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Synthesis of a novel inhibitor against MRSA and VRE: Preparation from zerumbone ring opening material showing histidine-kinase inhibition

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Abstract—Zerumbone ring-opening derivative **2** inhibited autophosphorylation of the essential histidine protein kinase (HPK), YycG, existing in *Bacillus subtilis* constituting a two-component system (TCS). However, it did not inhibit drug-resistant bacterium such as MRSA and VRE. Tryptophan derivative **34** also could be regulated by a TCS system like **2**. In addition, **34** showed good inhibition against MRSA and VRE.

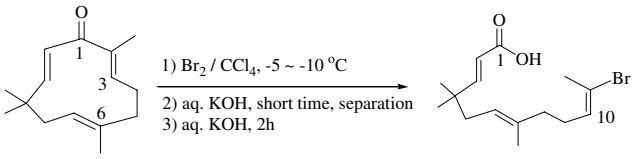
Zerumbone[1] **1**, one of the important natural materials having potent ability as natural materials-related diversity-oriented synthesis "*NMRDOS*"[2], is a monocyclic sesquiterpene. It is the major component of the essential oil of the wild ginger *Zingiber zerumbet* Smith. Not only does zerumbone show attractive reactivity[3a–f], but also zerumbone[4] and its derivatives[5] have a broad array of biological activities.

As shown in Scheme 1, this remarkable ring-cleavage system was shown in previous reports.[3d] Compound **2** was discovered as the first inhibitor of the autophosphorylation of the essential histidine protein kinase (HPK), YycG[5].

HPK, one of the major components in the two-component regulatory system, plays a key role in prokaryotic signal transduction to various environmental stresses. One of the two-component signal transduction systems consists of a histidine-kinase YycG and its cognate response regulator YycF. The YycG–YycF system was found in *Bacillus subtilis*,[6] *Staphylococcus aureus*,[7] and *Streptococcus pneumoniae*.[8] YycG–YycF is also conserved among other Gram-positive pathogen bacteria and is consideredU:/AP/DTD501/BMCL/10984 a novel target for antibacterial agents.

Further investigation of the inhibition activity against drug-resistant bacterium and the preparation of the derivatives for the biological activities resulted in tryptophan derivative **34**. This compound regulated the TCS system like compound **2** and also showed inhibition against Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE).

As shown in Scheme 2 and Table 1, the ring-cleavage product **2** was investigated to regulate the stereoisomers.



zerumbone 1 **2**

Scheme 1.

Keywords: Zerumbone; Inhibitor; Two-component system; Histidine kinase; YycG; Drug-resistant bacterium; MRSA; VRE.
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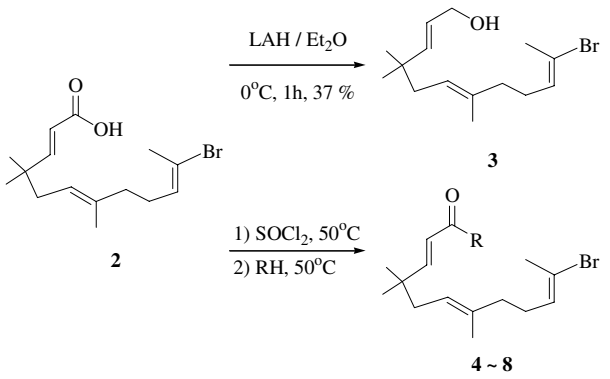


Scheme 2.

Table 1. Syntheses of esters and amides of **2**

Compound	RH	Yield (%)
4	MeOH	92
5	$C_8H_{17}OH$	99
6	$(C_4H_9)_2NH$	94
7	$(C_8H_{17})_2NH$	85
8	Piperidine	79

Treatment of **2** with LAH in dry diethyl ether at 0 °C for 1 h gave corresponding alcohol **3** in 37% yield. Thionyl chloride was added to **2** in various alcohols and amines, and stirred at 50 °C for several hours to afford **4**, **5**, **6**, **7**, and **8** in 79–99% yield.

