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Synthesis of potent and selective inhibitors of *Candida albicans* *N*-myristoyltransferase based on the benzothiazole structure

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Abstract—Two parallel synthetic methods using solid-supported reagents were established to examine the rapid optimization of weak hit compound **1**. Several compounds showed high potency in the low nanomolar range against *N*-myristoyltransferase. The structure–activity relationship (SAR) and antifungal activities of a series of novel 2-aminobenzothiazole *N*-myristoyltransferase inhibitors are presented.

1. Introduction

Myristoylation by the myristoyl–CoA:protein *N*-myristoyltransferase (Nmt) is an important lipid anchor modification of eukaryotic and viral proteins. These enzymes catalyze the covalent attachment of the C_{14} fatty acid to the N-terminal glycine on substrate proteins. According to eukaryote genome analyses, the predicted rate of substrate proteins is about 0.5% of all encoded proteins.[1] The incidence of systemic fungal infections caused by *Candida albicans* has been observed during the last two decades, particularly in immunocompromised patients. Several classes of antifungal drugs have been used, but for the best treatment of life-threatening infections, new, effective drugs are urgently needed. Genetic experiments have shown Nmt is an essential enzyme for survival of *Candida*. Therefore, Nmt is an attractive target for new fungicidal drugs.[2] Several Nmt inhibitors have already been reported as shown in Figure 1,[3] but further investigations of in vivo efficacy are necessary.[4] In this paper, we describe a solution phase parallel synthesis and SAR of a series of novel benzothiazole *N*-myristoyltransferase inhibitors. Figure 1 also shows the identified weak hit compound **1** (IC_{50}: 1.5 μM) by virtual screening[5] followed by CaNmt screening of selected compounds.

2. Chemistry

Scheme 1 shows the combinatorial synthetic route. Diversity was initially introduced to carboxylic acid **3** using a commercially available set of *N*-Boc protected diamines **4a**–**k**. After deprotection of the Boc group, reductive amination with aldehydes **6a**–**k** using a macroporous triacetoxyborohydride MP-$BH(OAc)_3$ was performed.[6] Then, a catch and release purification using macroporous *p*-toluenesulfonic acid (MP-TsOH) afforded the final products **7aa**–**7kk**. (examples: **3** + **4a**→**5a**, **5a** + **6a**→**7aa**; **3** + **4k**→**5k**, **5k** + **6k**→**7kk**). The amide junction modified compounds **10ai**–**10ck** (examples: **9a** + **6i**→**10ai**; **9c** + **6k**→**10ck**) were prepared using a protocol Benzothiazole carboxamide regio-isomers **11a**,**b** (Table 4) were also prepared from benzothiazole 5- or 7-carboxylic acid[7] by the above synthetic route (Table 1).

In order to optimize the effects of C2 substitution of benzothiazole, the synthetic route was changed as shown in Scheme 2. *N*-Boc protected amine **12** was reacted with 2-naphthaldehyde under general reductive amination conditions, followed by *N*-protection with an Alloc group and deprotection of the Boc group to give **13**. Condensation of **13** with 2-*N*-Boc-aminobenzothiazole-6-carboxylic acid gave **14b**. After the general transformations, palladium-catalyzed deprotection of

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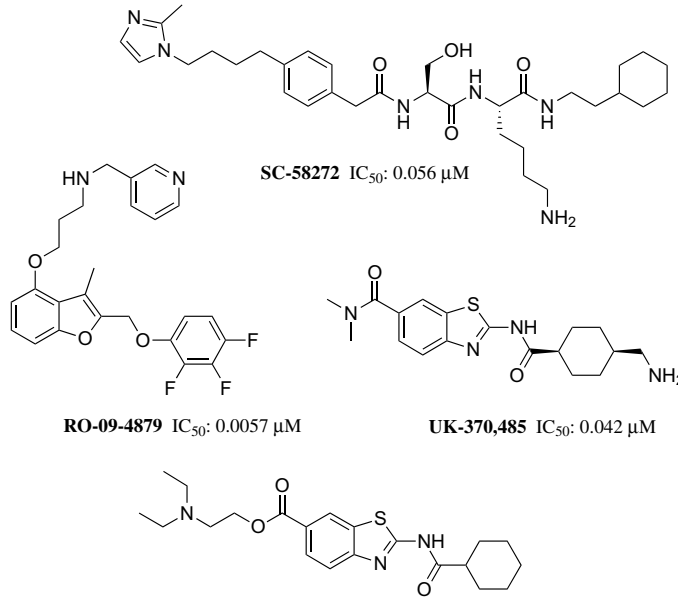
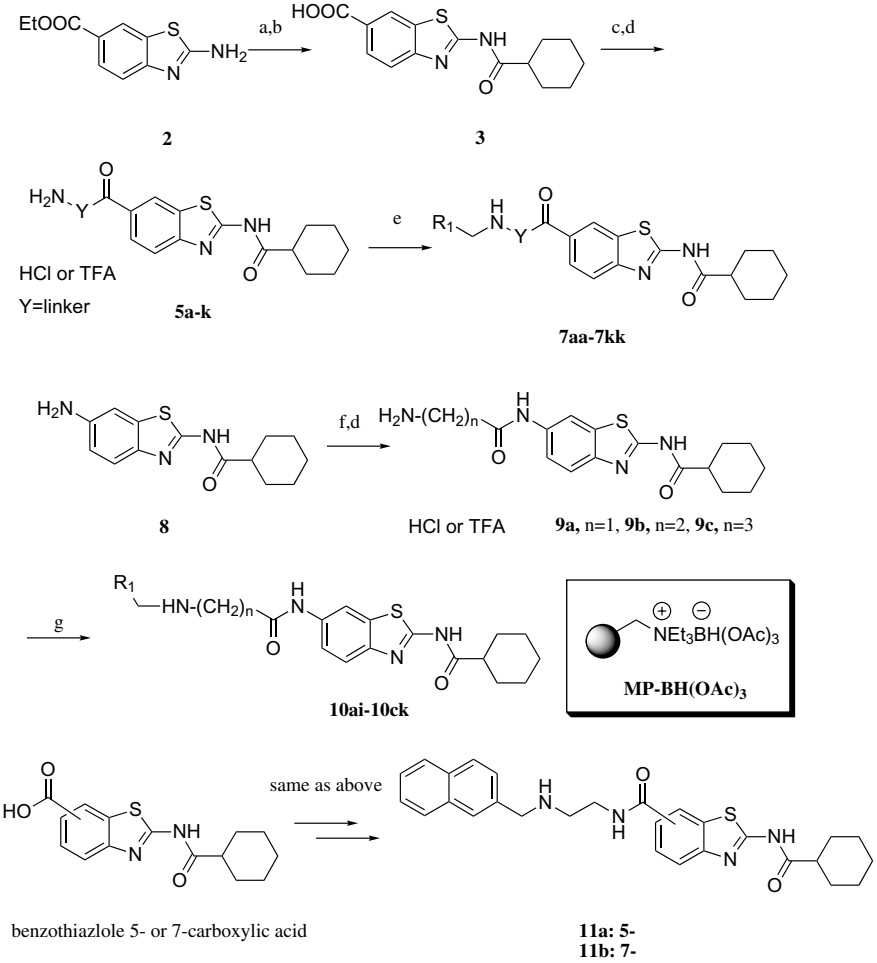
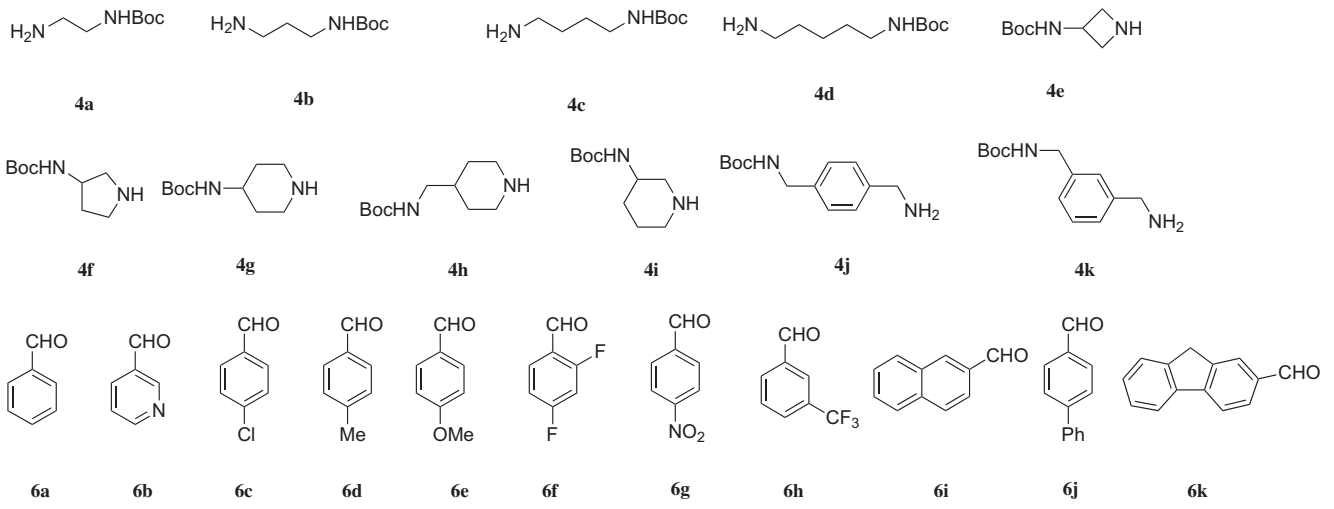


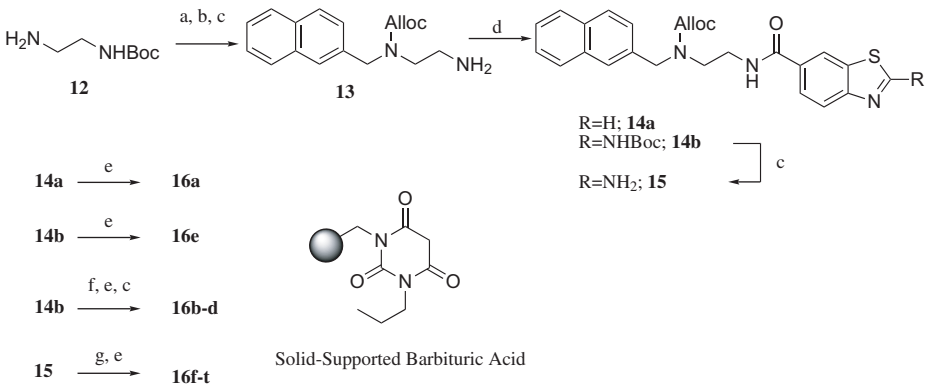
Figure 1.



