Send Orders of Reprints at bspsaif@emirates.net.ae

222 *Mini-Reviews in Medicinal Chemistry*, 2013, *13*, 222-236

Chitin Synthase Inhibitors as Antifungal Agents

Preeti M. Chaudhary, Santosh G. Tupe and Mukund V. Deshpande*

Biochemical Sciences Division, National Chemical Laboratory, Pune 411 008, India

Abstract: Increased risk of fungal diseases in immunocompromised patients, emerging fungal pathogens, limited repertoire of antifungal drugs and resistance development against the drugs demands for development of new and effective antifungal agents. With greater knowledge of fungal metabolism efforts are being made to inhibit specific enzymes involved in different biochemical pathways for the development of antifungal drugs. Chitin synthase is one such promising target as it is absent in plants and mammals. Nikkomycin Z, a chitin synthase inhibitor is under clinical development. Chitin synthesis in fungi, chitin synthase as a target for antifungal agent development, different chitin synthase inhibitors isolated from natural sources, randomly synthesized and modified from nikkomycin and polyoxin are discussed in this review.

Keywords: Antifungal agents, Chitin synthase, Chitin synthase inhibitors, Nikkomycin, Peptidyl nucleoside antibiotics, Polyoxin.

INTRODUCTION

In recent years, the increase in the number of immunocompromised hosts such as patients with AIDS, autoimmune diseases, burns, radiotherapy, chemotherapy and transplantation has resulted in increase in the fungal infections [1]. The situation has led to change in epidemiology and emergence of fungal pathogens which were previously considered clinically insignificant [2-4]. Most infections are caused by *Candida albicans* and *Aspergillus* sp. (*A. fumigatus* and *A. flavus*). However, in the last 2-3 decades, their percent share has declined due to emergence of pathogenic fungi which include - *Acremonium* sp., *Acremonium terreus, Blastomyces dermatitidis,* non-albicans *Candida* sp. (*C. glabrata*, *C. parapsilosis*, *C. krusei*), *Coccidioides immitis, Cryptococcus neoformans, Fusarium* sp., *Histoplasma capsulatum, Microsporum* sp., *Paecilomyces* sp., *Penicillium marneffei, Rhizopus oryzae, Trichoderma* sp., *Trichophyton* sp., and *Trichosporon* sp., etc. [5-8]. Apart from the increased number of immuno compromised hosts, development of resistance against antifungals has aggravated the situation [9]. Therefore, there is a constant need for antifungal agents with new target specificities. Currently, five classes of compounds are in use to treat systemic mycoses (Table **1**).

Intensive use of ergosterol synthesis inhibitors for many years has resulted in resistance development. Secondly, due to the drawbacks of existing drugs such as acute and chronic side-effects, less clinical efficiency, effect on non-targeted cells; the development of new antifungal agents is of major importance (For more details on current status of antifungal drug development and challenges in the treatment of fungal infections, please refer [10-12]). To address above-mentioned issues and challenges, synthesis of different molecules such as triazole linked β-lactam bile acid conjugates, their dimmers, tetrapeptide linked cholic acid derivatives, etc. have also been attempted [13-15]. Alternatively, identification of molecules which can target enzymes and/or biochemical processes almost exclusive to fungi could be a useful approach.

Fungal cell walls are primarily composed of polysaccharides namely chitin, glucans, mannans and glycoproteins. Chitin, a β-1,4 linked *N*-acetyl-D-glucosamine polymer, is an important constituent of the cell wall. As chitin is absent in plants and animals, its synthesis is a promising target for development of antifungal agents. Present review focuses on various approaches for chitin synthesis inhibition to develop effective antifungal drugs.

CHITIN SYNTHESIS IN FUNGI

In fungi, the amount of chitin in the cell wall varies from 2% in few yeasts to 61% in some filamentous fungi. Irrespective of the proportion, chitin appears to be essential for fungal growth and survival [16]. In chitin metabolism the enzymes from both carbon and nitrogen metabolism are involved which synthesize uridine diphosphate-*N*-acetyl glucosamine (UDP-GlcNAc) from glucose. Chitin synthase (CS, EC 2.4.1.16) is crucial as it is involved in the synthesis of chitin from UDP-GlcNAc (Fig. **1**). In the cytoplasm, most of the zymogenic CS is accumulated in specialized structures called chitosomes. Chitosomes fuse to the plasma membrane, active CS is formed by proteolysis and eventually chitin is synthesized. Crystalline microfibrils have 20-400 chitin chains held together by hydrogen bonding [17, 18].

*Address correspondence to this author at the Biochemical Sciences Division, National Chemical Laboratory, Dr. Homi Bhabha Road, Pashan, Pune 411008, India; Tel: 020-25902246; Fax: 020-25902648;
E-mail: mv.deshpande@ncl.res.in

1875-5607/13 $58.00+.00

Table 1. Clinically Used Antifungal Drugs.

Class of Antifungals	Antibiotic	Target - Mode of Action
Polyenes	Amphotericin B, Nystatin	Ergosterol - Polyenes complex with ergosterol in the fungal cell membrane and compromise the integrity of the cell membrane.
Azoles	Imidazoles (e.g. ketoconazole, miconazole) Triazoles (e.g., fluconazole, itraconazole and voriconazole)	Ergosterol biosynthesis - Inhibition of lanosterol 14-α-demethylase (cytochrome P450 dependent) enzyme (EC 1.14.13.70) leading to ergosterol depletion and accumulation of intermediates in the pathway. It disrupts the structure of the cell membrane, alters the activities of several membrane-bound enzymes.
Allylamines	Terbinafine	Ergosterol biosynthesis - Inhibit the enzyme squalene epoxidase (EC 1.14.99.7) in the ergosterol biosynthesis pathway
Fluoro-pyrimidines	5-fluorocytosine (5-FC)	Nucleic acid synthesis - Inhibit DNA and RNA synthesis
Echinocandins	Caspofungin	Glucan synthesis - Inhibit-(1,3)-glucan synthesis resulting in disruption of the fungal cell wall

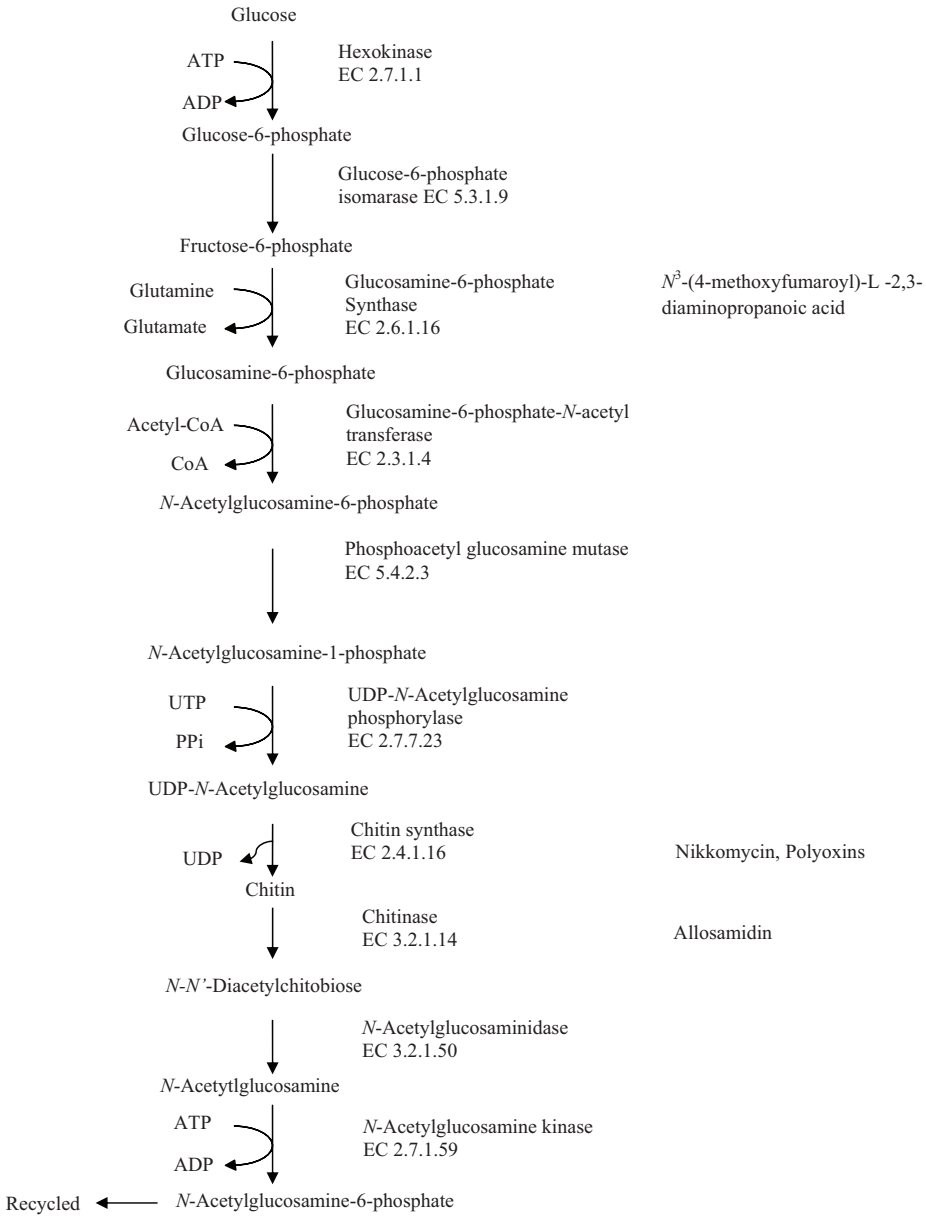


Fig. (1). Chitin synthesis pathway.

CHITIN SYNTHASE

Chitin synthase (CS) is present as isoezyme in fungi; the number varies from one in *Schizosaccharomyces pombe* [19], four in *C. albicans* [20], eight in *Benjiaminella poitrasii* [21] to ten in *Phycomyces blakesleeanus* [22]. CS, a glycosyltransferase type enzyme, catalyzes a transglycosylation reaction in which sugar residues are transferred from UDP-GlcNAc to the growing chitin chain releasing uridine diphosphate (UDP, Fig. **2**). The different isoenzymes are involved in chitin synthesis at different sites or different time points in the lifecycle of the fungus such as repair during cytokinesis, synthesis of primary septum, lateral cell wall integrity and hyphal tip growth soon and so forth. Roncero [23] reviewed different CS in fungi, their functions, genetic complexity and particularly its regulation in *S. cerevisiae*.

The universal motif of β-glycosyl transferases - amino acid residue sequence glutamine-any amino acid-any amino acid-arginine-tryptophan (QXXRW) is present in CS. Along with it, CS active site has two conserved domains A and B, with two aspartic acid residues at domain A and one at domain B which are required for catalytic activity [24]. Based on the alternating orientation of the GlcNAc residues within the chitin chain, it was proposed that CS possesses two active sites. According to the hypothesis, CS is a processive glycosyl transferase (that adds a number of sugar residues) which forms two glycosidic bonds simultaneously by a specific mechanism resulting in the inversion of the anomeric configuration and release of two UDP molecules [24].

