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Antimalarial Cation-dimers Synthesized in Two Steps from an Inexpensive Starting Material, Isonicotinic Acid

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*Malaria is one of the three major serious infectious diseases in the world. As the area affected by malaria includes a large proportion of developing countries, there is a need for new antimalarials that can be synthesized and supplied inexpensively. To generate low-cost antimalarials, the MAP series **6**–**10**, bis-cation dimers, synthesized by amidating the carboxyl group of isonicotinic acid (**11**) with various amines and by cationizing the nitrogen atoms of the pyridine ring with the corresponding alkyl bromides, were designed. This design enabled expansion of the structural variations of bis-cation-type antimalarial compounds. The compounds bearing alkyl or phenyl groups in the amide moieties showed remarkable antimalarial activities in vitro. Moreover, 1,1′-(1,12-dodecanediyl)bis[4-[(buthylamino)carbonyl]pyridinium bromide], MAP-412 (**6d**), exhibited a potent antimalarial activity ($ED_{50} = 8.2\ mg\,kg^{-1}$). Being prepared at low cost, our bis-cation-type antimalarial compounds may be useful as lead compounds for inexpensive antimalarials.*

Introduction

Malaria, one of the three major infectious diseases in the world, is caused by infection with malaria plasmodiums, which are transmitted by anopheles to infect erythrocytes. Malaria infection generally causes periodic fever, shaking chill, or excessive sweating. Cerebral malaria accompanied by cerebral thrombus, severe anemia, or metabolic acidosis sometimes occurs.[1] Every year, 300–500 million people suffer and 1.5–2.5 million people die from this disease.[2,3] Malaria-affected areas are approximately consistent with the habitats of anopheles, and most of the malaria-affected areas are in developing countries.[4] A striking correlation between malaria and poverty has been reported.[3] The reason for this relationship is thought to be the inability to prevent malaria infection because of poverty and economic activity disturbance caused by the disease.[4] Therefore, there is a need for new antimalarials that can be synthesized and supplied inexpensively. Although chloroquine (**1**) (Figure 1) and fansidar (sulfadoxine/pyrimethamine) are well-known antimalarial drugs available at low cost, the appearance of parasites resistant to these drugs[5,6] has led to the development of new antimalarials whose mechanisms of action are different.[7] Among antimalarials for drug-resistant strains, Vial et al. reported bis-cation-type antimalarial compounds, G25 (**2**)[8–10] and de novo phosphatidylcholine (PC) biogenesis inhibitors by the structural mimicry of choline, the ingredient of PC biogenesis used to generate phosphatidylcholine, which is a main ingredient of the malarial membrane.[12] In addition, there is no PC biosynthesis in mature erythrocytes.[13]

Furamidine (**4**), a well-known antiparasitic pentamidine derivative that can be cationized under the proper conditions, has also been developed as an antimalarial (Figure 1).[14–19] The antimalarial mechanism has been reported to be the inhibition

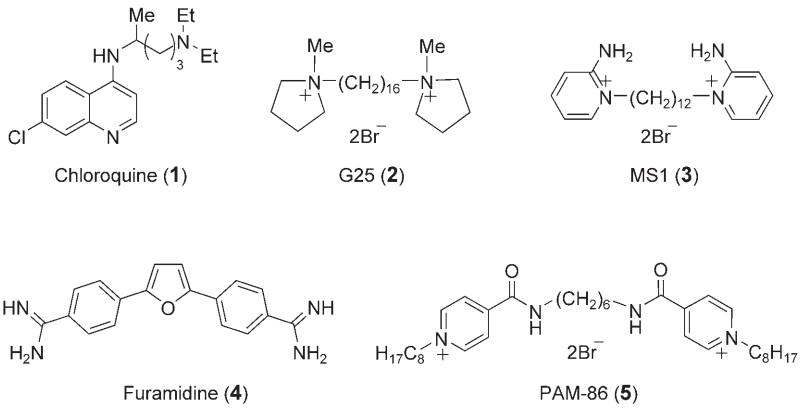

Figure 1. Chemical structures of well-known antimalarials **1**–**4** and PAM-86 (**5**).

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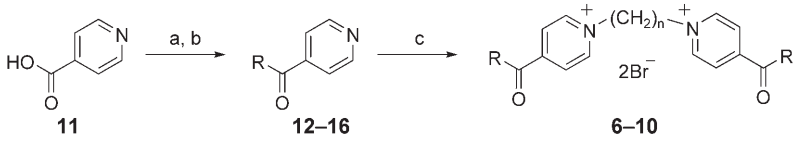
of hemozoin formation which is performed for detoxification of ferriprotoporphyrin IX (FPIX) produced by malaria parasites to obtain amino acids as their nutritional sources from erythrocytes.[20] The mechanism by which hemozoin synthesis is inhibited by G25 (**2**) has also been reported.[21] Furthermore, furamidine (**4**) has been shown to cause the collapse of the mitochondrial membrane potential.[22]