Scheme 1. Reagents and conditions: (a) cyclohexanecarboxylic acid, EDC, HOBt, DMF; (b) 1 N-NaOH, MeOH; (c) BocNH-linker-NHR (**4a**–**k**), EDC, HOBt, DMF; (d) 4 N-HCl/dioxane or 50% TFA–CH_2Cl_2; (e) R_1CHO (**6a**–**k**), MP-$BH(OAc)_3$, DMF, then PS-CHO, catch and release purification using MP-TsOH then 2 M-NH_3/MeOH; (f) $BocNH(CH_2)_nCOOH$, EDC, HOBt, DMF; (g) R_1CHO (**6i**–**k**), MP-$BH(OAc)_3$, DMF.

Table 1. List of diverse sets **4** and **6**





Scheme 2. Reagents and conditions: (a) 2-naphthaldehyde, $NaBH(OAc)_3$, DCE; (b) Alloc-Cl, K_2CO_3, $THF–H_2O$; (c) 50% $TFA–CH_2Cl_2$; (d) benzothiazole-6-carboxylic acid for **14a**, 2-*N*-Boc-aminobenzothiazole-6-carboxylic acid for **14b**, EDC, HOBt, DMF; (e) solid-supported barbituric acid, $Pd(PPh_3)_4$, THF; (f) Aryl CH_2Br or Alkyl CH_2Br, K_2CO_3, DMF; (g) Ac_2O, DMAP, CH_2Cl_2, or carboxylic acid, EDC, HOBt, DMF.

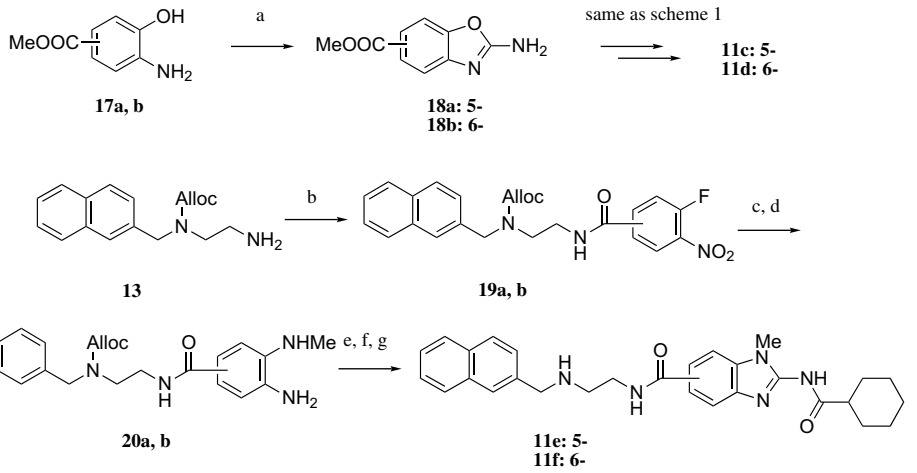
the Alloc group was conducted by the parallel mode using solid-supported barbituric acid.[8] This solid-supported reagent facilitated the isolation and purification of the deprotected polar and basic secondary amines.

Benzoxazole scaffolds (Table 4, **11c**,**d**) were prepared according to the literature.[9] Thus, the reaction of 2-aminophenol derivatives **17a**,**b** with di-(imidazole-1-yl)methanimine afforded methyl 2-aminobenzoxazole 5- and 6-carboxylate, respectively, which were converted to compound **11c**,**d** by the same procedure as shown in Scheme 1. Benzimidazole scaffolds (Table 4, **11e**,**f**) were prepared from *N*-protected diamines derivatives **13**. Condensation of **13** with fluoronitrobenzoic acid gave **19a**,**b**, which were treated with methylamine for the nucleophilic aromatic substitution and followed by the reduction of the nitro group to afford **20a**,**b**. A cyclization reaction using a literature procedure[10] followed by the palladium-catalyzed deprotection of the Alloc group gave **11e**,**f** (Scheme 3).

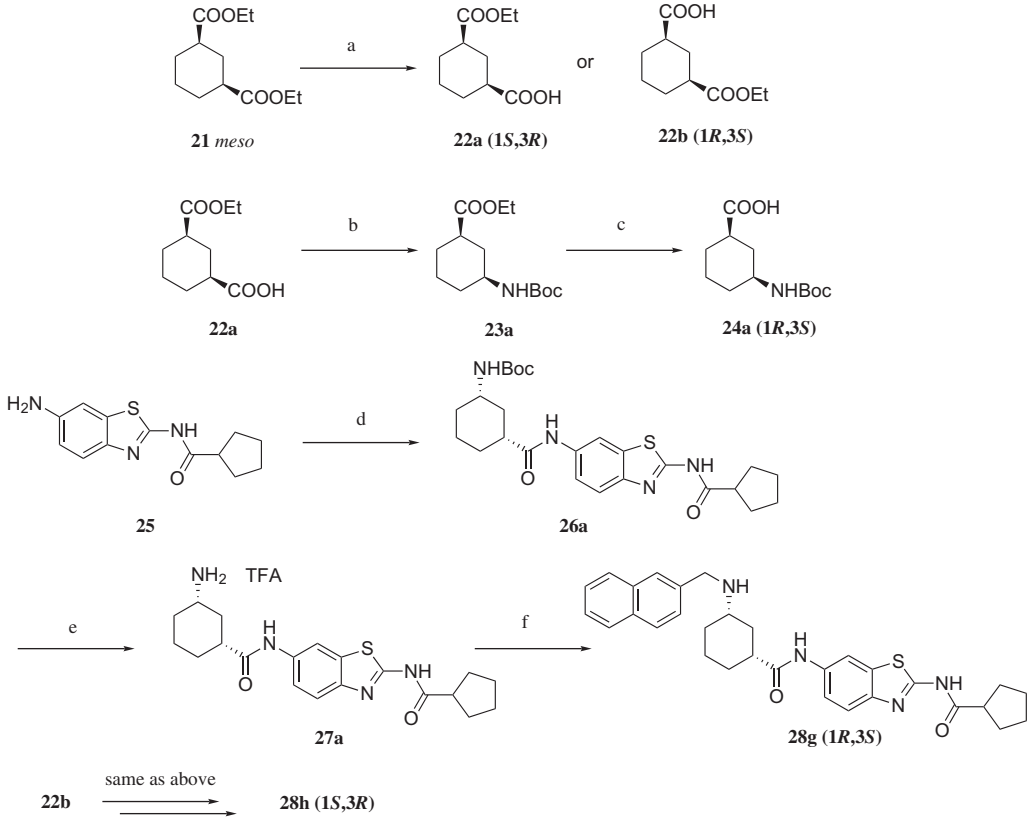
Compounds **28a**–**k** (Table 6) were prepared in a similar manner to that described for **10ai**–**10ck**. Enantiomers **28g** and **28e** were synthesized from the known (1*S*,3*R*)- or (1*R*,3*S*)-1,3-cyclohexanedicarboxylic acid mono ester.[11] Thus, enzyme-catalyzed desymmetrization of a *meso cis*-cyclohexanecarboxylic acid diester **21** was conducted using lipase AYS Amano or PS Amano to give monocarboxylic acid **22a** or **22b**, respectively. The Curtius rearrangement of **22a** was performed under general conditions, followed by hydrolysis to give (1*R*,3*S*)-3-Boc-aminocyclohexanecarboxylic acid **24a**. Condensation of **24a** with 6-aminobenzothiazole **25** gave **26a**. Deprotection of the Boc group was performed, followed by reductive amination to give (1*R*,3*S*)-enantiomer **28g** (98% ee). The (1*S*,3*R*)-enantiomer **28h** (93% ee) was synthesized by the same procedure from **22b** (Scheme 4).

3. Biology

The enzyme inhibitory activities of compounds against *C. albicans* Nmt (CaNmt) and human Nmt (HsNmt) were measured according to the literature.[12–14] In vitro antifungal assay against *C. albicans* (ATCC90028) was



Scheme 3. Reagents and conditions: (a) di-(imidazole-1-yl) methanimine, THF; (b) 4-fluoro-3-nitrobenzoic acid for **19a**, 3-fluoro-4-nitrobenzoic acid for **19b**, EDC, HOBt, DMF; (c) $MeNH_2$–THF; (d) $SnCl_2{\cdot}2H_2O$, 6 N-HCl, EtOH; (e) cyclohexanecarbonyl isothiocyanate, CH_2Cl_2; (f) EDC, DIEA, CH_2Cl_2; (g) solid-supported barbituric acid, $Pd(PPh_3)_4$, THF.



