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Design and Synthesis of Novel Benzofurans as a New Class of Antifungal Agents Targeting Fungal *N*-Myristoyltransferase. Part 1

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Abstract—Potent and selective *Candida albicans N*-myristoyltransferase (CaNmt) inhibitors have been identified through optimization of a lead compound **1** discovered by random screening. The inhibitor design is based on the crystal structure of the CaNmt complex with compound (*S*)-**3** and structure–activity relationships (SARs) have been clarified. Modification of the C-4 side chain of **1** has led to the discovery of a potent and selective CaNmt inhibitor **11** (RO-09-4609), which exhibits antifungal activity against *C. albicans* in vitro.

N-Myristoyltransferase (Nmt) is an enzyme that transfers the myristoyl group of myristoyl CoA to the N-terminal glycine of eukaryotic cellular proteins. *N*-Myristoylation of several G-proteins, Gpa1, Arf1, Arf2 and Vps15, which are essential for fungal growth, has been reported to be indispensable for their function in *Saccharomyces cerevisiae*.[1–4] Nmt has also been proven to be essential for the viability of fungi, including medically important pathogenic fungi such as *Candida albicans* (*C. albicans*) and *Cryptococcus neoformans*.[5,6] Therefore, Nmt is a good target for the development of novel fungicidal drugs with a new mode of action.

Although several peptidomimetic inhibitors of *C. albicans* Nmt (CaNmt) have been reported, their antifungal activity is only marginal.[7–9] Therefore, we set out to seek novel non-peptidic Nmt inhibitors having strong antifungal activity. We discovered a lead compound **1** that competitively inhibits CaNmt (IC_{50}: 0.98 μM) with high selectivity over human Nmt (IC_{50}: 194 μM), by a random screening of the Roche chemical libraries. However, **1** did not show potent antifungal activity in vitro (IC_{50} against *C. albicans* CY1002: 390 μM).

Compound **1** was originally reported by Grinev et al. to have antiarrhythmic and β-blocking action.[10] Thus we initiated the chemical modification study of **1** to identify a highly potent and safe antifungal agent by improving the enzyme inhibitory activity while eliminating the β-blocking action (IC_{50}: 0.098 μM).

Synthesis

The general synthesis of benzofuran derivatives is outlined in Scheme 1.[11] Acylresorcinol derivatives **I** were prepared by (1) *ortho* lithiation of 1,3-bis(methoxymethyl)resorcinol followed by acylation with acyl chloride, R^1COCl, or DMF and (2) removal of the methoxymethyl groups by acid hydrolysis. 4-Hydroxybenzofuran derivatives **II** were prepared by the same method reported by Atkinson et al., which includes mono-alkylation of intermediate **I** with ethyl bromoacetate and base catalyzed cyclization of the alkylated product.[12] Alkylation of intermediates **II** with excess alkylene dibromide gave bromoalkyl derivatives **III** in good yields. Amination of **III** with various amines at 70 °C gave the desired amino derivatives **IV** without affecting the ester group.

Results and Discussion

Since it is well known that an aromatic compound having a β-aminoalcohol moiety shows a β-blocking action, we removed the hydroxy group of **1** to eliminate this activity. As we expected, removing the hydroxy group lowered the β-adrenoceptor blockade significantly

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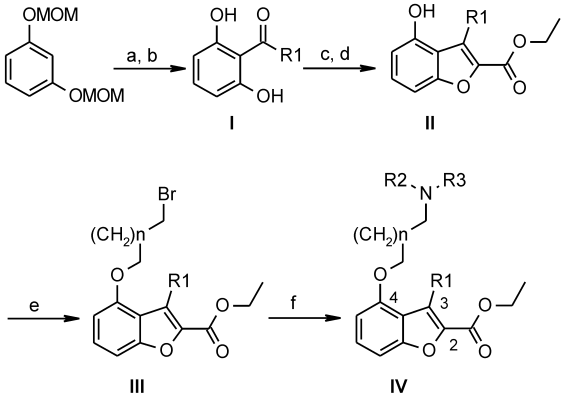
without affecting its CaNmt enzyme inhibitory activity (Table 1).[13–15] The IC_{50}s of **2** and **4** in the β-adrenoceptor blockade assay were 38 and 25 μM, respectively, whereas those of the corresponding hydroxy derivatives **1** and **3** were 0.098 and 0.0043 μM, respectively.[16] To find the optimal chain length of the side chain, compounds **5**–**7** were prepared and their enzyme inhibitory activity was compared with that of compound **4** (Table 2). The result indicated that trimethylene (**4**) was optimal.

