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ORIGINAL ARTICLE

Synthesis and biological activity of Citridone A and its derivatives

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Citridone A (1), originally isolated as a potentiator of antifungal miconazole activity from a fungal culture broth, has a phenyl-*R*-furopyridone structure. Because of its unique ring structure, 11 derivatives were chemically synthesized and their biological activity was evaluated. Derivatives 17, 20 and 21 potentiated miconazole activity against *Candida albicans*. Furthermore, 1, 14, 20 and 21 were found to inhibit yellow pigment production in methicillin-resistant *Staphylococcus aureus*.
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INTRODUCTION

Citridone A (**1**) (Figure 1), a potentiator of miconazole activity against *Candida albicans*, was originally isolated from the culture broth of *Penicillium* sp. FKI-1938.[1–3] Compound **1** has a rare phenyl-*R*-furopyridone skeleton (6-6/5/5 ring system) and **1** was the only natural compound having this ring system. Structurally related citridones **2**–**4** (Figure 1) were also isolated from the fungus, but they showed very weak miconazole-potentiating activity.[3] Because of its unique structure and biological properties, two groups have already completed its total synthesis.[4,5] These findings prompted us to synthesize new citridone A derivatives to understand the structure–activity relationship. Furthermore, the biological activity of the derivatives was re-evaluated in 15 in-house assay systems. Among 11 derivatives synthesized in this study (Figure 1), 3 derivatives, **17**, **20** and **21**, potentiated miconazole activity against *C. albicans*. Interestingly, **1**, **14**, **20** and **21** were found to inhibit yellow pigment production in methicillin-resistant *Staphylococcus aureus* (MRSA).

In this study, we described the synthesis of citridone derivatives and their biological activity, including the potentiating activity of antifungal miconazole and inhibitory activity of yellow pigment production in MRSA.

RESULTS AND DISCUSSION

Citridone A (**1**) and its 11 derivatives (**7**, **11**, **14**, **15**, **16**, **17**, **19**, **20**, **21**, **27** and **29**) were synthesized.[4] Derivatives **11**, **17** and **21** (enantiomer of **1**) were prepared as shown in Chart 1, according to the total synthesis of **1** we achieved previously.[4] Intermediate **5** was exposed to tetra-*n*-butylammonium fluoride to afford **7**, a new derivative without a dihydrofuran ring. The reactions of other intermediates **11** and **12** with *t*-BuOK proceeded via cyclobutane formation, followed by novel pyrolysis[6–8] to give alkenes **14** and **15**. Deprotection of **15** and reduction of aldehyde **17** gave the new derivatives **16** and **19**, respectively. In addition, **10**, which was synthesized by regioselective iodocyclization according to our established method,[4] was converted to regioisomer **20** (Chart 1). Derivatives **27** and **29** were also prepared as shown in Chart 2. The Pd(0)-catalyzed coupling reaction between **22** and **23**[4] followed by heating at 210 °C afforded pyridone **25**. Regioselective iodocyclization under different conditions produced the corresponding iodides **26** and **28**, from which E2 elimination then gave the desired derivatives **27** and **29**, respectively.

Potentiation of miconazole activity against *C. albicans* in combination with citridones and their derivatives (Figure 1) was assayed by the conventional method using paper disks.[1] None of the citridones themselves showed any inhibition against *C. albicans* at up to 20 μg per disk on plate A (without miconazole). Citridone A (**1**) and derivatives **17**, **20** and **21** (20 μg per 6 mm disk) were found to potentiate miconazole activity by forming inhibitory zones around the paper disks on plate B in contact with a small amount of miconazole (60 nM, which, at this level, had no effect on the growth of *C. albicans*; 21, 15, 16, 21 mm); however, the other compounds (20 μg per 6 mm disk) showed no potentiation activity. Citridone A and its enantiomer (**21**) showed the largest inhibition zone on plate B (Table 1).

As the structures of citridone derivatives are unique as small molecules, other biological activities of the derivatives were evaluated. Inhibitory activity of yellow pigment production in MRSA was assayed by our established method using paper disks.[9] Compounds **1**, **14**, **20** and **21** (20 μg per 6 mm disk) were found to inhibit yellow pigment production by forming white zones around the paper disks (16, 13, 15, 15 mm); however, the other compounds (20 μg per 6 mm disk) showed no activity. Similar results were obtained when using methicillin-sensitive *S. aureus* (MSSA) instead of MRSA (Table 1).

