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1,3,4-Oxadiazoline derivatives as novel potential inhibitors targeting chitin biosynthesis: Design, synthesis and biological evaluation

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ABSTRACT

Two series of 1,3,4-oxadiazoline heterocycle derivatives were designed, synthesized and identified. Bioactivity assays showed that all synthesized compounds inhibited chitin synthesis in yeast, suggesting they might be a novel class of potential inhibitors against chitin biosynthesis. The structure–activity relationships (SAR) of these compounds are discussed.

Chitin $(C_8H_{13}O_5N)_n$ is a linear polysaccharide (Fig. 1) chain consisting of more than thousands of *N*-acetylglucosamine (GlcNAc, 2-acetamido-2-deoxy-D-glucose) residues joined by β-1,4 glycosidic bonds.[1–3] It is widely distributed in invertebrates, anthropods and is an integral component in cell walls of fungi. Since it is absent from plants and vertebrates, the biosynthesis of chitin is believed to be a target pathway for the development of novel fungicides.[4,5] Biosynthesis of chitin is dependant on chitin synthase, which use uridine diphosphoryl-*N*-acetylglucosamine (UDP-GlcNAc) as substrate donor to synthesize β-1,4-linked *N*-acetyl-D-glucosamine (GlcNAc) (Fig. 1).[6–9] Chitin synthase is undoubtedly an ideal target for the development of drugs, pesticides and fungicides. However, the structure of the active site of membrane-bound chitin synthase is unknown due to its difficulties of the isolation and purification. Therefore most ‘rational designed’ drugs claimed to be chitin synthase inhibitors are more likely acting toward chitin biosynthesis.[4,10]

The investigation on chitin biosynthesis inhibitors could be dated to the discovery of fungicides such as the peptidyl nucleosides polyoxins and nikkomycins,[11,12] which are analogs of substrate donor, UDP-GlcNAc (Fig. 2). Structural modifications of these compounds have been reported, but with few success.[13–21] Very recently, other small organic molecules (Fig. 3) without any structural resemblance to UDP-GlcNAc, have been reported to have inhibitory activity toward chitin biosynthesis.[22–26] Most of these compounds are presumed to function through non-competitive inhibition, meaning that they bind reversibly to the sites away from the catalytic site of chitin synthase.[27] All the novel compounds are potential chitin synthase inhibitors whereas they are not chemically related. A common structural feature of these inhibitors is benzene rings or aromatic nitrogenated moieties, but fine details of the inhibitory mechanism of these candidates remain elusive and general rules cannot be drawn. On the other hand, their modest antifungal activities imply that further investigation would be needed.

An oxadiazole ring that improves pharmacokinetic and in vivo efficacy is often observed, which is known to be due to its higher hydrolytic and metabolic stability. Oxadiazole heterocycles are therefore important structural motifs in the design of novel drugs.[28–32] One example is DOW416 (Fig. 4),[33] which interferes with the formation of insect tissue and epicuticle and thus is considered to be a chitin synthase inhibitor. Other examples such as oxadiazolyl 3(2*H*)-pyridazinones derivatives (Fig. 4) with antifeedant activity, are presumed to affect chitin biosynthesis. It is believed that the oxadiazole moiety may block the incorporation of *N*-acetylglucosamine into newborn chitin chain.[34–36]

In this paper, two series of 1,3,4-oxadiazolines derivatives **C1–10** and **C11–20** (Scheme 1) were designed and synthesized. Since the oxadiazoline moiety may affect chitin biosynthesis, their inhibitory activities against yeast chitin synthase were expected. Their bioactivities indicated that they might be novel chitin biosynthesis inhibitors.

