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Synthesis and Structure–Activity Relationships of Novel Fungal Chitin Synthase Inhibitors

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Abstract—A novel *Candida albicans* chitin synthase 1 (CaChs1) inhibitor, **RO-41-0986** (**1**) was discovered by random screening. Systematic modification led to the identification of a highly potent CaChs1 inhibitor, **RO-09-3024** (**2**), having strong antifungal activity against *Candida* spp. in vitro.

Introduction

The medical need for safe and effective systemic antifungal agents with novel modes of action has been intensified due to an increasing number of deep-seated fungal infections and resistance development.[1] Chitin is a major and essential component of the fungal cell wall, which is widely distributed among yeast and mycelial fungi but absent in mammalian cells.[2] Thus, it is a very attractive target for designing antifungal agents. Polyoxins and nikkomicins[3] are known competitive inhibitors of chitin synthase, but they have shown only limited antifungal activity against *Coccidiodes* and *Blastomyces* spp.

A novel chitin synthase inhibitor, **RO-41-0986** (**1**: E:Z 2:1 mixture), was discovered from the Roche compound libraries by random screening (Fig. 1). It specifically inhibits *Candida albicans* chitin synthase 1[4,5] ($IC_{50} = 0.07$ μM) in a non-competitive manner. Though **1** is structurally similar to terbinafine[6] [IC_{50} (CaChs1) = 11.3 μM, IC_{50} (squalene epoxidase) = 0.03 μM)], it did not inhibit squalene epoxidase ($IC_{50} > 100$ μM) at all (Fig. 2). Furthermore, **1** showed only weak in vitro antifungal activity[7] ($IC_{50} = 3.3$ μg/mL) against *Candida albicans*, and was inactive against *Cryprococcus* and *Aspergillus* spp. Despite its strong CaChs1 inhibitory activity, **1** did not exhibit in vivo efficacy in a murine systemic candidiasis model. Therefore, we initiated a chemical modification study of **1** with the aim of identifying a novel chitin synthase inhibitor that has potent antifungal activity against *Candida albicans, Cryprococcus neoformans,* and *Aspergillus fumigatus* in vivo. In this paper, we describe the synthesis and structure–activity relationships (SAR) of a novel series of chitin synthase inhibitors.

Chemistry

Since we found that 3,4-dihydro derivatives of **1** retained both the chitin synthase inhibitory activity and in vitro antifungal activity against *Candida albicans*, we selected 5-amino-3,4-dihydro-1H-quinolin-2-one **5** as the representative core structure. The general synthetic procedure for the synthesis of 3,4-dihydro derivatives of **1** is illustrated in Scheme 1.

Compound **5** was synthesized from 2,6-dinitro-benzaldehyde **3** by treatment with methyl (triphenyl-phosphoranylidene) acetate in refluxing benzene followed by hydrogenation over 10% $Pd(OH)_2$-C in 78% yield. The *N*-monoalkylation of the aromatic amine of **5** was carried out by the conventional alkylation with the appropriate alkyl halide (e.g., 1-bromo-6,6-dimethyl-2-hepten-4-yne[8]) in the presence of K_2CO_3 or the reductive alkylation with the appropriate aldehyde such as 6,6-dimethyl-hepta-2,4-diynal and $NaBH_3CN$. Then, the second *N*-alkyla-

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tion, *N*-acylation or *N*-sulfonylation was carried out by the reductive alkylation, conventional acylation or sulfonylation, respectively, in a similar manner.

The hydrophilic derivatives (**11**–**14**) were synthesized from **8** as outlined in Scheme 2.

Treatment of **8** with glyoxylic acid in the presence of Cs_2CO_3 followed by methylation with $Me_2NCH(OMe)_2$ gave the hydroxylester **9** in 80% yield. The bicyclic compound **10** was synthesized from **9** by hydrogenation over 20% $Pd(OH)_2$-C in 98% yield. Diyne **11** was obtained by successive reductive alkylation with 6,6-dimethyl-hepta-2,4-diynal and $NaBH_3CN$ followed by treatment with formaldehyde solution and $NaBH_3CN$. Conversion of the hydroxyl group of **11** to the amine **12** was carried out in 3 steps; (i) *O*-mesylation, (ii) substitution of the resulting mesylate with azide group, and (iii) reduction of the azide with Ph_3P. The L-valylester **13** was synthesized by treatment with Boc-(L)-Val-OpNP followed by deprotection with TFA. The phosphate derivative **14** was prepared by treatment of **11** with diethyl chloro-phosphate in 14% yield.

