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4-Hydroxy-3-methyl-2(1*H*)-quinolone, originally discovered from a *Brassicaceae* plant, produced by a soil bacterium of the genus *Burkholderia* sp.: determination of a preferred tautomer and antioxidant activity

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Abstract

4-Hydroxy-3-methyl-2(1*H*)-quinolone (**1**), an old synthetic molecule and recently discovered from a plant without providing sufficient evidence to support the structure,

was isolated from a fermentation extract of *Burkholderia* sp. 3Y-MMP isolated from a soil by a Zn^{2+} enrichment culture. Detailed spectroscopic analyses by MS and NMR, combined with ^{13}C chemical shift comparison with literature values of the related compounds and a synthetic preparation of **1**, allowed its first full NMR characterization and identification of 4-hydroxy-2-quinolone but not 4-hydroxy-2-quinolinol as the preferred tautomer for this heterocyclic system. While the metal-chelating activity was negligible, **1** at 100 μM quenched hydroxy radical-induced chemiluminescence emitted by luminol by 86%. Because some *Burkholderia* species are pathogenic to plants and animals, the above result suggests that **1** is a potential antioxidant to counteract reactive oxygen species-based immune response in the host organisms.

Keywords

antioxidant; *Burkholderia* sp.; quinolone; soil bacterium; Zn^{2+} enrichment culture

Introduction

4-Hydroxy-2(1*H*)-quinolone is a unique structural motif mostly found in alkaloids from rutaceous plants (family *Rutaceae*) [1,2]. This motif has several tautomeric forms including 2,4-dihydroxyquinoline [3], although which tautomer to be taken seems not always be identified in some of the studies. Currently, 229 compounds are known to contain this unit as a part or a whole of the structure, among which only 12

originated from organisms other than rutaceous plants [4]. Examples from microbes include chymase inhibitors SF2809-I to VI from an actinomycete of the genus *Dactylosporangium* [5], a quorum sensing signaling molecule 2,4-dihydroxyquinoline (DHQ, **2**) from Gram-negative bacteria *Pseudomonas aeruginosa* and *Burkholderia thailandensis*, [6], and 4-*O*-β-D-glucopyranosyl-2,3,4-trihydroxy-2-quinoline from an ascomycete of the genus *Alternaria* [7].

The genus *Burkholderia* sensu lato, within the class *Betaproteobacteria*, represents a polyphyletic group of bacteria, which undergoes reclassification into several lineages [8]. Members of this group are basically free-living aerobes inhabiting soil and freshwater, but some are also found in the tissues of animals, plants, or fungi as pathogens or beneficial symbionts [9]. Not only as the subjects of human/animal health care and plant pathology [10], but they are now gathering significant attention as an emerging source of bioactive molecules. Many new structure classes, even after being spun off as a new genus from *Pseudomonas* in 1992 [11], have been discovered from this group, which, along with their large genomes comparable to those of actinomycetes or myxobacteria, demonstrates a higher capacity of secondary metabolism [12].

In the course of our continuing study on bioactive metabolites of less studied bacterial taxa [13], *Burkholderia* sp. 3Y-MMP, isolated from soil by an exhaustive enrichment culture under Zn^{2+}-load, was selected for a detailed chemical study, which resulted in the isolation of 4-hydroxy-3-methyl-2(1*H*)-quinolone (**1**, Fig. 1). This compound was recently reported from the root of woad (*Isatis tinctoria*) with no

details of structure characterization [14]. Here we describe isolation, unequivocal structure characterization, and antioxidant activity of **1**.

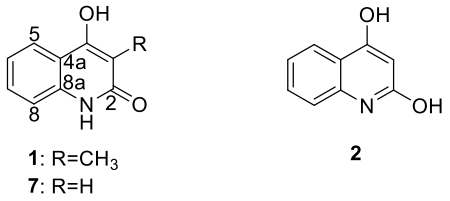

Figure 1: Structures of **1, 2**, and **7**.

Results and Discussion

The producing strain 3Y-MMP was cultured in King's B medium [15] for 4 days and the production culture was extracted with 1-BuOH. The butanolic extract was partitioned between CH_2Cl_2 and 60% MeOH, and the latter layer was flash-chromatographed on ODS followed by reversed phase HPLC to yield **1** (5.2 mg).

