Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 87–91

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Novel Benzofurans as a New Class of Antifungal Agents Targeting Fungal *N*-Myristoyltransferase. Part 3

Ken-ichi Kawasaki,[a] Miyako Masubuchi,[a] Kenji Morikami,[a] Satoshi Sogabe,[a] Tsunehisa Aoyama,[a] Hirosato Ebiike,[a] Satoshi Niizuma,[a] Michiko Hayase,[b] Toshihiko Fujii,[b] Kiyoaki Sakata,[b] Hidetoshi Shindoh,[c] Yasuhiko Shiratori,[a] Yuko Aoki,[b] Tatsuo Ohtsuka[a,*] and Nobuo Shimma[a]

[a]*Department of Chemistry, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan*
[b]*Department of Mycology, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan*
[c]*Department of Preclinical Science, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan*

Received 6 August 2002; accepted 1 October 2002

Abstract—A new series of acid-stable antifungal agents having strong inhibitory activity against *Candida albicans N*-myristoyltransferase (CaNmt) has been developed starting from acid-unstable benzofuranylmethyl aryl ether **2**. The inhibitor design is based on X-ray crystallographic analysis of a CaNmt complex with aryl ether **3**. Among the new inhibitors, pyridine derivative **8b** and benzimidazole derivative **8k** showed clear antifungal activity in a murine systemic candidiasis model.

Introduction

N-Myristoyltransferase (Nmt) is an enzyme that transfers the myristoyl group of myristoyl CoA to the N-terminal glycine of various eukaryotic cellular proteins. *N*-Myristoylation of 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*[5] and *Cryptococcus neoformans*.[6] Therefore, Nmt is a good target for the development of novel fungicidal drugs with a new mode of action. Since the mechanism of action is novel, Nmt inhibitors might show antifungal activity against even azole resistant fungal strains and might not cause the drug-drug interactions that are a drawback of azole antifungal agents.[7]

So far, peptidomimetic inhibitors,[8] benzothiazole inhibitors,[9,10] *p*-toluenesulfonamide inhibitors[11] and benzofuran inhibitors[12,13] of *C. albicans* Nmt (CaNmt) have been identified. Among them only the benzofuran inhibitors have been reported to show clear antifungal activity in vivo. In our previous papers, we reported the chemical modification work that yielded new benzofuran Nmt inhibitors **1** and **2**.[12,13] The inhibitor design was mainly based on X-ray crystallographic analysis of CaNmt complex with a benzofuran inhibitor and the modification was guided by various biological investigations including in vitro antifungal assay in the presence of 80% calf serum (quasi in vivo assay) and pharmacokinetic (PK) study. Such a multi-dimensional optimization resulted in the identification of fungicidal benzofuranylmethyl aryl ether **2**, which showed both strong CaNmt inhibitory activity (IC_{50}: 0.0056 μM) and in vitro antifungal activity against *C. albicans* CY1002 (IC_{50}: 0.035 μM). This compound also showed a good PK profile and in vivo efficacy in a murine systemic candidiasis model (ED_{50}: 7.1 mg/kg), but it was unstable in an artificial gastric fluid (pH 1.2). This labile nature of **2** should be improved for developing an orally active antifungal drug. To obtain acid-stable antifungal agents we designed benzofuranyl heteroaromatic ketones **8a–8k** by analyzing the crystal structure of the

*Corresponding author. Tel.: +81-467-45-4356; fax: +81-467-45-6824; e-mail: ohtsukatto@chugai-pharm.co.jp

0960-894X/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00844-2

CaNmt complex with **3**. In the present paper, we wish to report the design and synthesis of acid-stable benzofuran derivatives having inhibitory activity against CaNmt, and their biological activities.

