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Novel pyridobenzimidazole derivatives exhibiting antifungal activity by the inhibition of β-1,6-glucan synthesis

Hiroshi Takeshita [a,*], Jun Watanabe [a], Yoichi Kimura [b], Katsuhiro Kawakami [a], Hisashi Takahashi [a], Makoto Takemura [a], Akihiro Kitamura [c], Kazuhiko Someya [d], Ryohei Nakajima [e]

[a] Medicinal Chemistry Research Laboratories II, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan
[b] Exploratory Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan
[c] R&D Planning Department, Daiichi Sankyo Co., Ltd, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[d] Biological Research Laboratories IV, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan
[e] Vaccine Business Planning Department, Daiichi Sankyo Co., Ltd, 3-5-1, Nihonbashihoncho, Chuo-ku, Tokyo 103-8426, Japan

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ABSTRACT

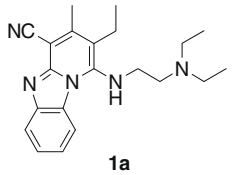
Based on the HTS hit compound **1a**, an inhibitor of β-1,6-glucan synthesis, we synthesized novel pyridobenzimidazole derivatives and evaluated their antifungal activity. Among the compounds synthesized, we identified the potent compound **15e**, which exhibits excellent activity superior to fluconazole against both *Candida glabrata* and *Candida krusei*. From the SAR study, we revealed essential moieties for antifungal activity.

Recently, the threat of fungal infection has increased due to a growing number of immunocompromised patients, which is caused by the development of cancer chemotherapy and organ transplant, and the growth of human immunodeficiency virus (HIV) infection.[1] There are several antifungal agents,[1b,2] but these agents are not considered to satisfy medical needs because of their toxicity,[2b] limited routes,[2b] and the emergence of drug-resistant[3] and drug-low-susceptible[4] strains. Given these observations, the development of new antifungal agents having a novel mode of action has been keenly desired.

In the course of our study, we discovered pyridobenzimidazole derivative **1a**[5] (Fig. 1) inhibiting the synthesis of fungal cell wall, which is absent from mammalian cells. Compound **1a** was the high-throughput screening (HTS) hit compound discovered from our chemical library, and we reported that **1a** exhibited antifungal activity by inhibiting the synthesis of β-1,6-glucan, which is a component of fungal cell wall. To our knowledge, **1a** is the first compound bearing antifungal activity by inhibiting β-1,6-glucan synthesis. These results prompted us to evaluate the potency of pyridobenzoimidazole scaffold as an antifungal agent. In this report, we describe the synthesis and the structure–activity relationships (SAR) of a series of pyridobenzimidazole derivatives with modification at the C-1 to C-4 positions.

As outlined in Scheme 1, chloropyridobenzimidazole **5**, the precursor of target compounds, was prepared according to a known method[6] for the synthesis of pyridobenzimidazole 4-carbonitriles. Thus, benzimidazole-2-acetonitrile **2** and corresponding β-ketoester **3** were heated in the presence of ammonium acetate to give tricyclic compound **4**, and chlorination of **4** in $POCl_3$ gave **5**. Compound **4f** without any substituent at the C-3 position was synthesized from **2** and β-methoxyacrylate **6** obtained by the Wittig reaction of ethyl phenylglyoxylate **7**. β-Ketoester **3g** was obtained by benzoylation of ethyl butyrate **9**. As shown in Schemes 2 and 3, chloride **5** was treated with various amines to afford target compounds.

The synthesis of **15i** and **15h** possessing oxy functional groups at the C-2 position was started from the known compound **4h**[6a]



1a

Figure 1.

* Corresponding author. Tel.: +81 3 3680 0151; fax: +81 3 5696 8344.
E-mail address: takeshita.hiroshi.km@daiichisankyo.co.jp (H. Takeshita).