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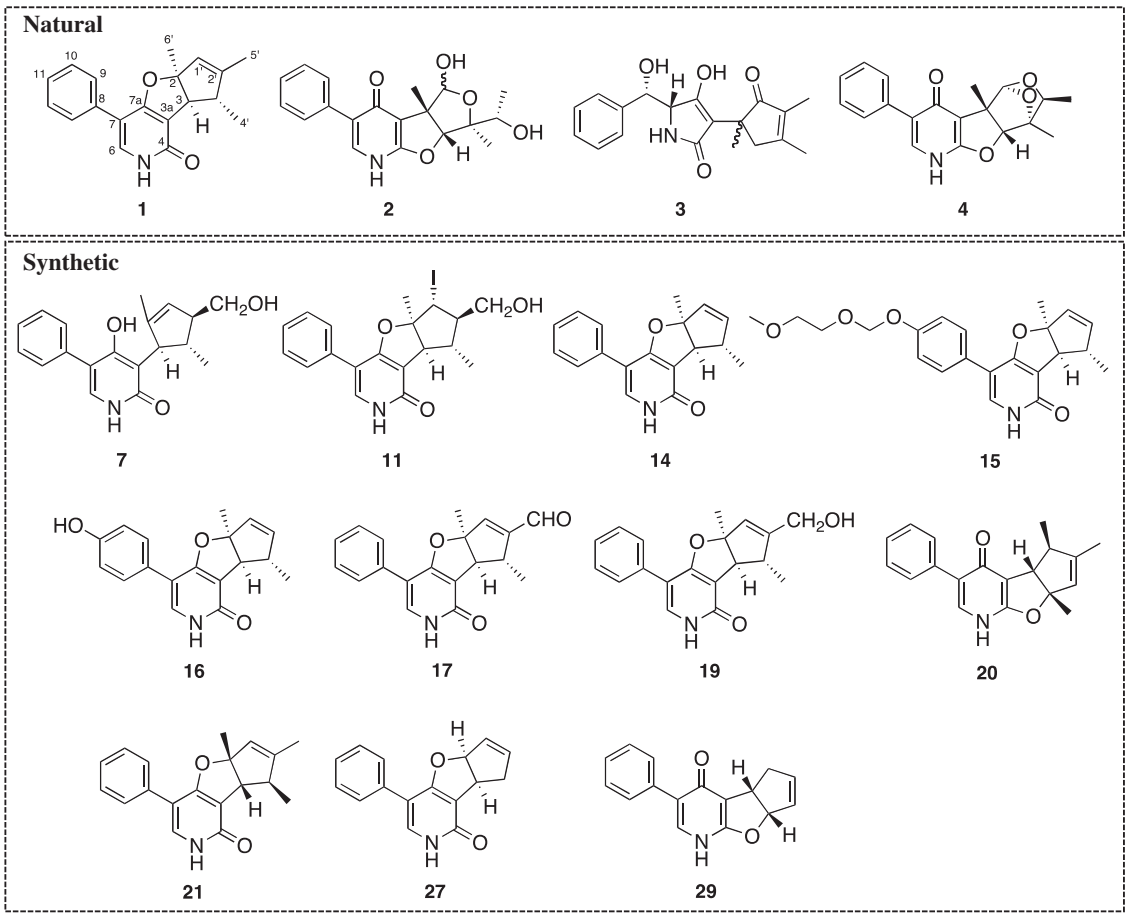

Figure 1 Structures of natural and synthetic citridones.

To confirm the inhibition of MRSA yellow pigment production by these compounds, they were evaluated by the liquid culture method.[9] As summarized in Table 1, compounds **1**, **14**, **20** and **21** inhibited yellow pigment production with IC_{50} values of 11.1 μg ml^{-1}, 30.0 μg ml^{-1}, 22.5 μg ml^{-1} and 22.7 μg ml^{-1}, respectively, without any effect on the growth of MRSA at 30 μg ml^{-1}.

It is well known that *S. aureus* produces a yellow pigment called staphyloxanthin.[10,11] Recently, several research groups reported that staphyloxanthin is one of the important virulent factors of *S. aureus*.[12,13] Staphyloxanthin acts as an antioxidant with its numerous conjugated double bonds, which enable *S. aureus* to survive by detoxification of host-generated reactive oxgen species such as O_2^-, H_2O_2 and HOCl.[14,15] Staphyloxanthin develops in the cell membrane of *S. aureus* and is associated with enhancing *S. aureus* survival and infection.[16]

Staphyloxanthin is composed of a glucose core to which a prenyl residue and a fatty acyl residue are attached. The biosynthetic pathway of staphyloxanthin was reported previously.[11] Importantly, a CrtM-deficient mutant, which lacks an enzyme involved in synthesis of the prenyl residue and cannot produce staphyloxanthin, was reported to fail to survive in the host mouse.[17,18] Recently, several squalene synthase inhibitors, BPH-652,[19] zaragozic acid,[20] rhodomyrtone[21] and tylopilusins,[22] were found to inhibit staphyloxanthin production in *S aureus*. Furthermore, BPH-652 was demonstrated to block infection of the host mouse with *S. aureus*.[19] Therefore, the staphyloxanthin biosynthetic pathway of *S aureus* is expected to offer a new potential target to combat MSSA and MRSA infection.