Synthesis

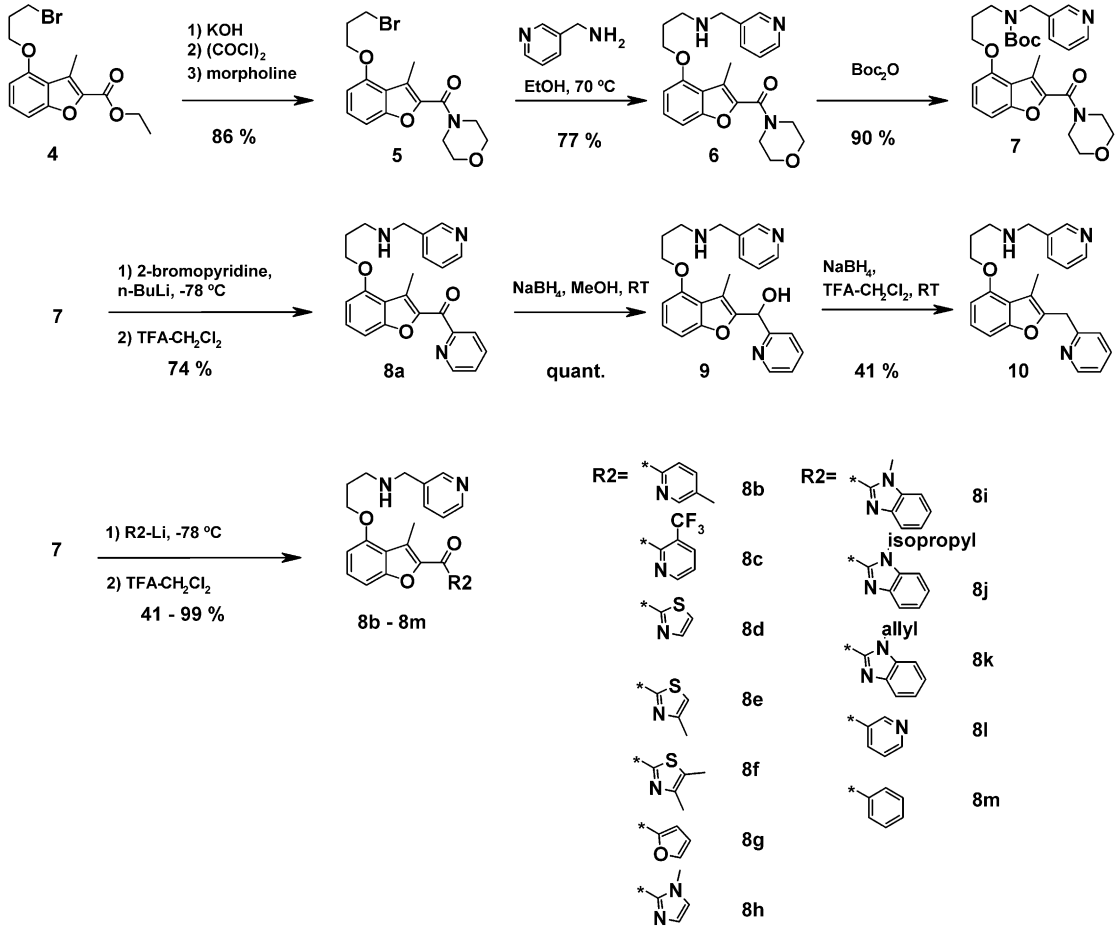
The general synthesis of the designed inhibitors (**8a–8k**) and reference compounds (**8l**, **8m**, **9** and **10**) is outlined in Scheme 1.

Ester **4**[13] was hydrolyzed by potassium hydroxide, and the resulting acid was converted into its acid chloride by treatment with oxalyl chloride, which was immediately subjected to the next amide formation reaction with morpholine. The resulting amide **5** was heated to 70 °C with 3-aminomethylpyridine in EtOH to give amine **6**. Amine **6** was converted to its *t*-butoxycarbonyl (Boc) derivative **7** by treatment with Boc_2O. The addition of 2-lithiated pyridine generated in situ to **7** at −78 °C and subsequent deprotection of Boc group with trifluoroacetic acid afforded pyridyl ketone **8a** in a good yield. Other heteroaromatic ketone derivatives **8b–8m** were synthesized in a similar manner from the common starting material **7** and an appropriate lithiated heteroaromatic compound. Treatment of **8a** with sodium borohydride gave alcohol **9** quantitatively and then it was converted into methylene derivative **10** by treatment with sodium borohydride in a mixture of trifluoroacetic acid and dichloromethane.[14]

Results and Discussion

Design of acid-stable inhibitors

The crystallographic analysis of a CaNmt complex with **3** and SARs obtained so far suggested that following interactions are important for the binding: (1) the hydrogen bonding between amide–NH_2 of Asn 392 and the ether oxygen of **3**, and (2) the hydrophobic interaction between the difluorophenyl group of **3** and phenylalanine residues, Phe 115, Phe 240 and Phe 339 (Fig. 1).[13] Since the arylmethyl ether moiety of **2** was thought to be unstable under acidic conditions, we designed compound **8a** that has an acid-stable ketone group instead of the ether group. The nitrogen atom of the pyridine ring would be a good hydrogen bond acceptor and the pyridine ring would be able to situate in the lipophilic pocket composed of the three phenylalanine residues. To locate the pyridine ring and its nitrogen atom to the appropriate positions, a carbonyl group was inserted between the pyridine ring and the benzofuran ring. The repulsive Coulomb force between the carbonyl oxygen and the benzofuran oxygen was



Scheme 1.