As shown in Table 2, the derivatives were tested for the inhibition of the autophosphorylation of YycG and for growth inhibition against MRSA and VRE.

YycG inhibition assay. The purified YycG was contained in 50 mM Tris–HCl (pH 7.5), 50 mM KCl, and 10 mM $MgCl_2$. The mixture was then incubated for 10 min at room temperature. The reaction was stopped by an equal volume of 2× sample buffer (120 mM Tris–HCl, pH 6.8, 20% glycerol, 4% SDS, 10% β-mercaptoethanol, and 0.1% BPB) and then analyzed on an SDS–polyacrylamide gel.

MIC assay. The minimum inhibitory concentration (MICs) was determined by the broth microdilution method using Mueller–Hinton broth (Becton–Dickinson and Company, Sparks, MD, USA). MRSA 870307 clinical isolate and VRE NCTC 12201 were used at each inoculum of 10^5 CFU/mL. After incubation at 37 °C for 18 h, MICs were defined as the lowest drug concentrations that prevented visible growth of bacteria.

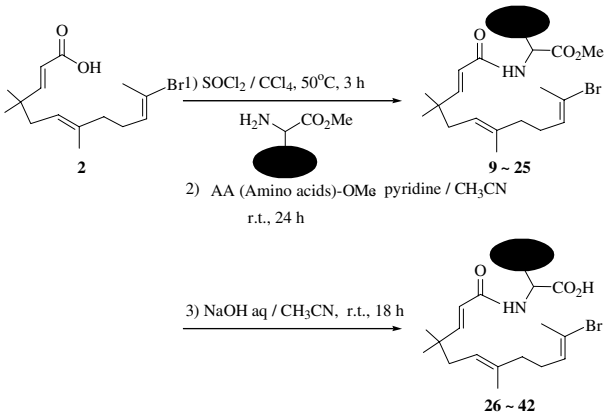
Table 2. IC_{50} and MIC of zerumbone derivatives

Compound	HK[a] inhibition IC_{50} (μg/mL (μM))	MRSA 870307 MIC (μg/mL)	VRE NCTC12201 MIC (μg/mL)
1	>500	>100	>100
2	20.0(63.5)	>100	>100
3	>500	>100	>100
4	>500	>100	>100
5	>500	>100	>100
6	>500	>100	>100
7	>500	>100	>100
8	>500	>100	>100

[a] YycG from *B. subtilis*.

Except for derivative **2**, all analogs including zerumbone **1** did not show any inhibition activity of HPK and growth inhibition against drug-resistant bacterium, MRSA 870307 and VRE NCTC12201. This result suggested that the carboxylic acid group in the inhibitor played a key role in appearance of these inhibition activities. Compound **2** had good inhibition activity for HPK, however, did not show the growth inhibition against MRSA and VRE. The problem of the physical properties like the penetration of the cell membrane might have influenced the evaluation in vivo.

As shown in Scheme 3 and Table 3, 17 amino acid (AA) derivatives of **2** with a methyl ester and carboxylic acid group were synthesized. Compound **2** in CCl_4 was reacted with $SOCl_2$ at 50 °C for 3 h to afford acyl chloride. Then, the resulting intermediate was added to a mixture of AA methyl ester and pyridine in CH_3CN. The



Scheme 3.

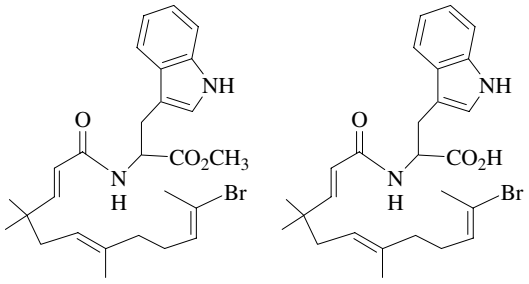
Table 3. Syntheses of amino acid (AA) derivatives