The molecular formula of **1** was determined to be $C_{10}H_9NO_2$ based on a sodium adduct ion peak at *m/z* 198.0525 observed by a HRESITOFMS measurement (calcd 198.0526). Broad IR absorption band around 3100 cm^{-1} and intense peak at 1600 cm^{-1} indicated the existence of hydroxy and aromatic groups, respectively.

^1H and ^{13}C NMR spectra displayed 6 and 10 resonances, respectively, and by combining with the results of ^1H-^1H coupling constants and COSY and HSQC spectroscopic analysis, following 8 molecular pieces were revealed: a consecutive four aromatic methines (δ_C 122.7/δ_H 7.85 dd, *J*=7.9, 1.0 Hz; 121.2/7.12 ddd, *J*=7.9, 7.2, 0.7 Hz; 129.8/7.41 ddd, *J*=8.1, 7.2, 1.2 Hz; 115.0/7.23 d, *J*=8.1 Hz), two

heteroatom-substituted non-protonated sp^2 carbons (δ 164.0 and 157.4), three sp^2 nonprotonated carbons (δ_C 137.4, 115.8, and 106.9), an allylic methyl group (δ_C 9.6/δ_H 1.98 s), and a singlet exchangeable proton (δ_H 11.3). The four methine unit was connected to the two quaternary carbons (δ_C 137.4 and 115.8: C8a and C4a) to form a disubstituted benzene ring by HMBC correlations H5/C8a, H6/C4a, H7/8Ca, H8/C4a, and H8/C8a. On the other hand, the remaining parts were assembled into a C_4 enol-amidyl or enol-imidic acyl unit based on HMBC correlations from the methyl proton $H_3$9 to the three non-protonated carbons C4 (δ 157.4), C3 (δ 106.9), and C2 (δ 164.0). Connection of this unit to C4a of the benzene ring was implied by an HMBC correlation from $H_3$9 to C4a, and correlations from the exchangeable proton to C4a and C3 supported this linkage as well as hydroxylation at the benzylic position. Finally, the chemical shift of C8a at 137.4 ppm was in favor of *N*-substitution, and comparison with the literature values from 4-methoxy-1,3-dimethyl-2(1*H*)-quinolone (**3**: δ 138.4) [16], *N*-methyl-2-pyridone (**4**: δ 139.5) [17], 2,4-dimethoxy-3-methylquinoline (**5**: δ 147.0) [18], and 2-methoxypyridine (**6**: δ 147.2) [19] supported a 2-quinolone form but not 2-quinolinol of **1** (Fig. 2). The same structure was synthesized from diethyl malonate and aniline (see Supporting Information File 1 for full experimental data), which substantiated this assignment.

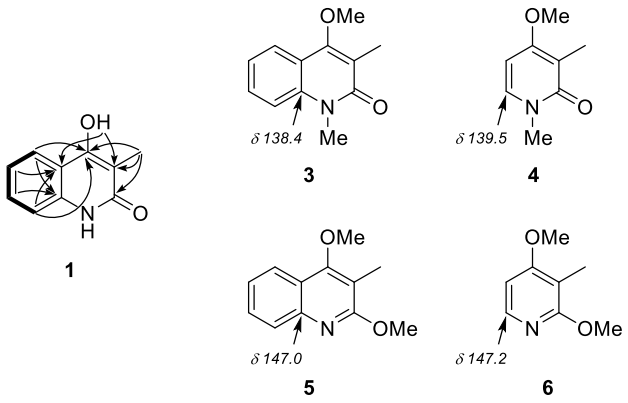

Figure 2: COSY-deduced spin-system (bold lines) and key HMBC correlations (arrows) for **1**, and structures of reference compounds **3**-**6** for ^{13}C chemical shift comparison.