CHITIN SYNTHASE INHIBITORS

Polyoxin and nikkomycin were the first reported CS inhibitors isolated from culture filtrates of *Streptomyces* sp., thereafter number of CS inhibitors were isolated from natural sources like plants, microorganisms. Whereas, synthetic chitin synthase inhibitors have been designed and prepared mainly based on diversity-oriented synthesis of UDP-GlcNAc analogs.

Screening for Chitin Synthase Inhibitors

The potential of antifungal agent is mainly adjudged by growth inhibition or spore germination inhibition assays. The assays are performed by disc diffusion method or broth microdilution technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Most of the pathogenic fungi (e.g. *C. immitis, B. dermatitidis, H. capsulatum, C. albicans*) change their morphology reversibly between yeast and filamentous hyphal form for survival and proliferation in the host, which in other words, is the change from saprophytic to pathogenic form. Therefore, the compound which inhibits this reversible transition may have a potential as an antifungal drug. Usually in a dimorphic fungus, chitin contents increase during yeast to hypha transition [25]. Therefore, inhibition of transition can be used as a test for screening of potential CS inhibitors [26].

The filamentous fungi grow apically. Usually hypal tip is plastic, thin walled and a site for the deposition of nascent chitin. In the presence of specific chitin synthase inhibitor in osmotically stabilized medium, the hyphal tip bursting can be observed within few minutes under the microscope [27].

It has been reported earlier that initial chitin synthesis is essential for protoplast regeneration in *B. poitrasii,* and specific CS inhibitors like nikkomycin have been shown to hamper the process of regeneration [28]. Similarly, Carrano *et al.* [29] developed a method to detect CS inhibitors based on regeneration of *C. albicans* protoplast using fluorescent chitin precursor dansyl GlcNAc.

A yeast genome-wide drug induced haploinsufficiency screen using *S. cerevisiae* as a model organism has been developed by Giaever *et al.* [30]. Lowering the dosage of a single gene from two copies to one copy in diploid yeast results in a heterozygote. The resulting mutant strain becomes sensitive to any drug that acts on the product of this gene as compared to wild type strain. This haploinsufficient phenotype thereby identifies the gene product of the heterozygous locus as the likely drug target [31]. For instance, diploid wild type *S. cerevisiae* strain and its mutant (haploid for the targeted gene *Chs2* – chitin synthase 2 gene) are used for the screening of the CS inhibitors. As the mutant has only one copy of *Chs2,* the effect of chitin synthase inhibitors on growth will be more pronounced on it as compared to the wild type, whereas other agents will exert similar effect on both the strains. *S. cerevisiae* genes essential for growth and with no homologs in humans may be selected as specific antifungal target. However, around 30% of the genes essential for yeast to filamentous transition in *C. albicans*, a switch central to its pathogenicity, lack homologs in *S. cerevisiae* and thus are better candidate antifungal targets [32]. Therefore, Roemer *et al.* [33] employed a pathogen-focused approach for drug discovery using haploinsufficiency in *C. albicans* (termed as CaFT – *C. albicans* Fitness Test) and screened 45% of the *C. albicans* genome for the molecular targets of growth inhibitory compounds. Using >2,800 heterozygous deletion strains and known inhibitors gene function elucidations were done. CaFT as screening test

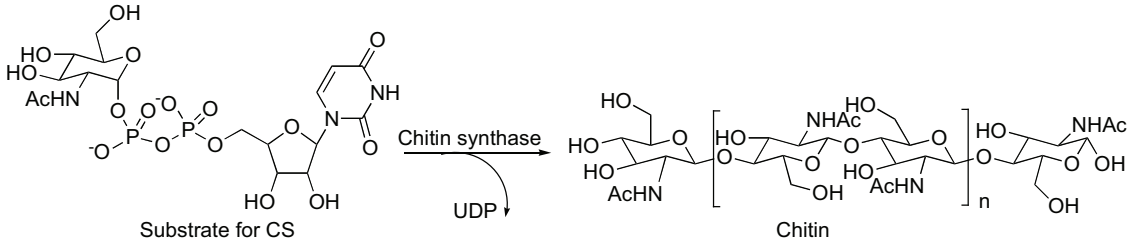


Fig. (2). Mechanism of chitin synthesis.

for drug discovery will confirm the mechanism of action of new compounds and their therapeutic potential as antifungal agents. Few such molecular targets are mentioned in Table **2** and except *Chs2*; other targets are new with no known inhibitor.

Chitin is present in pure form only in diatoms. The extracellular β-chitin spines of the diatom, *Thalassiosira fluviatilis* are completely acetylated. The spines act as flotation device. Action of chitinase on these β-chitin spines resulted in increased sinking rate of the cells [34]. Nikkomycin was reported to inhibit the β-spine formation in exponentially growing diatoms, which resulted in the sedimentation of treated cells. This sedimentation rate was found to be dose dependent.

For CS inhibitors screening, *in vitro* radiometric enzyme assay is routinely used in which CS activity in presence and absence of the inhibitor is estimated using radiolabelled substrate UDP-^{14}C-*N*-acetylglucosamine and by measuring the incorporation of ^{14}C in the formed chitin [35]. CS activity inhibition can also be estimated by non-radioactive CS assay using either horse radish peroxidase-WGA conjugate [36] or Fluorescein isothiocyanate (FITC) labelled WGA.

THE STORY OF POLYOXIN AND NIKKOMYCIN

Polyoxins

Isono *et al.* [37] reported the protective and curative effects of the culture filtrates of three strains of *Streptomyces* against sheath blight disease of rice caused by *Rhizoctonia solani*. Purification of the culture filtrates resulted in isolation of polyoxins A and B, which showed antifungal activities against other phytopathogenic fungi *Alternaria kikuchiana, Cochliobolus miyabeanus, Pyricularia oryzae* and *Pellicularia filamentosa* with minimum inhibitory concentration (MIC) in the range of 0.2-12.5 μg/ml [37, 38]. Subsequently, polyoxins A to L (Fig. **3**) were isolated and purified from the culture broths of *Streptomyces cacaoi* var. *asoensis* [39]. Polyoxins were designated as a new class of peptide nucleoside antibiotics based on the similarity of the core structure of all active polyoxins with CS substrate UDP-GlcNAc [39]. Suzuki *et al.* patented polyoxins A, B (U. S. patent 3,700,655) and polyoxins J, K, L (U. S. patent 3,625,940) for their use as agricultural fungicides in 1971-72. Polyoxins are still an important fungicide group and used in different compositions for treating plant diseases (U. S. patent 20110028419A1).

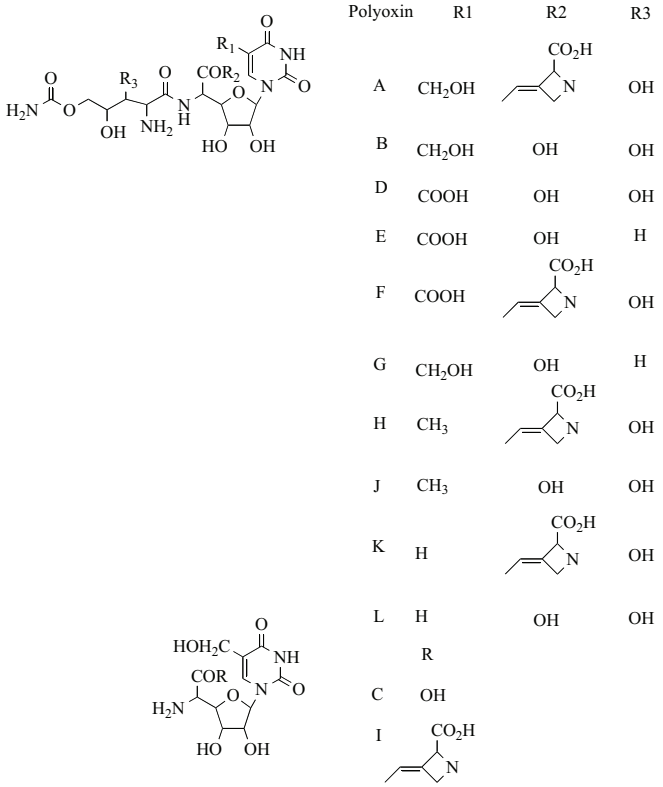


Fig. (3). Structures of polyoxins.

In vitro enzyme inhibition studies using *Saccharomyces carlsbergensis* CS preparation confirmed that the mode of action of the polyoxins was through competitive inhibition of the CS enzyme [40]. Further study on the relationship of structures of polyoxins to *P. oryzae* CS inhibition indicated that the oxygen atom at C-1', amino group at C-2', hydroxyl groups at C-3' and C-4', aliphatic carbon chain and terminal carbamoyloxy group moiety of polyoxins helps to stabilize the polyoxin-enzyme complex [41]. pKi (-log dissociation constant) versus pH plots for polyoxin C and other polyoxins

Table 2. Few New* Targets in Yeasts and Filamentous Fungi for the Development of Antifungal Agents.

Name of the Target	Biological Role	Additional Remarks
TRL1	Required for tRNA splicing and has phosphodiesterase, polynucleotide kinase, and ligase activities	Yeast is not viable due to deletion of the targeted gene.
ALR1	Plasma membrane Mg^{2+} transporter	Yeast is not viable due to deletion of the targeted gene.
RSC9	Component of the RSC chromatin remodeling complex; DNA-binding protein involved in the synthesis of rRNA	Yeast is not viable due to deletion of the targeted gene.
YPD1	Osmo-sensory signaling pathway via two component system	Yeast is not viable due to deletion of the targeted gene.
CHS2	Chitin synthesis	In yeast, *CHS2* deletion results in loss of well-defined septa and growth is arrested.

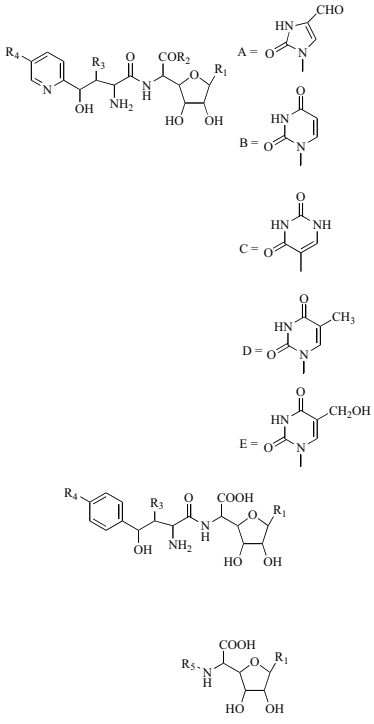
*except *CHS2*.

indicated that the ionized amino group at C-2' position plays an important role in the binding of polyoxins to CS. Whereas, the carbonyl oxygen atoms at C-1' and of the carbamoyloxy group might have participated in the hydrogen bond formation with the enzyme [42]. Few studies demonstrated *in vivo* effects of polyoxin D on cell wall of different fungi [43-45]. Polyoxin D treatment to *S. cerevisiae* resulted in pairs of abnormal cells either consisting two lysed cells from which the cytoplasm had been extruded at the cell junction, or consisting of two highly refractile cells joined by a thin bridge. Electron microscopy showed that secondary septa of varying thicknesses were laid down in an abnormal pattern with no primary chitin septum formation in both these cell types [45]. Though polyoxins were effective *in vitro* against CS, they were ineffective *in vivo* against yeast cells. The reasons were inability of polyoxins to cross the cellular membrane and accumulate intracellularly to required inhibitory concentrations and presence of other peptides in the growth medium which inhibited polyoxin transport and action [45]. Though naturally occurring polyoxin D was completely resistant to degradation by cell extracts, many other polyoxins were rapidly degraded by intracellular peptidases to products that were not inhibitors of CS [46].

