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Novel Nikkomycin Analogues: Inhibitors of the Fungal Cell Wall Biosynthesis Enzyme Chitin Synthase

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Abstract—A series of novel nikkomycin analogue inhibitors of the chitin synthase of fungal cell wall was synthesized and evaluated for their inhibitory activities. Among them, the compound having a phenanthrene group at the terminal amino acid was found to possess strong anti-chitin synthase activity. © 2000 Elsevier Science Ltd. All rights reserved.

The opportunistic infections caused by various pathogenic fungi have progressively increased and become a serious problem in chemotherapy. The reasons for this serious problem include the increasing resistance of fungi against antifungal drugs, toxicity of antifungal drugs, and an increasing number of immunocompromised hosts.[1] There is a clear need to identify new antifungal agents with different targets/modes of action that are highly selective for the fungus.[2]

The component of the fungal cell wall is the chitin unit, which is biosynthesized from uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) by chitin synthase (Chs). The natural product nikkomycin Z (NZ: **1**) is a well known Chs inhibitor which acts as a competitive inhibitor, presumably due to its structural similarity to the UDP-GlcNAc (**2**), a substrate of Chs[3] (Fig. 1).

Some research groups have been modifying a terminal amino acid of NZ (**1**) as di/tri-peptide to improve its biological activity.[4] Recently, a synthetic study of NZ (**1**) was reported by a Schering–Plough group, but the structure–activity relationship of Chs inhibition is still unclear.[5] Thus, our efforts have been focused on designing/synthesizing of novel NZ (**1**) analogues with enhanced Chs inhibitory activity. As a result, we have discovered novel NZ derivatives which have potent Chs inhibitory activities comparable to that of NZ (**1**).

Synthesis

The synthesis of novel NZ derivatives is outlined in Scheme 1.

Uracil polyoxcin C (UPOC: **3**)[6] is the starting material in the synthesis of novel NZ derivatives. The 5′-amino group of UPOC (**3**) was acylated with variety of *N*-*tert*-butoxycarbonyl protected amino acid derivatives (**4**) in the presence of *N*-hydroxysuccinimide/DCC to furnish protected NZ derivatives (**5**) in 16–60% yield.

In the final step, the protecting groups of **5** were removed with TFA, and the resulting TFA salt of **6** was subjected to ion-exchange resin (Dowex XFS-43279). After lyophylization of the products, white–pale yellow powders were obtained as novel NZ derivatives (**6**) in 19–98% yield.[7]

Biological Properties

The newly synthesized NZ analogues were designed to have a simplified chemical structure as a variety substituent at the terminal amino acid moiety, e.g., *S*-alkyl-L-Cys groups (**6a–d**), *S*-aryl-L-Cys groups (**6e–j**) and *S*-arylmethyl groups (**6k–q**).

Their Chs [isolated from *Candida albicans*] inhibition rate (%) at a concentration of 100 μg/mL and IC_{50} values are summarized[8] and nikkomycin Z (NZ:**1**) is also presented as a reference compound[9] in Table 1.

The Chs inhibitory activity of the *S*-alkyl-L-Cys derivatives (**6a–d**) and *S*-aryl-L-Cys derivatives (**6e–j**) were weak.

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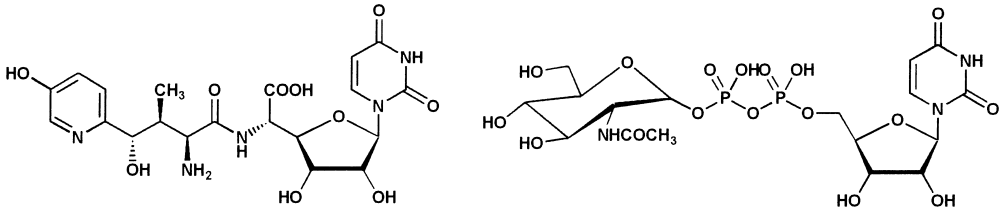
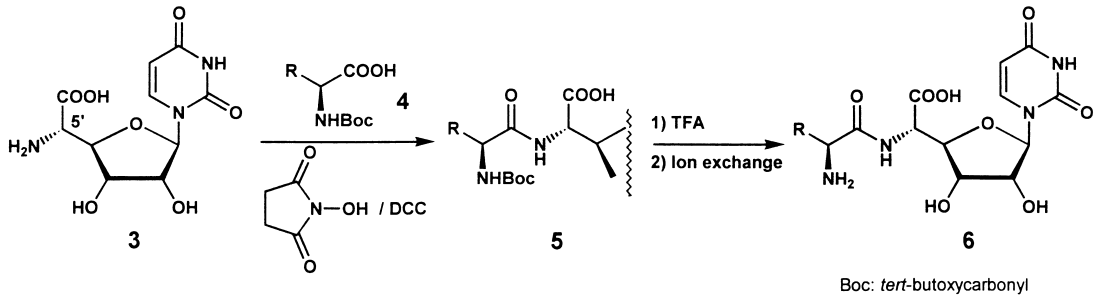


Figure 1.