Citridone A and derivatives **20** and **21**, having a common 4,5,6a-trimethyl-4,6a-dihydro-3a*H*-cyclopentafuran skeleton, showed both biological activities. It is very difficult to imagine that both activities are derived from the same mechanism of action. Further precise analysis is necessary to demonstrate the mechanisms.

MATERIALS AND METHODS

General experimental procedures

Ultraviolet spectra were recorded on a spectrophotometer (8453 UV–Visible spectrophotometer; Agilent Technologies Inc., Santa Clara, CA, USA). Optical rotations were measured with a digital polarimeter (DIP-1000; JASCO, Tokyo, Japan). HR-ESI-TOF-mass spectra were recorded on a mass spectrometer (JMS-T100LP; JEOL, Tokyo, Japan). Various NMR spectra were measured with a spectrometer (XL-400; Varian, Inc., Palo Alto, CA, USA).

Experimental procedures and characterization data

Stocked natural citridones B to D (**2**–**4**) used for this investigation were isolated from a culture broth of *Penicillium* sp. FKI-1938.[1,3]

All experimental procedures for the synthesis of compounds (**7**, **11**, **14**, **15**, **16**, **17**, **19**, **20**, **21**, **27** and **29**), including citridone A (**1**), are summarized in Supplementary Information.

*(5aS,8R,8aS)-5a,7,8-Trimethyl-4-phenyl-2,5a,8,8a-tetrahydro-1H-cyclopenta[4,5] furo[3,2–c]pyridin-1-one (citridone A, **1**).* $[\alpha]_D^{21}$ –76.9 (*c* 1.0, CH_3OH). IR (KBr) cm^{-1}: 2926, 1653, 1431, 1231, 1030, 763, 697. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.55–7.51 (m, 2H), 7.45 (s, 1H), 7.42–7.36 (m, 2H), 7.33–7.27 (m, 1H), 5.39 (dq, J = 1.7 Hz, 1H), 3.27 (d, J = 1.7 Hz, 1H), 2.91 (bq, J = 7.2