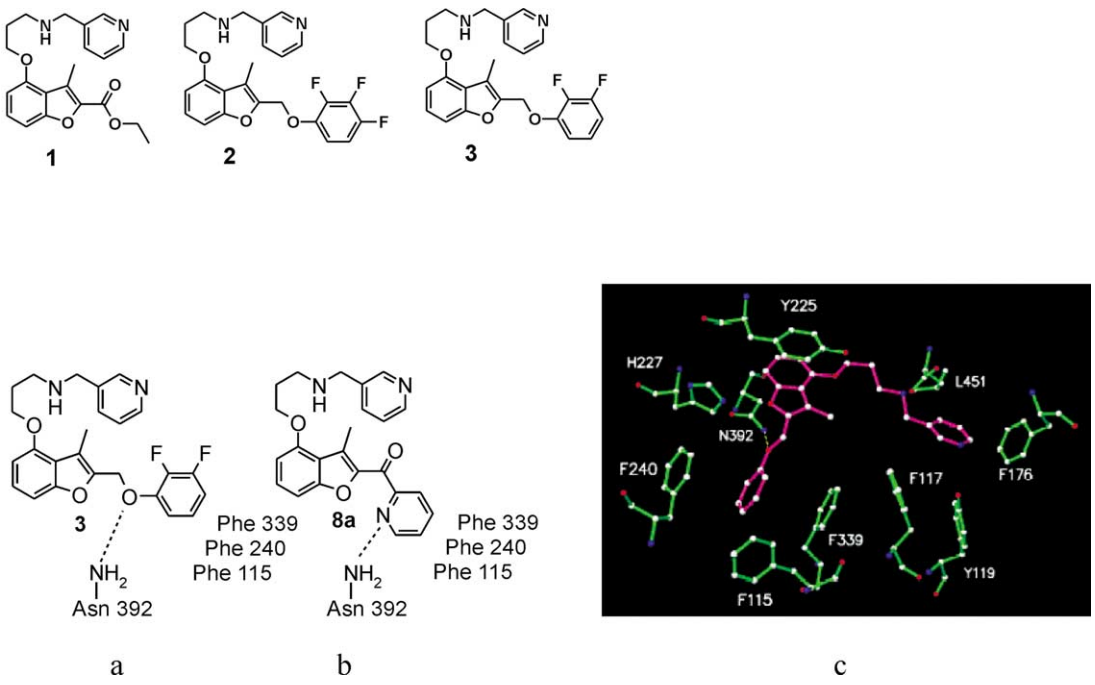


Figure 1. (a) Interaction between Asn 392 of CaNmt and **3** suggested by X-ray crystallographic analysis; (b) hypothetical interaction between Asn 392 of CaNmt and **8a**; (c) the crystal structure of the binding site of a CaNmt complex with **3**.

