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A New Class of Type I Protein Geranylgeranyltransferase (GGTase I) Inhibitor

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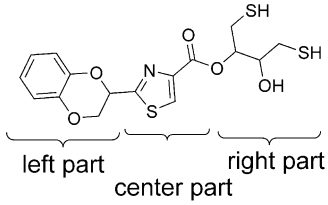
Abstract—Replacement of the thiol groups in **1**, a potent and highly selective *Candida albicans* GGTase I inhibitor discovered through screening, with an imidazole ring was achieved by using solid phase synthesis. A non-thiol compound, **7**, was found as a representative of a new class of potent *C. albicans* GGTase I inhibitor with high selectivity against human GGTase I.

Protein prenylation is a post-translational modification that confers a lipophilic nature (C15 farnesyl or C20 geranylgeranyl) and is essential for many proteins to be localized at proper membrane sites[1] and to interact with target proteins.[2]

G-proteins Rho1p[3] and Cdc42p,[4] which are Rho family proteins, were reported to be essential for viability in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*[5,6] and are known to be involved in cell polarity[7] and bud formation,[8] respectively. In addition, Rho1p has also been found to be a regulatory component of (1,3)-β-D-glucan synthase, which synthesizes essential cell wall components,[9–13] and these proteins show their functions only after geranylgeranylation of the cysteine residue by GGTase I.[14]

Recently, *RHO1*[13] gene and *CDC42*[15] gene were cloned from *Candida albicans*, a major fungal pathogen. Although the essentiality of Rho1p has not been clarified, *C. albicans* Rho1p was also found to be a regulatory component of (1,3)-β-D-glucan synthase.[13] Inhibitors of (1,3)-β-D-glucan synthase[16,17] have been proven to be excellent antifungal agents for treatment of human deep mycoses. Therefore, *C. albicans* GGTase I inhibitors are expected to act as antifungal agents against *C. albicans* by disrupting Rho1 function.

In random screening for *C. albicans* GGTase I inhibitors,[18] we found that **1** showed potent activity and high selectivity against human GGTase I, as shown in Figure 1. Although this compound showed weak antifungal activity, we chose **1** as a lead compound because of its great potency, low molecular weight, and excellent selectivity. GGTase I was reported to be an α/β heterodimer containing Mg^{2+} and Zn^{2+} in its structure,[19–22] and the DTT moiety of **1** would have important roles in the high potency by interacting with the metals. In spite of this hypothesis, this weak antifungal activity would be due to a dithiothreitol (DTT) moiety which may cause inactivation in the cells by either non-specific binding or metabolism. In order to improve the antifungal activities, we tried to replace the troublesome



1

IC50 (μM)			Antifungal activity MIC (μg / mL)
GGTase I		FTase	
Candida	Human	Human	*Candida*
0.017	40	>1000	>120

Figure 1. Structure and activity of compound **1**.

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thiol groups with other structures without a loss of activity or selectivity.

Because of the strong coordinating ability of the thiol group with metals, it seemed very difficult to replace the thiol group without a loss of activity. We planned to replace the thiol group after finding the more efficient left and center parts leaving the thiol group to which activity is guaranteed in the right part. As the first step, the thiazole carboxylic acid that was in the center part was replaced with a variety of amino acid derivatives with the assumption that the thiazolyl carboxylate structure would be a mimic of amino acids (Fig. 2). The solid phase synthetic method, with a variety of amino acid derivatives, was used to quickly study SAR around the center part. Furthermore, the thiol group of the right part seemed suitable for attaching to resins used in the solid phase synthesis but failed to attach the DTT to the resin. First, we studied the SAR in the right part to find structures that could attach to the resin.

The structures and activities are shown in Table 1. The R_3 groups of these compounds (**2**–**4**) have one thiol group and are partial structures of DTT. The result showed that one of the thiol groups in DTT was not always essential. Among these compounds, the 3-mercaptopropyl derivative (**3**) was the most acceptable for solid phase synthesis because of the moderate potency and simplicity of its structure. On the other hand, in order to search for more stable structures than the ester bond, a corresponding amide compound (**5**) was synthesized and found to have a slightly lower potency than the original ester compound (**3**). This result suggested that the ester bond could be replaced with other structures.

