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Research paper

# Synthesis and biological evaluation of novel phosphoramidate derivatives of coumarin as chitin synthase inhibitors and antifungal agents

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ABSTRACT

A series of novel phosphoramidate derivatives of coumarin have been designed and synthesized as chitin synthase (CHS) inhibitors. All the synthesized compounds have been screened for their chitin synthase inhibition activity and antimicrobial activity in vitro. The bioactive assay manifested that most of the target compounds exhibited good efficacy against CHS and a variety of clinically important fungal pathogens. In particular, compound **7t** with $IC_{50}$ of 0.08 mM against CHS displayed stronger efficiency than the reference Polyoxin B with $IC_{50}$ of 0.16 mM. In addition, the apparent $K_i$ values of compound **7t** was 0.096 mM while the $K_m$ of Chitin synthase prepared from *Candida tropicalis* was 3.86 mM for UDP-N-acetylglucosamine, and the result of the $K_i$ showed that the compounds was a non-competitive inhibitor of the CHS. As far as the antifungal activity is concerned, compounds **7o**, **7r** and **7t** were highly active against *Aspergillus flavus* with MIC values in the range of 1 μg/mL to 2 μg/Ml while the results of antibacterial screening showed that these compounds have negligible actions to the tested bacteria. These results indicated that the design of these compounds as antifungal agents was rational.



## 1. Introduction

Invasive fungal infections have become alarming recently due to the high morbidity and mortality rates in patients who received stem cell transplantation, antineoplastic chemotherapy, organ transplant or suffered human immunodeficiency virus (HIV) infection [1]. Many fungal infections are caused by opportunistic pathogens from *Candida*, *Cryptococcus* and *Aspergillus* [2]. However, effective and safe antifungal agents are very limited. Representative antifungal agents used in Clinical therapy include triazoles (*i.e.* fluconazole, voriconazole, itraconazole and posaconazole), polyenes (*i.e.* amphotericin B, nystatin), glucan synthesis inhibitors (*i.e.* echinocandins, caspofungin, micafungin, anidulafungin), chitin synthesis inhibitors (*i.e.* nikkomycin, polyoxins) and flucytosine, but each of them had certain limitation [3–6]. Furthermore, as the increasing emergence of multidrug-resistant strains, intractable pathogenic microorganisms and newly arising pathogens [7], there is a continuous demand for the discovery of novel antifungal agents to treat fungal infections.

The fungal cell wall is a unique organelle and required for the growth and the maintenance of osmotic stability of the cell [8]. Chitin is an important structural component of the cell wall of many fungi, which is a linear β-(1–4)-linked polymer of N-acetyl-glucosamine (GlcNAc) and responsible for imparting shape, strength and rigidity to the cell wall. Chitin synthase (CHS) plays an important role in the process of biosynthesis of chitin that is absent in plant and human [9,10]. Thus the chitin synthase is a valuable and attractive target to design new fungicide [11]. The earliest inhibitors of chitin synthase are the naturally occurring polyoxins and nikkomycins (Fig. 1), which possess some of structural features of the natural substrate UDP-GlcNAc. They are the most potent chitin synthase inhibitors [12,13]. However, despite excellent in vitro results, clinical utility of these inhibitors is compromised by their metabolic instability and poor cellular uptake, resulting in a

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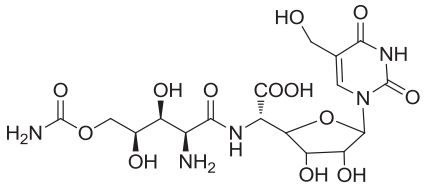
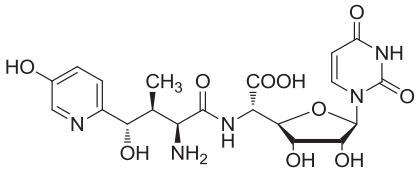



Polyoxin B




Nikkomycin Z

**Fig. 1.** The structures of chitin synthase inhibitor Polyoxin B and Nikkomycin Z.

decrease in efficacy. In addition, many derivatives of nikkomycins and polyoxins were designed and synthesized as natural substrate analogs, transition state mimesis or mechanistic inhibitors through changing some features of these molecules. These changes can be grouped into several categories, including changes of the terminal amino acid, the nucleoside moiety and the bridging unit of them. But none of these analogues has entered into clinical trial [14]. Therefore, there is a really perceived need to develop novel compounds with completely distinct skeleton structure as new CHS inhibitors with better antifungal activities, which are much desired.