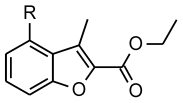


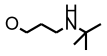
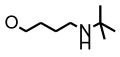
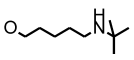
n=0 - 2; R^1=H, Me, Et, cyclopropyl, isopropyl;
NR^2R^3=isopropylamino, *tert*-butylamino, 1-piperidinyl, phenylamino, benzylamino, (pyridin-3-ylmethyl)amino

Scheme 1. Reagents and conditions: (a) *n*-BuLi, acyl chloride, THF, −78 °C to rt, 1 h, 25–68%, or *n*-BuLi, DMF, 0 °C to rt, 17 h, 51%; (b) 4 N HCl, MeOH/1,4-dioxane = 1:1, 50 °C, 2–4 h, 77–93%; (c) ethyl bromoacetate, K_2CO_3, acetone, 60 °C, 15 h, or rt, 15 h, 38–73%; (d) NaOEt, EtOH, 0 °C, 20 h, 9–76%; (e) alkylene dibromide, K_2CO_3, DMF, rt, 2–4 h, 43–88%; (f) amine, EtOH, 70 °C, 12–48 h, 29–100%.

For further optimization, we analyzed the X-ray crystal structure (3.5 Å) of a binary complex of CaNmt and (*S*)-**3** that was obtained by soaking experiments[17] (Fig. 1). The details of the X-ray crystallographic analysis will be reported in a separate paper.[18] The important interactions between (*S*)-**3** and CaNmt are summarized in Figure 1. The C-4 substituent on the benzofuran ring extends to a C-terminal leucine (Leu 451) of the polypeptide chain. The C-2 substituent is surrounded by

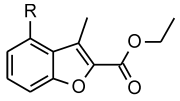
Table 2. Optimization of the C-4 substituent

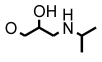
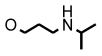
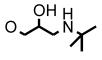
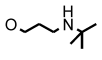
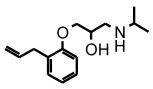


Compd	R	Enzyme inhibition[13]		Antifungal activity[14]
		CaNmt	HsNmt	*C. albicans* CY1002
5		50	>630	590
4		1.7	200	300
6		4.4	>580	190
7		15	>550	53

IC_{50}: μM.

Table 1. Enzyme inhibitory activity, antifungal activity and β-adrenoceptor blockade of benzofuran derivatives



Compd	R	Enzyme inhibition[13]		Antifungal activity[14]	β-Adrenoceptor blockade[15]
		CaNmt	HsNmt	*C. albicans* CY1002	
1		0.98	194	390	0.098
2		4.4	380	470	38
3		1.2	470	210	0.0043
4		1.7	200	300	25
Alprenolol		NT	NT	NT	0.0023

NT, not tested; IC_{50}: μM.

three phenylalanine residues, Phe 115, Phe 240 and Phe 339. The amino group in the C-4 side chain interacts with the carboxyl group of Leu 451. This interaction should be essential for the enzyme inhibitory activity because the C-terminal carboxylate was reported to play an important role in the catalytic mechanism.[19] The *tert*-butyl amino group in the C-4 side chain is surrounded by hydrophobic aromatic amino acid residues, Tyr 107, Tyr 119 and Phe 176. This structural analysis suggested that the replacement of the *tert*-butyl group by another hydrophobic group would increase the enzyme inhibitory activity. Thus, we designed and synthesized compounds **8**–**11** having a hydrophobic group on the amino group.