Scheme 1. Synthesis of novel nikkomycins.

Despite having the same atoms as a spacer in the terminal amino acid in the NZ (**1**), the *S*-aryl-L-Cys derivatives (**6e–j**) showed the weakest anti-Chs activity. While **6g** has the same functional group (3-hydroxypyridine) as NZ (**1**), its IC_{50} value was 100 times less than NZ (**1**).

On the other hand, *S*-arylmethyl-L-Cys derivatives (**6k–p**) showed the most potent anti-Chs activity in this series. Anti-Chs activity was enhanced by introducing a β-dimethyl group by using L-penicillamine instead of L-Cys of **6k** as the β-methyl group of NZ (**1**). Furthermore, the introduction of a hydroxyl group into the terminal aryl moiety resulted in significantly enhanced anti-Chs activity. However, the compound **6q**, a replacement of the hydroxyphenyl group in the terminal amino acid with the 3-hydroxypyridine group as NZ (**1**), showed very weak anti-Chs activity. Conformational analysis of **6q** and NZ (**1**) using a molecular mechanics calculation[10] showed that the acidic hydroxyl group of 3-hydroxypyridine moiety on NZ (**1**) could coincide with the basic nitrogen atom of the 3-hydroxypyridine moiety on **6q**. We concluded that the character of the Chs inhibition site on **6q** was reverse to the corresponding NZ (**1**), and that its anti-Chs activity of **6q** was therefore less than that of the other phenolic compounds (**6m–o**).

In order to find an active compound with high anti-Chs activity, we investigated a receptor site model study for NZ (**1**).[11] The receptor site model cannot postulate a three-dimensional arrangement of atoms or functional groups common to the binding compounds, but can directly represent the essential features of the receptor site in view of complementarity between the shape and properties of the receptor site and the binding compounds.

The outcome of the above study is shown in Figure 2. The surface is colored brown to map the hydrophobic areas of the model. Areas that were not hydrophobic are white. In this study, areas from the γ-methyl group to the pyridine moiety of NZ (**1**) were classified as hydrophobic. Therefore, assuming complementarity between the hydrophobicity of Chs and its inhibitors, we thought that compounds having such aryl groups as phenyl, naphthyl and phenanthrenyl etc., might enhance Chs inhibitory activity and designed/synthesized compounds having a series of hydrophobic groups at the β-position of the terminal amino acid.

As expected, newly designed hydrophobic compounds (**6r–u**) exhibited strong anti-Chs activity. The compound with a phenanthrene group at the terminal amino acid (**6u; KFC-431**) showed anti-Chs activity as strong as that of natural NZ (**1**).

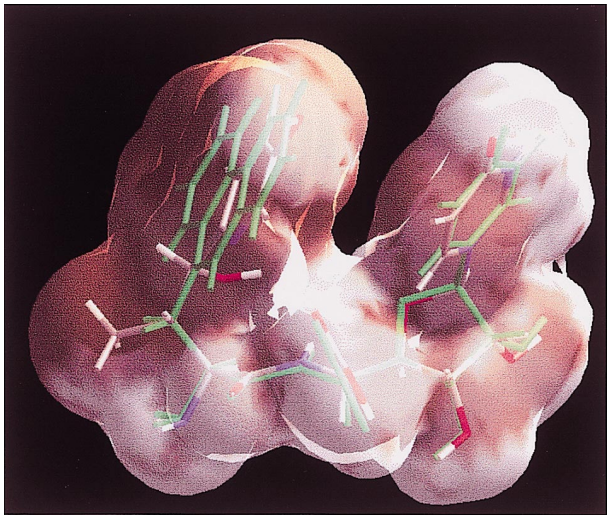
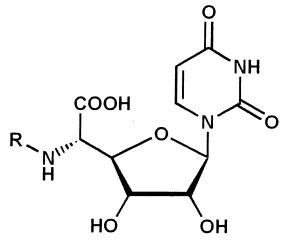
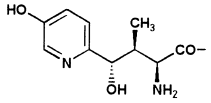
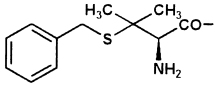
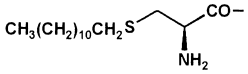
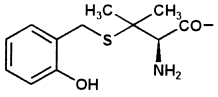
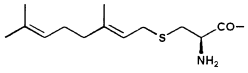
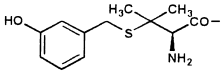
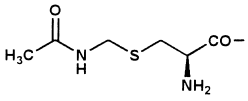
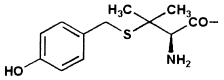
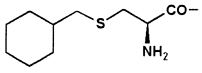
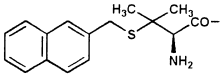
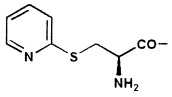
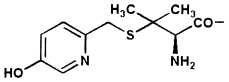
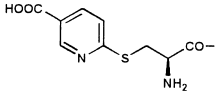
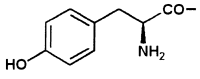
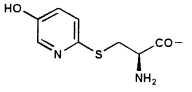
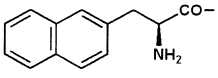
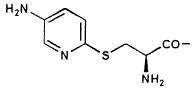
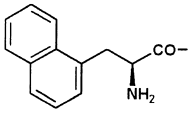
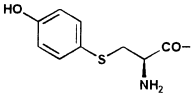
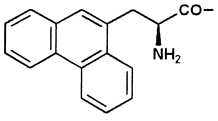
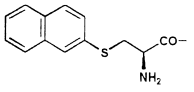
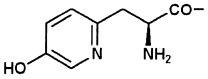
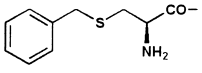
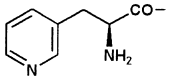