The target compounds 3-acetyl-5-aryl-2,3(2*H*)-1,3,4-oxadiazoles **C1–10** and 5-aryl-1,3,4-oxadiazole-3(2*H*)-carboxamide

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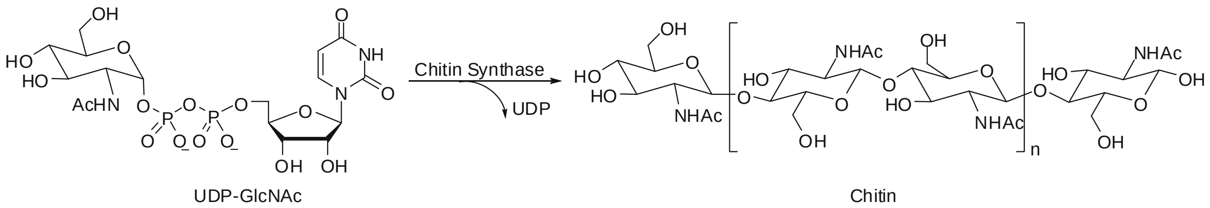


Figure 1. The biosynthesis of chitin. UDP-GlcNAc is as GlcNAc donor and chitin synthase is responsible for glycosidic bond.

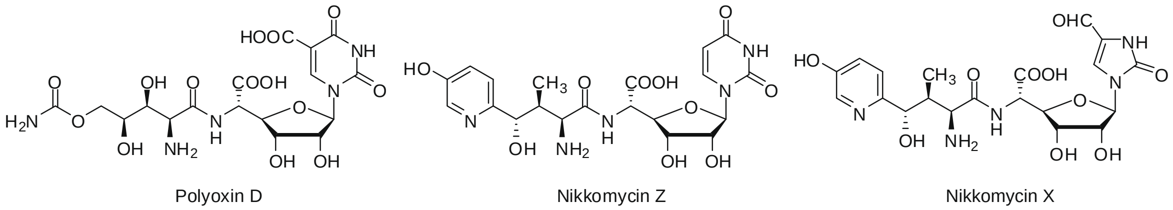


Figure 2. Representative structures of naturally occurring polyoxins and nikkomycins.

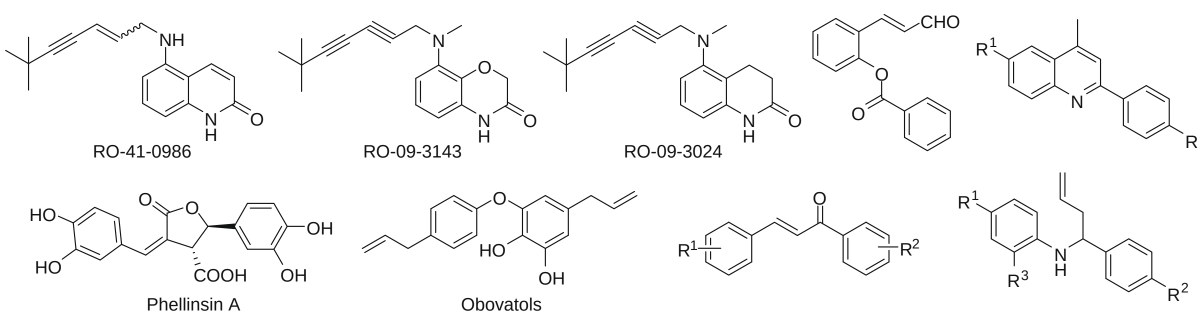


Figure 3. Some non-nucleoside compounds as chitin biosynthesis inhibitors.

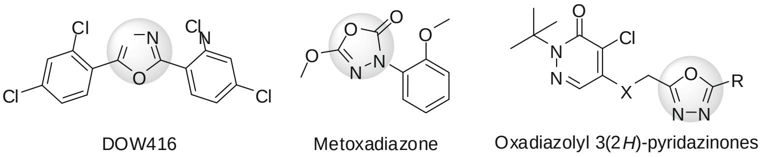
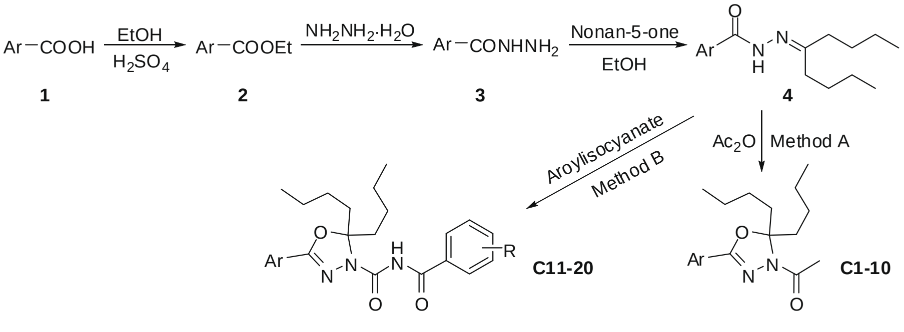


Figure 4. Bioactive compounds containing 1,3,4-oxadiazole moiety.