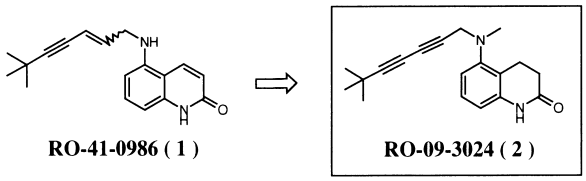


Figure 1.

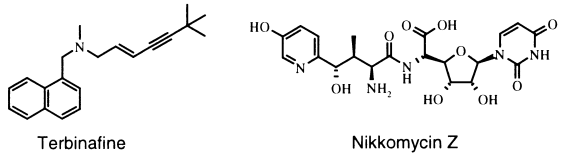
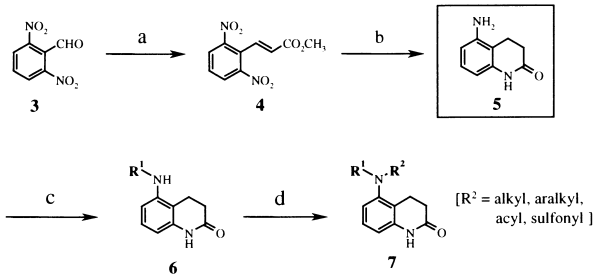
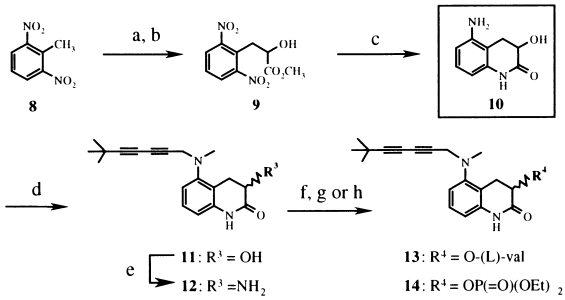


Figure 2.



Scheme 1. General synthetic route of **RO-41-0986** derivatives. (a) $Ph_3P{=}CHCO_2CH_3$, benzene, reflux (100%); (b) H_2, 10% $Pd(OH)_2$-C, MeOH, rt (78%); (c) RCH_2Br, K_2CO_3, DMF, rt or RCHO, $NaBH_3CN$, AcOH, MeOH, rt (~62%); (d) R′CHO, $NaBH_3CN$, AcOH, MeOH, rt (~100%) or R′COCl, or $R'SO_2Cl$.



Scheme 2. Synthetic route of the hydrophilic derivatives. (a) OHC-CO_2H, Cs_2CO_3, MeOH, reflux (88%); (b) $Me_2NCH(OMe)_2$, CH_2Cl_2, rt (91%); (c) H_2, 20% $Pd(OH)_2$-C, MeOH, rt (98%); (d) (i) 6,6-dimethyl-hepta-2,4-diynal, $NaBH_3CN$, AcOH, MeOH, rt; (ii) HCHO, $NaBH_3CN$, AcOH, MeOH, rt (90%); (e) (i) MsCl, collidine, CH_2Cl_2, 0 °C; (ii) NaN_3, DMF, rt; (iii) Ph_3P, THF, rt then H_2O, 50 °C (51%); (f) Boc-(L)-Val-OpNP, DMAP, HOBT, THF, rt (49%); (g) TFA, CH_2Cl_2, rt (100%); (h) $(EtO)_2POCl$, DMAP, Pyridine, CH_2Cl_2, rt (14%).

Results and Discussion

Preliminary SAR obtained from the random screening result

The preliminary SAR (CaChs1 inhibitory activity) obtained from the random screening result indicated that the important structural elements of **1** are the bicyclic structure (cf. compounds **19** and **20**), both N(1)-H and C-2 carbonyl group (cf. compounds **15**, **16** and **17**), and the unsaturated alkyl chain (cf. compound **18**) (Fig. 3).

In addition, we found that saturation of the 3,4-double bond of **1** did not affect the enzyme inhibitory activity.

Modification of the C-5 amino group: introduction of R^2

The results of the modification of the C-5 amino group are summarized in Table 1. When an additional alkyl group (R^2) was introduced on the C-5 amino group, their chitin synthase inhibitory activity was dramatically improved. The *N*-butyl derivative **26** showed the maximum inhibitory activity: 200 times more active than **1**. However, the in vitro antifungal activity was retained only when the alkyl group was methyl or ethyl. The introduction of an acyl or sulfonyl group (R^2) resulted in lost activity.

