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Synthesis and biological activity of 5-(4-methoxyphenyl)-oxazole derivatives

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ABSTRACT

5-(4′-Methoxyphenyl)-oxazole (MPO), originally reported as a synthetic compound, was isolated from fungal culture broth as an inhibitor of hatch and growth of *Caenorhabditis elegans*. Nineteen MPO derivatives were chemically synthesized, but showed no effect on *C. elegans* hatch and growth. These findings strongly suggested that the whole structure of MPO is essential for anti-*C. elegans* activity.

Small-molecule inhibitors of protein function are powerful tools for biological analysis[1] and can lead to the development of new drugs. It is important in chemical biological study to characterize the targets of small molecules with biological functions. Recently, the nematode *Caenorhabditis elegans* has been established as a useful model organism for studying developmental and metabolic processes.[2,3] The whole genome sequence of *C. elegans* was completed;[4] therefore we focused on the phenotypic abnormality of *C. elegans* caused by a small molecule, and started forward genomic screening for microbial metabolites which caused the phenotypic abnormality of *C. elegans* to obtain small molecules with a new mechanism of action. In our previous study, we screened a library of 315 known microbial compounds and 9,156 culture broths for small molecules that induced phenotypic abnormality of wild-type *C. elegans* and identified 7 active compounds.[5] Among them, we focused on 5-(4′-methoxyphenyl)-oxazole (MPO; **1**) for two reasons; (1) compound **1** was isolated from fungal metabolites for the first time, and (2) **1** showed unique activity in our screening assay using C. *elegans*. Namely, **1** caused abnormal arrangement of germ cells and inhibited the hatch of eggs newly spawned by adult *C. elegans* (100% hatch inhibition) at 17 μM on day 3. Moreover, the growth of *C. elegans* stopped at the L1 stage (egg to Larvae 1 stage; it takes 8 hours from the laying of egg) on day 3 after incubation in the presence of **1** at 17–100 μM without growth inhibition of *Escherichia coli*. There are few information about **1**. It was reported to be an octopamine receptor agonist.[6] However, octopamine (1.0 mM) had no effect on hatch and growth of *C. elegans*. In this study, twenty MPO derivatives were synthesized and their biological activities including effect on phenotype of *C. elegans* were investigated.

To investigate structure–activity relationships (SAR) of the MPO derivatives, regioisomers **2**–**4**, oxazole derivatives **5**–**15** with chemical modifications at the 5-position, oxazole derivatives **16**–**18** with an aryl group at the 2-position, a thiazole **19** and an imidazole **20** were synthesized according to the known procedures.[7,8] Most derivatives (except for **4**,[9] **16**,[10] **19**[11] and **20**[12]) were constructed by the coupling reaction of the corresponding aldehydes with *p*-toluenesulfonylmethylisocyanide (TosMIC) as shown in Scheme 1.

The effect of the synthetic MPO derivatives (**1**–**20**) on phenotype of *C. elegans* was tested[5,13] (see Supplementary data). As shown in Figure 1a and b, germ cells were orderly arranged and normal eggs were spawned in control *C. elegans*, respectively. However, synthetic **1** was found to cause a growth inhibition at 17–100 μM (data not shown) and abnormal arrangement of germ cells of *C. elegans* at 17 μM (Fig. 1c). And newly spawned eggs appeared labile and the contents seep out (Fig. 1d). These abnormality (growth inhibition and hatch inhibition) caused by synthetic **1** was the same as those caused by natural **1**. Among the derivatives, only **12** showed moderate hatch inhibition of *C. elegans* at 100 μM. However, the other derivatives lost the growth inhibition and the hatch inhibition of *C. elegans* (Table 1). For example, derivative **6** (OCH_3 moiety in **1** was replaced with OH in **6**) showed no growth