Scheme 4. (a) Lipase AYS Amano for **22a** or Lipase PS Amano for **22b**, pH 7.2 phosphate buffer; (b) $(PhO)_2PON_3$, Et_3N, toluene, then *t*-BuOH; (c) 5 N-NaOH, THF; (d) **24a**, EDC, HOBt, DMF; (e) TFA, CH_2Cl_2; (f) 2-naphthaldehyde, MP-$BH(OAc)_3$, DMF.

performed by a broth microdilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) method M27-A2. The minimum inhibitory concentration (MIC) of a compound was defined as the lowest concentration at which there was 80% inhibition of growth compared to the growth of a drug-free control.

4. Results and discussion

Based on the proposed reaction mechanism,[15] we took particular interest in the polar amine moiety of **1**. To increase enzymatic inhibitory activity, substitutes at C6 of benzothiazole **1** were initially examined. Several cyclic and acyclic amides that contain primary, secondary,

Table 2. Matrix of CaNmt inhibitory activity (%) at 10 μM of **7aa**–**7kk**

From	6a	6b	6c	6d	6e	6f	6g	6h	6i	6j	6k
4a											
4b											
4c											
4d											
4e											
4f											
4g											
4h											
4i											
4j											
4k											
		>90%		50-90%		0-49%					

and tertiary amines as the C6 substituents were synthesized using standard methods and it was determined that the amides with secondary amines displayed the highest potency (data not shown). Therefore, we focused on synthesizing secondary amine utilizing solution phase combinatorial chemistry. Table 2 summarizes the CaNmt inhibitory activities at 10 μM of the first library compounds.

From the matrix, hydrophobic groups such as 2-naphthyl (derived from **6i**), 4-chrolophenyl (**6c**), and 4-biphenyl (**6j**) seem suitable for high potency, although the matrix does not show accurate potencies. In most cases, 2-naphthyl derivatives showed high potency. Fluorine compounds such as 3-trifluoromethylpheyl (derived from **6h**) and 2,4-difluorophenyl (**6f**) groups had poor potency. Furthermore, shorter alkyl chain lengths (**4a**,**b**) seem suitable. Table 3 summarizes the close-up SAR of the 2-naphthyl derivatives.

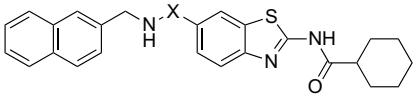
Table 3 clearly shows that even-numbered alkyl chain lengths (**7ai**, **7ci**) are more potent than odd-numbered ones (**7bi, 7di**). Reversed amide (**10bi**) resulted in a slight loss of activity. The selectivity against the HsNmt is generally good. Based on the above results, compound **7ai** was selected as the starting point for further optimization.

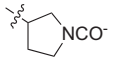
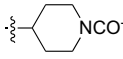
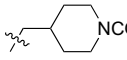
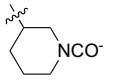
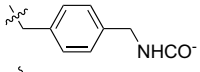
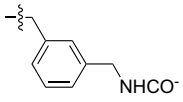
Next, the effects of modifying the carboxamide regioisomer and the core scaffold were investigated. Table 4 summarizes the results. The position of the amide group that contains the secondary amine is crucial for potent enzyme inhibitory activity (**7ai** vs **11a**,**b**). Benzoxazole (**11c**,**d**) and benzimidazole (**11e**,**f**) resulted in a significant loss of activity.

In order to further optimize the potency, the effects of C2 substitution of benzothiazole were examined. Table 5 summarizes the assay results. Activity was not observed when the amide group was removed (compounds **15**, **16a**–**e**). Less lipophilic alkyl amides decreased the potency (**16f**,**g**). In contrast, more lipophilic acyclic and cyclic alkyl amides (**16h**–**j**) showed potency equal or greater than **7ai**, but inserting heteroatoms resulted in a significant loss of activity (**16k**,**l**). Our experiments indicated that replacing the cycloalkyl groups with aryl groups decreased the potency.

Then all compounds were screened for the antifungal activity against *C. albicans* (ATCC90028). Surprisingly, all the compounds had weaker antifungal activities than

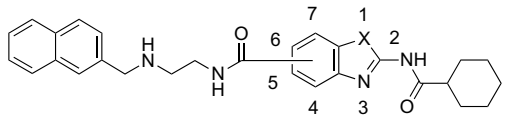
Table 3. SAR of the substitutes at position 6 of the benzothiazole



Compd	X	CaNmt inhibition IC_{50} (μM)	HsNmt inhibition (%) at 10 μM
7ai	$-(CH_2)_2NHCO-$	0.034	49
7bi	$-(CH_2)_3NHCO-$	0.11	61
7ci	$-(CH_2)_4NHCO-$	0.074	43
7di	$-(CH_2)_5NHCO-$	0.3	46
7ei		0.065	14
7fi		0.19	36
7gi		0.5	27
7hi		0.026	32
7ii		0.21	27
7ji		>10	N.T.
7ki		1.9	N.T.
10ai	$-CH_2CONH-$	2.3	8
10bi	$-(CH_2)_2CONH-$	0.18	32

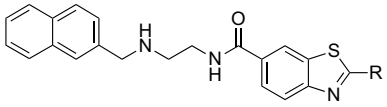
N.T.: not tested.

Table 4. SAR for core scaffolds



Compd	X	Position	CaNmt inhibition IC_{50} (μM)
7ai	S	6	0.034
11a	S	5	6.8
11b	S	7	1.0
11c	O	5	1.1
11d	O	6	0.29
11e	NMe	5	>10
11f	NMe	6	>10

Table 5. SAR for C2 substitution of benzothiazole



Compd	R	CaNmt inhibition IC_{50} (μM)	HsNmt inhibition (%) at 10 μM
16a	H	>10	N.T.
15	NH_2	>10	N.T.
16b	$NHCH_2Ph$	>10	N.T.
16c	$NHCH_2$–2-pyridyl	>10	N.T.
16d	$NHCH_2$–*c*-hexyl	>1	N.T.
16e	NHCOO–*t*-Bu	>10	N.T.
16f	NHCOMe	>10	N.T.
16g	NHCOEt	0.13	4
16h	NHCO–*i*-Pr	0.046	14
16i	NHCO–*c*-butyl	0.028	22
16j	NHCO–*c*-pentyl	0.023	32
7ai	NHCO–*c*-hexyl	0.034	49
16k	NHCO–tetrahydro-pyran-4-yl	0.89	5
16l	NHCO–piperidine-4-yl	>10	N.T.
16m	NHCOPh	0.16	32
16n	NHCO–2-pyridyl	>10	37
16o	NHCO–2-Cl–3-pyridyl	>10	16
16p	NHCO–2-pyrazine	0.73	11
16q	NHCOPh–4-Cl	>10	8
16r	NHCOPh–3,4-Cl	>10	22
16s	NHCOPh–4-CF_3	>10	1
16t	NHCOPh–2,5-OMe	0.31	71

N.T.: not tested.

expected from the potent enzyme inhibitory activity. One of them, the reversed amide **10bj** showed moderate antifungal activity as shown in Figure 2, but **10bj** showed less potent enzyme inhibitory activity than **7aj**. Therefore, we returned to reversed amide **10bj** to explore the effect and the role of the reversed amide on the antifungal activity.

Several analogues, which were prepared by the same procedure described above, and the reversed amide series were screened for CaNmt inhibitory activities and antifungal activities against *C. albicans*. Table 6 summarizes the results.

Linear alkyl series **28b** showed greater enzyme inhibitory activity than **10bj**, but the antifungal activity was disappointing. A correlation between the enzyme inhibitory activity and the antifungal activity was not observed. However, introducing a cycloalkyl linker, in particular, a cyclohexyl linker improved both the CaNmt inhibitory activity and antifungal activity. The antifungal activity seems to be associated with CaNmt inhibitory activity only in the cycloalkyl linker series (**28c**–**m**). The (1*R*,*S*)-enantiomer **28g** exhibited the most potent CaNmt inhibitory activity (IC_{50}: 0.49 nM) with an excellent selectivity (>10,000-fold) over the HsNmt (IC_{50}: 5.4 μM) and antifungal activity (MIC: 0.78 μM). Table 7 summarizes more screening results of **28g** and known inhibitors. The MIC value of **28g** was about 2–6 times lower than RO-09-4879 and UK-370753.[3c] There was a large difference between the effective concentrations of **28g** that were required for enzyme inhibition and fungal growth inhibition compared to RO-09-4879 and UK-370753. One possible explanation is that **28g** has a low ability to enter the cell, but it is unclear how many Nmt molecules must be deactivated before growth is inhibited. The permeation of most drugs across the cell membrane by passive diffusion or via a transporter is a critical step in the interaction of a drug with its cellular target and can influence its overall efficacy. Increased antifungal activity requires drug interaction with a transporter to increase the intracellular concentration. For example, caspofungin acetate is taken up by a high-affinity transpoter.[16]

The serum effect of **28g** was not observed in an in vitro antifungal assay and **28g** did not inhibit other strains such as *Candida krusei* (ATCC 6258; MIC: >25 μM), *Candida glabrata* (TIMM3171; MIC: >25 μM) and *Aspergillus fumigatus* (IFM40808; MIC: >25 μM). Unfortunately, the cytotoxicity of **28g** was about ten times stronger than RO-09-4879 and UK-370753, but **28g** exhibited an excellent selectivity against the HsNmt, which suggests that it may have an alternative mechanism for cytotoxicity.[17] Further investigations are necessary to determine the usefulness of the Nmt inhibitors.