Figure 2. Receptor site model of nikkomycin *Z* (**1**) and **6u** (green). Brown: hydrophobic area white: non hydrophobic area.

Table 1. Chitin synthases inhibition activity of novel nikkomycins



No.	R	Chs inhibition		No.	R	Chs inhibition	
		Rate (%)	IC_{50} (μg/mL)			Rate (%)	IC_{50} (μg/mL)
1		—	0.393	**6l**		97	9.83
6a		64	—	**6m**		88	13.8
6b		22	—	**6n**		100	4.91
6c		65	—	**6o**		99	5.89
6d		67	—	**6p**		94	1.42
6e		48	—	**6q**		58	—
6f		64	52.5	**6r**		99	4.83
6g		73	36.0	**6s**		96	6.43
6h		42	—	**6t**		99	4.69
6i		44	—	**6u**		99	0.31
6j		40	—	**6v**		34	—
6k		78	—	**6w**		82	19.8

In conclusion, molecular design of novel NZ (**1**) analogues with hydrophobic groups at the β-position created novel NZ analogues with strong anti-Chs activity without the β-methyl and γ-hydroxyl groups which are the original functional groups in NZ (**1**). As a result, we found the phenanthrene derivative **6u** (**KFC-431**) by the receptor site model study. Finally, these investigations could reduce the two stereo centers from terminal amino acid in NZ (**1**) and exploit an easy way to develop novel NZ analogues.

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References and Notes

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7. **6u**: ^{1}H NMR (400 MHz, D_2O) δ: 3.30–3.52 (2H, m), 3.85–4.00 (3H, m), 4.13 (1H, t, $J=5.9$ Hz), 4.26 (1H, d, $J=3.0$ Hz), 5.38–5.43 (2H, m), 7.19 (1H, d, $J=7.8$ Hz), 7.38–8.00 (6H, m), 8.00–8.19 (1H, m), 8.45–8.85 (2H, m). FAB–MS (negative, *m*/*z*): 533[M-H]$^-$.
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9. Nikkomycin Z (NZ: **1**) as reference compound was purchased from Calbiochem Inc.
10. Energy computations: A UDP-GlcNAc structure were used in a molecular dynamics (MD) simulation at 3000 K to sample conformational space. The structures lying lowest in potential energy were fully optimized with CHARMm version 22 (Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M., *J. Comp. Chem*., **1983**, *4*, 187.) as available through the QUANTA molecular modeling system version 4.0. The energy computation of NZ (**1**) was performed in the same manner. The structure with anti $C_{3'}$-endo in uridine ring conformation was used for the superposition. The structures of two Chs inhibitors (**6q, 6u**) were also generated through the conformational search. The lowest-energy structure was fully optimized each. During the CHARMm calculation, the dielectric constant for Chs was taken to be 10.0. The energy computations were performed on a Silicon Graphics 4D/35TG workstation. The resulting models were visualized on a Silicon Graphics O2/R5000 workstation. Flexible fitting: The flexible fitting NZ (**1**) to the template UDP-GlcNAc was performed such that the positions between the corresponding 5 atoms coincide with each other. The matched NZ (**1**) structure was within a 12 kcal/mol spread of energy from the global minimum. Two Chs inhibitors (**6q, 6u**) were also flexibly fitted to the template NZ (**1**) using the corresponding atoms. The structures of two inhibitors matched to NZ (**1**) were within a 10 kcal/mol spread of energy from the global minimum.
11. Receptor site model. The receptor site model was calculated from structures of NZ (**1**) and **6u** using CERIUS^2 version 3.5 (Molecular Simulations Inc., 9685 Scranton Road, San Diego, CA 92121–3752, USA). The receptor surface was colored brown to map the hydrophobic areas of the model and areas that were not hydrophobic were white.