The assay results are shown in Table 3. Among them, the (pyridin-3-ylmethyl)amino derivative **11** (RO-09-4609) showed the strongest enzyme inhibitory activity

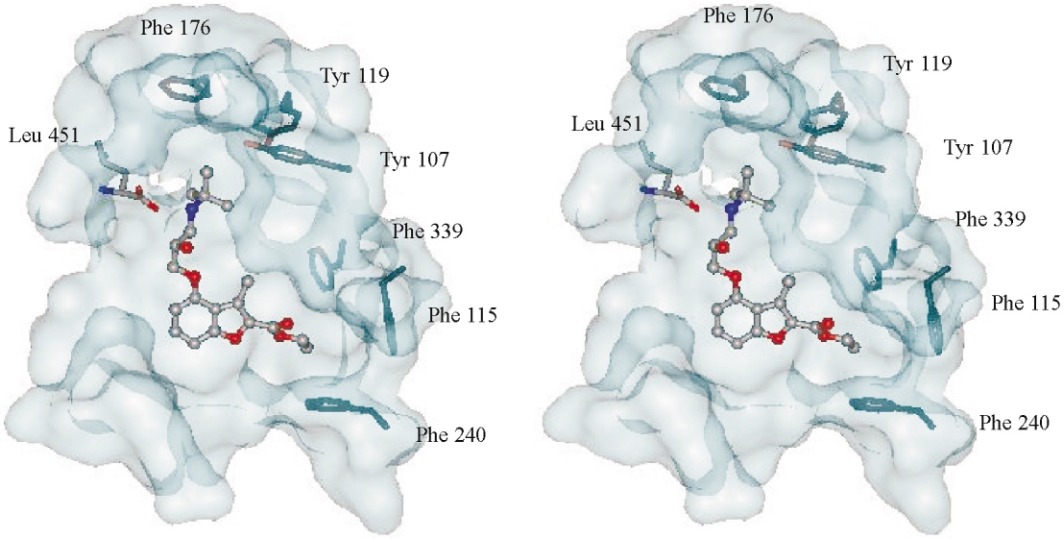
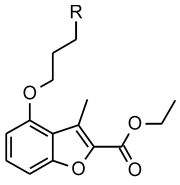


Figure 1. X-ray crystal structure of a binary complex of CaNmt and (*S*)-**3** (only the binding site is shown).

Table 3. Optimization of the amino substituent in the C-4 side chain

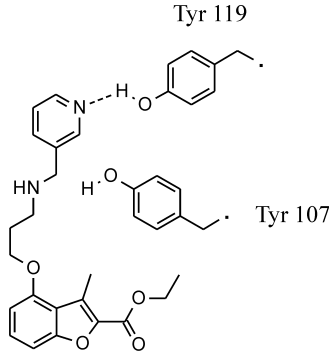


Compd	R	Enzyme inhibition[13]		Antifungal activity[14]
		CaNmt	HsNmt	*C. albicans* CY1002
4		1.7	200	300
8		1.6	170	85
9		> 570	> 570	> 280
10		3.3	530	10
11 (RO-09-4609)		0.1	> 540	1.6
12		> 540	> 540	> 270

IC_{50}: μM.

against CaNmt with extremely high selectivity (>5000-fold) over human Nmt. The high selectivity can be explained by the differences between CaNmt and HsNmt of the amino acid sequence alignment. The identity of the amino acid sequence alignment of the two Nmts is only 48%, which makes 26 amino acid residues around the benzofuran binding site different between the two Nmts (data not shown). It also showed moderate antifungal activity against *C. albicans* CY1002 in vitro. The increased enzyme inhibitory activity of **11** over the benzyl derivative **10** can be explained by an additional favorable hydrogen bonding between the pyridine nitrogen and the Tyr 119 hydroxy group (Fig. 2). This interpretation was supported by the finding that the inhibitory activity of **11** against a mutant CaNmt having alanine for Tyr 119 was 14 times weaker than that against the wild CaNmt.[20]

Since aniline derivative **9** and pyridin-3-ylmethoxy derivative **12** showed no activity at all, it is suggested that (1) the basicity of the aniline is too weak to interact with the C-terminal carboxylate (Leu 451) and (2) strong basicity of the aliphatic amino group is essential for ionic interaction between the carboxylate and the basic amino group of the inhibitor.[21]



Compound **11** (RO-09-4609)

Figure 2. Hypothetical interaction between **11** and Tyr 119 of CaNmt.