Nikkomycins

Dahn *et al.* [47] isolated chemical nikkomycin from the fermentation broth of *Streptomyces tendae* Tü 901, which inhibited the growth of several fungi by affecting chitin biosynthesis. Its structure was identified as a nucleoside-peptide antibiotic consisting of uracil, an amino hexuronic acid and a new amino acid containing a pyridine ring. Bayer AG first evaluated nikkomycin as fungicide (U.S. Patent Nos. 4,315,922 and 4,158,608) and then patented a method for treating fungal infections caused by dermatophytes, yeasts and dimorphic fungi such as *Epidemophyton floccosum*, *C. albicans*, *H. capsulatum,* etc. with nikkomycin derivatives (U.S. Patent 5019560).

Further from the fermentation studies with wild type and mutant strains of *S. tendae* Tü 901, 21 biologically active and six inactive nikkomycins were isolated and purified [48, 49]. Nikkomycins X, Z, pseudo-Z, I, J, N, M, D, E, B_Z, B_X, C_Z, C_X (Fig. **4**) are produced by wild type *S. tendae* Tü 901. Mutant strains *S. tendae* Tü 901/395, Tü 901/172 and Tü 901/C37 produced the remaining nikkomycins O_Z, O_X, W_Z, W_X, K_Z, K_X, Q_Z, Q_X, R_Z, R_X, ZT, Z_H, J_T, J_H (Fig. **4**). Based on *in vitro Coprinus cinereus* CS inhibition, *Ki* (inhibition constant) for the nikkomycins were found in the order B_X>X>Z_H>Z_T>B_Z>O_X>Z>W_X. The nikkomycins B_X and X exhibited better antifungal activity against different fungi including *C. albicans, Yarrowia lipolytica, Paecilomyces variotii, Mucor hiemalis* than nikkomycin Z [50]. Nikkomycin Z also showed poor antifungal activity against *S. cerevisiae* as it inhibited only CS 3 and was ineffective against CS 2 [51]. Whereas in *C. albicans,* nikkomycin Z inhibited vegetative growth by inhibiting all three CS with IC_{50} value for CS 1, CS 2, CS 3 were 7.4, 0.4 and 6.4 μg/ml respectively. Growth inhibition was accompanied by the absence of septum and cell wall chitin, which in turn brought about cell lysis [52]. Microscopic and cytochemical study indicated that *Gigaspora margarita* and *Glomus intraradices* CS inhibition by nikkomycin Z induces alterations in hyphal morphology, changes in the amount or distribution of chitin oligomers, a reduction in fungal wall thickness, and several other changes in the hyphal wall structure and organization [53]. Nikkomycins were most effective against dimorphic fungi *C. immitis, B. dermatitidis* due to high CS activity present in the cells. In the mouse models of coccidioidomycosis and blastomycosis, nikkomycin Z was found to be more effective than nikkomycin X and majority of azoles, while moderately effective in histoplasmosis [54]. The problems associated with the polyoxins, *i.e*. transport inside the cell and degradation by intracellular peptidases were not significant in nikkomycins. Nikkomycin Z, X and B_X were resistant to enzymatic hydrolysis, but were unstable at neutral and alkaline pH, which can be attributed to presence of hydroxypyridyl residue. Replacement of hydroxypyridyl residue with hydroxyphenyl ring as in nikkomycins W, W_Z, B_X and B_Z resulted in stability at neutral and alkaline pH. The β-methyl group of the N-terminal amino acid of dipeptide nikkomycins might be protecting the molecule against peptidase activity in crude cell-extracts of different fungi. All the investigated nikkomycins except C_Z and O_X* were transported via the peptide transport system in *Y. lipolytica* with more affinity for dipeptide nikkomycins than tripeptide [50].



Nikkomycin	R_1	R_2	R_3	R_4
X	A	OH	CH_3	OH
Z	B	OH	CH_3	OH
Pseudo-Z	C	OH	CH_3	OH
Z_T	D	OH	CH_3	OH
Z_H	E	OH	CH_3	OH
I	A	Glu	CH_3	OH
J	B	Glu	CH_3	OH
O_X	A	OH	H	OH
O_X*	A	OH	H	OH
O_Z	B	OH	H	OH
K_X	A	OH	H	H
K_Z	B	OH	H	H

	R_1	R_3	R_4
B_X	A	CH_3	OH
B_Z	B	CH_3	OH

	R_1	R_5
W_x	A	Tyr
W_Z	B	Tyr

Fig. (4). Structures of nikkomycins.

SYNTHETIC MODIFICATIONS OF POLYOXINS AND NIKKOMYCINS

Number of reports are available on modification of polyoxins and nikkomycins for the improvement of their properties. Being multifunctional molecules these nucleoside peptides offers different sites for the modification. General

structure of the polyoxin and nikkomycin with different possible sites for modifications is given in (Fig. **5**).

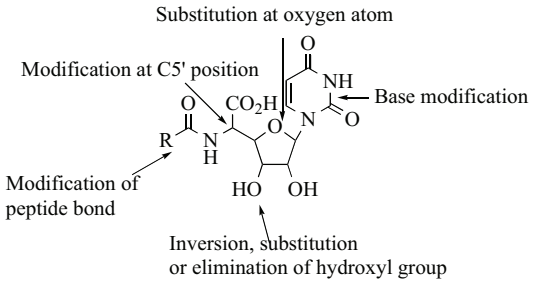


Fig. (5). Different sites for the modification of the nikkomycins and polyoxins.

Peptide Derivatives of Polyoxins

A series of amino acyl derivatives of polyoxin C and L were synthesized and evaluated for chitin synthase inhibitory activity by Isono *et al.* [55-57]. Initially, they prepared 5'-decarboxylated analogs of polyoxins by synthesizing ω-substituted aminoacyl derivatives of 5'-amino-5'-deoxyuridine. No antimicrobial activity was observed for all 7 derivatives when tested against 15 different human and phytophatogenic fungi indicating the importance of 5′-carboxyl group for activity. 5′-carboxyl group was thought to be essential as it would place a negative charge in a position analogous to that of the phosphate groups in UDP-GlcNAc [55]. Therefore, *N*-aminoacyl derivatives of polyoxin C with amino acids such as glycine, alanine, ornithine and norvaline as the side chains were synthesized. Structure activity relationship based on *in vivo* inhibition of phytopathogenic *A. kikuchiana*, *P. oryzae* and *C. miyabeanus* showed that: 1) the α-L-amino and 5′-carboxyl group were essential for the activity. 2) The alkyl chain length and the nature of ω-substituent affected the activity; polar groups such as ureido or carbamoyl increased the activity, whereas ionic groups such as amino or carboxyl groups decreased activity. However, these types of synthetic modifications did not show enhanced CS inhibition than the natural polyoxins [56, 57].

Polyoxins were ineffective against *C. albicans* due to inability of the antibiotic to penetrate the cell. Shenbagamurti *et al.*[58] structurally altered the polyoxin molecule by introducing hydrophobic amino acids in the side chain so as to enhance the penetration into *C. albicans*. The most active derivatives (**1-3**, Fig. **6**) were comparable in activity to ployoxin D (IC_{50} 0.45 μg/ml). Khare *et al.* [59] reported the synthesis and testing of polyoxins containing α-amino fatty acids (**4,** Fig. **6**). Compound **4** inhibited growth of C. *albicans* in culture at 40-80 μg/ml, whereas all other analogs were found to be less potent than polyoxin D (MIC 320 μg/ml).

Polyoxins found to be unstable in the internal cell environment, to overcome this problem dipeptide and tripeptidyl analogs of polyoxins were synthesized [60]. However, these analogs were rapidly metabolized to inactive form uracil polyoxin C (UPOC), releasing corresponding amino acids inside the cells. In order to improve metabolic stability, *Nc*-Oct-Lys-UPOC and *Nc*-Oct-Gln-UPOC (**5** and **6**, Fig. **6**) were synthesized and found to be more resistant to dipeptidase than above di- and tripeptidyl derivatives. But their antifungal efficacy against *C. albicans* (> 525 μg/ml) was still less than that of polyoxin D (MIC 26 μg/ml) [46]. Emmer *et al.* [61] synthesized two L-alanine-UPOC analogs in order to obtain resistant analogs to peptidase hydrolysis. However, these modified molecules were ineffective in inhibiting CS activity.

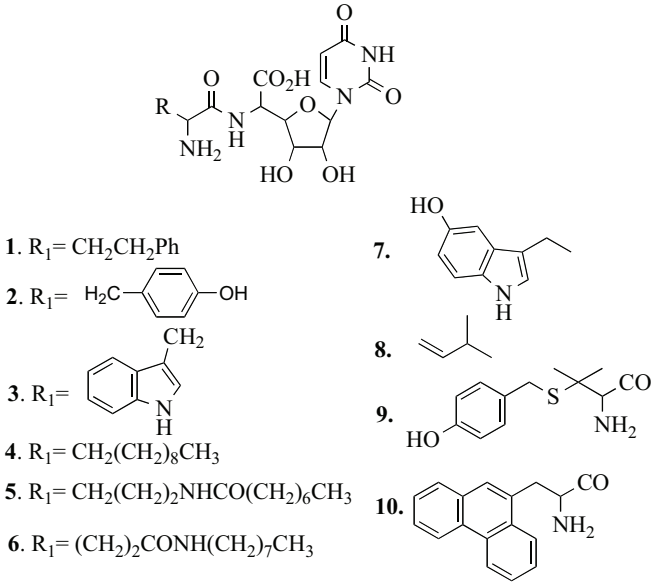


Fig. (6). Peptide derivatives of polyoxin and nikkomycin.

Peptide Derivatives of Nikkomycins

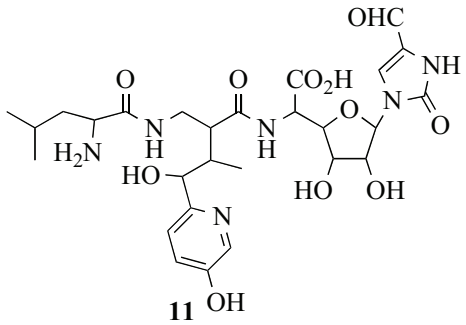
Cooper and coworkers [62] reported a synthetic study of nikkomycin Z containing different amino acyl groups (**7** and **8,** Fig. **6**). The chitin synthase and growth inhibitory activities of these derivatives were found to be much lower than that of nikkomycin Z against *Candida* strains. Obi *et al.* [63] synthesized nikkomycin Z analogs with different groups at the terminal amino acid moiety, e.g., *S*-aryl-L-Cys, *S*-aryl-L-methyl groups and phenanthrene (**9** and **10,** Fig. **6**). Among them, compound having phenanthrene group **10** was found to be a better *C. albicans* CS inhibitor with IC_{50} about 0.31 μg/ml (IC_{50} for nikkomycin was 0.393 μg/ml).