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** in DMSO-d_6

	1		
No.	^{13}C	^{1}H mult. (*J* in Hz), integration	HMBC ($\rightarrow^{13}C$)
1			
2	164.0		
3	106.9		
4	157.4		
4-O*H*		11.30 brs, 1H	3, 4a
4a	115.8		
5	122.7	7.85 dd (7.9, 1.0), 1H	4, 7, 8a
6	121.2	7.12 ddd (7.9, 7.2, 0.7), 1H	4a, 5, 7, 8, 8a
7	129.8	7.41 ddd (8.1, 7.2. 1.2), 1H	5, 8, 8a
8	115.0	7.23 d (8.1), 1H	4, 4a, 6, 7, 8a
8a	137.4		
9	9.6	1.98 s, 1H	2, 3, 4, 4a

Although **1** has repeatedly been synthesized since 1921 [20] and enumerated chemical shifts for ^{1}H and ^{13}C resonances were available [21], one-on-one assignments of the resonances to each structural part have not been made until this work. In addition, HMBC correlations from the enol proton and comparison of the chemical shift of the

carbon adjacent to nitrogen with the literature values unequivocally determined 2-quinolone to be a preferred tautomer of this heterocyclic system. The same C8a carbons of DHQ and *Alternaria*-derived glycoalkaloid resonate at 139.2 [22] and 133.4 ppm [7], respectively, which indicates that both also exist as 2-quinolone and hence should more precisely be called as 4-hydroxy-2-quinolone (4HQ, **7**) and 4-*O*-β-D-glucopyranosyl-3,4-dihydroxy-2-quinolone, respectively.

Though not alkylated, the close structural similarity to **7** suggests that **1** is also a member of 2-alkyl-4-quinolone class signaling molecules/antibiotics known from *Pseudomonas aeruginosa* and some *Burkholderia* species [23,24]. Quinolones of this class are classified into two lineages, those with or without a 3-methyl group, and the latter lineages are unique to *Burkholderia* producers [25]. These metabolites are shown to be biosynthesized by head-to-head condensation of anthranilate and β-ketoacylate precursors, followed by modification at C3 or nitrogen by putative monooxygenases or methyltransferase [24]. Entry of malonate as the acylate precursor into this pathway is proposed to yield 4HQ [26]. Thus, **1** is very likely to be biosynthesized by the same mechanism followed by additional methylation on C3.

Compound **1** is reportedly inhibitory to *Mycobacterium tuberculosis* H37Ra at IC_{90} 6.8 μM while weakly cytotoxic to MRC-5 human lung-derived fibroblasts with GI_{50} 84.7 μM [27]. It did not inhibit the production of nitric oxide in RAW 264.7 murine macrophage-like cells [28]. In our hands, **1** was inactive against any of tested strains including *Staphylococcus aureus* FDA209P JC-1 (Gram-positive bacterium), *Rhizobium radiobacter* NBRC14554, *Ralstonia solanacearum* SUPP1541,

Tenacibaculum maritimum NBRC16015 (Gram-negative bacteria), *Candida albicans* NBRC0197, and *Saccharomyces cerevisiae* S100 (yeasts).

Oxidative burst, which is transient production of massive reactive oxygen species (ROS), is implemented in eukaryotic cells, including protists [29], as an innate immune response to deactivate foreign substances or as part of phagocytic digestion of internalized nutrients [30]. Pathogenic microbes are equipped with a multitude of strategies to circumvent host immunity [30], among which redox enzymes and antioxidants are the direct countermeasures to neutralize the toxicity of ROS [31]. Limited examples of antioxidants include catecholamine melanin from a fungus *Cryptococcus neoformans* [32], 1,8-dihydroxynaphthalene melanin from fungi *Wangiella dermatitidis* and *Alternaria alternata* [33], staphyloxanthin from a fermicute *Staphylococcus aureus* [34], vitamin B_6 from fungi *Cercospora nicotianae* [35] and *Rhizoctonia solani* [36], and a melanin-like pigment from *Burkholderia cenocepacia* [37].

The antioxidant activity of **1** was evaluated using the luminol chemiluminescence extinction assay [38,39]. This assay quantifies the presence of the most detrimental ROS, hydroxy radical [40,41], as intensity of luminescence emitted by oxidation of luminol. Compound **1** at 100 μM decreased luminescence to 14% of the control reaction, which was comparable to a positive control agent, ethylenediaminetetraacetic acid, showing extinction to 16% at the same concentration (Fig. 3). Because Fenton reaction catalyzed by Cu^{2+} was used to generate hydroxy radical, entrapment of Cu^{2+} by **1** was initially suspected as the mechanism of

chemiluminescence inhibition. However, this speculation was ruled out by a titration experiment using Chrome Azurol S-Fe^{3+} (CAS) [42], which required 1600 times higher concentration for metal-chelation. Thus, **1** was found to be another example of the antioxidant from *Burkholderia*. Detailed studies on the antioxidation mechanism of **1** is now underway.