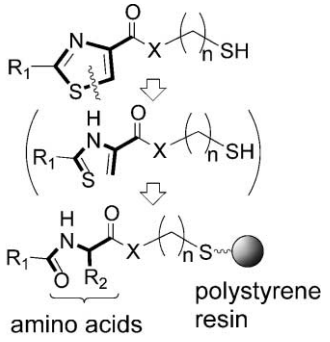
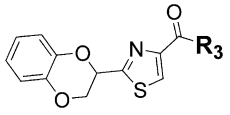
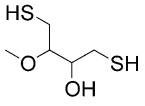
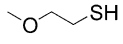
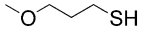
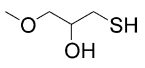
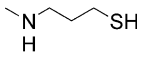


Figure 2. Transforming the center to amino acids.

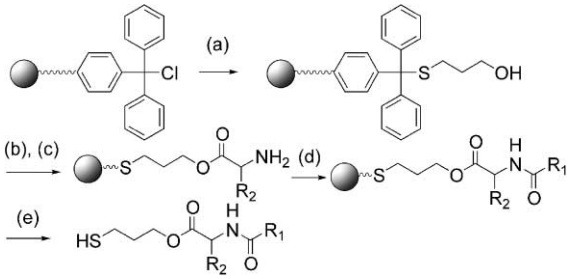
Table 1. SAR around DTT (right part: R_3)



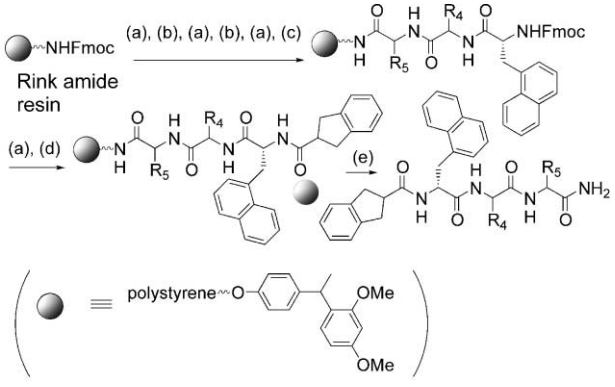
Compd	R_3	*Candida* GGTase I (IC_{50}: nM)
1		17
2		1300
3		240
4		31
5		780

After we found a suitable right part for the solid phase synthesis, a variety of compounds, which differed in R_1 and R_2, were prepared by combinatorial chemistry (Scheme 1). The thiol group of 3-mercaptopropanol was attached to a trityl resin, and a variety of amino acids were connected and acylated with several carboxylic acids.

We synthesized 70 compounds within 3 days in 70–80% purity, and the selected compounds are listed in Table 2. Aromatic rings, such as the R_2 moiety, were suggested to be essential for the potency. Structure–activity relationships from this library suggested that a D-1-naphthylalanine [D-Ala(1-naph)] structure in the center part was very good for the potency, and the IC_{50} value of the 1-indane derivative, the most potent compound (**6**), was determined to be 7.3 nM after purification.



Scheme 1. Reagents and conditions: (a) 3-mercaptopropanol, triethylamine, CH_2Cl_2, rt, 2 h; (b) Fmoc-amino acids, DCC, DMAP, DMF, rt, 15 h; (c) 20% piperidine/DMF, rt, 0.5 h; (d) R-COOH, DIPCl, HOBt, DMF, rt, 15 h; (e) 50% TFA/5% TIPS/CH_2Cl_2, rt, 0.5 h.



Scheme 2. Reagents and conditions: (a) 20% piperidine/DMF, rt, 0.5 h; (b) Fmoc-amino acids, PyBop, HOBt, DIEA, DMF, rt, 15 h; (c) Fmoc-D-Ala(1-naph)-OH, PyBop, HOBt, DIEA, DMF, rt, 15 h; (d) 2-indancarboxylic acid, PyBop, HOBt, DIEA, DMF, rt, 15 h; (e) 50% TFA/CH_2Cl_2, rt, 1 h.