Scheme 1. Synthesis of 1,3,4-oxadiazolines derivatives **C1–10** and **C11–20**.

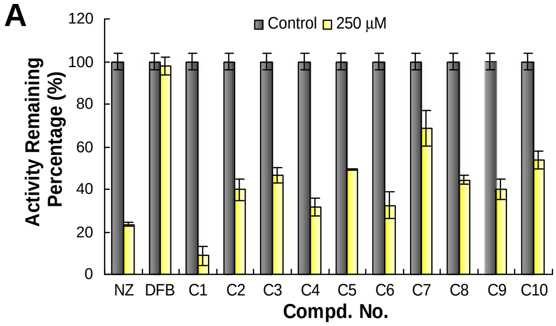
derivatives **C11–20** were prepared as depicted in Scheme 1. The condensation reaction between acylhydrazides **3** and nonan-5-one led to the formation of the important building blocks, the substituted aroylhydrazones **4**. The intermediates aroylhydrazones **4** undergo convenient heterocyclization reaction in the presence of acetic anhydride to provide 3-acetyl-5-aryl-2,3(2*H*)-1,3,4-oxadiazoles **C1–10** using method A. For synthesis of **C11–20** by method B, the reaction of substituted aroylhydrazone **4** with various substituted benzoylisocyanate was at about 75–80 °C in toluene for about 0.5–1.5 h to obtain the target compounds **C11–20** characterized as

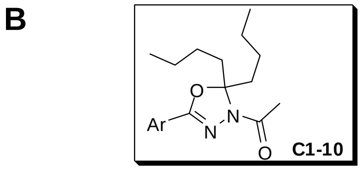
N-(substituted-benzoyl)-1,3,4-oxadiazole-3(2*H*)-carboxamide. All the structures were determined by ^{1}H NMR, ^{13}C NMR, IR, EIMS and high-resolution mass spectra (HR-MS) data.[37]

The inhibition activity against chitin biosynthesis was assayed using yeast cell extracts by the modified procedure described by Lecuro and Bulik.[38] All these compounds were evaluated. Nikkomycin Z (NZ), the well-known fungicide isolated from *Treptomyces tendae*, was applied as positive control. And diflubenzuron (DFB), the well-known insecticide, was used as negative control. Figures 5 and 6 indicated all the compounds, **C1–10** and **C11–20**, were bioactive.

To explore the influence of structural changes on activity, we introduced various electron-withdrawing groups (such as halogen atoms) and electron-donating substituents (Me, MeO, Et, etc.) into the aromatic ring. As shown in Figure 5, all the synthesized compounds 3-acetyl-5-aryl-2,3(2*H*)-1,3,4-oxadiazoles **C1–10** displayed moderate to high inhibitory activities on chitin biosynthesis at 250 μM, and compounds **C1**, **C4**, and **C6** exhibited significant inhibitory effect on enzyme activity up to 91%, 69%, and 68% at 250 μM, respectively. Among all the compounds containing electron-withdrawing groups, the compound containing *o*-chloro-substituent **C1** is the comparably highest potent, which led to 91% inhibition. This may be due to the incorporation of halogen atoms into the aromatic ring that increases lipophilicity. Nevertheless, position changes of the chloro-substituent within the same ring (compounds **C1** and **C4**) dramatically changed the inhibition activity, suggesting that the presence of a group in the *ortho*-position of benzene ring attached to oxadiazoline heterocycle could introduce important steric effects. The compound containing *ortho*-position substituent aromatic ring may increase the potent activity. On the other hand, methyl group bound to benzene rings in compound **C6** is better for inhibitory activity than alkyl- or alkoxy-substituted analogs. However, analogs with naphtalene and furane as aromatic ring (compounds **C9**, **C10**) showed moderate inhibitory activities.