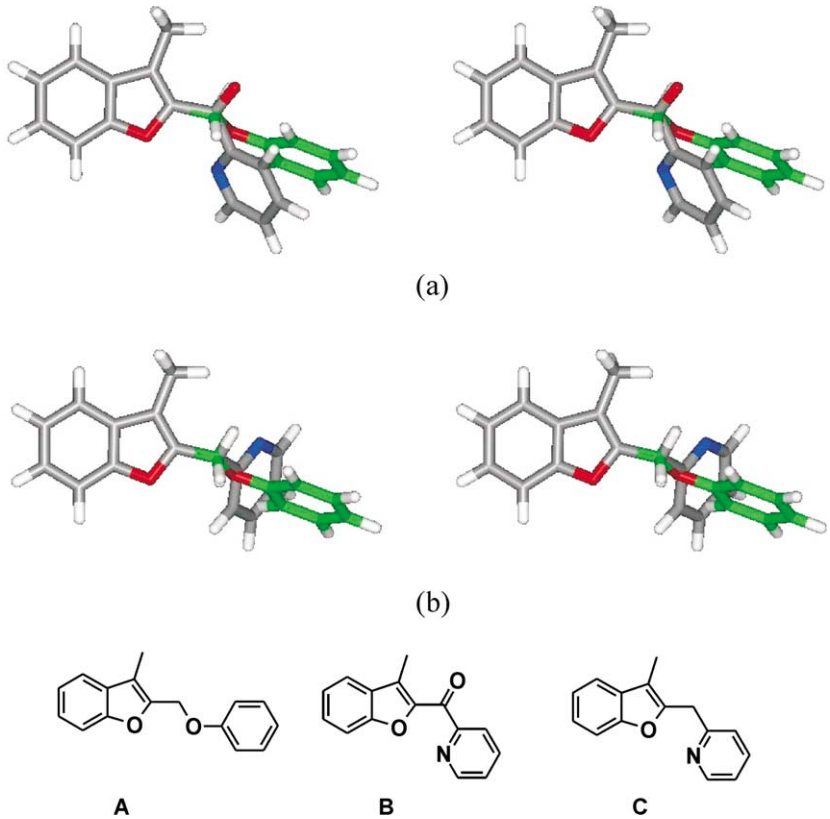


Figure 2. Comparison of the lowest energy conformations of **A**, **B** and **C** at the HF/6-31G level. Atoms denoted by red and blue are oxygen and nitrogen atoms, respectively. Phenyl ether moiety of **A** is depicted in green. (a) Superimposition of the lowest energy conformations of **A** and **B**. The conformation of **A** was calculated to be similar to that of the corresponding part of **3** bound with CaNmt.[15] (b) Superimposition of the lowest energy conformations of **A** and **C**.

Table 1. Enzyme inhibitory activity against CaNmt[a]

Compd	**8a**	**9**	**10**	**8l**	**8m**
IC_{50} (μM)	0.0035	0.047	1.9	0.62	1.2

[a]Potency against *C. albicans* Nmt (CaNmt) as assessed by IC_{50} using substrate peptide GLTISKLFRR-NH_2 (0.5 μM) and myristoyl-CoA at 0.5 μM.

expected to align these heteroatom *s-trans* and to make the conformation rigid. To verify our supposition, conformational analysis was performed on structures **A**, **B** and **C** that are the model compounds of **3**, **8a** and **10**, respectively. Their stable conformations generated by ab-initio molecular orbital calculation are shown in Figure 2. The analysis suggested that the pyridine nitrogen of **B** would occupy almost the same position as the ether oxygen of **A**. On the other hand, the position and orientation of the pyridine nitrogen of **C** were calculated to be completely different from those of the oxygen of **A**. Thus, **8a** was expected to be a good binder, whereas **10** was not.

Inhibitory activity against CaNmt

The enzyme inhibitory activity against CaNmt was measured by the same methods as described in previous papers[12,13] and the results are summarized in Table 1. As we expected, compound **8a** bearing 2-pyridyl carbonyl group showed strong inhibitory activity (IC_{50}: 0.0035 μM) against CaNmt, whereas its methylene analogue **10** was 540 times less active. On the other hand, **8l**, a position isomer of **8a**, was 180 times less active than **8a**. These results suggested that the position of the pyridine nitrogen is crucial for strong binding to CaNmt.

Thus, a new type of strong enzyme inhibitor **8a** was obtained and this success prompted us to synthesize other heteroaromatic ketones for further improvement of the activity. The pyridine ring of **8a** was replaced by various heteroaromatic rings as shown in Scheme 1. The enzyme inhibitory activities of the new analogues against CaNmt, human Nmt (HsNmt) and *Aspergillus fumigatus* Nmt (AfNmt), their in vitro antifungal activity and PK properties are summarized in Table 2.

Furan derivative **8g** showed very weak CaNmt inhibitory activity, whereas the nitrogen-containing heteroaromatic derivatives showed strong inhibitory activity. Since the furan oxygen is expected to be a poor hydrogen acceptor, it is reasonable that the inhibitory activity of **8g** was weaker than that of **8a**. Introduction of methyl group(s) to the thiazole ring enhanced the activity significantly (**8e** and **8f** vs **8d**). A similar increase of potency was also observed when a methyl group was introduced to the pyridine ring (**8b** vs **8a**). Introduction of methyl groups, the electron-donating groups, increases the electron density on thiazole nitrogen or pyridine nitrogen and thus would increase the hydrogen bond interaction with Asn 392. However, there is another factor that enhanced the activity of the methyl derivatives. The methyl group(s) might fit into a favorable position in the lipophilic pocket composed of Phe 115, Phe 240 and Phe 339.