Coumarin and its derivatives, a class of quite important lactones compounds containing a structure of benzene ring and α-pyrone, occupy an important position in medicinal chemistry, which have latent ability to exert noncovalent interactions (π-π, hydrophobic, electrostatic interactions, hydrogen bonds, metal coordination and van der Waals force *etc.*) with the various active sites in organisms [15]. As medicines, many of them display a wide range of bioactivities such as antibacterial, antifungal [16], anticoagulant [17], antioxidant [18], anti-inflammatory [19], analgesic [20], anticancer [21], anti-HIV [22] and antiviral [23] efficacies. For instance, the 7-substituted coumarin (**I**) showed a stronger efficacy against *Fusarium oxysporum* with MIC value of 19 μg/mL [24]; the 4-chloro-3-thiadiazole-imino coumarin (**II**) exhibited significant activity against *Candida albicans* with inhibitory zone diameter of 15 mm in 10 μg/mL [25]; and the 4-azidomethyl coumarin sulfonamides derivatives (**III**) showed excellent antifungal efficacies against *C. albicans* and *F. oxysporum* with MIC values of 1 μg/mL, which were 8 times more potent than fluconazole (MIC = 8 μg/mL) [26]. Hence, the diverse biological nature of coumarin derivatives has made it become a privileged structure in medicinal chemistry and much attention has been focused on the synthesis of numerous substituted coumarin derivatives.

The substituted phosphoramidates, as a class of orgnophosphorus compounds, possess relative low stability and rapid metabolic breakdown favorable properties in plants, animals, soil, and other components in environment, which have been used as impressive frameworks in drug and prodrug design in a number of fields [27,28]. Moreover, phosphoramidate moiety was widely used to enhance the water solubility of moleculars that had been proven to exhibit a wide range of biological activities such as anticancer [29], antiviral [30,31], anti-HIV [32], and antimicrobial [33,34] activities. For example, Bis(N,N-dimethylamino) pentachlorophenyl phosphate (**IV**) as organophosphorus fungicide showed moderate efficacy against *Blumeria graminis*; Phosphonamidate derivatives of 6-chloropurine (**V**) had good antifungal activity against *C. albicans* with inhibitory zone diameter of 16.5 mm in 100 μg/mL; N-aryl-*O*-ethyl phosphorodiamidates (**VI**) exhibited moderate antifungal activity against *Fusarium solani* and *Rhizoctonia solani*. (Fig. 2).

Keeping the biological importance of phosphonamidates and coumarin derivatives in mind and as part of our continuing research on the development of new chitin synthase inhibitors, we combined coumarin moiety and arylalkoxy-amino acid phosphoramide moiety with N-methylethanolamine which can afford a distance of 2–3 atoms between the two moieties to produce novel phosphoramidate derivatives of coumarin in hope of obtaining better chitin synthase inhibitors. Herein we report the screening results of chitin synthase inhibition activity and antifungal activity in vitro of the synthesized compounds.

## 2. Results and discussion

### 2.1. Chemistry

4-Hydroxycoumarin derivatives (**2a-e**) were synthesized from commercially available substituted phenol which combined with malonic acid in the presence of zinc chloride in phosphorus oxychloride at 65 °C in 41–66% yields [35]. Chlorination of compounds **2a-e** with phosphorus oxychloride gave 3-chloro-substitutedcoumarins (**3a-e**) in 60–80% yields in the presence of triethylamine at 65 °C [36]. Then the compounds **4a-e** were prepared by combining compounds **3a-e** with 2-(methylamino) ethanol in acetontrile at 65 °C in high yields ranging from 63% to 90% [37]. See in Scheme 1.

The amino acid ester salts (**5a-d**) were synthesized from the appropriate amino acid and thionyl chloride [38], which was showed in Scheme 2. The coupling with the appropriate amino acid ester salt (**5a-d**) and phenyl dichlorophosphate had been performed in the presence of $Et_3N$, giving the product (**6a-d**) as an oil. Finally the target compounds **7a-t** were obtained through coupling the compounds **4a-e** with **6a-d**, using *tert*-butylmagnesium chloride ($^tBuMgCl$) as a coupling reagent and THF as a solvent [39] (Scheme 3).

All the compounds were characterized by $^1H$ NMR, $^{13}C$ NMR and Mass spectra. In the $^1H$ NMR of **2a-e**, the singlet at δ12.30–12.60 ppm represented the OH group at C-4 which was disappeared after chlorination in the NMR spectrum of **3a-e**, while the singlet at δ5.50–5.65 ppm represents the CH group at C-3 which was shifted to δ6.45–6.60 ppm due to the substitution of OH group with Cl. In the $^1H$ NMR spectrum of **4a-e**, the singlet at δ2.00–2.11 ppm for one proton and singlet at δ3.00 ppm for three protons indicated presence of OH group and N–$CH_3$ group respectively. In the $^1H$ NMR spectrum of **7a-t**, the singlet at δ2.95–3.05 ppm and δ3.60–3.70 ppm for three protons each indicated presence of N–$CH_3$ group and $COOCH_3$ group respectively and the NH group was confirmed at δ2.01 ppm, which were further confirmed by their $^1H$ NMR,$^{13}C$ NMR, $^{31}P$ NMR, HRMS spectra.

### 2.2. Biological activity

#### 2.2.1. Chitin synthase inhibitory activity

All the newly synthesized compounds **7a-t** were evaluated for their in vitro chitin synthase inhibitory activities in comparison with commercially available polyoxin B as standard drug [40–42]. The CHS inhibitory activities were summarized in Table 1 and Fig. 3.