I.; Kohno, T. *Chem. Biol.* **2007**, *14*, 359. (b) Wanibuchi, K.; Morita, H.; Noguchi, H.; Abe, I. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2083. (c) Morita, H.; Shimokawa, Y.; Tanio, M.; Kato, R.; Noguchi, H.; Sugio, S.; Kohno, T.; Abe, I. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 669. (d) Abe, I.; Utsumi, Y.; Oguro, S.; Morita, H.; Sano, Y.; Noguchi, H. *J. Am. Chem. Soc.* **2005**, *127*, 1362. (e) Abe, I.; Oguro, S.; Utsumi, Y.; Sano, Y.; Noguchi, H. *J. Am. Chem. Soc.* **2005**, *127*, 12709. (f) Austin, M. B.; Bowman, M. E.; Ferrer, J.-L.; Schröder, J.; Noel, J. P. *Chem. Biol.* **2004**, *11*, 1179. (g) Jez, J. M.; Austin, M. B.; Ferrer, J.-L.; Bowman, M. E.; Schröder, J.; Noel, J. P. *Chem. Biol.* **2000**, *7*, 919. (h) Shi, S.-P.; Wanibuchi, K.; Morita, H.; Endo, K.; Noguchi, H.; Abe, I. *Org. Lett.* **2009**, *11*, 551. (i) Morita, H.; Yamashita, M.; Shi, S.-P.; Wakimoto, T.; Kondo, S.; Kato, R.; Sugio, S.; Kohno, T.; Abe, I. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 13504.

(4) (a) Heeb, S.; Fletcher, M. P.; Chhabra, S. R.; Diggle, S. P.; Williams, P.; Cámara, M. *FEMS Microbiol. Rev.* **2011**, *35*, 247. (b) Rho, T. C.; Bae, E. A.; Kim, D. H.; Oh, W. K.; Kim, B. Y.; Ahn, J. S.; Lee, H. S. *Biol. Pharm. Bull.* **1999**, *22*, 1141. (c) Déziel, E.; Lépine, F.; Milot, S.; He, J.; Mindrinos, M. N.; Tompkins, R. G.; Rahme, L. G. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 1339. (d) Lin, C.-C.; Lu, Y.-P.; Lou, Y.-R.; Ho, C.-T.; Newmark, H. H.; MacDonald, C.; Singletary, K. W.; Huang, M.-T. *Cancer Lett.* **2001**, *168*, 125. (e) Esatbeyoglu, T.; Huebbe, P.; Ernst, I. M. A.; Chin, D.; Wagner, A. E.; Rimbach, G. *Angew. Chem., Int. Ed.* **2012**, *51*, 5308. (f) Patro, B. S.; Rele, S.; Chintalwar, G. J.; Chattopadhyay, S.; Adhikari, S.; Mukherjee, T. *ChemBioChem* **2002**, *3*, 364.

(5) (a) Xiong, W.; Chen, J.-X.; Liu, M.-C.; Ding, J.-C.; Wu, H.-Y.; Su, W.-K. *J. Braz. Chem. Soc.* **2009**, *20*, 367. (b) Nigam, S.; Joshi, Y. C.; Joshi, P. *Heterocycl. Commun.* **2003**, *9*, 405. (c) Huo, C.; Tang, J.; Xie, H.; Wang, Y.; Dong, J. *Org. Lett.* **2016**, *18*, 1016. (d) Pyatakov, D. A.; Sokolov, A. N.; Astakhov, A. V.; Chernenko, A. Y.; Fakhrutdinov, A. N.; Rybakov, V. B.; Chernyshev, V. V.; Chernyshev, V. M. *J. Org. Chem.* **2015**, *80*, 10694. (e) Saini, R. K.; Joshi, Y. C.; Joshi, P. *Heterocycl. Commun.* **2007**, *13*, 219. (f) Song, L.; Zhu, S. *J. Fluorine Chem.* **2001**, *111*, 201. (g) Sun, X.; Li, P.; Zhang, X.; Wang, L. *Org. Lett.* **2014**, *16*, 2126.

(6) (a) Kel'in, A. V. *Curr. Org. Chem.* **2003**, *7*, 1691. (b) Kim, J. K.; Shokova, E.; Tafeenko, V.; Kovalev, V. *Beilstein J. Org. Chem.* **2014**, *10*, 2270. (c) Heller, S. T.; Natarajan, S. R. *Org. Lett.* **2006**, *8*, 2675. (d) Sada, M.; Matsubara, S. *J. Am. Chem. Soc.* **2010**, *132*, 432. (e) Iida, A.; Osada, J.; Nagase, R.; Misaki, T.; Tanabe, Y. *Org. Lett.* **2007**, *9*, 1859. (f) Lim, D.; Fang, F.; Zhou, G.; Coltart, D. M. *Org. Lett.* **2007**, *9*, 4139. (g) Sato, K.; Yamazoe, S.; Yamamoto, R.; Ohata, S.; Tarui, A.; Omote, M.; Kumadaki, I.; Ando, A. *Org. Lett.* **2008**, *10*, 2405.

(7) Katsuyama, Y.; Kita, T.; Funa, N.; Horinouchi, S. *J. Biol. Chem.* **2009**, *284*, 11160.

(8) (a) Katsuyama, Y.; Matsuzawa, M.; Funa, N.; Horinouchi, S. *J. Biol. Chem.* **2007**, *282*, 37702. (b) Morita, H.; Wanibuchi, K.; Nii, H.; Kato, R.; Sugio, S.; Abe, I. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 19778.

(9) Wanibuchi, K.; Zhang, P.; Abe, T.; Morita, H.; Kohno, T.; Chen, G.; Noguchi, H.; Abe, I. *FEBS J.* **2007**, *274*, 1073.

(10) Abe, I.; Takahashi, Y.; Morita, H.; Noguchi, H. *Eur. J. Biochem.* **2001**, *268*, 3354.

(11) Resmi, M. S.; Verma, P.; Gokhale, R. S.; Soniya, E. V. *J. Biol. Chem.* **2013**, *288*, 7271.

(12) Lee, K.-H.; Ab Aziz, F. H.; Syahida, A.; Abas, F.; Shaari, K.; Israf, D. A.; Lajis, N. H. *Eur. J. Med. Chem.* **2009**, *44*, 3195.

(13) da Silva, M. F. G. F.; Soares, M. S.; Fernandes, J. B.; Vieria, P. C. *Alkaloids Chem. Biol.* **2007**, *64*, 139.

(14) Dulcey, C. E.; Dekimpe, V.; Fauvelle, D.-A.; Milot, S.; Groleau, M.-C.; Doucet, N.; Rahme, L. G.; Lépine, F.; Déziel, E. *Chem. Biol.* **2013**, *20*, 1481.

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