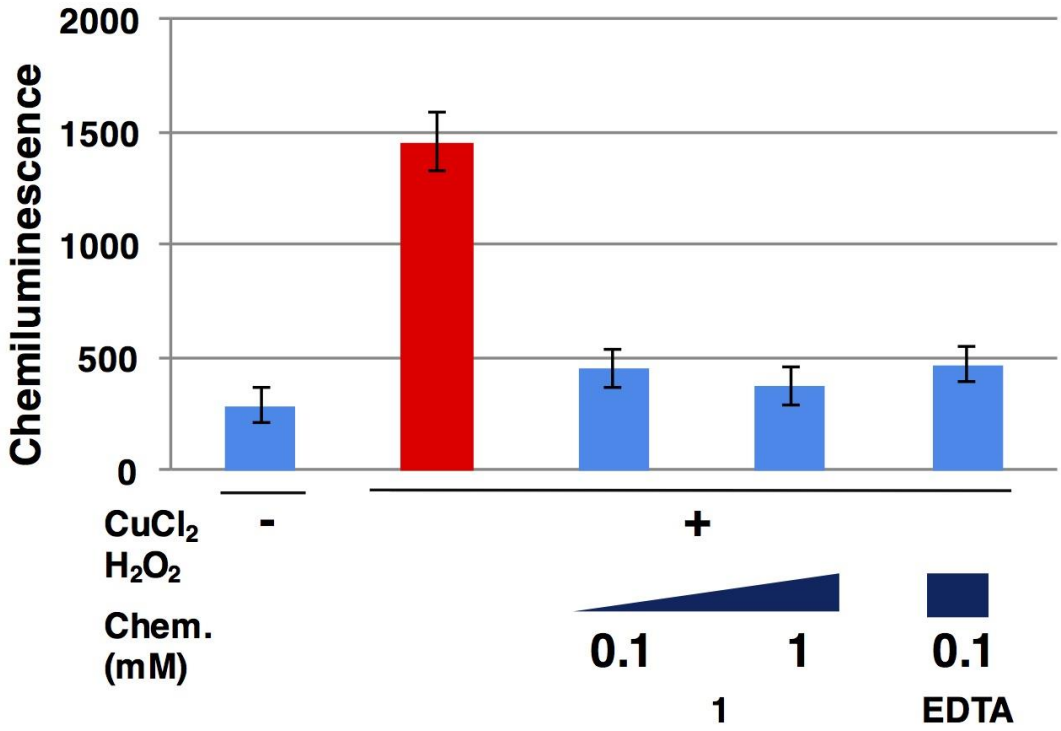

Figure 3: Extinction of luminol chemiluminescence by **1**.

Experimental

General experimental procedures

UV and IR spectra were obtained on a Hitachi U-3210 and a Perkin Elmer Spectrum 100, respectively. NMR spectra were collected on a Bruker AVANCE 500 spectrometer in DMSO-d_6 and $CDCl_3$ referenced at δ_H/δ_C 2.49/39.8 and 7.27/77.0,

respectively. HR-ESITOFMS were recorded on a Bruker micrOTOF focus mass spectrometer. Chemiluminescence was measured on a Molecular Devices SpectraMax M2 microplate reader.

Microorganism

Burkholderia sp. 3Y-MMP was isolated as one of siderophore-producers from soil by an exhaustive enrichment culture in the presence of Zn^{2+} followed by the modified CAS agar plates [42]. Approximately 0.1 g of soil samples, collected in Toyama, central Japan, in June 2015, were separately added to 10 mL of nutrient broth (Becton Dickinson Microbiology Systems, UK) containing 1 mM $ZnCl_2$ in a large test tube (ϕ 25 × 200 mm), and reciprocally shaken (70 times min^{-1}) at 28-30°C. When growth was observed, 10 µL aliquots of each culture was transferred into 10 mL of fresh medium. Following five rounds of enrichment, the culture was spread onto the same medium solidified with 1.5% (w/v) agar. After incubation at 28-30°C for 3 days, the emerging colonies were isolated from the plate.