Most inhibitors in Table 2 showed very strong enzyme inhibitory activities against CaNmt with extremely high selectivity over HsNmt. However, none of them showed strong inhibitory activity against AfNmt.

Antifungal activity and acid-stability

The strong CaNmt inhibitors, **8a–8f** and **8h–8k**, also showed strong antifungal activity against *C. albicans* CY1002 in vitro (Table 2). Among them, **8b** and **8k** were further evaluated by in vivo experiments, since **8b**

Table 2. Enzyme inhibitory activity, in vitro antifungal activity and pharmacokinetic (PK) properties in rats

Compd	Enzyme inhibition (μM) CaNmt[a]	Enzyme inhibition (μM) HsNmt[b]	Enzyme inhibition (μM) AfNmt[c]	Antifungal activity (μM) serum (−)[d]	Antifungal activity (μM) serum (+)[e]	PK parameters in rats[f] AUC (ng h/mL)	$t_{1/2}$ (h)
8a	0.0035	340	42	0.065	0.47	410	0.54
8b	0.00039	>480	4.1	0.036	0.082	440	0.58
8c	0.043	>430	11	0.34	5.1	590	0.56
8d	0.091	25	63	0.54	8.3	NT[g]	NT
8e	0.0020	130	18	0.14	0.74	470	0.34
8f	0.00071	83	4.3	0.039	0.19	320	0.65
8g	1.4	280	150	4.6	25	NT	NT
8h	0.0013	69	30	0.12	3.0	530	0.45
8i	0.00040	94	13	0.035	1.2	450	0.59
8j	0.0013	99	0.48	0.019	2.7	810	0.86
8k	0.00058	85	5.1	0.048	0.42	570	0.80
Fluconazole	NT	NT	NT	0.72	0.5	19,600	4.0

[a]Potency against *C. albicans* Nmt (CaNmt) as assessed by IC_{50} using substrate peptide GLTISKLFRR-NH_2 (0.5 μM) and myristoyl-CoA at 0.5 μM.
[b]Potency against human Nmt (HsNmt) as assessed by IC_{50} using substrate peptide GNAASARR-NH_2 (0.5 μM) and myristoyl-CoA at 0.5 μM.
[c]Potency against *A. fumigatus* Nmt (AfNmt) as assessed by IC_{50} using substrate peptide GLTISKLFRR-NH_2 (0.5 μM) and myristoyl-CoA at 0.5 μM.
[d]Antifungal activity against *C. albicans* CY1002 as assessed by IC_{50} in YNBPB medium (1% glucose, 0.25% K_2HPO_4, pH 7).
[e]Antifungal activity against *C. albicans* CY1002 as assessed by IC_{50} in 80% calf serum (80% calf serum supplemented with 10 μM $FeCl_3 \cdot 6H_2O$, 10 μM deferoxamine, 2% dextrose).
[f]Cassette dosing: Five compounds were intravenously administered to a rat (2 mg/kg) and the plasma concentration of each compound was measured by LC–MS.[16]
[g]NT, not tested.

showed the strongest antifungal activity against *C. albicans* CY1002 in the presence of 80% calf serum and **8k** showed a good PK profile. The antifungal assay in 80% serum should be more predictive for in vivo efficacy than the conventional in vitro antifungal assay, since the former assay system includes a factor of drug-protein binding.[17,18] Their in vivo efficacy was determined as follows: (1) Fisher rats ($n=5$) were infected intravenously with a lethal dose of *C. albicans* (CY1002); (2) The infected rats were treated iv with multiple doses (three times per day for 2 days) of the test compound; and (3) efficacy of the compounds was calculated as the effective dose (mg/kg) for 50% survival (ED_{50}) on day 7. The compounds **8b** and **8k** showed clear antifungal activity in this model with ED_{50}s of 15 and 8.7 mg/kg, respectively. However, the efficacy was still weaker than that of fluconazole. This can be explained by a poorer PK profile than that of fluconazole. We still need to improve the PK profile to obtain a strong antifungal drug.

As we expected, these compounds were stable under acidic conditions. HPLC analysis showed 100, 86, 87 and 99% of **8a**, **8b**, **8j** and **8k** remained unchanged even after 2-h treatment in artificial gastric fluid (pH 1.2) at 37 °C, whereas 94% of **2** decomposed after 2-h treatment under the same condition. Thus, we successfully designed and synthesized acid-stable inhibitors that showed clear antifungal activity against *C. albicans* both in vitro and in vivo. The PK properties, however, were not much improved by the modification. Although the benzimidazole derivatives showed slightly higher AUC than did the aryl ether **2** (AUC = 330 ng·h/mL), their elimination half-lives ($t_{1/2}$) were shorter than that of **2** ($t_{1/2}=2.0$ h).