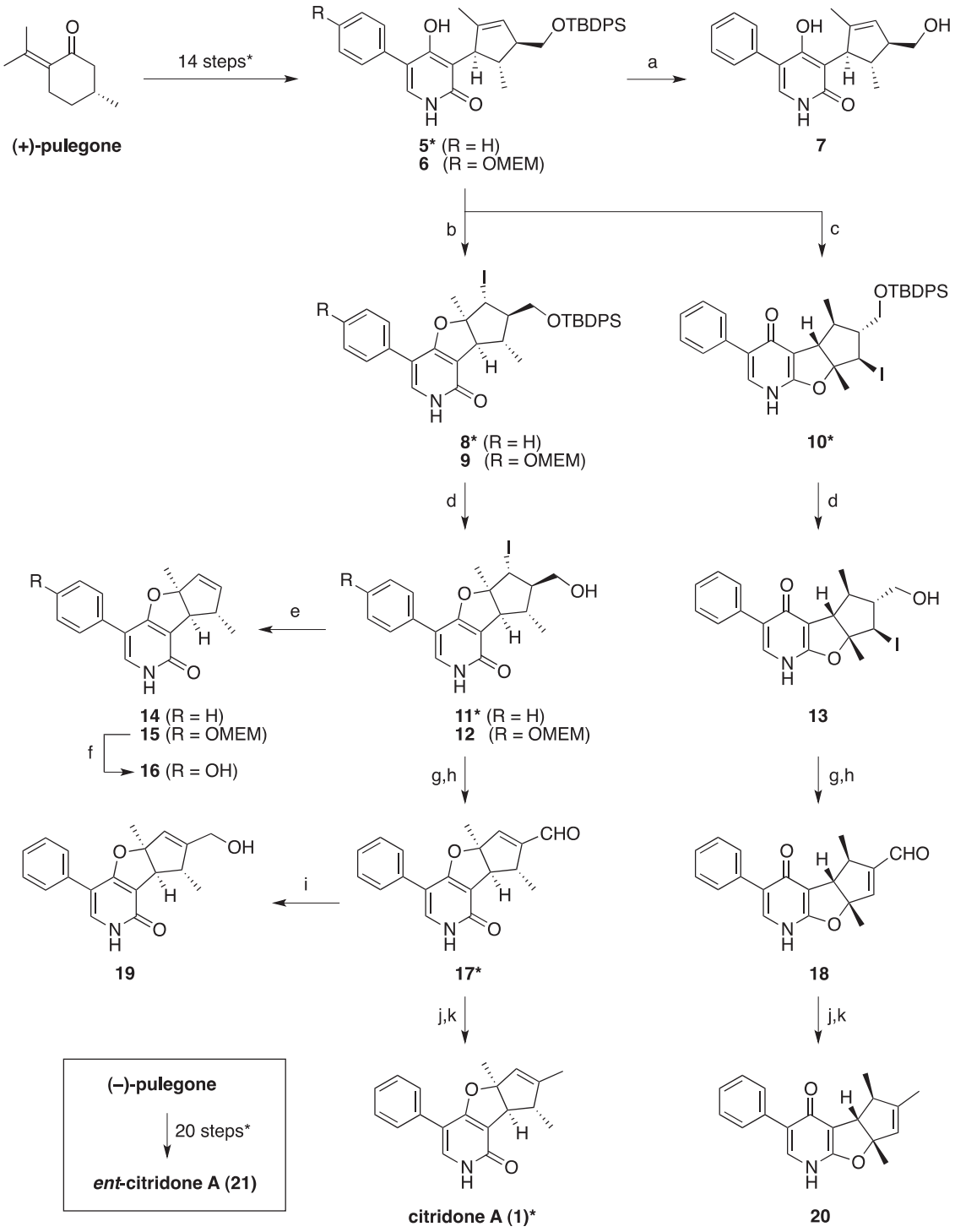

Chart 1 Preparation of compounds **7**, **11**, **14**, **15**, **16**, **17**, **19**, **20** and **21**. Reagents and conditions: (a) tetra-*n*-butylammonium fluoride, tetrahydrofuran, room temperature, 88%; (b) *N*-iodosuccinimide, pyridine, CH_2Cl_2, room temperature*; (c) I_2, CH_2Cl_2, room temperature*; (d) tetra-*n*-butylammonium fluoride, tetrahydrofuran, room temperature, 88% for **11***, 89% for **12**, 77% for **13**; (e) *t*-BuOK, *N*,*N*-dimethylformamide. 100 °C, 88% for **14**, 77% for **15**; (f) trifluoroacetic acid, CH_2Cl_2, 0 °C, 92%; (g) Dess-Martin Periodinane, CH_2Cl_2, dimethyl sulfoxide, pyridine, room temperature; (h) 1,8-diazabicyclo[5.4.0]undec-7-ene, CH_2Cl_2, room temperature, 99% (2 steps) for **17***, 65% (2 steps) for **18**; (i) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, −78 °C, 77%; (j) $(TMSSCH_2)_2$, ZnI_2, CH_2Cl_2, Et_2O; (k) Bu_3SnH, azobisisobutyronitrile, benzene, 55% (2 steps) for **1***, 40% (2 steps) for **20**. *See, ref. 1.