5. Conclusion

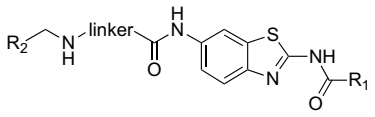
In summary, the synthesis and optimization of a series of 2-aminobenzothiazole *N*-myristoyltransferase inhibitors using solution phase combinatorial chemistry are described. The present study demonstrates the efficiency of parallel synthesis using solid-supported reagents in a rapid synthesis and SAR evaluation. Members of this class of compounds exhibit potent enzyme inhibitory activity and antifungal activity. Studies on further modifications of **28g** (FTR1335) are currently underway.

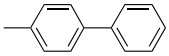
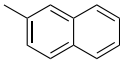
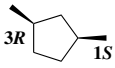
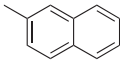
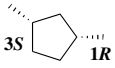
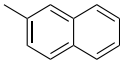
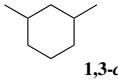
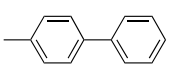
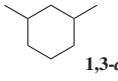
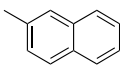
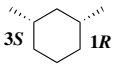
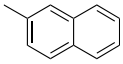
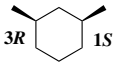
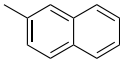
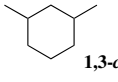
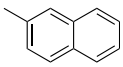
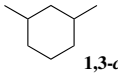
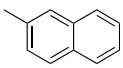
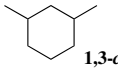
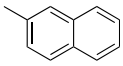
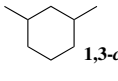
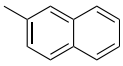
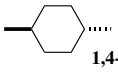
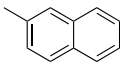
6. Experimental

6.1. General comments

All the solvents and reagents were purchased from commercial sources and were used without further purifica-

Table 6. SAR for reversed amide series



Compd	Linker	R_1	R_2	CaNmt inhibition IC_{50} (μM)	MIC (μM) *C. albicans* ATCC90028
28a	$-(CH_2)_2-$			0.16	6.25
28b	$-(CH_2)_3-$			0.015	50
28c				0.015	>12.5
28d				0.011	6.25
28e				0.13	>12.5
28f				0.0012	0.78
28g				0.00049	0.78
28h				>0.01	3.13
28i				0.0033	3.13
28j				0.0024	3.13
28k				0.0023	1.56
28l				0.0081	3.13
28m				0.034	6.25

tion. MP-$BH(OAc)_3$ (Part Number 800414), MP-TsOH cartridge (Part Number 800477-0050-C) and PS-Benzaldehyde (Part Number 800360) were purchased from Argonaut Technologies. Solid-supported barbituric acid was prepared according to a literature procedure.[8] Parallel syntheses were performed on a Quest 210 (Argonaut) or on an SY-1000 (Shimadzu). ^1H NMR (400 MHz) spectra were recorded on JNM-EX400 using tetramethylsilane as an internal standard. FAB mass spectra were recorded on JEOL JMS-700 spectrometer. The LC-LRMS system consisted of an SCL-10Avp series (Shimadzu) coupled to an LCMS-QP8000α (Shimadzu, atmospheric pressure chemical ionization, APCI). All the HPLC analyses were carried out using an Inertsil ODS3 column, 1.5 mm × 150 mm (GL Sciences). A flow rate of 0.12 mL/min and a gradient of (5–95)% B over 5 min were used (solvent A, water/0.05% formic acid; solvent B, acetonitrile/0.05% formic acid). The enantiomeric excess (ee) was determined by HPLC analysis. HPLC was performed on a Hitachi HPLC L-4200 (detector), L-6000 (pump), L-7300 (column oven) using a Daicel Chiralcel OD-H column; mobile phase hexane/EtOH/Et_2NH = 60:40:0.1; flow rate, 0.5 mL/min; temperature, 25 °C; detection, UV at 254 nm. Optical resolutions were recorded on a high sensitive polarimeter SEPA-300 (Horiba).

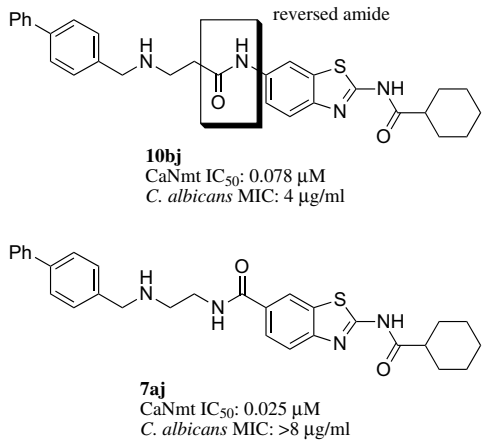


Figure 2.

6.2. Ethyl 2-amino-benzothiazole-6-carboxylate (2)

A mixture of ethyl 4-aminobenzoate (16.5 g, 0.1 mol), KSCN (97.18 g, 1 mol), and $CuSO_4$ (80 g, 0.5 mol) in MeOH (270 mL) was stirred for 3 h at 70 °C. After cooling, the suspension was filtered and the filtrate diluted with water (400 mL) and heated to boiling. Ethanol was added to the boiling filtrate until a clear slight yellow solution resulted. Cooling of this solution resulted in crystallization of **2** (11 g, 50%) as a pale yellow solid.

^{1}H NMR (DMSO-d_6) δ (ppm): 8.34 (d, J = 1.4 Hz, 1H), 7.87 (dd, J = 1.4, 8.3 Hz, 1H), 7.42 (d, J = 8.3 Hz, 1H), 4.30 (q, J = 7.3 Hz, 2H), 1.32 (t, J = 7.3 Hz, 3H). MS (FAB): 223 (M+1).

6.3. 2-[(Cyclohexanecarbonyl)amino]-benzothiazole-6-carboxylic acid (3)

To a solution of **2** (50 g, 0.225 mol) and cyclohexanecarboxylic acid (35 g, 0.27 mol) in DMF (200 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide [EDC] (65 g, 0.338 mol) and 1-hydroxybenzotriazole [HOBt] (46 g, 0.338 mol) at room temperature. The mixture was stirred at 50 °C for 16 h, and then poured into water (1.5 L). The resulting solid was collected by filtration and washed with water. The solid was dried, then washed with hot isopropyl ether to afford 33 g (41%) of ethyl 2-[(cyclohexanecarbonyl)amino]-benzothiazole-6-carboxylate, which was used in the next step without further purification.

^{1}H NMR ($CDCl_3$) δ (ppm): 10.87 (br s, 1H), 8.57 (d, J = 1.4 Hz, 1H), 8.14 (dd, J = 8.3, 1.4 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 4.43 (q, J = 6.8 Hz, 2H), 2.28–2.45 (m, 1H), 1.09–1.95 (m, 13H). MS (FAB): 333 (M+1). HRMS (FAB) Calcd for $C_{17}H_{21}N_2O_3S$: 333.1273, Found: 333.1281.

The mixture of the above compound (33 g, 0.1 mol), 1 N-NaOH (1 L), and MeOH (200 mL) was stirred at room temperature for 4 h and then the solvent was evaporated. The resulting aqueous layer was washed with $CHCl_3$. The aqueous layer was separated and cooled to 5 °C, 10% aqueous HCl was added to lower the pH 2. The resulting solid was collected by filtration and washed with water. The solid was dried to afford 25 g (84%) of **3**. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.58 (d, J = 1.4 Hz, 1H), 7.99 (dd, J = 8.3, 1.4 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 3.26 (br s, 1H), 2.48–2.60 (m, 1H), 1.09–1.90 (m, 10H). MS (FAB): 305 (M+1). HRMS (FAB) Calcd for $C_{15}H_{17}N_2O_3S$: 305.0960, Found: 305.0965.

6.4. 2-[(Cyclohexanecarbonyl)amino]-*N*-(2-aminoethyl)-benzothiazole-6-carboxamide hydrochloride (5a)

To a solution of **3** (1 g, 3.48 mmol) and *tert*-butyl *N*-(2-aminoethyl)carbamate (0.56 g, 3.48 mmol) in DMF (15 mL) was added EDC (1 g, 5.22 mmol) and HOBt (0.7 g, 5.22 mmol) at room temperature. The mixture was stirred for 16 h at room temperature and then poured into water (100 mL). The resulting solid was collected by filtration and washed with water and then the solid was dried. The crude product was purified by chromatography on silica gel to afford 1.28 g (86%) of 2-[(cyclohexanecarbonyl)amino]-*N*-[(2-*tert*-butoxycarbonyl)aminoethyl]-benzothiazole-6-carboxamide as a pale yellow solid, which was used in the next step. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.34 (s, 1H), 8.42 (s, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 8.3 Hz, 1H), 6.83 (br s, 1H), 3.25–3.38 (m, 2H), 3.08–3.19 (m, 2H), 2.50–2.62 (m, 1H), 1.16–1.91 (m, 19H). MS (FAB): 447 (M+1). HRMS (FAB) Calcd for $C_{22}H_{31}N_4O_4S$: 447.2066, Found: 447.2097.

To the above compound (210 mg, 0.47 mmol) was added 4 N-HCl-dioxane (10 mL). The mixture was stirred at room temperature for 16 h and then the resulting solid was collected by filtration and washed with dioxane. The solid was dried to afford 179 mg (quant.) of **5a** as a colorless solid.