In summary, crystallographic analysis of the CaNmt complex with benzofuranylmethyl aryl ether **3** led us to design a new series of acid-stable antifungal agents, heteroaromatic ketones **8a**–**8k**. Among them, **8b** and **8k** showed clear antifungal activity in a murine systemic candidiasis model. We have overcome the acid labile nature of benzofuranylmethyl aryl ether **2**, keeping its strong antifungal activity. Nevertheless, since the inhibitors do not strongly inhibit AfNmt, further modification work is required to obtain compounds that inhibit both AfNmt and CaNmt and thus would inhibit the growth of both *A. fumigatus* and *C. albicans*.

References and Notes

1. Gordon, J. I.; Rudnick, D. A.; Adams, S. P.; Gokel, G. W. *J. Biol. Chem.* **1991**, *266*, 8647.
2. Johnson, D. R.; Bhatnagar, R. S.; Knoll, L. J.; Gordon, J. I. *Annu. Rev. Biochem.* **1994**, *63*, 869.
3. Stearns, T.; Kahn, R. A.; Botstein, D.; Hoyt, M. A. *Mol. Cell. Biol.* **1990**, *10*, 6690.
4. Herman, P. K.; Stack, J. H.; DeModena, J. A.; Emr, S. D. *Cell* **1991**, *64*, 425.
5. Weinberg, R. A.; McWherter, C. A.; Freeman, S. K.; Wood, D. C.; Gordon, J. I.; Lee, S. C. *Mol. Microbiol.* **1995**, *16*, 241.
6. Lodge, L. K.; Jackson-Machelski, E.; Toffaletti, D. L.; Perfect, J. R.; Gordon, J. I. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12008.
7. Benedetti, M. S.; Bani, M. *Drug Metab. Rev.* **1991**, *31*, 665.
8. Devadas, B.; Gordon, J. I.; Sikorski, J. A. *J. Med. Chem.* **1998**, *41*, 996, and reference cited therein.
9. Armour, D. R.; Bell, A. S.; Kemp, M. I.; Edwards, M. P.; Wood, A. *Abstracts,* 221st ACS National Meeting, San Diego, April 1–5, 2001; MEDI-349.
10. Bell, A. S.; Armour, D. R.; Edwards, M. P.; Kemp, M. I.; Wood, A. *Abstracts*, 221st ACS National Meeting, San Diego, April 1–5, 2001; MEDI-350.
11. Ganesh, K. R.; Madhvaro, K. V. *Indian Drugs* **2001**, *38*, 406.
12. Masubuchi, M.; Kawasaki, K.; Ebiike, H.; Ikeda, Y.; Tsujii, S.; Sogabe, S.; Fujii, T.; Sakata, K.; Shiratori, Y.; Aoki, Y.; Ohtsuka, T.; Shimma, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1833.
13. Ebiike, H.; Masubuchi, M.; Liu, P.; Kawasaki, K.; Morikami, K.; Sogabe, S.; Hayase, M.; Fujii, T.; Sakata, K.; Shindoh, H.; Shiratori, Y.; Aoki, Y.; Ohtsuka, T.; Shimma, N. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 607.
14. Gribble, G. W.; Kelly, W. J.; Emery, S. E. *Synthesis* **1978**, 763.
15. Meunier, F.; Lambert, C.; Van der Auwera, P. *J. Antimicrob. Chemother.* **1990**, *25*, 227.
16. Frick, L. W.; Adkison, K. K.; Wells-Knecht, K. J.; Woollard, P.; Higton, D. M. *Pharm. Sci. Technol. Today* **1998**, *1*, 12.
17. Masubuchi, K.; Okada, T.; Kohchi, M.; Sakaitani, M.; Mizuguchi, E.; Shirai, H.; Aoki, M.; Watanabe, T.; Kondoh, O.; Yamazaki, T.; Satoh, Y.; Kobayashi, K.; Inoue, T.; Horii, I.; Shimma, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 395.
18. Masubuchi, K.; Okada, T.; Kohchi, M.; Murata, T.; Tsukazaki, M.; Kondoh, O.; Yamazaki, T.; Satoh, Y.; Ono, Y.; Tsukaguchi, T.; Kobayashi, K.; Ono, N.; Inoue, T.; Horii, I.; Shimma, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1273.