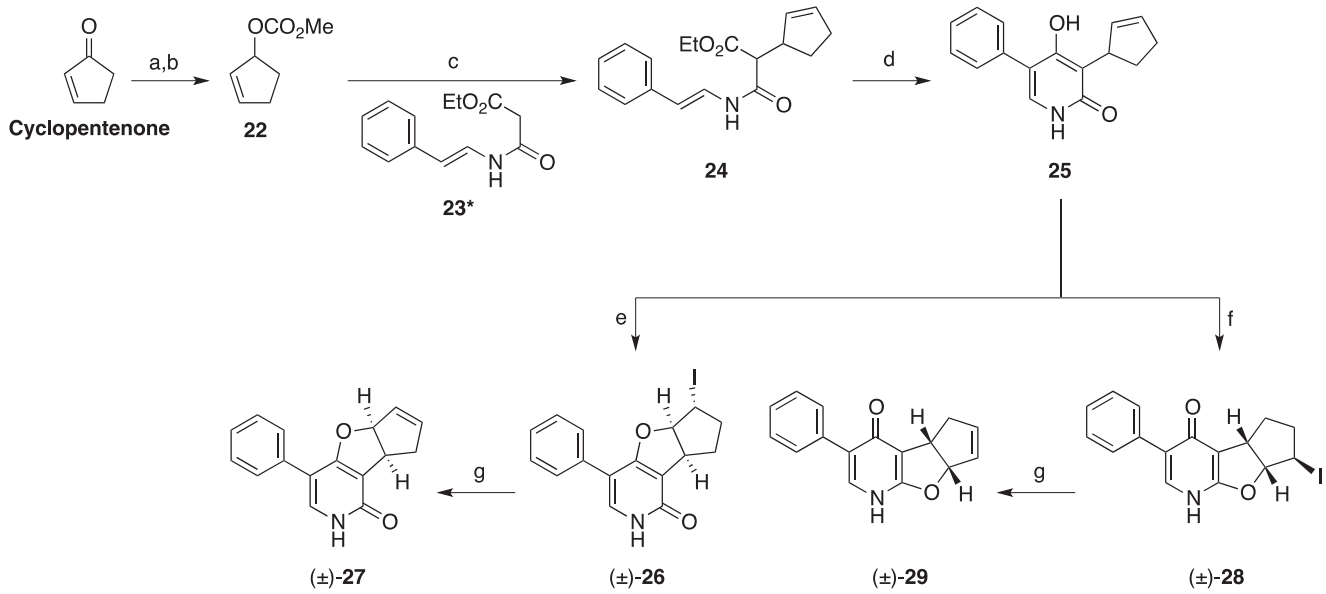

Chart 2 Preparation of compounds **27** and **29.** Reagents and conditions: (a) diisobutylaluminium hydride, CH_2Cl_2, −78 °C; (b) $ClCO_2Me$, *N,N,N′,N′*-tetramethylethylenediamine, CH_2Cl_2, 0 °C, 92% (2 steps); (c) $(allylPdCl)_2$, 1,1′-bis(diphenylphosphino)ferrocene, NaH, tetrahydrofuran, room temperature, quant.; (d) Ph_2O, 210 °C, 86%; (e) *N*-iodosuccinimide, pyridine, CH_2Cl_2, room temperature, 61%; (f) I_2, CH_2Cl_2, room temperature, 83%; (g) *t*-BuOK, *N,N*-dimethylformamide, 100 °C, 95% for (±)-**27**, 93% for (±)-**29**.

Table 1 Summary of biological activity of citridone derivatives

	Miconazole-potentiating activity		*Inhibition of yellow pigment production*			
	Inhibition zone against C. albicans		*MSSA*	*MRSA*		
Compound	*Plate A*[a]	*Plate B (60 nM MZ)*[a]	*White zone*[a]	*White zone*[a]	*Growth*[b]	*Y. P. prod.*[b]
	(mm)	(mm)	(mm)	(mm)	(IC_{50} μg ml^{-1})	
1	—	21	17	16	>30	11.1
14	—	—	15	13	>30	30.0
17	—	15	—	—	>30	>30.0
20	—	16	15	15	>30	22.5
21	—	21	15	15	>30	22.7

Abbreviations: -, No inhibition zone or no white zone; MRSA, methicillin-resistant *Staphylococcus aureus;* MZ, miconazole; Y. P. prod., yellow pigment production.
[a]Paper disk method (20 μg per sample per 6 mm disk).
[b]Liquid culture method.

Hz, 1H), 1.73 (s, 3H), 1.65 (s, 3H), 1.30 (d, J=7.9 Hz, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 164.9, 163.1, 150.5, 133.9, 133.6, 128.5, 127.6, 127.2, 126.3, 113.4, 111.1, 103.8, 56.7, 49.0, 26.3, 20.3, 14.8. high resolution mass spectrum (HRMS) (ESI) $[M+Na]^+$ calcd for $C_{19}H_{19}NNaO_2$ = 316.1314, found = 316.1331.

*4-Hydroxy-3-((1R,4S,5R)-4-(hydroxymethyl)-2,5-dimethylcyclopent-2-en-1-yl)-5-phenylpyridin-2(1H)-one (**7**).* $[\alpha]_D^{27}$ +39.2 (*c* 0.5, $CHCl_3$). IR (KBr) cm^{-1}: 3373, 2925, 2361, 1639, 1433, 1214. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.54–7.34 (m, 6H), 5.53 (s, 1H), 4.04 (bd, J=6.8 Hz, 1H), 3.87 (dd, J=12.0, 2.2 Hz, 1H), 3.67 (dd, J=12.0, 2.2 Hz, 1H), 2.61–2.57 (m, 1H), 2.35–2.29 (m, 1H), 1.64 (s, 3H), 1.28 (d, J=6.8 Hz, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 166.7, 160.8, 140.2, 132.9, 131.6, 129.8, 129.3, 128.8, 128.6, 122.5, 112.3, 63.8, 55.2, 51.9, 41.2, 20.9, 15.1. HRMS (ESI) $[M+Na]^+$ calcd for $C_{19}H_{21}NNaO_3$ = 334.1521, found = 334.1518.

*(5aR,6R,7R,8R,8aS)-7-(Hydroxymethyl)-6-iodo-5a,8-dimethyl-4-phenyl-2,5a,6,7,8,8a-hexahydro-1H-cyclopenta[4,5]furo[3,2–c]pyridin-1-one (**11**).* $[\alpha]_D^{20}$ −56.0 (*c* 1.0, CH_3OH). IR (KBr) cm^{-1}: 3321, 2960, 2872, 1656, 1429, 1379, 1033. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.53–7.50 (m, 2H), 7.41 (1H), 7.40–7.36 (m, 2H), 7.33–7.28 (m, 1H), 4.47 (d, J=14.3 Hz, 1H), 3.76 (dd, J=11.8, 2.6 Hz, 1H), 3.72 (dd, J=11.8, 2.3 Hz, 1H), 3.14 (d, J=9.8 Hz, 1H), 2.10–2.00 (m, 1H), 1.73–1.63 (m, 1H), 1.67 (s, 3H), 1.40 (d, J=6.7 Hz, 3H). ^{13}C-NMR (100 MHz, CD_3OD) δ: 166.1, 163.3, 135.6, 134.2, 129.7, 128.7, 128.5, 114.6, 112.9, 99.0, 58.8, 57.0, 56.5, 41.6, 39.9, 29.6, 19.6. HRMS (ESI) $[M+H]^+$ calcd for $C_{19}H_{21}INO_3$ = 438.0566, found = 438.0551.