Since we could find very potent left and right parts, we tried to replace the thiol group, which might be unfavorable for the drugs, with other structures as the next stage. According to our strategy of using combinatorial chemistry, we planned to transform the 3-mercaptopropanol moiety to a dipeptide (AA1-AA2) (Fig. 3) to make a tripeptide [D-Ala(1-naph)-AA1-AA2] library in the solid phase (Scheme 2), fixing the most potent 1-indane and D-Ala(1-naph) structures found in the former library.

In this library, we synthesized 266 compounds using a variety of L-, D-, natural and unnatural amino acids for AA1 and AA2 parts. We found that several compounds containing at least one D-histidine (D-His) on the AA1 or AA2 positions showed much better potencies than other tripeptide analogues. This fact indicates that the thiol group could be replaced with an imidazole structure, probably because of its ability to coordinate with metals like the thiol group. According to the result that the compounds which have only one D-His moiety in the structure showed good potency, we expected that the AA2 moiety could be removed and designed the dipeptide analogues that have a D-His for the right part (Fig. 4).

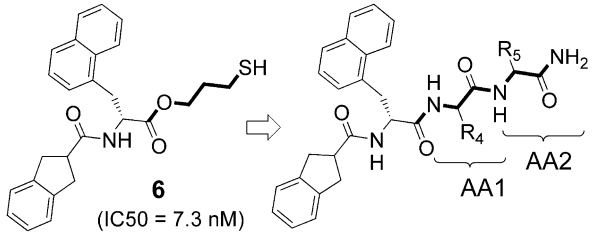
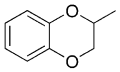
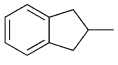
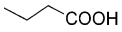
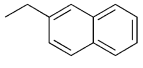
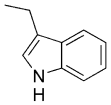


Figure 3. Tripeptide analogues by transformation of 3-mercaptopropanol to dipeptide.

Table 2. Structure and activity of the center part library (inhibition % @ 3 μM)

R_2/R_1		
H	< 10	< 10
	< 10	< 10
	< 10	< 10
	< 10	< 10
	55	63
	**89**	**90**
	25	36
	72	72

Some of the R_1 moieties of the dipeptide analogues showed excellent potencies. Activity of the selected compounds are shown in Table 3. Although the compounds did not have high levels of purity (≅60%), the 9-xanthenyl and diphenylmethyl analogues showed very high potencies.

Furthermore, assuming that the imidazole ring is an essential functional group for the activity and that the

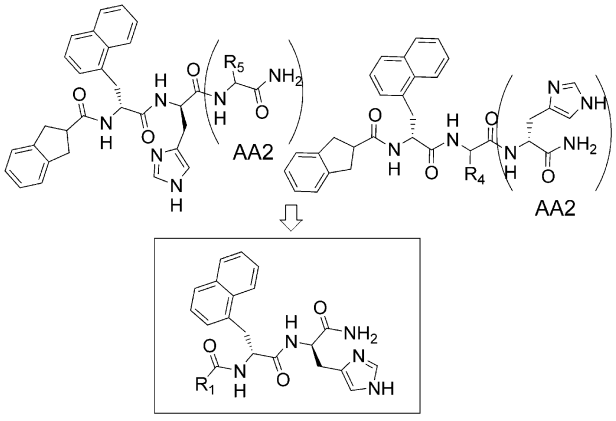
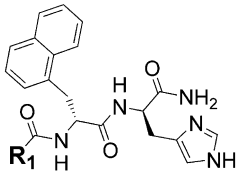
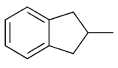
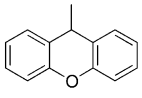
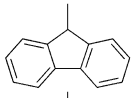
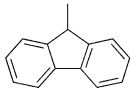


Figure 4. Design of the dipeptide analogue by removing the AA2 moiety.