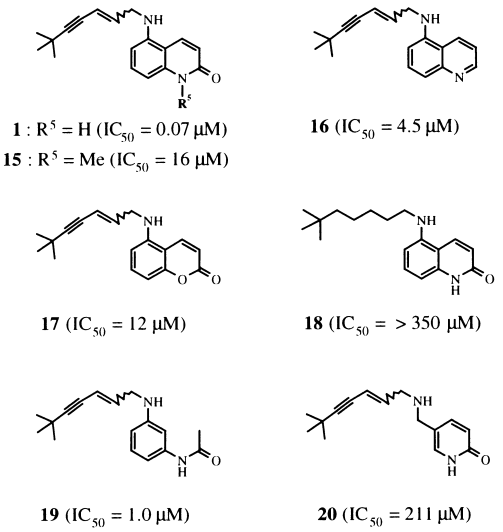


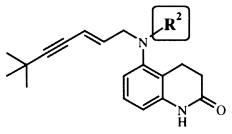
Figure 3. Preliminary data obtained from the random screening. (CaChs1:IC_{50} = μM).

Modification of the ene-yne side chain

Since the unsaturated side chain was found to be important for chitin synthase inhibitory activity from the screening result, the further optimization of this moiety was carried out. The results of the side chain modification are summarized in Tables 2 and 3. Since **1** is an *E*/*Z* mixture, the preferable geometry of the double bond was investigated with the 3,4-dihydro derivatives of **1**. The result indicates that both isomers **22** and **31** showed strong enzyme inhibitory activity, but the *E*-isomer was 4 times more active than the *Z*-isomer. The saturation or replacement of this double bond with a phenyl group resulted in reduction of inhibitory activity. Surprisingly, the replacement of the double bond with the triple bond (**2**) significantly increased the CaChs1 inhibitory activity ($IC_{50} = 0.14$ nM) as well as in vitro antifungal activity as compared with **22**. Thus, the unsaturated moiety was fixed as a diyne group, and the terminal *tert*-butyl group was further modified (Table 3).

The replacement of one methyl of the *tert*-butyl group of **2** with OH, MeO or phenyl resulted in significant loss of activity. From these data, the best side chain was identified as 6,6-dimetyl-hepta-2,4-diynyl group.

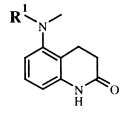
Table 1. Modification of C-5 amino group: R[2a]

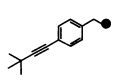


Compound	R^2	CaChs1 IC_{50} (nM)	In vitro antifungal activity *C. albicans* CY1002 IC_{50} (μg/mL)
21	H	70.9	>200
22	Methyl	2.77	0.19
23	Ethyl	0.9	0.27
24	*n*-Propyl	0.68	>200
25	iso-Propyl	1.23	>200
26	*n*-Butyl	0.35	>200
27	*n*-Pentyl	1.1	>200
28	Benzyl	54	>200
29	Acetyl	775	>200
30	Mesyl	391	>200
1		71.3	3.30
Nikkomycin Z		3200	12

[a]Medium: YPD broth, Incubation: 27 °C, 24 h; Inoculum size: $1–2\times10^4$ (CFU/mL).

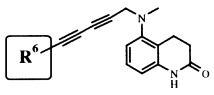
Table 2. Modification of the side chain: R[1a]



Compound	R^{1a}	Enzyme inhibition CaChs1 IC_{50} (nM)	In vitro antifungal activity IC_{50} (μg/mL) *Candida albicans* CY1002
22		2.77	0.19
31		13.2	95
32		8.95	>200
33		7.22	>200
2		0.14	0.07
Nikkomicin Z		3200	12

[a]Medium: YPD broth, Incubation: 27 °C, 24 h; Inoculum size: $1–2\times10^4$ (CFU/mL).

Table 3. Modification of the side chain: R[6a]



Compound	R^6	Enzyme inhibition CaChs1 IC_{50} (nM)	In vitro antifungal activity IC_{50} (μg/mL) *Candida albicans* CY1002
2		0.14	0.07
34		7.88	200
35		213	>200
36		1031	3.5
37		13,700	> 200
Nikkomicin Z		3200	12

[a]Medium: YPD broth, Incubation: 27 °C, 24 h; Inoculum size: $1–2\times10^4$ (CFU/mL).