Krainer *et al.* [64] synthesized nine tripeptidyl and dipeptidyl analogs of polyoxin and nikkomycin as anticandidal prodrugs. Inside the cell, these analogs were expected to release active moiety upon hydrolysis. Out of these analogs only **11** (Fig. **7**) was found to release active component on hydrolysis inside the cells and act as prodrug. None of the compounds proved better anticandidal agents than nikkomycin.

Heteryl Nucleoside Derivatives of Nikkomycin Z

Different heteryl nucleoside derivatives were synthesized by Behr and co-workers [65] which conformationally mimic the transition state in presumed glycosyl transfer reaction. In these derivatives the methyl quinoline and 2-methyl-5-hydroxypyridine served as the glycopyranosyl surrogate and

malonic acid, tartaric acid and carbohydrate based non-hydrolysable linkers were chosen as a surrogate for the phosphate portion of the nucleoside (**12-13,** Fig. **8**). These linkers mimic the six membered ring pyrophosphate-M^{2+} complex formed in the active site of the CS enzyme. However, all the compounds showed weak *S. cerevisiae* CS inhibitory activity as compared to nikkomycin Z.



11

Fig. (7). Peptidyl analog of nikkomycin and polyoxin as a prodrug.

C5′ Modification of Polyoxin and Nikkomycin

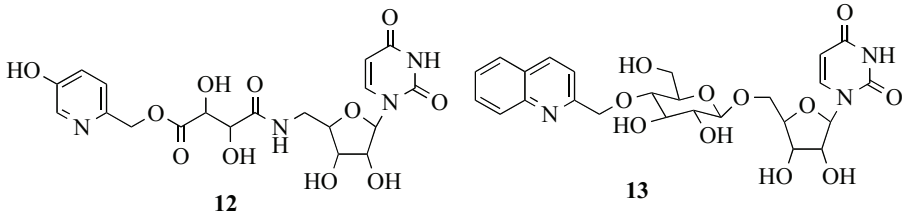
Using combinatorial concept and Ugi 4 Component Coupling reaction, Suda *et al.* [66] synthesized active antifungal analogs of polyoxins. All the Ugi products obtained were a mixture of diastereomers at C5' position. Out of all these compounds diasteriomer **14** (Fig. **9**) was found to be most active against *C. albicans* CS 1 with IC_{50} value 3.69 μg/ml (for nikkomycin 4.69 μg/ml). Chaudhary *et al.* [26] used the click chemistry approach for the synthesis of 1,2,3-triazolyl derivatives of uridine (**15,** Fig. **9**). Two sets of aryl ether and aryl ester 1,2,3-triazolyl derivatives of uridine were synthesized. These compounds were tested for their antifungal activity and *in vitro* CS inhibition in *Benjaminiella poitrasii,* a zygomycetous fungus. All the tested compounds showed CS inhibition in the range of 70-95% at 4 μg/ml concentration as compared to 96.5% for nikkomycin Z.

C-Glycosyl Nucleoside Derivatives

A novel *C*-glycosyl nucleoside with tartarate as a linker residue between uridine moiety and GlcNAc was synthesized [67]. The IC_{50} value for the CS inhibition by synthesized compound **16** (Fig. **10**) was $12.06x10^3$ μg/ml, which appeared to be a very poor inhibitor of CS 1. Chang *et al.* [68] reported the first synthesis of the C1-phosphonate analog of UDP-N-GlcNAc, as C-glycosyl phosphonate are well-known isostere of corresponding phosphate. Compound **17** (Fig. **10**) was weak inhibitor of CS (Ki > 10 mM) with competitive mode of action.

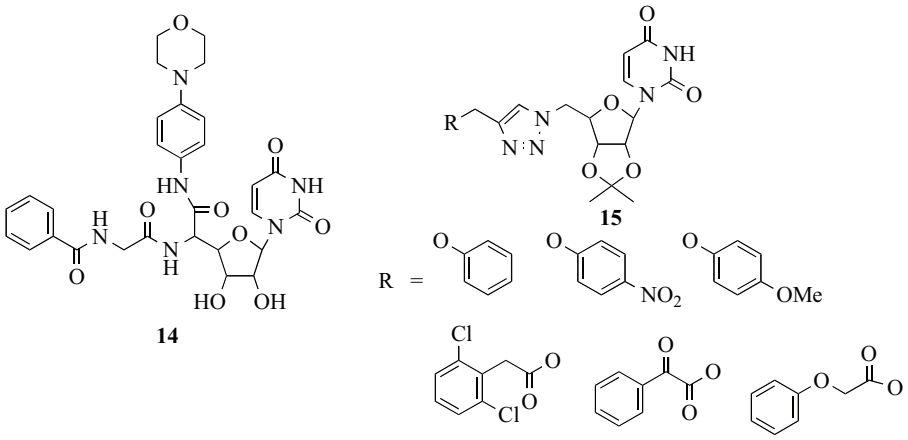
SYNERGISTIC INTERACTION OF NIKKOMYCIN WITH OTHER DRUGS

Nikkomycin Z exhibited additive and synergistic interactions when used in combination with either fluconazole or itraconazole against *C. albicans, C. parapsilosis, C. neoformans* and *C. immitis* [69]. Significant synergism was observed between nikkomycin and itraconazole against *A. fumigatus* and *A. flavus*. Powerful synergy for inhibition, killing, or both between nikkomycin Z and a glucan synthase inhibitor LY 303366 (semisynthetic analog of echinocandian B) was observed against *A. fumigatus* and few other pathogens [70]. Nikkomycin Z (2 to 32 μg/ml) in



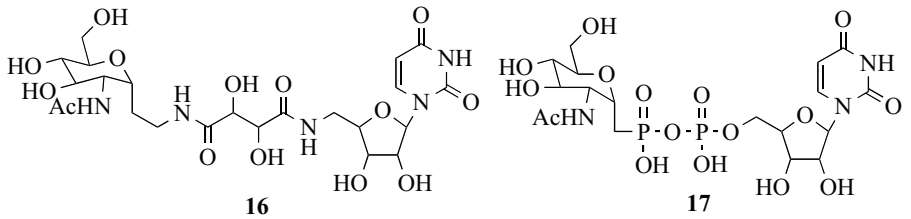
12 **13**

Fig. (8). Heteryl nucleoside derivatives of nikkomycin Z.



14 **15**

Fig. (9). C5′ modified nikkomycin derivatives.



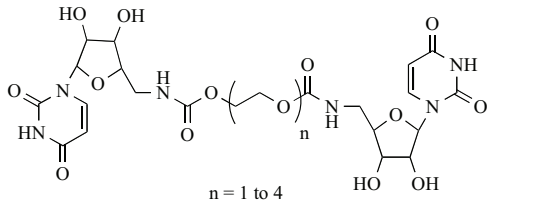
16 **17**

Fig. (10). C-Glycosyl nucleoside derivatives of UDP-*N*-acetylglucosamine.

combination with echinocandin FK463 (0.03 to 0.5 µg/ml) caused significant synergistic hyphal damage in filamentous fungi over a wide range of concentrations [71]. It also showed synergism with voriconazole and caspofungin against *C. albicans* [72]. The therapeutic efficacy of nikkomycin Z in *Candida vaginitis* murine model was enhanced when used in combination with zeamatin, a protein produced in corn which protects plants against fungal pathogens [73]. Synergism of nikkomycin with other drugs indicates that joining of two drug molecules may lead to a hybrid drug with dual mode of action and better efficacy.

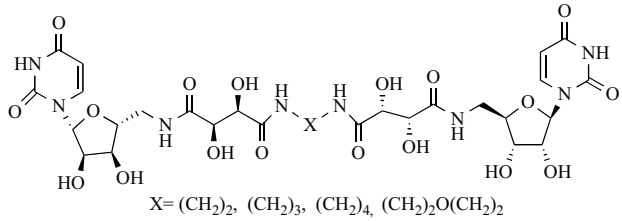
Dimeric Inhibitors

Finney *et al.* [74] tested the hypothesis of presence of two active sites in CS by synthesizing dimeric nucleoside inhibitors with the view that if two adjacent active sites are present in close proximity, then dimeric inhibitors should show bivalent inhibition. Dimeric inhibitors were synthesized by connecting 5'-deoxy-5'-aminouridine fragments by carbamate linkers of different lengths (**18,** Fig. **11**). It was found that efficacy of the dimeric inhibitors was dependent on the length of the spacer used, with shorter spacer (~14 Å) showing better efficacy. In the continuation of this work, uridine analogs joined by tartarate amide spacer (**19,** Fig. **11**) were synthesized and evaluated for their CS inhibition [75]. Use of tartarate amide spacer added incentives to the inhibitory activity with shortest dimer (~12Å) showing significant inhibition of activity. The results indicated the possibility of presence of two active sites in CS.



n = 1 to 4

18



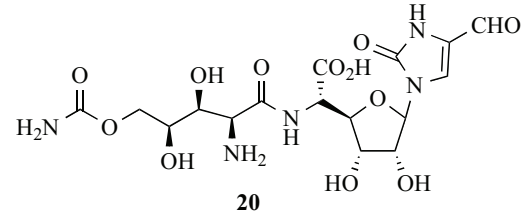
X= $(CH_2)_2$, $(CH_2)_3$, $(CH_2)_4$, $(CH_2)_2O(CH_2)_2$

19

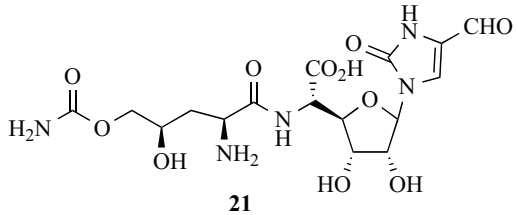
Fig. (11). Dimeric nucleoside inhibitors of chitin synthase.

Combinatorial Biosynthesis Approach for Hybrid CS Inhibitors

Recently, Li *et al.* [76] used the combinatorial biosynthesis approach for the generation of new derivatives of polyoxin and nikkomycin. Since the sturcutre of these two peptide nucleosides are similar, gene responsible for the biosynthesis of dipeptide of polyoxin from *S. cacaoi* was introduced in the *S. ansochromogenes* mutant, which produced nucleoside moiety of nikkonycin X. With this production of two hybrid antibiotics was achieved, out of which one was identified as polyoxin N **20** and another named as polynik A **21** (Fig. **12**). These two hybrid antibiotics have better stability at neutral and alkaline conditions as compared to naturally occurring nucleosides. These antibiotics showed better antifungal activity against *C. albicans* and five different phytopathogenic fungi including *A. kikuchiana* and *Botrytis cinerea* as compared to polyoxin B.