*(5aS,8S,8aS)-5a,8-Dimethyl-4-phenyl-2,5a,8,8a-tetrahydro-1H-cyclopenta[4,5]furo[3,2–c]pyridin-1-one (**14**).* $[\alpha]_D^{27}$ −129.8 (*c* 1.0, $CHCl_3$). IR (KBr) cm^{-1}: 2926, 1739, 1655, 1501, 1459, 1428, 1369, 1263, 1227, 1150, 1096. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.54–7.50 (m, 3H), 7.43–7.36 (m, 1H), 7.34–7.28 (m, 2H), 5.95 (dd, J=5.6, 2.1 Hz, 1H), 5.73 (dd, J=5.4, 1.5 Hz, 1H), 3.25 (s, 1H), 3.14-3.12 (m, 1H), 1.69 (s, 3H), 1.29 (d, J=7.5 Hz, 3H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 141.4, 134.5, 133.2, 131.1, 128.5, 127.6, 127.4, 127.4, 104.8, 55.5, 46.3, 29.5, 26.0, 21.6. HRMS (ESI) $[M+Na]^+$ calcd for $C_{18}H_{17}NNaO_2$ = 302.1157, found = 302.1142.

*(5aS,8S,8aS)-4-(4-((2-Methoxyethoxy)methoxy)phenyl)-5a,8-dimethyl-2,5a,8,8a-tetrahydro-1H-cyclopenta[4,5]furo[3,2–c]pyridin-1-one (**15**).* $[\alpha]_D^{25}$ −59.2 (*c* 1.0, $CHCl_3$). IR (KBr) cm^{-1}: 3401, 2925, 1652, 1614, 1514, 1445, 1228, 1103, 1000, 833. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.58–7.56 (m, 1H), 7.45–7.41 (m, 2H), 7.09–7.06 (m, 2H), 5.94 (dd, J=7.6, 1.8 Hz, 1H), 5.72 (dd, J=7.6, 1.8 Hz, 1H), 5.29 (s, 2H), 3.85–3.82 (m, 2H), 3.58–3.55 (m, 2H), 3.38 (s, 3H), 3.24 (s, 1H), 3.13–3.08 (m, 1H), 1.69 (s, 3H), 1.27 (d, J=6.8 Hz, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 165.5, 161.2, 156.7, 141.3, 133.9, 131.0, 128.8, 126.5, 116.3, 104.9, 93.4, 71.6, 67.6, 59.0, 55.4, 46.4, 26.2, 21.5. HRMS (ESI) $[M+H]^+$ calcd for $C_{22}H_{26}NO_5$ = 384.1811, found = 384.1824.

*(5aS,8S,8aS)-4-(4-Hydroxyphenyl)-5a,8-dimethyl-2,5a,8,8a-tetrahydro-1H-cyclopenta[4,5]furo[3,2–c]pyridin-1-one (**16**).* $[\alpha]_D^{25}$ −9.91 (*c* 0.5, CH_3OH). IR (KBr) cm^{-1}: 3435, 1646, 1516, 1430, 1270, 1108, 1042, 834, 786, 630. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.35–7.30 (m, 3H), 6.81–6.77 (m, 2H),

5.98 (dd, $J=7.6$, 2.4 Hz, 1H), 5.73 (dd, $J=7.6$, 1.8 Hz, 1H), 3.18 (d, $J=1.6$ Hz, 1H), 3.04–2.97 (m, 1H), 1.67 (s, 3H), 1.25 (d, $J=7.2$ Hz). ^{13}C-NMR (100 MHz, CD_3OD) δ: 166.8, 163.0, 158.0, 133.9, 132.0, 129.7, 125.3, 116.1, 114.5, 113.0, 105.4, 56.7, 26.2, 21.6. HRMS (ESI) $[M+Na]^+$ calcd for $C_{18}H_{17}NNaO_3=318.1106$, found = 318.1108.