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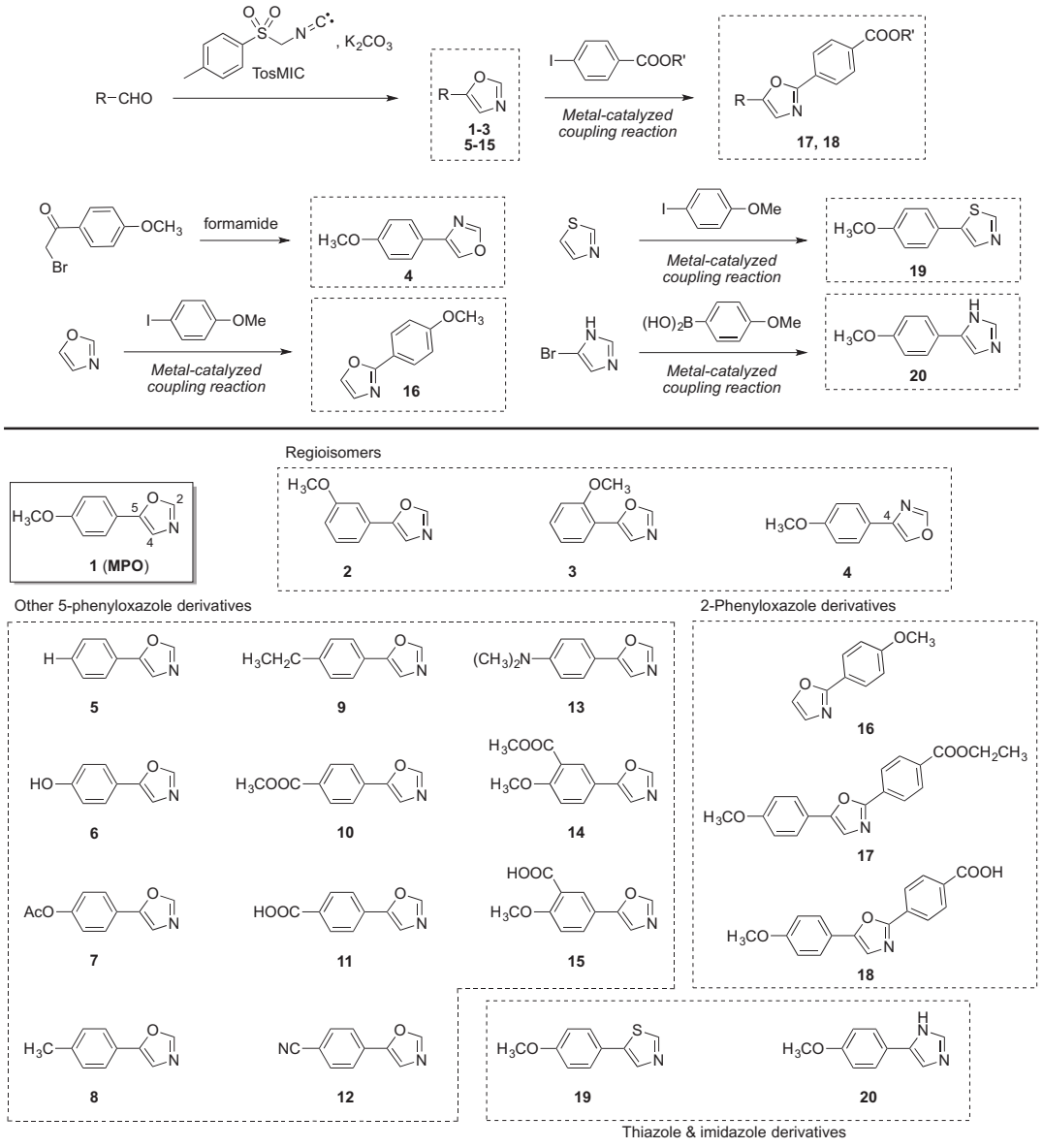

Scheme 1. Synthetic methods of MPO derivatives.

inhibition and no hatch inhibition of *C. elegans* even at 100 μM. Derivatives **13**, **16** and **19** showed weaker growth inhibition than **1**.

Therefore, other biological activities were compared between active **1** and inactive derivative **6**, and the results are summarized in Table 2. Regarding the antimicrobial activity (see Supplementary data),[14,15] both **1** and **6** showed antifungal activity only against *Magnaporthe oryzae* with EC_{50} values of 4.0 and 15 μM, respectively. They exhibited no activity against *E. coli*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and *Candida albicans* even at 100 μM. Both **1** and **6** showed no activity against *P. falciparum* K1 even at 100 μM. Regarding the cytotoxicity, **1** and **6** showed very weak activity against HeLa cells with EC_{50} values of 30 and 51 μM, respectively. Furthermore, previous study demonstrated that *C. elegans daao-1* gene encode a functional D-amino acid oxidase (DAO), while the *ddo-1*, *ddo-2*, and *ddo-3* genes encode functional D-aspartate oxidases (DDOs; DDO-1, DDO-2, and DDO-3, respectively), and these enzymes were found to play important roles in the development and maturation of germ cells in *C. elegans*.[16,17] Therefore, it was investigated whether or not **1** and **6** inhibited *C. elegans* DAO and DDO activity (see Supplementary data),[18] However, both **1** and **6** showed no inhibitory activity of them even at 100 μM (Table 2).