^{1}H NMR (DMSO-d_6) δ (ppm): 12.38 (br s, 1H), 8.75–8.87 (m, 1H), 8.56 (d, J = 1.4 Hz, 1H), 8.05–8.30 (br s, 3H), 8.00 (dd, J = 8.3, 1.4 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.17 (br s, 2H), 3.48–3.63 (m, 2H), 2.92–3.08 (m, 2H), 2.48–2.65 (m, 1H), 1.13–1.91 (m, 10H). MS (FAB): 347 (M+1). HRMS (FAB) Calcd for $C_{17}H_{23}N_4O_2S$: 347.1542, Found: 347.1585.

Table 7. In vitro antifungal activity and cytotoxicity of selected compound **28g** with the known inhibitors

Compd	CaNmt inhibition IC_{50} (μM)	MIC (μM) *C. albicans* ATCC90028	MIC (μM) *C. albicans* ATCC90028 (with 10% serum)	Cytotoxicity assay HeLa 229 CC_{50} (μM)
28g	0.00049	0.78	0.78	1.0
RO-09-4879	0.0029	0.1	0.2	13.3
UK-370753	0.01	0.39	0.39	14.4

6.5. Analogues 5b–k

These compounds were prepared in a similar manner to that described for **5a**. The products were adequately characterized by ^{1}H NMR and HRMS spectrum.

6.6. 2-[(Cyclohexanecarbonyl)amino]-*N*-[(2-naphthylmethyl)amino]ethyl-benzothiazole-6-carboxamide (7ai)

A DMF solution (1 mL) of 2-naphthaldehyde (0.24 mmol) was added to a DMF solution of **5a** (1 mL, 0.3 mmol). The macroporous triethylammonium methylpolystyrene triacetoxyborohydride [MP-$BH(OAc)_3$] (1.8 mmol/g, 430 mg, 0.9 mmol) was then added and the mixture was agitated at room temperature for 16 h. After which PS-Benzaldehyde (1.08 mmol/g, 185 mg, 0.2 mmol) and DMF (1 mL) were added and the mixture was further agitated for a period of 6 h. The solution was filtered and the resin was washed with DMF. The combined filtrate was added onto the macroporous *p*-toluenesulfonic acid (MP-TsOH) cartridge, preconditioned by washing with CH_2Cl_2, followed by washing with DMF (3×5 mL), CH_2Cl_2 (3×5 mL), and MeOH (3×5 mL) to remove any nonbasic impurities. The amine was released from cartridge by the addition ammonia in MeOH (2 M, 5 mL). The resulting solution was concentrated to afford crude product. The crude product was thrown a short pad of silica gel (1 g) to afford 43 mg (37%) of **7ai**.

^{1}H NMR ($CDCl_3$) δ (ppm): 8.26 (d, $J = 1.4$ Hz, 1H), 7.75–7.85 (m, 5H), 7.71 (d, $J = 8.2$ Hz, 1H), 7.41–7.47 (m, 3H), 6.95–7.04 (m, 1H), 4.02 (s, 2H), 3.58–3.65 (m, 2H), 2.95–3.05 (m, 2H), 2.35–2.45 (m, 1H), 1.18–2.01 (m, 10H). LC-LRMS (APCI): 487 (M+1). HRMS (FAB) Calcd for $C_{28}H_{31}N_4O_2S$: 487.2168, Found: 487.2162.

6.7. Analogues 7aa–7kk and 11a,b

These compounds were synthesized by the reductive amination of **5a**–**k** with a series of aromatic aldehyde **6a**–**k**. The products were analyzed by TLC and LC-LRMS (>90% pure as judged by LC-LRMS analysis). The expected molecular ion was confirmed by LC-LRMS (APCI).

6.8. *N*-[6-(3-[(2-Naphthylmethyl)amino]propionylamino)-benzothiazol-2-yl]cyclohexanecarboxamide (10bi)

Titled compound **10bi** was prepared in a similar manner to that described for **7ai**.

^{1}H NMR ($CDCl_3$) δ (ppm): 10.70 (s, 1H), 8.27 (d, $J = 1.4$ Hz, 1H), 7.87–7.90 (m, 4H), 7.63 (d, $J = 8.3$ Hz, 1H), 7.42–7.55 (m, 3H), 7.35 (d, $J = 8.3$ Hz, 1H), 4.06 (s, 2H), 3.08–3.15 (m, 2H), 2.54–2.60 (m, 2H), 2.30–2.39 (m, 1H), 1.20–1.97 (m, 10H). MS (FAB): 487 (M+1). HRMS (FAB) Calcd for $C_{28}H_{31}N_4O_2S$: 487.2168, Found: 487.2175.

6.9. Analogues 10ai–10ck

These compounds were synthesized by the reductive amination using MP-$BH(OAc)_3$. The products were analyzed by TLC and LC-LRMS (>90% pure as judged by LC-LRMS analysis). The expected molecular ion was confirmed by LC-LRMS (APCI).

6.10. *tert*-Butyl 2-[(naphthalene-2-ylmethyl)amino]ethyl-carbamate (13)

To a stirred solution of *tert*-butyl *N*-(2-aminoethyl)carbamate (25.95 g, 162 mmol) and 2-naphthaldehyde (21.09 g, 135 mmol) in DCE (540 mL) was added sodium triacetoxyborohydride (42.9 g, 202.5 mmol). The mixture was stirred at room temperature for 4 h, and then saturated aqueous $NaHCO_3$ was added (300 mL). The organic phase was separated and aqueous phase was extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated to afford the crude product. The crude product was purified by chromatography on silica gel to afford 30.78 g (76%) of *tert*-Butyl 2-[(naphthalene-2-ylmethyl)-amino]ethyl-carbamate as pale yellow oil, which was used in the next step. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.80–7.87 (3H, m), 7.74 (1H, s), 4.95 (1H, br), 3.95 (2H, s), 3.22–3.32 (2H, m), 2.78–2.83 (2H, m), 1.44 (9H, s). MS (FAB): 301 (M+1). HRMS (FAB) Calcd for $C_{18}H_{25}N_2O_2$: 301.1916, Found: 301.1899.

To a THF solution of the above compound (140 mL, 23.2 g, 77.3 mmol) was added aqueous K_2CO_3 (2 M, 80 mL, 160 mmol) and then cooled to 0 °C. To the stirred solution was added allyl chloroformate (9.95 g, 79.27 mmol). The mixture was stirred at room temperature for 2 h and then poured into water. The organic phase was separated and aqueous phase was extracted with AcOEt. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated to afford the crude product. The crude product was purified by chromatography on silica gel to afford 29.7 g (quant.) of allyl (2-*tert*-butoxycarbonylaminoethyl)-naphthalene-2-ylmethyl-carbamate as pale yellow oil, which was used in the next step. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.78–7.84 (m, 3H), 7.60–7.73 (m, 1H), 7.26–7.50 (m, 3H), 5.91–6.02 (m, 1H), 5.22–5.36 (m, 2H), 4.69 (s, 4H), 3.27–3.43 (m, 4H), 1.42 (s, 9H). MS (FAB): 385 (M+1). HRMS (FAB) Calcd for $C_{22}H_{29}N_2O_4$: 385.2127, Found: 385.2133.

To a CH_2Cl_2 solution (100 mL) of the above compound (29 g, 77 mmol) was added TFA (100 mL). The mixture was stirred at room temperature for 2 h and then evaporated to dryness. The residue was dissolved in $CHCl_3$, washed with saturated aqueous $NaHCO_3$ and brine, dried over K_2CO_3, and evaporated to afford the crude product. The crude product was purified by chromatography on aluminum oxide to afford 16.27 g (74%) of **13** as an yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 7.77–7.86 (m, 3H), 7.61–7.74 (m, 1H), 7.29–7.55 (m, 3H), 5.83–6.03 (m, 1H), 5.16–5.40 (m, 2H), 4.68 (s, 4H), 4.25 (br s, 2H) 3.39–3.54 (m, 2H),

2.91–3.07 (m, 2H). MS (FAB): 285 (M+1). HRMS (FAB) Calcd for $C_{17}H_{21}N_2O_2$: 285.1603, Found: 285.1640.

6.11. Allyl-*N*-{2-[(2-*N*-*tert*-butoxycarbonylamino-benzothiazol-6-yl)carbonyl]-aminoethyl}-*N*-(2-naphthylmethyl) carbamate (14b)

To a solution of **13** (7.1 g, 25 mmol) and 2-*N*-*tert*-butoxycarbonylamino-benzothiazole-6-carboxylic acid (6.85 g, 25 mmol) in DMF (100 mL) was added EDC (7.17 g, 37.5 mmol) and HOBt (5.07 g, 37.5 mmol) at room temperature. The mixture was stirred at room temperature for 16 h, and then poured into water (500 mL). The resulting solid was collected by filtration and washed with water. The crude product was purified by chromatography on silica gel to afford 11.2 g (80%) of **14b** as a colorless solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.26 (s, 1H), 7.77–7.89 (m, 5H), 7.62–7.71 (m, 1H), 7.32–7.49 (m, 3H), 5.88–5.98 (m, 1H), 5.14–5.33 (m, 2H), 4.64–4.75 (m, 4H), 3.54–3.68 (m, 4H), 1.61 (s, 9H). MS (FAB): 561 (M+1). HRMS (FAB) Calcd for $C_{30}H_{33}N_4O_5S$: 561.2172, Found: 561.2129.