The siderophore productivity was screened by color reaction on modified CAS agar plates, which was prepared by mixing modified minimal medium (4.0 g/L succinate, 1.0 g/L ammonium sulfate, 0.2 g/L KH_2PO_4, 0.2 g/L $MgSO_4 \cdot 7H_2O$, pH 7.0) and Chrome Azurol S (CAS) solution [42] in a ratio of 9:1 with solidification by 1.5% agar. The isolates were spread onto the CAS plates and incubated at 28-30°C for 3 days. Upon production of siderophores, agar medium in contact with colonies turns orange due the removal of Fe^{3+} from a blue Fe–CAS complex. Strain 3Y-MMP gave a

positive response to this test.

The 16S rDNA sequence of strain 3Y-MMP was determined by a DNA analysis service (Tsuruga Bio, Toyobo Co. Ltd., Osaka, Japan) using a primer set 10F (5′-GTTTGATCCTGGCTCA-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′). A partial sequence with a length of 800 bp (accession number LC508727) thus read was queried against the Basic Local Alignment Search Tool program (BLAST) available at the DNA Data Bank of Japan (DDBJ) website, which reported 99.9% homology to *Burkholderia cepacia* strain N1_1_43 (accession number MN691134).

Fermentation and isolation

A cell mass of *Burkholderia* sp. 3Y-MMP, scraped off from an agar plate, was inoculated into 500 mL K-flasks each containing 100 mL King's B medium composed of peptone 2%, glycerin 1%, K_2HPO_4 0.41%, and $MgSO_4 \cdot 7H_2O$ 0.15%. The production cultures thus made were rotary shaken at 200 rpm at 30 ℃ for 4 days. After fermentation, an equal amount of 1-butanol was added to each flask, shaken for additional 1 h, and then centrifuged at 6000 rpm. The butanol layer was collected and dried *in vacuo* to give a solid (2.7 g) from a 2L culture. The extract was partitioned between 60% aqueous MeOH and CH_2Cl_2, and the former layer was fractionated on ODS eluted sequentially with a step gradient of MeCN-0.1% HCOOH mixed in ratios of 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2. A fraction eluted by 30% MeCN was evaporated to provide 69.4 mg of a solid, which was purified by HPLC on an ODS column (Cosmosil AR-II, 1 × 25 cm) eluted with 16% MeCN containing 0.1%

HCO_2H at a flow rate of 4 mL/min, which yielded **1** as a white powder (5.2 mg, t_R 31.3 min).

*4-Hydroxy-3-methyl-2(1H)-quinolone (**1**):* UV (MeOH) λ_{max} nm (ε): 312 (2300), 226 (12000); IR (ATR) ν_{max} 3268, 3186, 2958, 2927, 1595, 1486, 1387, 1354, 1243, 1026, 772, 761, 692, 664 cm^{-1}; HR-ESITOFMS *m*/*z* 198.0525 $[M+Na]^+$ (calcd for $C_{10}H_9NONa_2$, 198.0526); ^1H and ^{13}C NMR data are shown in Table 1.

Evaluation of Fe^{3+} binding activity

The iron-binding activity was evaluated by the CAS assay developed by Schwyn and Neilands [42]. Two-point-five mg of **1** in DMSO (20 μL) was mixed with a blue-colored CAS stock solution (50 μL) and further brought up to 100 μL with H_2O (final concentration of **1**: 160 mM). After 10 min at an ambient temperature, the solution turned orange due to the loss of Fe^{3+} from the indicator CAS dye, indicating positive to the iron-binding ability of **1**. A prolonged reaction caused biphasic separation of the mixture.

Antimicrobial assay

Antimicrobial activity was evaluated by the method described previously [13].

Antioxidant assay

Antioxidant activity was evaluated by the method described in reference 38. Briefly, luminol (10 μM), H_2O_2 (1000 μM), and vehicle solvent with or without test compounds were mixed in 50 mM boric acid-sodium hydroxide buffer (pH 9.0). To

this mixture was added $CuCl_2$ (100 μM) to initiate Fenton reaction, and after 5 min incubation, chemiluminescence at 500 nm was recorded on a microplate reader. The experiments were run triplicate, and the mean ratio of light extinction was expressed as the potency of antioxidant activity.