20



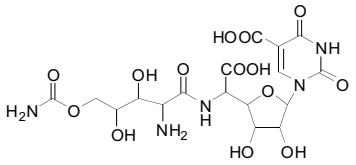
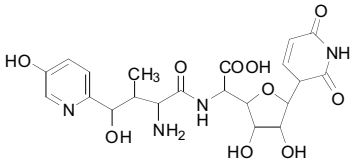
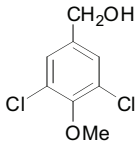
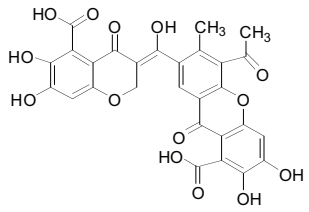
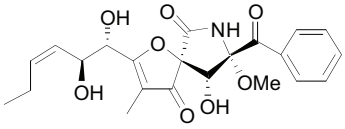
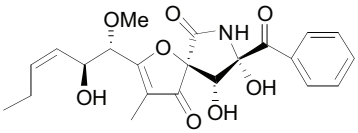
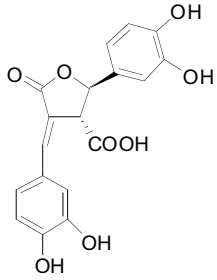
21

Fig. (12). Hybrid derivatives polyoxin N and polynik A.

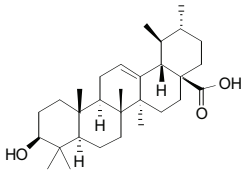
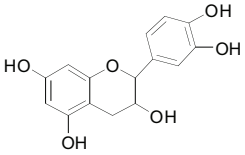
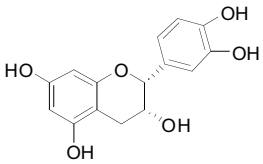
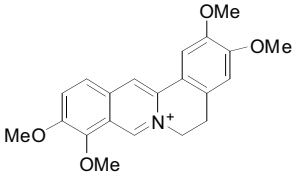
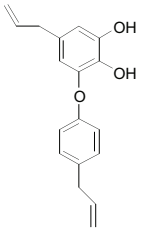
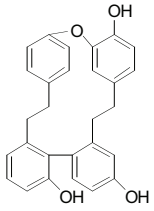
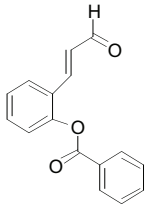
OTHER NATURALLY OCCURRING CHITIN SYNTHASE INHIBITORS

CS inhibitors isolated from natural sources, their structure and CS inhibitory activity are given in Table **3**. Modification of few naturally occurring CS inhibitors were carried out to enhance their antifungal potential. The phenolic OH groups in Phellinsin A determined the CS inhibitory activity. The antifungal activity with respect to the CS inhibition increased with increase in number of phenolic OH groups with maximum activity for compound having three phenolic OH groups [77]. Chlorinated compounds

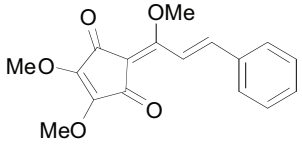
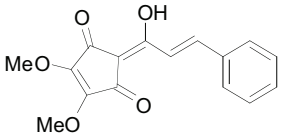
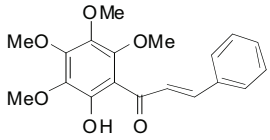
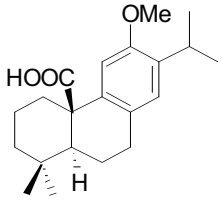
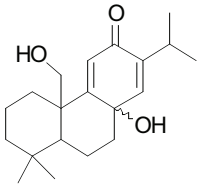
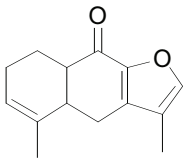
Table 3. Natural Scaffolds for the Possible Development of Chitin Synthase Inhibitors.

Structure and Name of Natural Scaffold	Source	Chitin Synthase Inhibition
Bacterial Source		
 Polyoxin D	*Streptomyces cacaoi* var. *asoensis*	IC_{50} for *C. albicans* CS 0.45 µg/ml [39, 58]
 Nikkomycin Z	*S. tendae* Tü 901	IC_{50} for *C. albicans* CS 1, CS 2 and CS 3 - 7.4, 0.4 and 6.4 µg/ml respectively [47, 52].
Fungal source		
 3,5-Dichloro-4-methoxybenzyl alcohol	Submerged cultures of *Stropharia* sp.	IC_{50} for *Coprinus cinereus* CS 51.70 µg/ml [78].
 Xanthofulvin	*Eupenicillium sp.*	IC_{50} for *S. cerevisae* CS 2 - 1.27 µg/ml [80].
 Pseurotin A	*Aspergillus fumigatus*	IC_{50} for *C. cinereus* CS 31.4 µg/ml [81].
 8-*O*-demethylpseurotin A	*Aspergillus fumigatus*	IC_{50} for *C. cinereus* CS 82.78 µg/ml [81].
 Phellinsin A	Cultured broth of *Phellinus* sp. PL3	IC_{50} for *S. cerevisae* CS 1 and CS 2 - 76 and 28 µg/ml respectively [77, 82].

(Table 3) contd….

Structure and Name of Natural Scaffold	Source	Chitin Synthase Inhibition
Plant Source		
 Ursolic acid	*Crataegus pinnatifida* Bunge leaves	IC_{50} for *S. cerevisiae* CS 2 0.84 µg/ml [83].
 Catechin	Stem bark of *Taxus cuspidata*	IC_{50} for CS 15 µg/ml [84].
 Epicatechin	Stem bark of *Taxus cuspidata*	IC_{50} for CS 29 µg/ml [84].
 Palmatine	*Phellodendron amurense*	IC_{50} for *C. albicans* CS 137.28 µg/ml [85].
 Obovatol	*Magnolia obovata*	IC_{50} for *S. cerevisiae* CS 2 - 10.86 µg/ml [86].
 Plagiochin E (PLE)	Liverwort *Marchantia polymorpha* L	IC_{50} for *C. albicans* CS 2 and 3 - 16 µg/ml [87, 88].
 2-benzoyloxycinnamaldehyde (2-BCA)	Aerial parts of *Pleuropterus ciliinervis*	IC_{50} for CS 2 of *S. cerevisiae,* 70.8 µg/ml which was 2.5 times better than nikkomycin Z (175.6 µg/ml) [79].

(Table 3) contd….

Structure and Name of Natural Scaffold	Source	Chitin Synthase Inhibition
 Methyllinderone	Stem bark of *L. erythrocarpa* Makino (Lauraceae)	IC_{50} for CS 2 *S. cerevisiae* 23.3 µg/ml [89].
 Linderone	Stem bark of *L. erythrocarpa* Makino (Lauraceae)	IC_{50} for CS 2 *S. cerevisiae* 21.4µg/ml [89].
 Kanakugiol	Stem bark of *L. erythrocarpa* Makino (Lauraceae)	IC_{50} for *S. cerevisiae* CS 2- 23.8 µg/ml [89].
 *O*-methyl pisiferic acid	Leaves of *Chamaecyparis pisifera*	IC_{50} for CS 2 of *S. cerevisiae* 1.91 µg/ml [90].
 8,20-dihydroxy-9(11),13-abietadien-12-one	Leaves of *Chamaecyparis pisifera*	IC_{50} for *S. cerevisiae* CS 2 - 71.99 µg/ml [90].
 3,4,8a-trimethyl-4a,7,8,8a-tetrahydro-4a-naphto[2,3-b]furan-9-one	Whole plant of *Chloranthus japonicus* SIEB	IC_{50} for *S. cerevisiae* CS 2 - 39.6 µg/ml [91].

related to 3,5-dichloro-4-methoxybenzyl alcohol (DCMB) isolated from a mushroom *Stropharia* sp. were compared with DCMB for CS inhibition. Hexachlorophene and pentachlorophenol showed better CS inhibitory activity than DCMB [78]. From aerial parts of *Pleuropterus ciliinervis,* 2-benzoyloxycinnamaldehyde (2'-BCA) was isolated and its hydroxy, methoxy and halogenated derivatives were synthesized. Chloro and bromo derivatives of 2'-BCA exhibited 1.9 and 2.7 fold stronger CS 2 inhibitory activity than 2'-BCA, respectively [79].

CHEMICALLY SYNTHESIZED CS INHIBITORS

Many chemically synthesized compounds structurally not related to the UDP-GlcNAc, but possessing antifungal and CS inhibitory activities have been reported. Ke *et al.* [92] designed and synthesized two series of 1,3,4-oxadiazoline derivatives C1–10 and C11–20 with the rationale that an oxadiazole ring improves pharmacokinetic and *in vivo* efficacy of drugs due to its higher hydrolytic stability. All the compounds were found to inhibit CS activity *in vitro*. Structure-activity relationship study was done by introducing

different electron donating (methyl, methoxy, ethyl, etc.) and withdrawing groups (such as halogen atoms) into the aromatic ring. Compound C1 **(22,** Fig. **13**) containing *o*-chloro-substituent was most potent with 91% *S. cerevisiae* CS inhibition at 250 μM concentration, which may be due to increased lipophilicity due to the incorporation of halogen atoms into the aromatic ring. Introduction of acylurea moiety into 1,3,4-oxadiazolines heterocyclic structure resulted in lowered activities. Compounds C1 and C15 **(23,** Fig. **13**) exhibited CS inhibition with IC_{50} values of 2.66 and 2.85 μg/ml respectively.

Forty one novel chalcone derivatives were synthesized by Claisen-Schmidt condensation and tested for antifungal activity against different human pathogens [93]. All these chalcones were inactive against *Candida* and *Cryptococcus* sp., but found to be active against dermatophytes such as *Microsporum* sp., *Trichophyton* sp. and *E. floccosum*. For structure activity relationship study, modification in A, B ring and enone linkage of chalcones was done. Introduction of electron donating groups decreased the antifungal activity, while electron withdrawing group specifically NO_2 at para position (**24,** Fig. **13**) increased the antifungal activity against *M. floccosum* with MIC value of 0.25 μg/ml. Replacement of benzene ring A by naphthalene resulted in loss of antifungal activity, while enone linkage was found to be important for antifungal activity. The IC_{50} values for CS inhibition was <220 μg/ml.

Urbina *et al.* [94] synthesized a series of new 4-aryl- or 4-alkyl-N-arylamine-1-butenes and converted few of them into 2-substituted 4-methyl-tetra-hydroquinolines and quinolines. Unsubstituted homoallylamines found to inhibit dermatophytes such as *Microsporum* sp., *Tricophyton* sp. and *Epidermophyton floccosum* with MIC <50 μg/ml. The most sensitive species was *E. floccosum*. When tested for *S. cerevisiae* CS 1 inhibition, it was observed that all homoallylamines were able to inhibit CS acitivity with IC_{50} <200 μg/ml. The derivative quinolines (**25**, Fig. **13**) were more effective with MIC values ranging from 0.75-25 μg/ml against above dermatophytes. The IC_{50} of the quinolines for *S. cerevisiae* CS were 20-40 μg/ml.

Considering the biological activity of the cyanopyridine derivatives Gholap *et al.* [95] reported the synthesis of novel 2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile and its derivatives. Out of all the synthesized compounds **26** and **27** (Fig. **13**) at 4 μg/ml concentration showed 91-95% *B. poitrasii* CS inhibition, while other compounds showed CS inhibition in the range of 60-90%. All the compounds exhibited antifungal activity against *C. albicans* and *Mucor* sp.