6.12. Allyl-*N*-{2-[(2-amino-benzothiazol-6-yl)carbonyl]-aminoethyl}-*N*-(-2naphthylmethyl) carbamate (15)

To a CH_2Cl_2 solution (20 mL) of **14b** (5.8 g, 9.8 mmol) was added TFA (10 mL). The mixture was stirred at room temperature for 2 h. The mixture was evaporated to dryness and then dissolved in $CHCl_3$. A saturated aqueous $NaHCO_3$ was added to neutralized. The resulting solid was collected by filtration and washed with $CHCl_3$ to afford 4.5 g (99%) of **15** as a colorless solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.10 (s, 1H), 7.82–7.91 (m, 3H), 7.66–7.78 (m, 3H), 7.30–7.53 (m, 3H), 5.89–5.97 (m, 1H), 5.10–5.36 (m, 2H), 4.54–4.71 (m, 4H), 3.37–3.49 (m, 4H). MS (FAB): 461 (M+1). HRMS (FAB) Calcd for $C_{25}H_{25}N_4O_3S$: 461.1647, Found: 461.1645.

6.13. 2-(Acetylamino)-*N*-2-[(2-naphthylmethyl)-amino]ethyl-benzothiazole-6-carboxamide 16f

To a solution of **15** (138 mg, 0.3 mmol) and 4-dimethylaminopyridine (37 mg, 0.3 mmol) in CH_2Cl_2 (10 mL) was added Ac_2O (92 mg, 0.9 mmol). The mixture was stirred at room temperature for 4 h and then washed with 3 N-HCl, water and brine, dried over Mg_2SO_4, and evaporated to afford the crude product. The crude product was purified by chromatography on silica gel to afford 137 mg (91%) of allyl {2-[(2-acetylamino-benzothiazole-6-carbonyl)-amino]-ethyl}-naphthalene-2-ylmethyl carbamate as a colorless solid, which was used in the next step. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.30 (s, 1H), 7.35–7.84 (m, 10H), 5.86–5.97 (m, 1H), 5.15–5.34 (m, 2H), 4.66–4.75 (m, 4H), 3.62–3.69 (m, 4H), 2.30 (s, 3H). MS (FAB): 503 (M+1). HRMS (FAB) Calcd for $C_{27}H_{27}N_4O_4S$: 503.1753, Found: 503.1736.

A mixture of the above compound (50 mg, 0.1 mmol), $Pd(PPh_3)_4$ (6 mg, 0.005 mmol), and solid-supported barbituric acid (100 mg, 0.1 mmol) in THF (1 mL) were agitated at 50 °C for 12 h. The solution was filtered and the resin was washed with THF. The combined filtrate was added onto the MP-TsOH cartridge, preconditioned by washing with CH_2Cl_2, followed by washing with DMF (3×5 mL), CH_2Cl_2 (3×5 mL), and MeOH (3×5 mL) to remove any nonbasic impurities. The amine was released from cartridge by the addition ammonia in MeOH (2 M, 5 mL). The resulting solution was concentrated to afford crude product. The crude product was thrown a short pad of silica gel (1 g) to afford 33 mg (79%) of **16f** as a colorless solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.24 (s, 1H), 7.65–7.84 (m, 7H), 7.43–7.52 (m, 3H), 3.98–4.04 (m, 2H), 3.56–3.63 (m, 2H), 2.93–3.01 (m, 2H), 2.28 (s, 3H). MS (FAB): 419 (M+1). HRMS (FAB) Calcd for $C_{23}H_{23}N_4O_2S$: 419.1541, Found: 419.1527.

6.14. *N*-{2-[(2-Naphtylmethyl)amino]ethyl}-2-[(tetrahydro-2*H*-pyran-4-ylcarbonyl)amino]-benzothiazole-6-carboxamide 16k

To a solution of **15** (92 mg, 0.2 mmol) and tetrahydorpyran-4-yl carboxylic acid (26 mg, 0.2 mmol) in DMF (10 mL) was added EDC (57 mg, 0.3 mmol) and HOBt (40 mg, 0.3 mmol) at room temperature. The mixture was stirred at room temperature for 48 h, and then poured into water. The resulting mixture was extracted with AcOEt, washed with water and brine, dried over Mg_2SO_4, and evaporated to afford the crude product. The crude product was purified by chromatography on silica gel to afford 75 mg (66%) of allyl carbamate as a colorless solid, which was used in the next step. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.30 (s, 1H), 7.35–7.84 (m, 10H), 5.85–5.96 (m, 1H), 5.15–5.32 (m, 2H), 4.59–4.73 (m, 4H), 4.06–4.10 (m, 2H), 3.58–3.65 (4H, m), 3.44–3.50 (m, 2H), 2.65–2.70 (m, 1H), 1.89–1.98 (m, 4H). MS (FAB): 573 (M+1). HRMS (FAB) Calcd for $C_{31}H_{33}N_4O_5S$: 573.2127, Found: 573.2125.

In a similar manner to that described for **16f**, palladium-catalyzed facile removal strategy was performed to afford **16k** in 92% yield.

^{1}H NMR ($CDCl_3$) δ (ppm): 8.25 (s, 1H), 7.44–7.83 (m, 10H), 3.92–4.05 (m, 4H), 3.57–3.63 (m, 2H), 3.45 (t, J = 11 Hz, 2H), 2.96–3.06 (m, 2H), 2.57–2.67 (m, 1H), 1.78–1.92 (m, 4H). LC-LRMS (APCI): 489 (M+1). HRMS (FAB) Calcd for $C_{27}H_{29}N_4O_3S$: 489.1961, Found: 489.1951.

6.15. Analogues 16g–t

These compounds were synthesized by palladium-catalyzed facile removal strategy using solid-supported barbituric acid. The products were analyzed by TLC and LC-LRMS (>90% pure as judged by LC-LRMS analysis). The expected molecular ion was confirmed by LC-LRMS (APCI).

6.16. Methyl 2-[(cyclohexylcarbonyl)amino]-benzoxazole-5-carboxylate (18a)

To a solution of 3-amino-4-hydroxybenzoate (4.7 g, 2.8 mmol) and di-(imidazole-1-yl)methanimine (4.5 g, 2.8 mmol) in THF was stirred at 70 °C for 4 h. The reaction mixture was evaporated to dryness. To the residue

was added water (100 mL) and the resulting solid was collected by filtration, washed with water, and then dried to afford 3.7 g (70%) of **18a** as a colorless solid.

^{1}H NMR (DMSO-d_6) δ (ppm): 7.23 (d, J = 2.0 Hz, 1H), 7.66 (dd, J = 8.3, 2.0 Hz, 1H), 7.57 (br s, 2H), 7.42 (d, J = 8.3 Hz, 1H), 3.84 (s, 3H). MS (FAB): 193 (M+1). HRMS (FAB) Calcd for $C_9H_9N_2O_3$: 193.0613, Found: 193.0621.

6.17. Methyl 2-[(cyclohexylcarbonyl)amino]-benzoxazole-6-carboxylate (18b)

Titled compound **18b** was prepared in a similar manner to that described for **18a**.

^{1}H NMR ($CDCl_3$) δ (ppm): 7.62–7.85 (m, 4H), 7.26 (d, J = 7.8 Hz, 1H), 3.80 (s, 3H). MS (FAB): 193 (M+1). HRMS (FAB) Calcd for $C_9H_9N_2O_3$: 193.0613, Found: 193.0606.

6.18. 2-[(Cyclohexylcarbonyl)amino]-*N*-[2-(2-naphthylamino)ethyl]-benzoxazole-5-carboxamide (11c)

Titled compound **11c** was prepared in a similar manner to that described for benzothiazole derivatives **7ai**.

^{1}H NMR ($CDCl_3$) δ (ppm): 7.99 (s, 1H), 7.62–7.85 (m, 5H), 7.38–7.50 (m, 4H), 6.90–7.02 (m, 1H), 4.01 (s, 2H), 3.56–3.41 (m, 2H), 2.80–3.08 (m, 2H), 2.58–2.70 (m, 1H), 1.22–2.08 (m, 10H). MS (FAB): 471 (M+1). HRMS (FAB) Calcd for $C_{28}H_{31}N_4O_3$: 471.2397, Found: 471.2419.

6.19. 2-[(Cyclohexylcarbonyl)amino]-*N*-[2-(2-naphthylamino)ethyl]-benzoxazole-6-carboxamide (11d)

Titled compound **11d** was prepared in a similar manner to that described for benzothiazole derivatives **7ai**.

^{1}H NMR ($CDCl_3$) δ (ppm): 8.05 (s, 1H), 7.70–7.85 (m, 4H), 7.40–7.63 (m, 5H), 6.90–7.05 (m, 1H), 4.02 (s, 2H), 3.48–3.61 (m, 2H), 2.90–3.01 (m, 2H), 2.58–2.72 (m, 1H), 1.20–2.05 (m, 10H). MS (FAB): 471 (M+1). HRMS (FAB) Calcd for $C_{28}H_{31}N_4O_3$: 471.2397, Found: 471.2406.

6.20. Allyl-*N*-2-[(4-fluoro-3-nitrobenzoyl)amino]ethyl-*N*-(2-naphthylmethyl)carbamate (19a)

To a solution of **13** (2.8 g, 10 mmol) and 4-fluoro-3-nitorobenzoic acid (1.8 g, 10 mmol) in DMF (20 mL) was added EDC (2.9 g, 15 mmol) and HOBt (2.1 g, 15 mmol) at room temperature. The mixture was stirred at room temperature for 1.5 h, and then poured into water. The resulting mixture was extracted with AcOEt, washed with water and brine, dried over Mg_2SO_4, and evaporated to afford the crude product. The crude product was purified by chromatography on silica gel to afford 3.9 g (86%) of **19a** as yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 8.41–8.53 (m, 1H), 7.20–8.08 (m, 10H), 5.85–6.08 (m, 1H), 4.60–4.80 (m, 4H), 5.20–5.43 (m, 2H), 3.60 (br s, 4H). MS (FAB): 453 (M+1). HRMS (FAB) Calcd for $C_{24}H_{23}FN_3O_5$: 452.1622, Found: 452.1646.