In conclusion, we first confirmed that synthetic **1** showed the same unique activity in *C. elegans* (Fig. 1) as well as natural **1** previously isolated from the fungal culture broth, and have demonstrated a SAR and biological activity for MPO derivatives. All MPO derivatives, except for **12**, showed no hatch inhibition of *C. elegans* although the 4-cyano derivative **12** exhibited weaker hatch inhibition than **1**. Moreover, derivatives **13**, **16** and **19** exhibited weaker growth inhibition than **1**. These results revealed that the whole structure of **1** is essential and is optimal for growth and hatch inhibitions of *C. elegans*. Thus, **1** seems to act on a confined and tight space of the active site, although its molecular target remains elusive.

Next, we compared other biological activities of **1** and inactive derivative **6**. The structural difference is that the methoxy residue in **1** was replaced with the hydroxy residue in **6**. Both compounds showed very similar biological (anti-*M. oryzae* and cytotoxicity

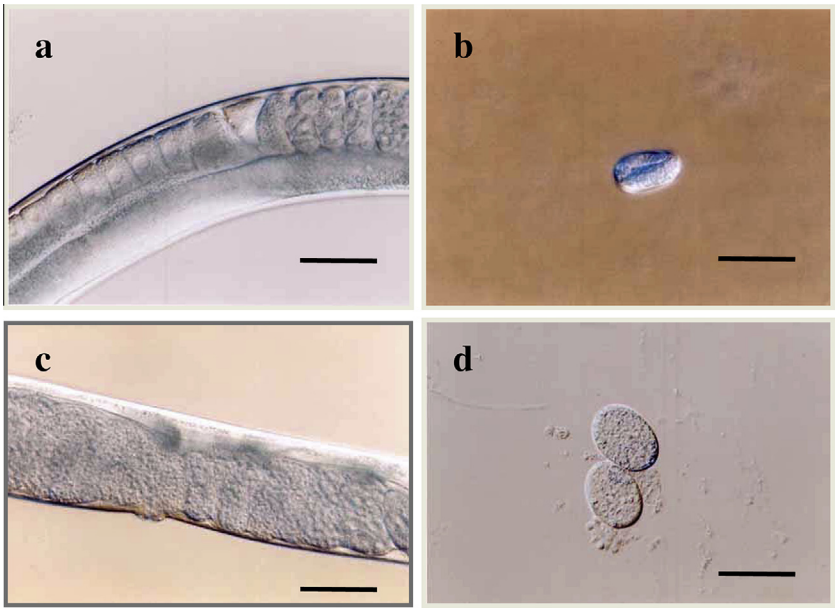

Figure 1. Effect of synthetic **1** and derivative **12** on phenotype of *C. elegans*. Representative images of *C. elegans* incubated in the presence of **1** on day 3 obtained from a microscope. (a) Control (MeOH): germ cells; (b) control (MeOH): one newly spawed egg; (c) **1** (17 μM): germ cells; (d) **1** (17 μM): two newly spawned eggs. Scale bars represent 50 μm.

Table 1
Inhibitory activities of MPO derivatives against growth and hatch of *C. elegans*

		MIC (μM)			MIC (μM)	
		Growth inhibition	Hatch inhibition		Growth inhibition	Hatch inhibition
1	(MPO)	34	17	**11**	>100	>100
2		>100	>100	**12**	>100	100
3		>100	>100	**13**	50	>100
4		>100	>100	**14**	>100	>100
5		>100	>100	**15**	>100	>100
6		>100	>100	**16**	100	>100
7		>100	>100	**17**	>100	>100
8		>100	>100	**18**	>100	>100
9		>100	>100	**19**	50	>100
10		>100	>100	**20**	>100	>100

Table 2
Biological activities of **1** and **6**

Assay system		EC_{50} (μM)	
		1	**6**
Bacteria			
	Escherichia coli	>100	>100
	Staphylococcus aureus	>100	>100
Fungi			
	Aspergillus fumigatus	>100	>100
	Candida albicans	>100	>100
	Magnaporthe oryzae	4	15
Malaria			
	Plasmodium falciparum K1	>100	>100
Mammalian cell lines			
	HeLa cells	30	51
	HL60 cells	>100	>100
Caenorhabditis elegans			
	D-Amino acid oxidase (DAO)	>100	>100
	D-Aspartate oxidase-1 (DDO-1)	>100	>100
	D-Aspartate oxidase-2 (DDO-2)	>100	>100
	D-Aspartate oxidase-3 (DDO-3)	>100	>100

against HeLa cell) activities, and their potency was analogous. These findings suggested that the structure of 5-phenyloxazole has a common molecule target in *M. oryzae* and HeLa cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.042.

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