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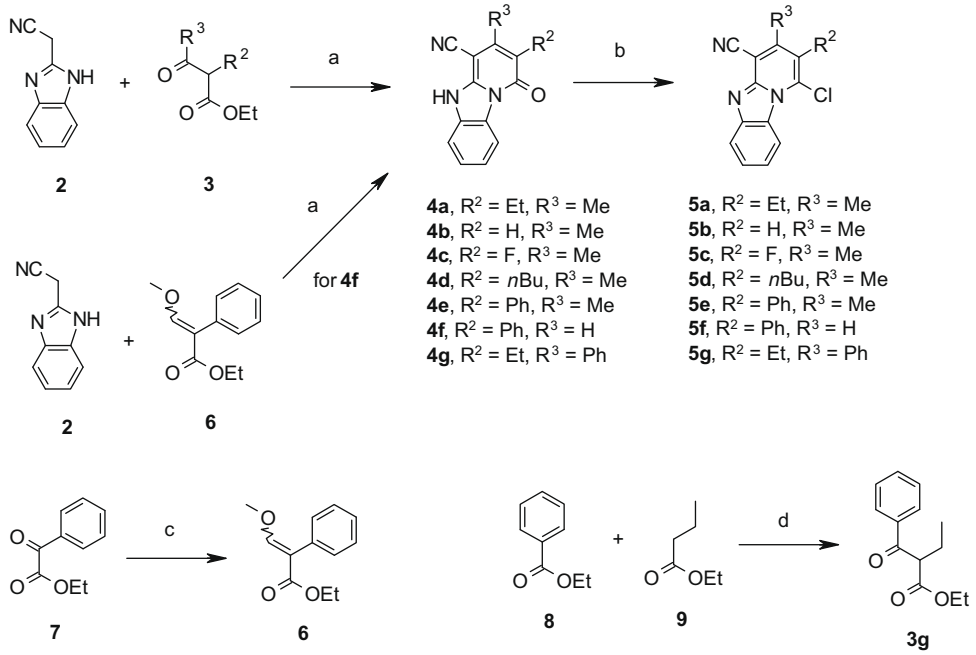
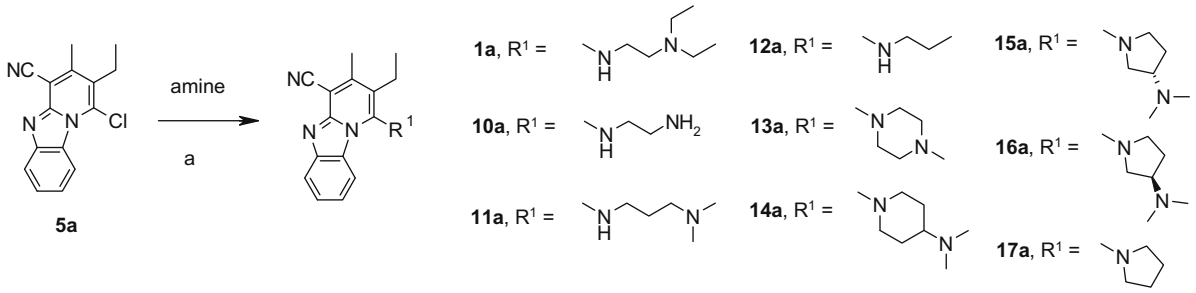
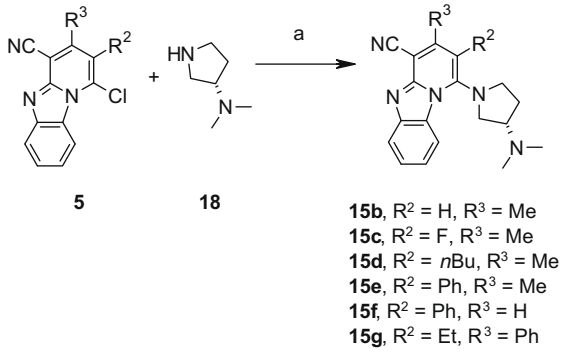


Scheme 1. Reagents and conditions: (a) $AcONH_4$, 140–150 °C (83% for **4a**, 85% for **4b**, 64% for **4c**, 90% for **4d**, 85% for **4e**, 19% for **4f**, 34% for **4g**); (b) $POCl_3$, reflux (90% for **5a**, 64% for **5b**, 82% for **5c**, 81% for **5d**, 64% for **5e**, 74% for **5f**, 33% for **5g**); (c) $MeOCH_2P^+Ph_3Cl^-$, NaH, THF (76%); (d) NaH, DME (48%).