6.21. Allyl-*N*-2-[(3-fluoro-4-nitrobenzoyl)amino]ethyl-*N*-(2-naphthylmethyl)carbamate (19b)

Titled compound **19b** was prepared in a similar manner to that described for **19a**.

^{1}H NMR ($CDCl_3$) δ (ppm): 8.00–8.18 (m, 1H), 7.30–7.83 (m, 10H), 5.82–6.05 (m, 1H), 5.19–5.39 (m, 2H), 4.65–4.79 (m, 4H), 3.60 (br s, 4H). MS (FAB): 453 (M+1). HRMS (FAB) Calcd for $C_{24}H_{23}FN_3O_5$: 452.1622, Found: 452.1640.

6.22. Allyl-*N*-(2-[3-amino-4-(methylamino)benzoyl]aminoethyl)-*N*-(2-naphthylmethyl)carbamate (20a)

A mixture of **19a** (2.04 g, 4.54 mmol) and 2 M-$MeNH_2$/THF (4.5 mL, 9 mmol) was stirred for 1.5 h at room temperature and then evaporated to dryness. To the residue was added $SnCl_2{\cdot}2H_2O$ (4.7 g, 20.9 mmol) and dissolved in EtOH (10 mL). To the solution was added 6 N-HCl (16 mL), stirred for 16 h at room temperature. The mixture was cooled to <10 °C and basified to pH 11 with 10% aqueous NaOH. The mixture was extracted with $CHCl_3$, washed with water and brine, dried over Mg_2SO_4, and evaporated to afford the crude product. The crude product was purified by chromatography on silica gel to afford 1.8 g (92%) of **20a** as pale yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 7.28–7.86 (m, 9H), 7.00–7.22 (m, 1H), 6.56 (d, J = 7.8 Hz, 1H), 5.80–6.01 (m, 1H), 5.12–5.43 (m, 2H), 4.62–4.80 (m, 4H), 3.42–3.71 (m, 4H), 3.26 (br s, 2H), 2.91 (s, 3H). MS (FAB): 433 (M+1). HRMS (FAB) Calcd for $C_{25}H_{29}N_4O_3$: 433.2240, Found: 433.2195.

6.23. Allyl-*N*-(2-[4-amino-3-(methylamino)benzoyl]aminoethyl)-*N*-(2-naphthylmethyl)carbamate (20b)

Titled compound **20b** was prepared in a similar manner to that described for **20a**.

^{1}H NMR ($CDCl_3$) δ (ppm): 7.60–7.90 (m, 5H), 6.85–7.56 (m, 5H), 6.64 (d, J = 7.3 Hz, 1H), 5.80–6.18 (m, 1H), 5.10–5.39 (m, 2H), 4.60–4.75 (m, 4H), 3.50–3.70 (m, 6H), 2.89 (s, 3H). MS (FAB): 433 (M+1). HRMS (FAB) Calcd for $C_{25}H_{29}N_4O_3$: 433.2240, Found: 433.2195.

6.24. 2-[(2-Cyclohexylcarbonyl)amino]-1-methyl-*N*-2-[(2-naphthylmethyl)amino]ethyl-1*H*-benzimidazole-5-carboxamide (11e)

To a solution of **20a** (879 mg, 2 mmol) in CH_2Cl_2 (10 mL) was added cyclohexanecarbonyl isothiocyanate (378 mg, 2.2 mmol). The mixture was stirred at room temperature. After 2 h, to the mixture was added EDC (582 mg, 3 mmol) and DIPEA (1.7 mL, 10.15 mmol), stirred for 16 h at room temperature. The mixture was washed with water and brine, dried over Mg_2SO_4, and

evaporated to afford the crude product. The crude product was purified by chromatography on silica gel to afford 503 mg (44%) of allyl-*N*-2-[(2-[(cyclohexylcarbonyl)amino]-1-methyl-1*H*-benzimidazol-5-ylcarbonyl)amino]ethyl-*N*-(2-naphthylmethyl)carbamate as a colorless solid, which was used in the next step. ^{1}H NMR ($CDCl_3$) (ppm): 12.20 (br s, 1H), 7.60–7.83 (m, 5H), 7.26–7.53 (m, 5H), 7.16 (d, J = 8.3 Hz, 1H), 5.84–6.20 (m, 1H), 5.16–5.43 (m, 2H), 4.70 (br s, 4H), 3.50–3.70 (m, 7H), 2.38–2.50 (m, 1H), 1.20–2.11 (m, 10H). MS (FAB): 568 (M+1). HRMS (FAB) Calcd for $C_{33}H_{38}N_5O_4$: 568.2924, Found: 568.2891.

The above compound was converted to **11e** by palladium-catalyzed facile removal strategy using solid-supported barbituric acid as described for **16f**.

^{1}H NMR ($CDCl_3$) δ (ppm): 7.60–7.90 (m, 8H), 7.42–7.54 (m, 3H), 7.15 (d, J = 8.3 Hz, 1H), 6.92 (br s, 1H), 4.00 (s, 2H), 3.62 (s, 3H), 3.50–3.60 (m, 2H), 2.90–3.00 (m, 2H), 2.38–2.51 (m, 1H), 1.21–2.01 (m, 10H). MS (FAB): 484 (M+1). HRMS (FAB) Calcd for $C_{29}H_{34}N_5O_2$: 484.2712, Found: 484.2725.

6.25. 2-[(2-Cyclohexylcarbonyl)amino]-1-methyl-*N*-2-[(2-naphthylmethyl)amino]ethyl-1*H*-benzimidazole-6-carboxamide (11f)

Titled compound **11f** was prepared in a similar manner to that described for **11e**.

^{1}H NMR ($CDCl_3$) δ (ppm): 7.70–7.83 (m, 5H), 7.41–7.50 (m, 5H), 7.20 (br s, 1H), 6.90–6.98 (m, 1H), 4.00 (s, 2H), 3.62 (s, 3H), 3.50–3.61 (m, 2H), 2.90–3.02 (m, 2H), 2.30–2.50 (m, 1H), 1.18–2.01 (m, 10H). MS (FAB): 484 (M+1). HRMS (FAB) Calcd for $C_{29}H_{34}N_5O_2$: 484.2712, Found: 484.2664.

6.26. (1*S*,3*R*)-3-(Ethoxycarbonyl)cyclohexanecarboxylic acid (22a)

A suspension of diethyl *cis*-1,3-cyclohexanesicarboxylate (2.28 g, 10 mmol) and lipase AYS Amano (1 g) in pH 7.2 phosphate buffer (100 mL) was stirred for 24 h at room temperature. A Celite (5.7 g) was added and the mixture was stirred thoroughly. The mixture was filtered a Celite pad, and the filtrate acidified to pH 1 with 3 N HCl. The resulting mixture was extracted with AcOEt, washed with water and brine, dried over Mg_2SO_4, and evaporated to afford 2 g (quant.) of **22a** as colorless oil.

$[\alpha]_D^{25}$ +4.2 (c 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ (ppm): 9.75 (br, 1H), 4.13 (q, J = 6.8 Hz, 2H), 2.20–2.40 (m, 3H), 1.57 (q, J = 12.7 Hz, 1H), 1.25–1.45 (m, 3H), 1.25 (t, J = 6.8 Hz, 3H). MS (FAB): 201 (M+1). HRMS (FAB) Calcd for $C_{10}H_{17}O_4$: 201.1127, Found: 201.1116.

6.27. Ethyl (1*R*,3*S*)-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylate (23a)

To a solution of **22a** (4.0 g, 20 mmol) and triethylamine (3.1 mL, 22 mmol) in toluene (100 mL) was added diphenylphosphoryl azide (4.8 mL, 22 mmol) at room temperature. The mixture was stirred at 110 °C for 1 h, and then cooled to 70 °C. To the mixture was added *tert*-BuOH (11 mL, 100 mmol) and then stirred at 95 °C for 12 h. The mixture was cooled to room temperature, washed with water and brine, dried over Mg_2SO_4, and evaporated to afford the crude product. The crude product was purified by chromatography on silica gel to afford 1.37 g (25%) of **23a** as a colorless solid. The product was recrystallized from isopropyl ether to produce colorless needles.

$[\alpha]_D^{25}$ −32.3 (c 0.5, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ (ppm): 4.42 (br, 1H), 4.11 (q, J = 6.8 Hz, 2H), 3.45 (m, 1H), 2.30–2.43 (m, 1H), 2.20–2.28 (m, 1H), 1.80–2.00 (m, 3H), 1.44 (s, 9H), 1.18–1.42 (m, 6H), 0.98–1.15 (m, 1H). MS (FAB): 272 (M+1). HRMS (FAB) Calcd for $C_{14}H_{26}NO_4$: 272.1862, Found: 272.1850.

6.28. (1*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]cyclohexanecarboxylic acid (24a)

To a solution of **23a** (1.35 g, 5 mmol) in THF (30 mL) was added 5 N aqueous NaOH (6 mL, 30 mmol) at room temperature. The mixture was stirred at 50 °C for 12 h, and then cooled to room temperature. The mixture was washed with diethyl ether, and the aqueous phase was cooled to 0 °C. The mixture was acidified to pH 1 with 3 N HCl water and extracted with $CHCl_3$, washed with water and brine, dried over Mg_2SO_4, and evaporated to afford the 1.15 g (95%) of **24a** as a colorless solid.