AA	Compound	Yield (%)	Compound	Yield (%)
G	**9**	62	**26**	34
A	**10**	77	**27**	98
V	**11**	92	**28**	73
L	**12**	30	**29**	76
I	**13**	45	**30**	69
F	**14**	68	**31**	90
P	**15**	33	**32**	32
M	**16**	56	**33**	91
W	**17**	64	**34**	93
S	**18**	76	**35**	97
T	**19**	95	**36**	99
N	**20**	82	**37**	91
Q	**21**	87	**38**	93
Y	**22**	92	**39**	38
D	**23**	67	**40**	86
E	**24**	45	**41**	89
H	**25**	40	**42**	67

Table 4. IC50 and MIC of amino acid (AA) derivatives

Compound	HK[a] inhibition IC_{50} (μg/mL(μM))	MRSA 870307 MIC (μg/mL)	VRE NCTC12201 MIC (μg/mL)
9	>500	>100	>100
10	>500	>100	>100
11	>500	>100	>100
12	>500	>100	>100
13	>500	>100	>100
14	>500	>100	>100
15	<250	>100	>100
16	>500	>100	>100
17	>500	>100	>100
18	<250	>100	>100
19	>500	>100	>100
20	500	>100	>100
21	500	>100	>100
22	>500	>100	>100
23	>500	>100	>100
24	>500	>100	>100
25	>500	>100	>100
26	<250	>100	>100
27	<250	>100	>100
28	<250	>100	>100
29	>500	>100	>100
30	>500	>100	>100
31	<250	>100	>100
32	<250	>100	>100
33	<250	>100	>100
34	**22.0 (43.9 μM)**	**100**	**50**
35	<250	>100	>100
36	>500	>100	>100
37	<250	>100	>100
38	<250	>100	>100
39	<250	>100	>100
40	<250	>100	>100
41	<250	>100	>100
42	<250	>100	>100

[a] YycG from *B. subtilis*.



17 **34**

Figure 1. Tryptophan derivatives of **2**.

reaction mixture was stirred at room temperature for 24 h to afford the AA methyl ester derivatives in 30–95% yield. The methyl ester derivatives were hydrolyzed with NaOH aq in CH_3CN at room temperature for 18 h to obtain corresponding carboxylic acid derivatives in 32–99% yield.

As shown in Table 4 and Figure 1, the derivatives were tested for the inhibition of the autophosphorylation of HPK (YycG) and growth inhibition against MRSA and VRE. Many of the carboxylic acid derivatives showed inhibition activities for YycG (<250 μg/mL), but most of the methyl ester derivatives did not show inhibition activities for YycG (>500 μg/mL). In addition, only tryptophan derivative **34** showed activity in the growth inhibition against MRSA and VRE.

The IC_{50} of **34** for YycG was 43.9 μM which was a little bit stronger than that of **2**. The MIC for MRSA and VRE were 100 and 50 μg/mL, respectively. Compound **34** had inhibition activity for YycG in almost parallel with growth inhibition against MRSA and VRE. This result was the first successful example that should get special attention.

These results suggest the most important factor is that the derivatives of **2** have a carboxylic acid group. Even when the new carboxylic acid group from the amino acid was introduced to afford **34**, the inhibition activity of **34** for YycG resulted in the same potential as **2**. When derivative **34** is interacted with YycG, both the hydrophobic part and the carboxylic acid of **34** affect the active site in YycG. It can be suggested that since YycG has a wide space at the active site, the difference in carbon chain length of the inhibitor hardly influences the inhibition for YycG. The important mechanism of the relationship between enzyme and inhibitor at the active site of YycG might be elucidated by computational chemical methods.

Compound **2** did not show any inhibition activity against MRSA and VRE in vivo. However, **34** with the tryptophan moiety showed good inhibition activity against both MRSA and VRE. The emergence of the in vivo biological activity of **34** is a noteworthy topic.

We believe by focusing on the structure–activity relationship of structures **2** and **34** for the inhibition effect of HPK, that this can play an important role in the development of medicine against drug-resistant bacterium.

Acknowledgments

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