Scheme 2. Reagents and condition: (a) Et_3N, DMF, 80 °C (45% for **1a**, 57% for **10a**, 56% for **11a**, 55% for **12a**, 54% for **13a**, 53% for **14a**, 67% for **15a**, 52% for **16a**, 51% for **17a**).



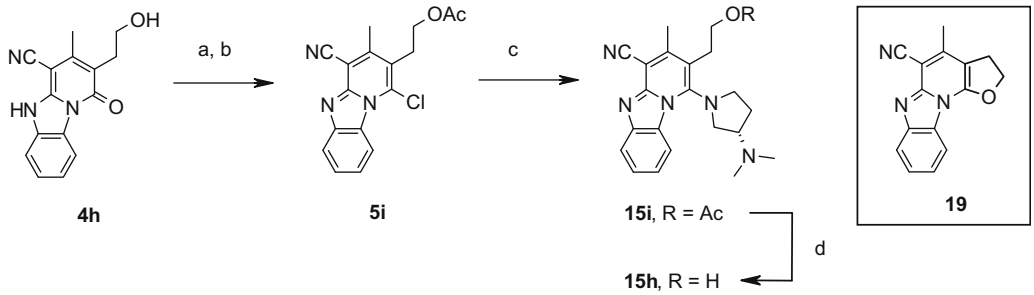
Scheme 3. Reagents and condition: (a) Et_3N, DMF, 80 °C (57% for **15b**, 65% for **15c**, 57% for **15d**, 56% for **15e**, 73% for **15f**, 71% for **15g**).

according to the method in Scheme 4. Acetylation of the hydroxyl group of **4h** and successive chlorination afforded chloride **5i**, which was treated with (3*S*)-(dimethylamino)pyrrolidine **18** to furnish 2-(acetoxyethyl) compound **15i**. Deacetylation of **15i** with 1 N NaOH afforded tetracyclic compound **19** as a main product and only a low amount of the target compound **15h**. To avoid this intramolecular cyclization, more weakly basic LiOH was used and then 2-(hydroxyethyl) compound **15h** was obtained in 83% yield.

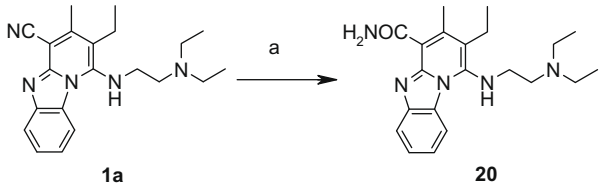
As illustrated in Scheme 5, compound **20** possessing a carbamoyl moiety at the C-4 position instead of a cyano group was obtained by treating with concd H_2SO_4 in low yield (16%).

The compounds synthesized were assayed for their in vitro antifungal activity against *Candida albicans* (*C. albicans*), *Candida glabrata* (*C. glabrata*), *Candida krusei* (*C. krusei*), *Candida tropicalis* (*C. tropicalis*) and *Aspergillus fumigatus* (*A. fumigatus*) by using the method described in our previous report.[5] The results are shown as the minimum inhibitory concentration (MIC) (the lowest drug concentration producing an optically clear well) against each fungus for our compounds and as the lowest drug concentration producing a prominent reduction in turbidity for fluconazole.

The activity of compounds substituted with various amines at the C-1 position was shown in Table 1. HTS hit compound **1a** exhibited moderate activity of MIC values that were 1 μg/ml against *C. glabrata* and 4 μg/ml against *C. krusei*, respectively. The activity of compound **10a** with a terminal primary amine instead of a tertiary amine decreased. Propylamino compound **12a** without a terminal amino moiety exhibited no inhibitory activity. It was considered that a terminal amine was essential for antifungal activity. Additionally, (dimethylamino)propylamino compound **11a** whose methylene carbon was extended was also inactive. This result indicated that the length between the terminal amine and pyridobenzimidazole scaffold was important for antifungal



Scheme 4. Reagents and conditions: (a) Ac_2O, DMAP, pyridine (77%); (b) $POCl_3$, reflux (83%); (c) **18**, Et_3N, DMF, 80 °C (41%); (d) LiOH, THF–H_2O (83%).