In order to improve the physicochemical properties, we introduced a urea moiety known as an important structural element for bioactivities,[39–44] into 1,3,4-oxadiazolines heterocyclic structure. Figure 6 shows the effect on chitin biosynthesis by 1,3,4-oxadiazole-3(2*H*)-carboxamide derivatives **C11–20**. Compounds **C13**, **C15, C19** and **C20** exhibited moderate inhibition activities at the concentration of 250 μM, while compound **C15** exhibited the highest inhibitory activity (about 86%). Other compounds displayed relatively weak inhibition. Comparing with compounds **C11** and **C4**, the introduction of acylurea moiety in **C11** significantly decreased the inhibitory activity. **C11–20** possess structural moiety similar to that of diflubenuron, which might explain their relatively lower activity. As for the substituents on two aromatic rings when the substituent R is 2,6-difluoro-substituent, the compound bearing 3,5-dimethylphenyl (**C20**) is more favorable for inhibition activities, which is in agreement with compound **C6**. Furthermore,

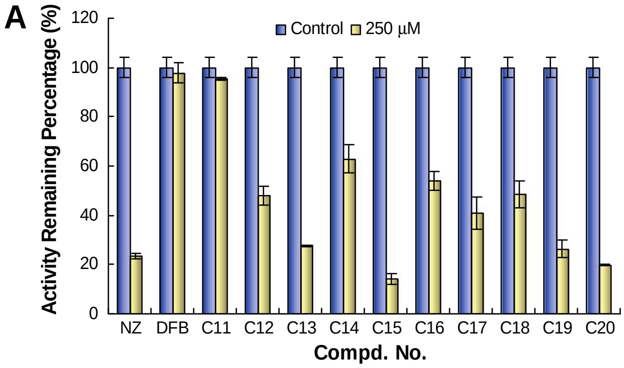


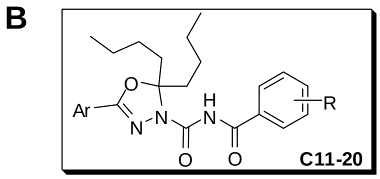


C1-10

Compd. No.	Substituents Ar	Compd. No.	Substituents Ar
C1	*o*-Cl-C_6H_4	**C6**	3,5-Me_2-C_6H_3
C2	3,4,5-$(MeO)_3$-C_6H_2	**C7**	*p*-Et-C_6H_4
C3	3-MeO-C_6H_4	**C8**	3,4-OCH_2O-C_6H_3
C4	*p*-Cl-C_6H_4	**C9**	Naphthalen-2-yl
C5	*p*-F-C_6H_4	**C10**	Furan-2-yl

Figure 5. The structures and relative activity relationships of 1,3,4-oxadiazolines derivatives **C1–10**. (A) the activity remaining in the presence of compounds; (B) the structures of compounds.





C11-20

Compd. No.	Substituents Ar	 R
C11	*p*-Cl-C_6H_4	2,6-F_2
C12	3,4,5-$(MeO)_3$-C_6H_2	2,6-F_2
C13	3,5-Me_2-C_6H_3	*o*-Cl
C14	3,4-OCH_2O-C_6H_3	*o*-Cl
C15	2,4-Cl_2-C_6H_3	*o*-Cl
C16	3,4-OCH_2O-C_6H_3	2,6-F_2
C17	2,4-Cl_2-C_6H_3	2,6-F_2
C18	Furan-2-yl	2,6-F_2
C19	2,4-Cl_2-C_6H_3	2,3,5-F_3-4-MeO
C20	3,5-Me_2-C_6H_3	2,6-F_2

Figure 6. The structures and relative activity relationships of 1,3,4-oxadiazole-3(2*H*)-carboxamides derivatives **C11–20**. (A) the activity remaining in the presence of compounds; (B) the structures of compounds.