*(5aS,8R,8aS)-5a,8-Dimethyl-1-oxo-4-phenyl-2,5a,8,8a-tetrahydro-1H-cyclopenta[4,5]furo[3,2–c]pyridine-7-carbaldehyde (**17**).* $[\alpha]_D^{20}$ –191.0 (*c* 1.0, $CHCl_3$). IR (KBr) cm^{-1}: 2969, 1689, 1652, 1456, 1430, 1373, 1227. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.83 (s, 1H), 7.53–7.30 (m, 6H), 6.58 (s, 1H), 3.48 (q, $J=7.2$ Hz, 1H), 3.41 (s, 1H), 1.80 (s, 3H), 1.38 (d, $J=7.1$ Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 190.0, 164.3, 162.9, 151.8, 147.2, 134.7, 133.0, 128.5, 127.5, 127.3, 112.3, 110.8, 101.3, 56.8, 42.4, 25.1, 20.4. HRMS (ESI) $[M+Na]^+$ calcd for $C_{19}H_{17}NNaO_3=30.1106$, found = 330.1077.

*(5aS,8R,8aS)-7-(Hydroxymethyl)-5a,8-dimethyl-4-phenyl-2,5a,8,8a-tetrahydro-1H-cyclopenta[4,5]furo[3,2–c]pyridin-1-one (**19**).* $[\alpha]_D^{29}$ –51.7 (*c* 0.5, CH_3OH). IR (KBr) cm^{-1}: 3460, 2849, 2150, 1650, 1453, 1430, 1112. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.50 (d, $J=8.4$ Hz, 2H), 7.44 (s, 1H), 7.37 (t, $J=8.4$ Hz, 2H), 7.31–7.27 (m, 1H), 5.65 (s, 1H), 4.18 (d, $J=15.6$ Hz, 1H), 4.10 (d, $J=15.6$ Hz, 1H), 3.28 (d, $J=0.8$ Hz, 1H), 3.01 (q, $J=7.2$ Hz, 1H), 1.68 (s, 3H), 1.30 (d, $J=7.2$ Hz, 3H). ^{13}C-NMR (150 MHz, CD_3OD) δ: 166.5, 163.3, 155.5, 135.1, 129.5, 128.7, 128.5, 128.4, 126.3, 114.8, 113.0, 104.7, 60.2, 58.0, 47.0, 26.3, 20.8. HRMS (ESI) $[M+Na]^+$ calcd for $C_{19}H_{19}NNaO_3=332.1262$, found = 332.1276.

*(4bS,5R,7aS)-5,6,7a-Trimethyl-3-phenyl-4b,5-dihydro-1H-cyclopenta[4,5]furo[2,3–b]pyridin-4(7aH)-one (**20**).* $[\alpha]_D^{25}$ + 107.6 (*c* 1.0, CH_3OH). IR (KBr) cm^{-1}: 2965, 1653, 1454, 1432, 1217, 1041. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.54–7.51 (m, 3H), 7.41–7.37 (m, 2H), 7.32–7.28 (m, 1H), 5.40 (bs, 1H), 3.28 (d, $J=1.0$ Hz, 1H), 2.90 (bq, $J=7.0$ Hz, 1H), 1.73 (s, 3H), 1.67 (s, 3H), 1.30 (d, $J=7.0$ Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 165.4, 162.7, 150.7, 134.3, 133.5, 128.7, 127.7, 127.4, 126.4, 111.8, 104.3, 56.6, 49.2, 26.4, 20.4, 14.9. HRMS (ESI) $[M+H]^+$ calcd for $C_{19}H_{20}NO_2=294.1494$, found = 294.1485.

*(5aR,8S,8aR)-5a,7,8-Trimethyl-4-phenyl-2,5a,8,8a-tetrahydro-1H-cyclopenta[4,5]furo[3,2–c]pyridin-1-one (ent-Citridone A, **21**).* $[\alpha]_D^{23}$ + 77.6 (*c* 0.1, CH_3OH). IR (KBr) cm^{-1}: 2924, 1652, 1431, 1204, 1034, 765, 697. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.54–7.51 (m, 2H), 7.47–7.34 (m, 4H), 5.43 (dq, $J=1.4$ Hz, 1H), 3.33 (bs, 1H), 2.88 (bq, $J=7.0$ Hz, 1H), 1.74 (s, 3H), 1.70 (s, 3H), 1.30 (d, $J=7.2$ Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.1, 163.1, 150.7, 133.7, 133.4, 128.7, 127.8, 127.5, 126.4, 113.1, 111.9, 104.2, 56.6, 49.2, 26.4, 20.4, 14.9. HRMS (ESI) $[M+H]^+$ calcd for $C_{19}H_{20}NO_2=294.1494$, found = 294.1488.