Random Screening of Libraries of Synthetic Compounds

From random screening of chemically synthesized compound libraries, a chitin synthase inhibitor RO-41-0986 (**28**, Fig. **14**) was obtained. The synthesis and structure activity relationship study of RO-41-0986 through modification of C-5 amino group, enediene side chain, pyridine-2-one moiety and side chain substituent has been reported. Derivative RO-09-3143 (**29,** Fig. **14**) showed maximum and specific inhibition of *Candida* CS 1 with IC_{50} value of 0.000041 μg/mL and *in vitro* antifungal activity against *C. albicans* CY1002 with IC_{50} value 0.07 μg/mL [96]. RO-09-3143 prevented septa formation in *C. albicans* and the cells displayed an aberrant morphology, confirming the role of the *C. albicans* CS 1 in septum formation [97].

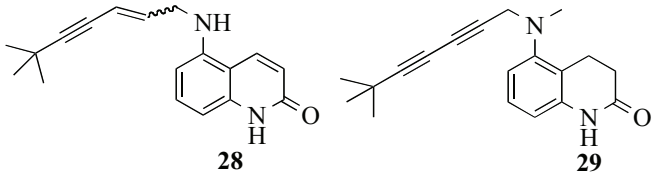


Fig. (14). Chitin synthase inhibitors from Roche compound library.

FUTURE PROSPECTS

Although nikkomycins were isolated in 1976, only recently nikkomycin Z is being developed as a specific drug for the treatment of coccidiomycosis, also known as Valley fever. The disease is caused by *C. immitis* and is endemic to

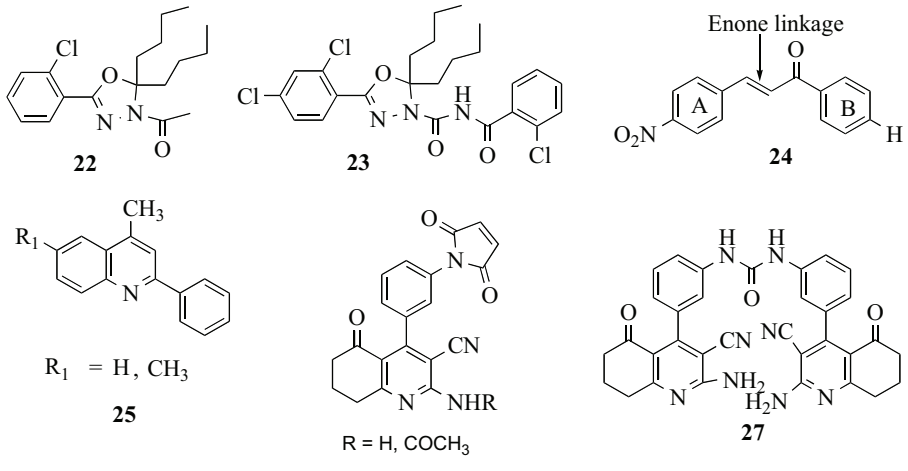


Fig. (13). Chemically synthesized chitin synthase inhibitors.

Southwestern United States. Currently, nikkomycin is in phase II clinical trials for the treatment of valley fever [12]. The delay in the development of nikkomycin as a drug may be due to its efficacy essentially confined to highly chitinous fungi such as *C. immitis* and *B. dermatitidis* [98]. One of the most dreadful fungal pathogen *C. albicans* is less susceptible and other *Candida* sp. are resistant to Nikkomycin Z [99].

The literature review signifies that the research on CS inhibitors has progressed steadily and many advances have been made. However, only efforts related to nikkomycin Z lead to the development of a potential candidate for the clinical treatment. The limited array and often associated side-effects of the existing antifungal drugs emphasize the search for novel antifungal agents with fungus-specific mode of action. Therefore, research for the development of CS inhibitors needs to be pursued further. Availability of different bioinformatics tools and recent report on homology modeling and molecular dynamics of the *Moniliophthora perniciosa*, a causative agent of 'Witches' broom disease of cocoa tree, CS active site [100], indicated the possibility of complete elucidation of active site of different CSs from pathogenic fungi. 3D-Quantitative Structure Activity Relationship (QSAR) studies of enzyme-ligand interactions could then lead to development of effective CS inhibitors. In accordance with two active site mechanism of CS, dimeric molecules of CS inhibiting monomers with different linkers can be synthesized and explored for improved inhibition. Synthesis of hybrid molecules with dual activity is an emerging strategy which involves conjugation of two separate pharmacological agents into a single molecule. Recently, the approach was used for the synthesis of hybrid molecules of two known anti-malarial drugs, chloroquine (CQ) and the non-sedating H1 antagonist astemizole [101]. The approach may be employed for synthesis of antifungal agents by joining two inhibitors of enzymes from same or different pathways using hydrolysable linker molecules. For instance, a CS inhibitor like nikkomycin can be joined to glucosamine-6-phosphate synthase inhibitor N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid or glucan synthase inhibitor caspofungin. This may tackle the problem of resistance development against one mechanism. Synergistic action of nikkomycin Z in combination of existing drugs indicates its therapeutic potential which needs to be assessed in different animal disease models. The combinatorial biosynthesis approach [76] may be further explored for the development of new hybrid CS inhibitors with improved characteristics. In the near future, directed efforts for CS inhibitors development may lead to a group of safe and effective antifungal drugs.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

PC for Senior Research Fellowship and SGT for Research Associateship thank Council for Scientific and Industrial Research, India.

REFERENCES

[1] Maertens, J.; Vrebos, M.; Boogaerts, M. Assessing risk factors for systemic fungal infections. *Eur. J. Cancer Care (Engl.)* **2001**, *10*, 56-62.

[2] Richardson, M.D. Changing patterns and trends in systemic fungal infections. *J. Antimicrob. Chemother.,* **2005**, *56*, i5-i11.

[3] Richardson, M.; Lass-Flörl, C. Changing epidemiology of systemic fungal infections. *Clin. Microbiol. Infect.,* **2008**, *14*, 5-24.

[4] Lass-Flörl, C. The changing face of epidemiology of invasive fungal disease in Europe. *Mycoses,* **2009**, *52*, 197-205.

[5] Walsh, T.J.; Groll, A.H. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. *Transpl. Infect. Dis.,* **1999**, *1*, 247-261.

[6] Nucci, M.; Marr, K.A. Emerging fungal diseases. *Clin. Infect. Dis.,* **2005**, *41*, 521-526.

[7] Walsh, T.J.; Groll, A.; Hiemenz, J.; Fleming, R.; Roilides, E.; Anaissie, E. Infections due to emerging and uncommon medically important fungal pathogens. *Clin. Microbiol. Infect.,* **2004**, *10*, 48-66.

[8] Pfaller, M.A.; Diekema, D.J. Rare and emerging opportunistic fungal pathogens: concern for resistance beyond *Candida albicans* and *Aspergillus fumigatus*. *J. Clin. Microbiol.,* **2004**, *42*, 4419-4431.

[9] Eliopoulos, G.M.; Perea, S.; Patterson, T.F. Antifungal resistance in pathogenic fungi. *Clin. Infect. Dis.,* **2002**, *35*, 1073-1080.

[10] Shao, P.-L.; Huang, L.-M.; Hsueh, P.-R. Recent advances and challenges in the treatment of invasive fungal infections. *Int. J. Antimicrob. Agents,* **2007**, *30*, 487-495.

[11] Petrikkos, G.; Skiada, A. Recent advances in antifungal chemotherapy. *Int. J. Antimicrob. Agents,* **2007**, *30*, 108-117.

[12] Ostrosky-Zeichner, L.; Casadevall, A.; Galgiani, J.N.; Odds, F.C.; Rex, J.H. An insight into the antifungal pipeline: selected new molecules and beyond. *Nat. Rev. Drug Discov.,* **2010**, *9*, 719-727.

[13] Vatmurge, N.S.; Hazra, B.G.; Pore, V.S.; Shirazi, F.; Chavan, P.S.; Deshpande, M.V. Synthesis and antimicrobial activity of beta-lactam-bile acid conjugates linked via triazole. *Bioorg. Med. Chem. Lett.,* **2008**, *18*, 2043-2047.

[14] Bavikar, S.N.; Salunke, D.B.; Hazra, B.G.; Pore, V.S.; Dodd, R.H.; Thierry, J.; Shirazi, F.; Deshpande, M.V.; Kadreppa, S.; Chattopadhyay, S. Synthesis of chimeric tetrapeptide-linked cholic acid derivatives: Impending synergistic agents. *Bioorg. Med. Chem. Lett.,* **2008**, *18*, 5512-5517.

[15] Vatmurge, N.S.; Hazra, B.G.; Pore, V.S.; Shirazi, F.; Deshpande, M.V.; Kadreppa, S.; Chattopadhyay, S.; Gonnade, R.G. Synthesis and biological evaluation of bile acid dimers linked with 1,2,3-triazole and bis-beta-lactam. *Org. Biomol. Chem.,* **2008**, *6*, 3823-3830.

[16] Ruiz-Herrera, J.; San-Blas, G. Chitin synthesis as a target for antifungal drugs. *Curr. Drug Targets Infect. Disord.,* **2003**, *3*, 77-91.

[17] Bartnicki-Garcia, S. Chitosomes: past, present and future. *FEMS Yeast Res.,* **2006**, *6*, 957-965.

[18] Cohen, E. Chitin biochemistry: synthesis and inhibition. *Ann. Rev. Entomol.*, **1987**, *32*, 71-93.

[19] Bowen, A.R.; Chen-Wu, J.L.; Momany, M.; Young, R.; Szaniszlo, P.J.; Robbins, P.W. Classification of fungal chitin synthases. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 519-523.

[20] Lenardon, M.D.; Milne, S.A.; Mora-Montes, H.C.M.; Kaffarnik, F.A.R.; Peck, S.C.; Brown, A.J.P.; Munro, C.A.; Gow, N.A.R. Phosphorylation regulates polarisation of chitin synthesis in *Candida albicans*. *J. Cell Sci.,* **2010**, *123*, 2199-2206.

[21] Chitnis, M.V.; Munro, C.A.; Brown, A.J.P.; Gooday, G.W.; Gow, N.A.R.; Deshpande, M.V. The zygomycetous fungus, *Benjaminiella poitrasii* contains a large family of differentially regulated chitin synthase genes. *Fungal Genet. Biol.,* **2002**, *36*, 215-223.

[22] Miyazaki, A.; Ootaki, T. Multiple genes for chitin synthase in the zygomycete fungus *Phycomyces blakesleeanus*. *J. Gen. Appl. Microbiol.,* **1997**, *43*, 333-340

[23] Roncero, C. The genetic complexity of chitin synthesis in fungi. *Curr. Genet.,* **2002**, *41*, 367-378.

[24] Saxena, I.M.; Brown, R.M., Jr.; Fevre, M.; Geremia, R.A.; Henrissat, B. Multidomain architecture of beta-glycosyl transferases: implications for mechanism of action. *J. Bacteriol.,* **1995**, *177*, 1419-1424.

[25] Khale, A.; Srinivasan, M.C.; Deshmukh, S.S.; Deshpande, M.V. Dimorphism of *Benjaminiella poitrasii* isolation and biochemical studies of morphological mutants. *Anton. Leeuw. Int. J. G.,* **1990**, *57*, 37-41.