$[\alpha]_D^{23}$ −32.8 (c 0.5, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ (ppm): 4.45 (br, 1H), 3.47 (m, 1H), 2.20–2.50 (m, 2H), 1.82–2.02 (m, 3H), 1.44 (s, 9H), 0.98–1.42 (m, 4H). MS (FAB): 244 (M+1). HRMS (FAB) Calcd for $C_{12}H_{22}NO_4$: 244.1549, Found: 244.1533.

6.29. *tert*-Butyl *N*-(1*R*,3*S*)-3-[(2-[(cyclopentylcarbonyl)amino]-benzothiazol-6-ylamino)carbonyl]cyclohexyl carbamate (26a)

Titled compound **26a** was prepared in a similar manner to that described for **5a**.

$[\alpha]_D^{25}$ −22.8 (c 0.45, DMF), ^{1}H NMR (DMSO-d_6) δ (ppm): 12.15 (s, 1H), 9.93 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 7.8, 2.0 Hz, 1H), 6.72 (br, 1H), 3.28–3.40 (m, 1H), 2.90–3.02 (m, 1H), 2.40–2.50 (m, 1H), 1.52–2.00 (m, 12H), 1.38 (s, 9H), 1.05–1.37 (m, 4H). MS (FAB): 487 (M+1). HRMS (FAB) Calcd for $C_{25}H_{35}N_4O_4S$: 487.2379, Found: 487.2351.

6.30. (1*R*,3*S*)-3-Amino-*N*-2-[(cyclopentylcarbonyl)-amino]-benzothiazol-6-ylcyclohexanecarboxamide trifluoroacetate (27a)

To a suspension of **26a** (187 mg, 0.39 mmol) in CH_2Cl_2 (2 mL) was added TFA (2 mL). The mixture was stirred for 1 h at room temperature then evaporated to dryness. The residue was added diethyl ether (10 mL) and the

resulting solid was collected, washed with diethyl ether, dried to give 180 mg (94%) of **27a** as a colorless solid.

$[\alpha]_D^{25}$ +5.0 (*c* 0.45, DMF), ^{1}H NMR (DMSO-d_6) δ (ppm): 12.27 (s, 1H), 10.13 (s, 1H), 8.31 (d, J = 2.0 Hz, 1H), 7.85 (br, 3H), 7.65 (d, J = 8.8 Hz, 1H), 7.49 (dd, J = 8.8, 2.0 Hz, 1H), 2.90–3.18 (m, 2H), 1.20–2.08 (m, 17H). MS (FAB): 387 (M+1). HRMS (FAB) Calcd for $C_{20}H_{27}N_4O_2S$: 387.1855, Found: 387.1842.

6.31. (1*R*,3*S*)-*N*-{2-[(Cyclopenthylcarbonyl)amino]-benzothiazol-6-yl}-3-[(2-naphthylmethyl) amino]cyclohexanecarboxamide (28g)

Titled compound **28g** was prepared in a similar manner to that described for **7ai** (98% ee).

$[\alpha]_D^{25}$ −72.6 (*c* 0.25, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ (ppm): 8.25 (d, J = 1.0 Hz, 1H), 7.75–7.92 (m, 4H), 7.74 (s, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.40–7.50 (m, 3H), 7.31 (dd, J = 8.3, 1.5 Hz, 1H), 4.01 (br s, 2H), 2.74–2.85 (m, 1H), 2.63–2.72 (m, 1H), 2.23–2.40 (m, 2H), 1.20–2.10 (m, 15H). LC-LRMS (APCI): 527 (M+1). HRMS (FAB) Calcd for $C_{31}H_{35}N_4O_2S$: 527.2481, Found: 527.2495.

6.32. Analogues 28a–k

These compounds were synthesized by the reductive amination using MP-$BH(OAc)_3$. The products were analyzed by TLC and LC-LRMS (>90% pure as judged by LC-LRMS analysis). The expected molecular ion was confirmed by LC-LRMS (APCI).

7. Expression in *E. coli* and purification of Nmts

CaNmt coding gene was obtained by PCR from *C. albicans* (ATCC 90028) genomic library using the primers 5′-NNNNNNNNCATATGTCGGGAGATAACACAGGGAATAAATC-3′ and 5′-NNNNNNGCGGCCGCTAATAAAACTACACCTATACCACTTGTTTGATCTTCG-3′ and using Advantage-HF PCR kit (CLONTECH). The PCR product was inserted into *E. coli* expression vector pET-15b (Novagen) between the NdeI and the BamHI site, to obtain CaNmt protein with the poly-histidine tag at N-terminus. In the same way, HsNmt1 coding gene was obtained from human lymphocyte cDNA library using the primers 5′-NNNNNNCATATGGCGGACGAGAGTGAGACAGC-3′ and 5′-NNNNNNCTCGAGTTATTGTAGCACCAGTCCAACCTTCTCTGC-3′, and inserted into pET-15b between the NdeI and the XhoI site. Both expression vectors were confirmed the sequences with PRISM 310 (ABI).

E. coli BL21(DE3) containing the CaNmt or HsNmt1 expression vector was grown at 37 °C in a medium (10 g/L trypton, 5 g/L yeast extract, 10 g/L NaCl, and 100 g/mL ampicilin) until the absorbance at 600 nm reached 0.8 at which time isopropyl β-D-thiogalactopyranoside was added to give a final concentration of 1 mM. The cultures were maintained at 28 °C for a further 4 h and the bacteria harvested by centrifugation. The bacteria was then suspended in PBS, and sonicated. The lysate was cleared by centrifugation and the supernatant was applied to TALON metal affinity resin (CLONTECH) to purify the poly-histidine tagged CaNmt according to the manufacturer protocol. The fraction containing the purified CaNmt or HsNmt1 was dialyzed overnight against the same buffer as for Nmt reaction (10 mM Tris–HCl pH 7.4, 1 mM DTT, 5 mM $MgCl_2$, 0.1 mM EGTA).

8. Measurement of inhibitory activities of compounds against CaNmt and HsNmt1

In vitro Nmt assay was performed as described methods previously[4c,13,14] with slight modification. The reagents were added in 100 μL to give a final concentration of 10 mM Tris–HCl pH 7.4, 1 mM DTT, 5 mM $MgCl_2$, 0.1 mM EGTA, 0.01% Triton X-100, 0.5 μM peptide substrate (GLTISKLFRR or GLTISKLFRRK-biotin for CaNmt, GNAASARR for HsNmt1), 32 nM [^{3}H]-myristoyl-CoA (Amersham Biosciences), the appropriate amount of enzyme and a compound at variable concentrations. After pre-incubation at 30 °C for 10 min, the reaction was initiated by the addition of enzyme and performed at 30 °C for 10 min. In the case of using GLTISKLFRRK-biotin as peptide substrate, the reaction was terminated with 0.1% phosphoric acid and the reaction solution was transferred into streptavidin-coated 96-well plate, followed by incubation at 30 °C with shaking for 30 min. After washes with PBS 5 times, the amount of bound 3H in each well was measured to quantify the reaction product [^{3}H]-myristoylated GLTISKLFRRK-biotin with the plate scintillation counter 1450 MicroBeta (PerkinElmer). In the case of using other peptides as peptide substrate, the reaction was terminated by the addition of 100 μL ice-cold methanol containing 2% tritonX-100 and 10% TCA. The reaction mixture (100 μL) was analyzed by HPLC in the conditions described previously[13] to quantify the [^{3}H]-myristoylated peptide.

In order to measure the inhibitory activity of compounds at each concentration, the quantity of [^{3}H]-myristoylated peptide in reaction with no compound was defined as control (100%), and with no enzyme was defined as background (0%). The effects of compounds at each concentration were calculated as percentage of control. The 50% inhibitory concentration (IC_{50}) of compound was calculated by nonlinear regression analysis using the Prism 3.0 software package (GraphPad Software, San Diego, CA).

9. In vitro antifungal activity

The MICs of the compounds and reference compounds for *C. albicans* were determined by a broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS) method M27-A2.[18] Compounds were dissolved in DMSO. The strains were cultured at 35 °C for 48 h. The MICs were read spectrophotometrically. The MICs of the compounds were

defined as the lowest concentration at which there was 80% inhibition of growth compared with the growth of a drug-free control.

10. Cytotoxicity assay

Human cervix adenocarcinoma HeLa 229 (HeLa) cells were purchased from Dainippon Pharmaceutical Co. Ltd (Osaka, Japan), and maintained in MEM medium containing 5% fetal bovine serum (FBS; GIBCO) at 37 °C under 5% CO_2 condition. Cytotoxicity of compounds was measured in HeLa cells. Cells were pre-incubated in 96-well plates at 5×10^3 cells/75 μL of medium per well for 24 h. Then, the serial dilutions of compounds in DMSO were added to the wells, with the final concentration of DMSO in all wells at 1%, in a total volume of 150 μL. After 2 days incubation, 50 μL of WST-8 (Dojindo, Kumamoto, Japan) that was diluted with medium into 5 times was added to each well, and the plates returned to the incubator for 2 h, after which the absorbance at 450 nm of each well was measured (reference: 650 nm). The survival rates were determined by comparing the absorbance of a well containing compound to that of control wells. Cytotoxicity (CC_{50} value) is defined as the concentration of each compound that produces a 50% reduction in the survival rate.

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