(5aS,8aS*)-4-Phenyl-2,5a,8,8a-tetrahydro-1H-cyclopenta[4,5]furo[3,2–c]pyridin-1-one (**27**).* IR (KBr) cm^{-1}: 3055, 2929, 2360, 2340, 1649, 1598, 1230, 1062, 1197, 762. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.65–7.61 (m, 1H), 7.52–7.50 (m, 2H), 7.42–7.38 (m, 2H), 7.34–7.30 (m, 1H), 6.15 (d, $J=5.2$ Hz, 1H), 6.05 (d, $J=5.2$ Hz, 1H), 5.89 (dd, $J=5.2$, 2.4 Hz, 1H), 4,15 (t, $J=7.8$ Hz, 1H), 2.90 (d, $J=7.8$ Hz, 1H), 2.85 (t, $J=2.4$ Hz, 1H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 166.2, 162.1, 137.4, 134.5, 132.9, 128.6, 128.0, 127.6, 127.6, 113.3, 112.2, 95.7, 40.9, 38.2. HRMS (ESI) $[M+Na]^+$ calcd for $C_{16}H_{13}NNaO_2=274.0844$, found = 274.0841.

(4bS,7aS*)-3-Phenyl-4b,5-dihydro-1H-cyclopenta[4,5]furo[2,3–b]pyridin-4(7aH)-one (**29**).* IR (KBr) cm^{-1}: 3020, 2929, 2857, 2400, 1642, 1597, 1477, 1216. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.48–7.45 (m, 3H), 7.39–7.35 (m, 2H), 7.32–7.28 (m, 1H), 6.20–6.17 (m, 1H), 5.98 (d, $J=8.0$ Hz, 1H), 5.92-5.89 (m, 1H), 4.14 (td, $J=8.0$, 2.4 Hz, 1H), 2.89 (ddt, $J=17.6$, 8.0, 2.4 Hz, 1H), 2.69 (dt, $J=17.6$, 2.4 Hz, 1H). ^{13}C-NMR (150 MHz, CD_3OD) δ: 169.4, 163.9, 138.8, 136.5, 130.4, 129.2, 129.0, 128.2, 111.1, 95.6, 41.8, 39.7. HRMS (ESI) $[M+H]^+$ calcd for $C_{16}H_{14}NO_2=252.1024$, found = 252.1012.

Assay for miconazole-potentiating activity against *C. albicans* using paper disks

C. albicans ATCC 64548 was inoculated into a 50-ml test tube containing 10 ml seed medium (potato extract containing peptone 0.50% and glucose 1.0%), and was grown for 24 h on a rotary shaker. In Method A, the seed culture of *C. albicans* (0.10%, v/v) was transferred to two different agar plates, GY agar (glucose 1.0%, yeast extract 0.50% and agar 0.80%; plate A) and GY agar plus miconazole (60 nM; plateB). The concentration (60 nM) of miconazole is one-fourth of the MIC value against *C. albicans*, and showed no effect on the growth of *C. albicans*. Paper disks (6 mm; ADVANTEC, Tokyo, Japan) containing a 20-μg sample were placed on plates A and B, which were incubated at 27 °C for 24 h. Samples showing inhibition zones selectively on plate B were selected as potentiators of miconazole activity against *C. albicans*.

Assay for inhibition of yellow pigment production in MRSA using paper disks

MRSA K-24 strain, a clinical isolate, was used as a yellow pigment-producing strain. MRSA was cultured in Mueller-Hinton broth at 37 °C for 20 h and adjusted to 1×10^8 CFU per ml. The inoculum (100 μl) was spread on 25 ml TYB agar (tryptone 1.7%, yeast extract 1.0%, NaCl 0.5%, K_2HPO_4 0.25%, agar 1.5% and glycerol monoacetate 1.5%) on a plate (100 × 140 mm). Paper disks (6 mm i.d.) containing a 20-μg sample were placed on the plate and incubated at 37 °C for 72 h. Inhibition of the production of yellow pigments by a sample is expressed as the diameter (mm) of the white zone on the plate.

Assay for growth and yellow pigment production in MRSA by liquid culture

A mixture containing TYB (980 μl), a sample (10 μl) and MRSA (10 μl, at a final concentration of 1×10^7 CFU per ml) in a total volume of 1000 μl was incubated on a rotary shaker at 210 r.p.m. for 72 h at 37 °C. (1) MRSA growth: the culture's turbidity was determined at 600 nm using a Power Wave x 340 (BIO-TEK Instruments Inc., Winooski, VT, USA). (2) Yellow pigment production: after the culture was centrifuged, yellow pigments in MRSA mycelia were extracted with methanol (500 μl) at 60 °C for 2 h in the dark. The absorbance of yellow pigments was determined at 450 nm using a Power Wave × 340. Inhibition of MRSA growth and yellow pigment production by a sample (% of control) is defined as (absorbance-sample/absorbance-control) × 100. The IC_{50} values are defined as the sample concentrations that cause 50% inhibition of MRSA growth and yellow pigment production.

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