[26] Chaudhary, P.M.; Chavan, S.R.; Shirazi, F.; Razdan, M.; Nimkar, P.; Maybhate, S.P.; Likhite, A.P.; Gonnade, R.; Hazara, B.G.; Deshpande, M.V.; Deshpande, S.R. Exploration of click reaction

for the synthesis of modified nucleosides as chitin synthase inhibitors. *Bioorg. Med. Chem.,* **2009**, *17*, 2433-2440.

[27] Patil, R.S.; Deshpande, A.M.; Natu, A.A.; Nahar, P.; Chitnis, M.; Ghormade, V.; Laxman, R.S.; Rokade, S.; Deshpande, M.V. Biocontrol of root infecting plant pathogenic fungus, *Sclerotium rolfsii* using mycolytic enzymes and chitin metabolism inhibitors singly and in combination. *J. Biol. Control.,* **2001**, *15*, 157-164.

[28] Chitnis, M.V.; Deshpande, M.V. Isolation and regeneration of protoplasts from the yeast and mycelial form of the dimorphic zygomycete *Benjaminiella poitrasii*: Role of chitin metabolism for morphogenesis during regeneration. *Microbiol. Res.,* **2002**, *157*, 29-37.

[29] Carrano, L.; Tavecchia, P.; Sponga, F.; Spreafico, F. Dansyl *N*-acetyl glucosamine as a precursor of fluorescent chitin: a method to detect fungal cell wall inhibitors. *J. Antibiot. (Tokyo),* **1996**, *50*, 177-179.

[30] Giaever, G.; Shoemaker, D.D.; Jones, T.W.; Liang, H.; Winzeler, E.A.; Astromoff, A.; Davis, R.W. Genomic profiling of drug sensitivities via induced haploinsufficiency. *Nat. Genet.,* **1999**, *21*, 278-283.

[31] Baetz, K.; McHardy, L.; Gable, K.; Tarling, T.; Reberioux, D.; Bryan, J.; Andersen, R.J.; Dunn, T.; Hieter, P.; Roberge, M. Yeast genome-wide drug-induced haploinsufficiency screen to determine drug mode of action. *Proc. Natl. Acad. Sci. U.S.A.,* **2004**, *101*, 4525-4530.

[32] Uhl, M.A.; Biery, M.; Craig, N.; Johnson, A.D. Haploinsufficiency-based large-scale forward genetic analysis of filamentous growth in the diploid human fungal pathogen *Candida albicans*. *EMBO J.,* **2003**, *22*, 2668-2678.

[33] Xu, D.; Jiang, B.; Ketela, T.; Lemieux, S.; Veillette, K.; Martel, N.; Davison, J.; Sillaots, S.; Trosok, S.; Bachewich, C.; Bussey, H.; Youngman, P.; Roemer, T. Genome-wide fitness test and mechanism-of-action studies of inhibitory compounds in *Candida albicans*. *PLoS Pathog.,* **2007**, *3*, e92.

[34] Gooday, G.W.; Woodman, J.; Casson, E.A.; Browne, C.A. Effect of nikkomycin on chitin spine formation in the diatom *Thalassiosira fluviatilis*, and observations on its peptide uptake. *FEMS Microbiol. Lett.,* **1985**, *28*, 335-340.

[35] Choi, W.J.; Cabib, E. The use of divalent cations and pH for the determination of specific yeast chitin synthetases. *Anal. Biochem.,* **1994**, *219*, 368-372.

[36] Lucero, H.A.; Kuranda, M.J.; Bulik, D.A. A nonradioactive, high throughput assay for chitin synthase activity. *Anal. Biochem.,* **2002**, *305*, 97-105.

[37] Isono, K.; Junsaku, N.; Yoshitsugu, K.; Saburo, S. Studies on polyoxins, antifungal antibiotics part I. isolation and characterization of polyoxins A and B. *Agric. Biol. Chem.,* **1965**, *29*, 848-854.

[38] Suzuki, S.; Isono, K.; Nagatsu, J.; Mizutani, T.; Kawashima, Y.; Mizuno, T. A new antibiotic, polyoxin A. *J. Antibiot. (Tokyo),* **1965**, *18*, 131.

[39] Isono, K.; Asahi, K.; Suzuki, S. Studies on polyoxins, antifungal antibiotics. XIII. The structure of polyoxins. *J. Am. Chem. Soc.,* **1969**, *91*, 7490-7505.

[40] Keller, F.A.; Cabib, E. Chitin and yeast budding. Properties of chitin synthetase from *Saccharomyces carlsbergensis. J. Biol. Chem.,* **1971**, *246*, 160-166.

[41] Hori, M.; Kakiki, K.; Misato, T. Further study on the relation of polyoxin structure to chitin synthetase inhibitions. *Agri. Biol. Chem.,* **1974**, *38*, 691-698.

[42] Hori, M.; Kakiki, K.; Misato, T. Interaction between polyoxin and active center of chitin synthetase. *Agri. Biol. Chem.,* **1974**, *38*, 699-705.

[43] Endo, A.; Kakiki, K.; Misato, T. Mechanism of action of the antifungal agent polyoxin D. *J. Bacteriol.,* **1970**, *104*, 189-196.

[44] Bartnicki-Garcta, S.; Lippman, E. Inhibition of *Mucor rouxii* by polyoxin D: effects on chitin synthetase and morphological development. *J. Gen. Microbiol.,* **1972**, *71*, 301-309.

[45] Bowers, B.; Levin, G.; Cabib, E. Effect of polyoxin D on chitin synthesis and septum formation in *Saccharomyces cerevisiae*. *J. Bacteriol.,* **1974**, *119*, 564-575.

[46] Smith, H.A.; Shenbagamurthi, P.; Naider, F.; Kundu, B.; Becker, J.M. Hydrophobic polyoxins are resistant to intracellular degradation in *Candida albicans*. *Antimicrob. Agents Chemother.,* **1986**, *29*, 33-39.

[47] Dähn, U.; Hagenmaier, H.; Hohne, H.; Konig, W.; Wolf, G.; Zahner, H. Stoffwechselprodukte yon Mikroorganismen 154. Mitteilung. Nikkomycin, ein neuer Hemmstoff der Chitinsynthese bei Pilzen. *Arch. Microbiol.,* **1976**, *107*, 143-160.

[48] Decker, H.; Walz, F.; Bormann, C.; Zähner, H.; Fiedler, H.; Heitsch, H.; König, W. Metabolic products of microorganisms. 255. Nikkomycins Wz and Wx, new chitin synthetase inhibitors from *Streptomyces tendae*. *J. Antibiot. (Tokyo)* **1990**, *43*, 43-48.

[49] Fiedler, H.P. Nikkomycins and polyoxins. In: *Natural products isolation. separation of antimicrobials, antivirals and enzyme inhibitors.* Wagman G.W.; Cooper, R., Eds.; Elsevier: Amsterdam, **1989;** pp. 153-189.

[50] Decker, H.; Zähner, H.; Heitsch, H.; Konig, W.A.; Fiedler, H.P. Structure-activity relationships of the nikkomycins. *J. Gen. Microbiol.,* **1991**, *137*, 1805-1813.

[51] Gaughran, J.P.; Lai, M.H.; Kirsch, D.R.; Silverman, S.J. Nikkomycin Z is a specific inhibitor of *Saccharomyces cerevisiae* chitin synthase isozyme Chs3 *in vitro* and *in vivo*. *J. Bacteriol.,* **1994**, *176*, 5857-5860.

[52] Kim, M.-K.; Park, H.-S.; Kim, C.-H.; Park, H.-M.; Choi, W. Inhibitory effect of nikkomycin Z on chitin synthases in *Candida albicans*. *Yeast,* **2002**, *19*, 341-349.

[53] Bago, B.; Chamberland, H.; Goulet, A.; Vierheilig, H.; Lafontaine, J.G.; Piché, Y. Effect of Nikkomycin Z, a chitin-synthase inhibitor, on hyphal growth and cell wall structure of two arbuscular-mycorrhizal fungi. *Protoplasma,* **1996**, *192*, 80-92.

[54] Hector, R.F.; Zimmer, B.L.; Pappagianis, D. Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis. *Antimicrob. Agents Chemother.,* **1990**, *34*, 587-593.

[55] Isono, K.; Azuma, T.; Suzuki, S. Polyoxin analogs. I. Synthesis of aminoacyl derivatives of 5'- amino-5'- deoxyuridine. *Chem. Pharm. Bull.,* **1971**, *19*, 505-512.

[56] Isono, K.; Suzuki, S.; Azuma, T. Semisynthetic polyoxins aminoacyl derivatives of polyoxin C and their *in vitro* activity. *Agri. Biol. Chem.,* **1971**, *35*, 1986-1989.

[57] Azuma, T.; Saita, T.; Isono, K. Polyoxin alalogues III synthesis and biological activity of aminoacyl derivatives of polyoxin C and L. *Chem. Pharm. Bull.,* **1977**, *25*, 1740-1748.

[58] Shenbagamurthi, P.; Smith, H.A.; Becker, J.M.; Steinfeld, A.; Naider, F. Design of anticandidal agents: synthesis and biological properties of analogs of polyoxin L. *J. Med. Chem.,* **1983**, *26*, 1518-1522.

[59] Khare, R.K.; Becker, J.M.; Naider, F.R. Synthesis and anticandidal properties of polyoxin L analogs containing α-amino fatty acids. *J. Med. Chem.,* **1988**, *31*, 650-656.

[60] Naider, F.; Shenbagamurthi, P.; Steinfeld, A.S.; Smith, H.A.; Boney, C.; Becker, J.M. Synthesis and biological activity of tripeptidyl polyoxins as antifungal agents. *Antimicrob. Agents Chemother.,* **1983**, *24*, 787-796.

[61] Emmer, G.; Ryder, N.S.; Grassberger, M.A. Synthesis of new polyoxin derivatives and their activity against chitin synthase from *Candida albicans*. *J. Med. Chem.,* **1985**, *28*, 278-281.

[62] Cooper, A.B.; Desai, J.; Lovey, R.G.; Saksena, A.K.; Girijavallabhan, V.M.; Ganguly, A.K.; Loebenberg, D.; Parmegiani, R.; Cacciapuoti, A. A convenient degradation of polyoxin D to uracil polyoxin C: access to key intermediates and synthesis of antifungal α-aminoacyl derivatives of UPOC. *Bioorg. Med. Chem. Lett.,* **1993**, *3*, 1079-1084.

[63] Obi, K.; Uda, J.-I.; Iwase, K.; Sugimoto, O.; Ebisu, H.; Matsuda, A. Novel nikkomycin analogs: inhibitors of the fungal cell wall biosynthesis enzyme chitin synthase. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 1451-1454.

[64] Krainer, E.; Becker, J.M.; Naider, F. Synthesis and biological evaluation of dipeptidyl and tripeptidyl polyoxin and nikkomycin analogs as anticandidal prodrugs. *J. Med. Chem.,* **1991**, *34*, 174-180.

[65] Behr, J.-B.; Gourlain, T.; Helimi, A.; Guillerm, G. Design, synthesis and biological evaluation of hetaryl-nucleoside derivatives as inhibitors of chitin synthase. *Bioorg. Med. Chem. Lett.,* **2003**, *13*, 1713-1716.