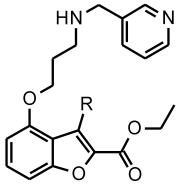
The crystal structure revealed that the benzofuran ring occupied the hydrophobic pocket of the binding site and little space was left for an extra substituent at the C-6 and C-7 positions. This observation was consistent with the SAR finding that mono-methylation at the C-7 position lowered CaNmt inhibitory activity by a factor of 252.

Since there seemed to be some space around the C-3 position, the methyl group at the C-3 position of **11** was replaced by a larger substituent. The assay results of the C-3 derivatives are shown in Table 4. However, the result indicated that methyl was still the best among them. The X-ray crystallographic analysis did not clearly explain the reason why neither ethyl derivative **13** nor cyclopropyl derivative **14** showed strong inhibitory activity against CaNmt, though there appeared to be enough space for them.

Compound **11** did not show antifungal activity in a mouse systemic candidiasis model, although the antifungal activity in vitro was nearly as strong as that of fluconazole.

In summary, the structural information of the CaNmt complex with compound (*S*)-**3** was effectively utilized for designing more potent inhibitors and led to the identification of **11** (RO-09-4609), a new class of antifungal agents, which showed strong competitive CaNmt inhibitory activity ($K_i = 0.27$ μM), extremely high selectivity as compared to HsNmt and moderate antifungal activity against *C. albicans* in vitro. The optimal substituents on C-3 and C-4 were revealed to be methyl and 3-[(pyridin-3-ylmethyl)amino]propoxy, respectively. Compound **11** was selected as a lead compound for further optimization. The mechanism of the antifungal activity is being investigated in detail.

Table 4. Optimization of the C-3 substituent



Compd	R	Enzyme inhibition[13]		Antifungal activity[14]
		CaNmt	HsNmt	*C. albicans* CY1002
11 (RO-09-4609)	CH_3	0.1	>540	1.6
13	CH_2CH_3	10	280	12
14	Cyclopropyl	4.4	91	11
15	Isopropyl	83	260	NT
16	H	79	>560	NT
SC-58272[22]		0.83	>140	200
Fluconazole		NT	NT	0.72

NT, not tested; IC_{50}: μM.

References and Notes

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13. Potency against *C. albicans* Nmt as assessed by IC_{50} using substrate peptide GLTISKLFRR-NH_2 (0.5 μM) and myristoyl–CoA at 0.5 μM. Potency against *Human* Nmt as assessed by IC_{50} using substrate peptide GNAASARR-NH_2 (0.5 μM) and myristoyl–CoA at 0.5 μM. NT, not tested.
14. Broth dilution method; medium: YNBPB (=YNB+1% glucose+0.25% K_2HPO_4), pH 7.0, inoculum size; 1×10^4 cfu/mL, incubation: 1 day at 27 °C.
15. Adrenergic, beta, non-selective assay, performed by NOVA SCREEN.
16. Treatment of 3-methyl-4-oxiranylmethoxy-benzofuran-2-carboxylic acid ethyl ester (Sangwan, N. K.; Rastogi, S. N.; Kar, K. *Eur. J. Med. Chem.* **1987**, *22*, 153) with *tert*-butylamine in ethanol at 70 °C for 2 h afforded **3**.
17. Compound (*S*)-**3** was the more active isomer (IC_{50} against CaNmt: 1.1 μM) and (*R*)-**3** was the less active isomer (IC_{50} against CaNmt: 150 μM).
18. Manuscript in preparation. The coordinates of the protein will be deposited with PDB.
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20. There are two tyrosine residues (Tyr 119 and Tyr 107) surrounding the pyridyl group of compound **11**. Alanine substitution of the tyrosine residues indicated that only Tyr 119 interacts with **11**. For wild type CaNmt, the K_i value of compound **11** was 0.27 μM. For Tyr119Ala and Tyr107Ala, the K_i values of compound **11** are 3.9 and 0.33 μM, respectively.
21. Compound **12** was prepared by treatment of compound **III** ($R^1=CH_3$) with 3-pyridinemethanol and NaH in DMF at room temperature for 19 h in 11% yield.
22. A dipeptide inhibitor of CaNmt, *N*-[[4-[4-(2-methyl-1*H*-imidazol-1-yl)butyl]phenyl]acetyl]-L-seryl-*N*-(2-cyclohexylethyl)-L-lysinamide, reported in refs 8, 9, and 19.