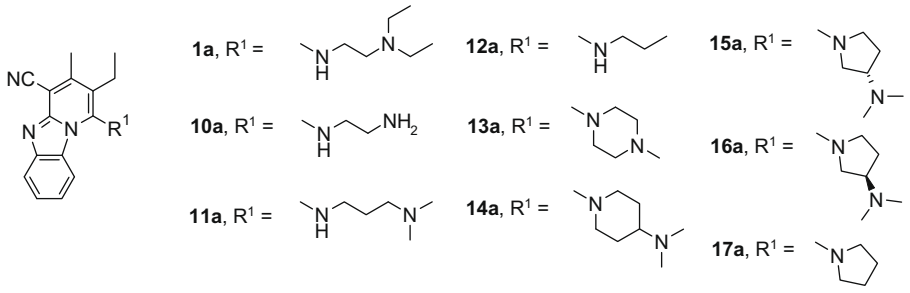


Scheme 5. Reagents and condition: (a) concd H_2SO_4, EtOH, reflux (16%).

activity. In regards to cyclic amines, though methylpiperazinyl compound **13a** and (dimethylamino)piperidinyl compound **14a** exhibited no activity, (3*S*)-(dimethylamino)pyrrolidinyl compound **15a** showed more potent activity than **1a**. The configuration of the dimethylamino moiety affected activity as shown in the less potent activity of (*R*)-form **16a** as compared to that of (*S*)-form **15a**. As in the case of acyclic amines, pyrroridinyl compound **17a** without a terminal amine exhibited no activity.

Table 2 shows the MIC values of the compounds with (3*S*)-(dimethylamino)pyrrolidinyl moiety at the C-1 position and

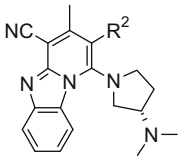
Table 1
In vitro antifungal activity of pyridobenzimidazole derivatives possessing various substituents at the C-1 position



Organism	MIC (μg/ml)									
	Fluconazole[a]	**1a**	**10a**	**11a**	**12a**	**13a**	**14a**	**15a**	**16a**	**17a**
Candida albicans ATCC24433	0.25	>128	>128	>128	>128	>32	>128	>128	>128	>64
Candida glabrata IFO0622	2	1	4	>128	>128	>32	>128	0.5	1	>64
Candida krusei TIMM0269	16	4	32	>128	>128	>32	>128	2	16	>64
Candida tropicalis TIMM0313	0.25	>128	>128	>128	>128	>32	>128	32	64	>64
Aspergillus fumigatus ATCC36607	>64	>128	>128	>128	>128	>32	>128	>128	>128	>64

[a] The lowest drug concentration producing a prominent reduction in turbidity was employed as end point.

Table 2
In vitro antifungal activity of pyridobenzimidazole derivatives possessing various substituents at the C-2 position



Organism	MIC (μg/ml)							
	Fluconazole[a] R^2=	**15a** Et	**15b** H	**15c** F	**15d** *n*Bu	**15e** Ph	**15h** $(CH_2)_2OH$	**15i** $(CH_2)_2OAc$
Candida albicans ATCC24433	0.25	>128	>128	>128	>128	>128	>128	>128
Candida glabrata IFO0622	2	0.5	8	4	⩽0.125	⩽0.125	32	1
Candida krusei TIMM0269	16	2	32	16	0.5	⩽0.125	16	4
Candida tropicalis TIMM0313	0.25	32	>128	>128	0.5	0.5	>128	16
Aspergillus fumigatus ATCC36607	>64	>128	>128	>128	>128	>128	>128	>128

[a] The lowest drug concentration producing a prominent reduction in turbidity was employed as end point.