Supporting Information

Supporting Information file 1

Synthetic procedure of **1**, UV, IR, ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC spectra for natural and synthetic **1**, and UV, IR, ^{1}H NMR, ^{13}C NMR spectra for synthetic intermediates.

File Name: Li et al. 4-OH-3-Me-2-quinolone SI.pdf

File Format: PDF

Title: Supporting Information for: 4-Hydroxy-3-methyl-2(1*H*)-quinolone, originally discovered from *Brassicaceae* plant, produced by a soil bacterium of the genus *Burkholderia* sp.: determination of a preferred tautomer and antioxidant activity

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References

1. Shang, X. F.; Susan, L.; Natschke, M.; Liu, Y. Q.; Guo, X.; Xu, X. S.; Goto M.; Li, J. C.; Yang, G. Z.; Lee, K. H. *Med. Res. Rev.* **2018,** *38,* 775–828.

2. Shang, X. F.; Susan, L.; Natschke, M.; Yang, G. Z.; Liu, Y. Q.; Guo, X.; Xu, X. S.; Goto, M.; Li, J. C.; Zhang, J. Yu.; Lee, K. H. *Med. Res. Rev*. **2018,** *38,* 1614–1660.

3. Abdou, M. M. *Arab. J. Chem*. **2017,** *10,* S3324–3337.

4. Dictionary of Natural Product 28.2 Chemical Search. http://dnp.chemnetbase.com/faces/chemical/ChemicalSearch.xhtml (accessed Feb 27, 2020).

5. Tani, N.; Harimaya, K.; Gyobu, Y.; Sasaki, T.; Takenouchi, O.; Kawamura, T.; Kamimura, T.; Harada, T. *J. Antibiot*. **2004,** *57,* 89–96.

6. Lépine, F.; Dekimpe, V.; Lesic, B.; Milot, S.; Lesimple, A.; Mamer, O. A.; Rahme, L. G.; Déziel, E. *Biol. Chem.* **2007,** *388,* 839–845.

7. Yang, X.; Yang, J. *Acta. Pharm. Sin*. **2008,** *43,* 1116–1118.

8. Estrada-de Los Santos, P.; Palmer, M.; Chávez-Ramírez, B.; Beukes, C.; Steenkamp, E. T.; Briscoe, L.; Khan, N.; Maluk, M.; Lafos, M.; Humm, E.; Arrabit, M.; Crook, M.; Gross, E.; Simon, M. F.; Dos Reis Junior, F. B.; Whitman, W. B.; Shapiro, N.; Poole, P. S.; Hirsch, A. M.; Venter, S. N.; James, E. K. *Genes* **2018,** *9,* 389.

9. Mannaa, M.; Park, I.; Seo, Y. S. *Int. J. Mol. Sci.* **2019,** *20,* 121.

10. Depoorter, E.; Bull, M. J.; Peeters, C.; Coenye, T.; Vandamme, P.; Mahenthiralingam, E. *Appl. Microbiol. Biotechnol.* **2016,** *100,* 5215–5229.

11. Yabuuchi, E.; Kosako, Y.; Oyaizu, H.; Yano, I.; Hotta, H.; Hashimoto, Y.; Ezaki, T.; Arakawa, M. *Microbiol. Immunol.* **1992,** *36,* 1251–1275.

12. Kunakom, S.; Eustáquio, A. S. *J. Nat. Prod.* **2019,** *82,* 2018-2037.

13. Li, D.; Oku, N.; Hasada, A.; Shumizu, M.; Igarashi, Y. *Beilstein J. Org. Chem.* **2018,** *14,* 1446–1451.

14. Zhang, D.; Shi, Y.; Li, J.; Ruan, D.; Jia, Q.; Zhu, W.; Chen, K.; Li, Y.; Wang, R. *Molecules* **2019,** *24,* 4033.

15. King, E. O.; Ward, M. K.; Raney, D. E. *J. Lab. Clin. Med*. **1954,** *44,* 301–307.

16. Reisch, J.; Mester, I. *Arch. Pharm*. **1980,** *313,* 751–755.

17. Still, I. W. J.; Plavac, N.; McKinnon, D. M.; Chauhan, M. S. *Can. J. Chem.* **1976,** *54,* 280–289.

18. Osborne, A. G.; Warmsley, J. F. *J. Nat. Prod.* **1992,** *55,* 589–595.

19. Chambers, R. D. Parsons, M.; Sandford, G.; Skinner, C. J.; Atherton, M. J.; Moilliet, J. S. *J. Chem. Soc. Perkin. Trans.* **1999,** *1,* 803–810.