[66] Suda, A.; Ohta, A.; Sudoh, M.; Tsukuda, T.; Shimma, N. Combinatorial synthesis of nikkomycin analogs on solid support. *Heterocycles,* **2001**, *55*, 1023-1028.

[67] Xie, J.; Thellend, A.; Becker, H.; Vidal-Cros, A. Synthesis and evaluation of a C-glycosyl nucleoside as an inhibitor of chitin synthase. *Carbohydr. Res.,* **2001**, *334*, 177-182.

[68] Chang, R.; Vo, T.-T.; Finney, N.S. Synthesis of the C1-phosphonate analog of UDP-GlcNAc. *Carbohydr. Res.,* **2006**, *341*, 1998-2004.

[69] Li, R.K.; Rinaldi, M.G. *In vitro* antifungal activity of nikkomycin Z in combination with fluconazole or itraconazole. *Antimicrob. Agents Chemother.,* **1999**, *43*, 1401-1405.

[70] Stevens, D.A. Drug interaction studies of a glucan synthase inhibitor (LY 303366) and a chitin synthase inhibitor (nikkomycin Z) for inhibition and killing of fungal pathogens. *Antimicrob. Agents Chemother.,* **2000**, *44*, 2547-2548.

[71] Chiou, C.C.; Mavrogiorgos, N.; Tillem, E.; Hector, R.; Walsh, T.J. Synergy, pharmacodynamics, and time-sequenced ultrastructural changes of the interaction between nikkomycin Z and the echinocandin FK463 against *Aspergillus fumigatus*. *Antimicrob. Agents Chemother.,* **2001**, *45*, 3310-3321.

[72] Sandovsky-Losica, H.; Shwartzman, R.; Lahat, Y.; Segal, E. Antifungal activity against *Candida albicans* of nikkomycin Z in combination with caspofungin, voriconazole or amphotericin B. *J. Antimicrob. Chemother.,* **2008**, *62*, 635-637.

[73] Stevens, D.A.; Calderon, L.; Martinez, M.; Clemons, K.V.; Wilson, S.J.; Selitrennikoff, C.P. Zeamatin, clotrimazole and nikkomycin Z in therapy of a *Candida vaginitis* model. *J. Antimicrob. Chemother.,* **2002**, *50*, 361-364.

[74] Yeager, A.R.; Finney, N.S. The first direct evaluation of the two-active site mechanism for chitin synthase. *J. Org. Chem.,* **2004**, *69*, 613-618.

[75] Yeager, A.R.; Finney, N.S. Second-generation dimeric inhibitors of chitin synthase. *Bioorg. Med. Chem.,* **2004**, *12*, 6451-6460.

[76] Li, J.; Li, L.; Tian, Y.; Niu, G.; Tan, H. Hybrid antibiotics with the nikkomycin nucleoside and polyoxin peptidyl moieties. *Metab. Eng.,* **2011**, *13*, 336-344.

[77] Lee, S.; Kim, S.-U.; Kim, E.; Hwang, E.-I.; Jung, S.-H.; Lee, H.K. Biological evaluation of phellinsin A analogs as chitin synthase II inhibitors. *Bull. Korean Chem. Soc.,* **2007**, *28*, 1824-1826.

[78] Pfefferle, W.; Anke, H.; Pfefferle, M.W. Inhibition of solubilized chitin synthase by chlorinated aromatic compounds isolated from mushroom cultures. *Agri. Biol. Chem.,* **1990**, *54*, 1381-1384.

[79] Kang, T.H.; Hwang, E.I.; Yun, B.S.; Park, K.D.; Kwon, B.M.; Shin, C.S.; Kim, S.U. Inhibition of chitin synthases and antifungal activities by 2'-benzoyloxycinnamaldehyde from *Pleuropterus ciliinervis* and its derivatives. *Biol. Pharm. Bull.,* **2007**, *30*, 598-602.

[80] Roche, H.-L. Xanthofulvin as an inhibitor of chitin synthase and its potential as an antifungal. *Expert Opin. Ther. Pat.,* **1993**, *3*, 1801-1802.

[81] Wenke, J.; Heidrun, A.; Olov, S. Pseurotin A and 8-*O*-demethylpseurotin A from *Aspergillus fumigatus* and their inhibitory activities on chitin synthase. *Biosci. Biotechnol. Biochem.,* **1993**, *57*, 961-964.

[82] Hwang, E.I.; Yun, G.S.; Kim, Y.K.; Kwon, B.M.; Kim, H.G.; Lee, H.B.; Jeong, W.J.; Kim, S.U. Phellinsin A, a novel chitin synthase inhibiting phenolic from the fungus *Phellinus* sp. *J. Antibiot.,* **2000**, *53*, 903-911.

[83] Jeong, T.-S.; Hwang, E.-I.; Lee, H.-B.; Lee, E.-S.; Kim, Y.-K.; Min, B.-S.; Bae, K.-H.; Bok, S.-H.; Kim, S.-U. Chitin synthase II inhibitory activity of ursolic acid, isolated from *Crataegus pinnatifida*. *Planta Med.,* **1999**, *65*, 261-262.

[84] Kim, S.-U.; Hwang, E.-I.; Nam, J.-Y.; Son, K.-H.; Bok, S.-H.; Kim, H.-E.; Kwon, B.-M. Inhibition of chitin synthase II by catechins from stem bark of *Taxus cuspidata*. *Planta Med.,* **1999**, *65*, 97-98.

[85] Park, K.-S.; Kang, K.-C.; Kim, J.-H.; Adams, D.J.; Johng, T.-N.; Paik, Y.-K. Differential inhibitory effects of protoberberines on sterol and chitin biosyntheses in *Candida albicans*. *J. Antimicrob. Chemother.,* **1999**, *43*, 667-674.

[86] Hwang, E.-I.; Kwon, B.-M.; Lee, S.-H.; Kim, N.-R.; Kang, T.-H.; Kim, Y.-T.; Park, B.-K.; Kim, S.-U. Obovatols, new chitin synthase 2 inhibitors of *Saccharomyces cerevisiae* from *Magnolia obovata*. *J. Antimicrob. Chemother.,* **2002**, *49*, 95-101.

[87] Niu, C.; Qu, J.B.; Lou, H.X. Antifungal bis[bibenzyls] from the chinese liverwort *Marchantia polymorpha* L. *Chem Biodivers,* **2006**, *3*, 34-40.

[88] Wu, X.-Z.; Cheng, A.-X.; Sun, L.-M.; Lou, H.-X. Effect of plagiochin E, an antifungal macrocyclic bis(bibenzyl), on cell wall chitin synthesis in *Candida albicans*. *Acta Pharmacol. Sin.,* **2008**, *29*, 1478-1485.

[89] Hwang, E.I.; Lee, Y.M.; Lee, S.M.; Yeo, W.H.; Moon, J.S.; Kang, T.H.; Park, K.D.; Kim, S.U. Inhibition of chitin synthase 2 and antifungal activity of lignans from the stem bark of *Lindera erythrocarpa*. *Planta Med.,* **2007**, *73*, 679-682.

[90] Kang, T.H.; Hwang, E.I.; Yun, B.S.; Shin, C.S.; Kim, S.U. Chitin synthase 2 inhibitory activity of *O*-methyl pisiferic acid and 8,20-dihydroxy-9(11),13-abietadien-12-one, isolated from *Chamaecyparis pisifera*. *Biol. Pharm. Bull.,* **2008**, *31*, 755-759.

[91] Yim, N.H.; Hwang, E.I.; Yun, B.S.; Park, K.D.; Moon, J.S.; Lee, S.H.; Sung, N.D.; Kim, S.U. Sesquiterpene furan compound CJ-01, a novel chitin synthase 2 inhibitor from *Chloranthus japonicus* SIEB. *Biol. Pharm. Bull.,* **2008**, *31*, 1041-1044.

[92] Ke, S.; Liu, F.; Wang, N.; Yang, Q.; Qian, X. 1,3,4-Oxadiazoline derivatives as novel potential inhibitors targeting chitin biosynthesis: Design, synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.,* **2009**, *19*, 332-335.

[93] López, S.N.; Castelli, M.V.; Zacchino, S.A.; Domínguez, J.N.; Lobo, G.; Charris-Charris, J.; Cortés, J.C.G.; Ribas, J.C.; Devia, C.; Rodríguez, A.M.; Enriz, R.D. *In vitro* antifungal evaluation and structure-activity relationships of a new series of chalcone derivatives and synthetic analogs, with inhibitory properties against polymers of the fungal cell wall. *Bioorg. Med. Chem.,* **2001**, *9*, 1999-2013.

[94] Urbina, J.M.; Cortés, J.C.G.; Palma, A.; López, S.N.; Zacchino, S.A.; Enriz, R.D.; Ribas, J.C.; Kouznetzov, V.V. Inhibitors of the fungal cell wall. Synthesis of 4-aryl-4-*N*-arylamine-1-butenes and related compounds with inhibitory activities on β-(1-3) glucan and chitin synthases. *Bioorg. Med. Chem.,* **2000**, *8*, 691-698.

[95] Gholap, A.R.; Toti, K.S.; Shirazi, F.; Kumari, R.; Bhat, M.K.; Deshpande, M.V.; Srinivasan, K.V. Synthesis and evaluation of antifungal properties of a series of the novel 2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile and its analogs. *Bioorg. Med. Chem.,* **2007**, *15*, 6705-6715.

[96] Masubuchi, K.; Taniguchi, M.; Umeda, I.; Hattori, K.; Suda, H.; Kohchi, Y.; Isshiki, Y.; Sakai, T.; Kohchi, M.; Shirai, M.; Okabe, H.; Sudoh, M.; Yamazaki, T.; Shimma, N. Synthesis and structure-activity relationships of novel fungal chitin synthase inhibitors. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 1459-1462.

[97] Sudoh, M.; Yamazaki, T.; Masubuchi, K.; Taniguchi, M.; Shimma, N.; Arisawa, M.; Yamada-Okabe, H. Identification of a novel inhibitor specific to the fungal chitin synthase. *J. Biol. Chem.,* **2000**, *275*, 32901-32905.

[98] Yadan J-C.; Gonneau, M.; Sarthou, P.; Le Goffic, F. Sensitivity to nikkomycin Z in *Candida albicans*: role of peptide permeases. *J. Bacteriol*., **1984**, *160*, 884-888.

[99] Hector, R. F.; Schaller, K. Positive interaction of nikkomycins and azoles against *Candida albicans in vitro* and *in vivo*. *Antimicrob. Agents Chemother.,* **1992**, *36*, 1284-1289.

[100] International Society of Computational Biology. http://www.iscb.org/uploaded/css/38/10508.pdf (Accessed November 4, 2011).

[101] Musonda, C.C.; Whitlock, G.A.; Witty, M.J.; Brun, R.; Kaiser, M. Chloroquine-astemizole hybrids with potent *in vitro* and *in vivo* antiplasmodial activity. *Bioorg. Med. Chem. Lett.,* **2009**, *19*, 481-484.

Received: November 23, 2011 Revised: April 03, 2012 Accepted: April 08, 2012