Table 3
In vitro antifungal activity of pyridobenzimidazole derivatives possessing various substituents at the C-3 and C-4 positions

Organism	MIC (μg/ml)			
	Fluconazole[a]	**15f**	**15g**	**20**
Candida albicans ATCC24433	0.25	>128	>128	>128
Candida glabrata IFO0622	2	⩽0.125	>128	>128
Candida krusei TIMM0269	16	2	>128	>128
Candida tropicalis TIMM0313	0.25	8	>128	>128
Aspergillus fumigatus ATCC36607	>64	>128	>128	>128

[a] The lowest drug concentration producing a prominent reduction in turbidity was employed as end point.

various substituents at the C-2 position. The activity of compound **15b** without any substituent at the C-2 position decreased, and the not sterically bulky fluoro compound **15c** also showed less potent activity than ethyl compound **15a**. Additionally, acetoxyethyl compound **15i** and hydroxyethyl compound **15h** showed less potent activity. In contrast, compound **15d** possessing a *n*-butyl moiety longer than an ethyl moiety, exhibited more potent activity against *C. glabrata*, *C. krusei* and *C. tropicalis*. Moreover, phenyl compound **15e**[7] also exhibited excellent activity of MIC values that were ⩽0.125 μg/ml against both *C. glabrata* and *C. krusei*, and 0.5 μg/ml against *C. tropicalis*. These results suggested that the introduction of lipophilic substituents to the C-2 position enhanced antifungal activity.

Next, the results of the substitution at the C-3 and C-4 positions were shown in Table 3. The activity of **15f** without a methyl group at the C-3 position was less potent than that of corresponding **15e**. Meanwhile, **15g** possessing a phenyl substituent did not show any activity. Therefore it was considered to be favorable that the substituent at the C-3 position was a measurably bulky lipophilic moiety. Compound **20** bearing a carbamoyl group instead of a cyano group at the C-4 position showed no activity.

Compound **15e** exhibited the most potent activity among the compounds synthesized and it was confirmed that **15e** inhibited β-1,6-glucan synthesis selectively.[8] Compared to fluconazole, **15e** was more active against both *C. glabrata* and *C. krusei* and showed almost equal activity against *C. tropicalis*. Although the MIC values of all the compounds synthesized against *C. albicans* were >128 μg/ml in the conditions used, the morphological change on the surface of the cell wall of *C. albicans* was observed under a microscope.[8] Therefore it was considered that the compounds had a certain effect on the synthesis of the cell wall. We are currently studying the evaluation of the growth inhibition of *C. albicans*. By contrast, neither antifungal activity nor a morphological change was observed against *A. fumigatus*. Kre6p is our primary target and its homologues have been found in *Aspergillus* species,[5] but it was reported that *Aspergillus* species contain no β-1,6-glucan polymer.[9] The functions of these homologues in *Aspergillus* species are not well understood, therefore further work is needed to understand the effect of Kre6p inhibition in *Aspergillus* species.

In summary, we have demonstrated that pyridobenzimidazole scaffold can be utilized for novel antifungal agents inhibiting β-1,6-glucan synthesis and we provide the following findings. (1) The basic substituent at the C-1 position and the cyano group at the C-4 position were essential for antifungal activity, (2) the lipophilic substituent at the C-2 position and the methyl moiety at the C-3 position were of importance to potent activity. Further chemical modifications and investigations of in vivo efficacy are currently under way.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.024.

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7. Analytical data of compound **15e**: Yellow solid. Mp 211–214 °C; IR (ATR) 2222, 1628, 1589, 1466, 1344, 1298, 1263, 1186, 1134 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.98–2.09 (3H, m), 2.12 (6H, s), 2.31 (3H, s), 2.72–3.69 (4H, m), 7.20–7.38 (3H, m), 7.48–7.60 (4H, m), 7.89–8.14 (1H, m), 8.02 (1H, d, J = 8.1 Hz); ^{13}C NMR (DMSO-d_6) δ 150.22, 147.39, 145.63, 144.59, 134.73, 130.63, 130.60, 129.35, 128.84, 128.79, 128.38, 125.64, 121.31, 121.27, 118.89, 115.92, 115.51, 95.43, 65.10, 54.21, 49.84, 43.41, 29.17, 19.95; $[\alpha]_D^{25.0}$ −56.4° (c 1.030, $CHCl_3$); MS (ESI), m/z 396 $[M+H]^+$; Anal. Calcd for $C_{25}H_{25}N_5$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.79; H, 6.32; N, 17.78.
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