20. Gabriel, S.; Gerhard, W. *Ber. Dtsch. Chem. Ges B*. **1921,** *54B,* 1067–1078.

21. Kawada, M.; Inoue, H.; Ohba, S.; Hatano, K.; Abe, H.; Hayashi, C.; Watanabe, T.; Igarashi, M. PCT Int. Appl. WO 2014/132902 A1.

22. AIST Spectral Database for Organic Compounds. SDBS No. 15669. https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi (accessed Feb. 23, 2020).

23. Heeb, S.; Fletcher, M. P.; Chhabra, S. R.; Diggle, S. P.; Williams, P.; Cámara, M. *FEMS. Microbiol. Rev*. **2011,** *35,* 247–274.

24. Coulon, P. M. L.; Groleau, M-C.; Déziel, E. *Front. Cell. Infect. Microbiol.* **2019,**

9, 33.

25. Vial, L.; Lépine, F.; Milot, S.; Groleau, M. C.; Dekimpe, V.; Woods, D. E.; Déziel, E. *J. Bacteriol.* **2008,** *190,* 5339–5352.

26. Zhang, Y. M.; Frank, M, W.; Zhu, K.; Mayasundari, A.; Rock, C. O. *J. Biol. Chem.* **2008,** *283,* 28788–28794.

27. de Maced, M. B.; Kimmel, R.; Urankar, D.; Gazvoda, M.; Peixoto, A.; Cools, F.; Torfs, E.; Verschaeve, L.; Lima, E. S.; Lyčka, A.; Milićević, D.; Klásek, A.; Cos, P.; Kafka, S.; Košmrlj, J.; Cappoen, D. *Eur. J. Med. Chem*. **2017,** *138,* 491–500.

28. Aoki, S.; Ye, Y.; Higuchi, K.; Takashima, A.; Tanaka, Y.; Kitagawa, I.; Kobayashi, M. *Chem. Pharm. Bull*. **2001,** *49,* 1372–1374.

29. Iriti, M.; Faoro, F. *Mycopathologia* **2007,** *164,* 57–64.

30. Siddiqui, R.; Khan, N. A. *Exp. Parasitol.* **2012,** *130,* 95–97.

31. Teng, T.; Ji, A.; Ji, X.; Li, Y. *J. Immunol. Res.* **2017,** *2017,* 9671604.

32. Jacobson, E. S.; H. S. Emery. *J. Bacteriol.* **1991,** *173,* 401–403.

33. Jacobson, E. S.; Hove, E.; Emery, H. S. *Infect. Immun.* **1995,** *63,* 4944–4945.

34. Clauditz, A.; Resch, A.; Wieland, K.-P.; Peschel, A.; Götz, F. *Infect. Immun.* **2006,** *74,* 4950-4953.

35. Bilski, P.; Li, M.Y.; Ehrenshaft, M.; Daub, M. E.; Chignell, C. F. *Photochem. Photobiol.* **2000,** *71,* 129-134.

36. Samsatly, J.; Chamoun, R.; Gluck-Thaler, E.; Jabaji, S. *Front. Microbiol.* **2016,** *6,* 1429.

37. Keith, K. E.; Killip, L.; He, P.; Moran, G. R.; Valvano, M. A. *J. Bacteriol*. **2007,**

189, 9057–9065.

38. Paejo, I.; Petrakis, C.; Kefalas, P. *J. Pharm. Toxicol. Methods* **2018,** *43,*183–190.

39. Georgetti, S. R.; Casagrande, R.; Di Mambro, V. M.; Azzolini, A. E.; Fonseca, M. *J. AAPS PharmSci*. **2003,** *5,* E20.

40. Sakihama, Y.; Cohen, M. F.; Grace, S. C.; Yamasaki, H. *Toxicology* **2002,** *177,* 67–80.

41. Stadtman, E. R.; Berlett, B. S. *J. Biol. Chem.* **1991,** *266,* 17201–17211.

42. Schwyn, B.; Neilands, J. B. *Anal. Biochem*. **1987,** *160,* 47–56.