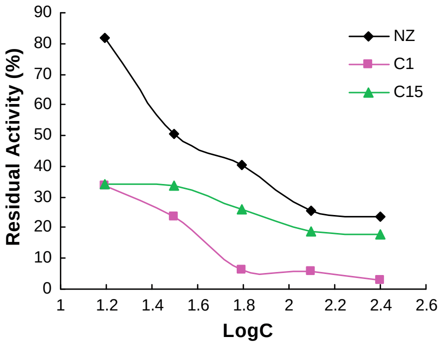


Figure 7. Semi-logarithmic plot of the inhibitory activity of compounds **C1** and **C15** against chitin biosynthesis.

compounds containing electron-withdrawing group such as 2,4-dichlorophenyl (**C15** and **C19**) is more efficient than those with alkoxy-substituents on the benzene ring. However, compound **C11** containing *p*-chlorophenyl showed lower activity, suggesting that the *ortho*-position steric effects of aromatic ring attached to oxadiazoline ring were important for inhibitory activity.

Since compounds **C1** and **C15** displayed the highest inhibitory activity, five serial dilutions were further tested for enzyme activity. As indicated in Figure 7, their inhibitory effects on chitin biosynthesis were concentration-dependent. Compounds **C1** and **C15** exhibited significant inhibition against chitin biosynthesis with the IC_{50} values of 7.93 and 5.60 μM, respectively.

In conclusion, the novel 1,3,4-oxadiazolines derivatives **C1–10** and **C11–20** were designed as potential antifungal reagents, which interfered with chitin biosynthesis. Compounds **C1** and **C15** showed the highest inhibitory activity at lower concentration. The understanding of structure–activity relationship and assay of these compounds may provide some insights into the rational design of new inhibitors of chitin biosynthesis.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.11.095.

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37. The selected analytical data of representative compounds **C1** and **C15** were as follows: **C1**. This compound was obtained as light yellow liquid following the standard procedures, yield 76%, IR (KBr): $\nu = 1665$ (C=O), 1617 (C=N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.78$ (dd, $^3J = 7.6$ Hz, $^4J = 2$ Hz, 1H, Ph-H), 7.50 (dd, $^3J = 8.2$ Hz, $^4J = 1.2$ Hz, 1H, Ph-H), 7.33–7.43 (m, 2H, Ph-H), 2.42–2.46 (m, 2H, CH_2), 2.34 (s, 3H, $COCH_3$), 1.94–2.02 (m, 2H, CH_2), 1.27–1.44 (m, 8H, CH_2), 0.90 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.29$, 152.93, 133.30, 131.67, 131.22, 130.40, 126.71, 124.00, 104.86, 36.06, 24.70, 22.39, 22.26, 13.98; MS: $m/z = 336$ (M^+), 279, 237, 210, 139, 111; EI-HRMS: calcd for $C_{18}H_{25}ClN_2O_2$ (M^+), 336.1605; found, 336.1605; **C15**. This compound was obtained as white solid following the standard procedures, yield 64%, mp 104.3–105.7 °C; IR (KBr): $\nu = 3362$ (N–H), 1710, 1691 (C=O), 1625 (C=N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.36$ (s, 1H, N–H), 7.74 (d, $J = 8.8$ Hz, 1H, Ph-H), 7.66 (d, $J = 7.2$ Hz, 1H, Ph-H), 7.54 (d, $^4J = 2$ Hz, 1H, Ph-H), 7.41–7.44 (m, 2H, Ph-H), 7.35–7.40 (m, 2H, Ph-H), 2.34–2.42 (m, 2H, CH_2), 1.96–2.05 (m, 2H, CH_2), 1.25–1.41 (m, 8H, CH_2), 0.89 (t, $J = 7$ Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.79$, 152.10, 146.04, 137.79, 134.76, 134.14, 131.78, 131.30, 131.08, 130.47, 129.99, 129.91, 127.34, 127.08, 121.62, 105.08, 36.33, 24.62, 22.31, 13.97; MS: $m/z = 509$, 328, 286, 271, 244, 180, 139, 111, 75; EI-HRMS: calcd for $C_{24}H_{26}Cl_3N_3O_3$ (M^+), 509.1040; found, 509.1025.
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