Table 4. In vitro antifungal spectrum of the compound **2** and nikkomycin Z[a]

Species	Compound **2** IC_{50} (μg/mL)	Nikkomycin Z IC_{50} (μg/mL)
C. albicans CY1003	0.10	81
C. albicans CY3003	0.09	7.0
C. albicans CY1124[b]	0.17	15
C. glabrata CY1014	0.47	>200
C. tropicalis CY1038	0.07	73
C. krusei CY1191	0.31	>200
C. parapsilosis CY1028	0.50	13
C. neoformans CY1059[c]	>200	>200
A. fumigatus CF1003	43	11

[a]Medium: YPD broth for yeast, YPDA for filamentous fungi; Incubation: 27 °C, Inoculum size: $1–2\times10^4$ (CFU/mL), 24 h.
[b]Azole resistant AD.
[c]5-FC resistant 422.

Table 5. In vitro and in vivo antifungal activities of the compound **2** derivatives at C-3 position

Compound	C-3 substituent	CaChs1	In vitro antifungal activity IC_{50} (μg/mL)			In vivo[a]
		IC_{50} (nM)	*C. albicans* CY1002	*C. neoformans* CY1057	*A. fumigatus* CF1003	T/C (%) at 5 mg/kg
2	H	0.14	0.07	>200	84	34
11	OH	3	0.13	>200	82	39
13	*O*-(L)-valyl	14.9	1.3	16	19	15
12	NH_2	12.3	20	15	35	23
14	$OP(=O)(OE)_2$	0.76	2.1	0.23	21	n.t.[c]
Flucanozole		n.t.[c]	1.2	3.4	50	38[b]

[a]TOKA assay in systemic candidiasis in mice; infection: *C. albicans* CY1002 5×10^5 cells/mouse; treatment: 0, 2, 4 h after infection by iv; vehicle: 5% DMSO 5% PEG300-30% HPCD; T/C = CFU (colony forming unit) of treatment/CFU of control×100.
[b]Treatment: 0, 2, 4 h after infection by iv (1 mg/kg).
[c]n.t. = not tested.

The antifungal spectra of the compound **2** and nikkomycin Z are shown in Table 4. **2** was active against a wide range of *Candida* species, including an azole-resistant strain, weakly active against *Aspergillus fumigatus*, and inactive against *Cryprococcus neoformans*. It was significantly better than nikkomycin Z.

Modification of pyrid-2-one moiety

Compound **2** exhibited only weak in vivo efficacy in the target organ kidney assay (TOKA)[9] in a systemic candidiasis model in mice (Table 5), despite its extremely potent CaChs1 inhibitory activity. The possible reasons for the weak in vivo activity are rapid metabolism (major metabolism: *N*-demethylation in mouse liver microsome/37 °C, 20 min), strong serum protein binding (>99%: mouse), and low water solubility.

Thus, we synthesized more hydrophilic analogues of **2** as summarized in Scheme 2. The (L)-valyl ester **13** showed the improved in vivo efficacy in the TOKA assay as compared with **2** (Table 5), though the efficacy of **13** was still weaker than that of fluconazole.

In summary, we have identified novel and specific CaChs1 inhibitors, **RO-09-3024** (**2**) and its analogues, which show in vitro antifungal activity against most of *Candida* spp., including azole-resistant spp. and exhibit in vivo efficacy in TOKA assay in mice systemic candidiasis model. Further modification study is in progress.

References and Notes

1. Tuite, M. F. *TIBTECH* **1996**, *14*, 219.
2. Ruiz-Herrera, J. *Fungal Cell Wall: Structure, Synthesis, and Assembly*; CRC Press, 1992; pp 1.
3. Zhang, D.; Miller, M. J. *Curr. Pharm. Des.* **1999**, *5*, 73.
4. Sudoh, M.; Watanabe, M.; Mio, T.; Nagahashi, S.; Yamada-Okabe, H.; Takagi, M.; Arisawa, M. *Microbiology* **1995**, *141*, 2676.
5. Mio, T.; Yabe, T.; Sudoh, M.; Satoh, Y.; Nakajima, T.; Arisawa, M.; Yamada-Okabe, H. *J. Bacteriol.* **1996**, *178*, 2416.
6. Ryder, N. S.; Mieth, H. *Curr. Top Med. Mycol.* **1991**, *4*, 220.
7. Antifungal susceptibility assays were performed by the broth microdilution method according to M27-A guidelines recommended by the National Committee for Clinical Laboratory Standards (NCCLS).
8. Stütz, A. *J. Med. Chem.* **1984**, *27*, 1539.
9. Abruzzo, G. K.; Flattery, A. M.; Gill, C. J.; Kong, L.; Smith, J. G.; Krupa, D.; Pikounis, V. B.; Kropp, H.; Bartizal, K. *Antimicrob. Agents Chemother.* **1995**, *39*, 1077.