Table 3. Structure and activity of dipeptide library



R_1	Inhibition % @ 3 μM	Compd
	48	**8**
	97	
	71	
	96	

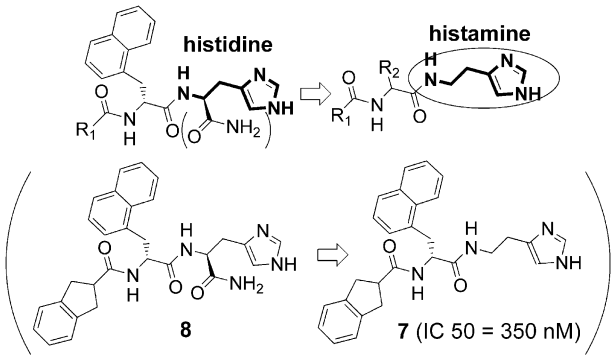
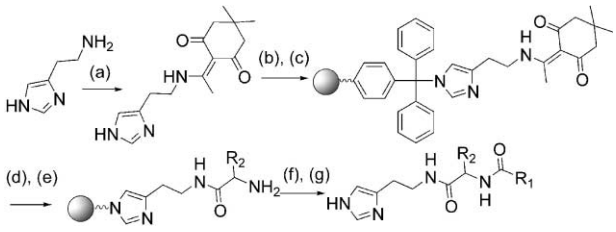


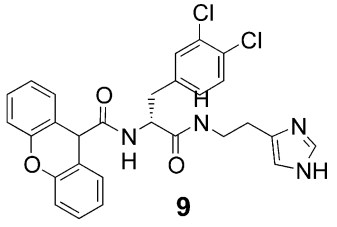
Figure 5. Removal of the carbamoyl moiety of the D-His.



Scheme 3. Reagents and conditions: (a) acetyldimedone, DMF, rt, 15 h; (b) trityl chloride polystyrene resin, triethylamine, benzene, 80 °C, 15 h; (c) 10% hydrazine/DMF, rt, 1 h; (d) Fmoc-amino acids, DIPCl, HOBt, DMF, rt, 15 h; (e) 20% piperidine/DMF, rt, 0.5 h; (f) R-COOH, DIPCl, HOBt, DMF, rt, 15 h; (g) 50% TFA/CH_2Cl_2, rt, 1 h.

carbamoyl group of the D-His moiety could be removed (Fig. 5), we synthesized decarbamoyl compound **7** to compare the potency with the corresponding carbamoyl compound **8**, which was synthesized in the former library. As we expected, compound **7** showed good potency (IC_{50} = 350 nM) and from this result, we constructed a histamine library using the solid phase synthetic method (Scheme 3).

We used 15 amino acids for the center part and 80 carboxylic acids for the left part. We found that not only the D-Ala(1-naph) group but also the D-3,4,-dichlorophenylalanine group were suitable structures for the center part and that compound **9**, having a 9-xanthenyl moiety in the left part, showed very high potency for the *C. albicans* GGTase I (IC_{50} = 10 nM) and had high selectivity against human GGTase I (> ×1000). It seemed difficult to replace the thiols of the lead compound (**1**) because of the strong ability of the thiols with the metals in GGTase I structure. However, making full use of the combinatorial chemistry methodology, we could find a non-thiol compound (**9**) that had almost the same potency and selectivity as compound **1**.



9

Recently, it was reported that *C. albicans* could sustain growth even when GGTase I was absent or limited, because Rho1p and Cdc42p could be prenylated by FTase.[23] Compound **9** does not show an antifungal activity against *C. albicans*, probably because it is a selective GGTase I inhibitor. Although we have not obtained the antifungal agent against *C. albicans* starting from compound **1**, compound **9** has the possibility to become a new drug lead against other pathogenic fungi because of its very high potency and selectivity. Also, this compound could become a new tool to investigate the roles of GGTase I in various fungi.

Acknowledgements

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References and Notes

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