We reported *N*,*N*′-hexamethylene[bis(4-carbamoyl-1-octylpyridinium)] dibromide (PAM-86: **5**), a potent antimalarial compound that can be synthesized inexpensively by using isonicotinic acid (**11**) as a starting material (Figure 1).[23] Although the in vitro antimalarial activity of PAM-86 (**5**) was found to be as potent as that of chloroquine (**1**) (IC_{50} was 10 nM when the FCR-3 strain was used as a malarial plasmodium),[23] the in vivo activity in mice was less potent than that of chloroquine (**1**) (unpublished data). In addition, it was difficult to modify the side-chain structure. However, the fundamental structure of PAM-86 (**5**) is attractive for creation of a low-cost antimalarial as it is possible to synthesize PAM-86 (**5**) from an inexpensive starting material, isonicotinic acid (**11**). Thus, for the novel design of bis-cation-type antimalarials prepared from inexpensive isonicotinic acid (**11**), we designed new bis-cation-type compounds, MAP series **6**–**10**, that contain an alkyl chain connecting the nitrogens of each pyridine and possess amide bonds in both ends, as shown in Figure 2. This design enables production of compounds containing various groups, not only acyclic hydrocarbon chains but also cyclic hydrocarbons or phenyl rings, to expand the structural variations of these bis-cation-type antimalarials. In this article, we describe the in vitro antimalarial activities of these compounds and present results of a structure–activity relationship study and in vivo activity.

Results and Discussion

Isonicotinic acid (**11**) was used as the starting material (Scheme 1). Compound **11** was treated with a solution of thionyl chloride in the presence of catalytic DMF to give the acid chloride intermediate, which was subsequently reacted with the corresponding amines to give isonicotinamide **12**–**16**. These were reacted with dibromoalkane to afford the target compounds **6**–**10**.



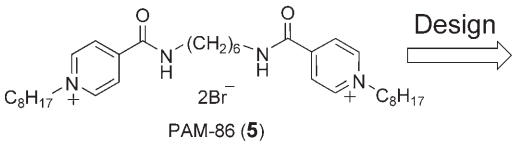
11 **12–16** **6–10**

	R
12	C_4H_9NH
13	$C_6H_{13}NH$
14	$C_8H_{17}NH$
15a	
15b	
16	PhNH

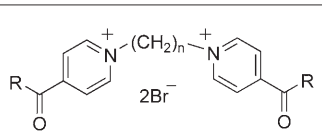
		R	n
6a	MAP-46	C_4H_9NH	6
6b	MAP-48	C_4H_9NH	8
6c	MAP-410	C_4H_9NH	10
6d	MAP-412	C_4H_9NH	12
7a	MAP-66	$C_6H_{13}NH$	6
7b	MAP-68	$C_6H_{13}NH$	8
7c	MAP-610	$C_6H_{13}NH$	10
7d	MAP-612	$C_6H_{13}NH$	12
8a	MAP-86	$C_8H_{17}NH$	6
8b	MAP-88	$C_8H_{17}NH$	8
8c	MAP-810	$C_8H_{17}NH$	10
8d	MAP-812	$C_8H_{17}NH$	12
9a	MAP-Mor10		10
9b	MAP-Pyr10		10
10	PMAP-H10	PhNH	10

Scheme 1. Reagents and yields: a) $SOCl_2$, DMF; b) amine, THF, 22–73% for two steps; c) dibromo alkanes, DMF, 31–89%.

One of the prominent features of MAP compounds is the ability to introduce various side chains, alkyl, morpholino, pyrrolidino, or phenyl groups into the side ends, which expands the structural variations of bis-cation-type antimalarial compounds. In addition, all compounds were simply prepared in just two steps.



PAM-86 (**5**)



	R	n
MAPs (**6**–**8**)	$C_mH_{2m+1}NH$ m = 4, 6, 8	6, 8, 10, 12
MAP-Mor10 (**9a**)		10
MAP-Pyr10 (**9b**)		10
PMAP-H10 (**10**)	PhNH	10

Figure 2. Strategy for the molecular design of MAP series (**6**–**10**) as new bis-cation-type antimalarials.

Antimalarial activity against FCR-3 strain (*P. falciparum*) and cytotoxicity against the FM3A F28-7 strain (cells derived from breast cancer of mice) were determined as shown in Table 1. Most of the compounds **6**–**10** (exceptions **6a**, **9a**, and **9b**) showed antimalarial activities.

Table 1. In vitro antimalarial activities and cytotoxicities of chloroquine (**1**) and compounds prepared in this study.[a]

Compounds	*m*	*n*	IC_{50} [nM] Antimalarial activity[b]	Cytotoxicity[c]
Chloroquine (**1**)	–	–	18	32000
PAM-86 (**5**)	–	–	10	5800
MAP-46 (**6a**)	4	6	17500	>500000
MAP-48 (**6b**)	4	8	1600	>500000
MAP-410 (**6c**)	4	10	400	>500000
MAP-412 (**6d**)	4	12	100	52879
MAP-66 (**7a**)	6	6	400	8600
MAP-68 (**7b**)	6	8	5	8000
MAP-610 (**7c**)	6	10	5	10500
MAP-612 (**7d**)	6	12	67	4700
MAP-86 (**8a**)	8	6	92	305
MAP-88 (**8b**)	8	8	18	94
MAP-810 (**8c**)	8	10	14	640
MAP-812 (**8d**)	8	12	90	580
MAP-Mor10 (**9a**)	–	10	>100000	>100000
MAP-Pyr10 (**9b**)	–	10	9930	>100000
PMAP-H10 (**10**)	–	10	29	42828

[a] In vitro antimalarial activities and cytotoxicities were determined as described in the Experimental Section. [b] Antimalarial activities against chloroquine-sensitive *P. falciparum* (FCR-3 strain) were examined. [c] Cytotoxicity against mouse mammary tumor FM3A cells was examined.

Table 2. In vivo antimalarial activities of chloroquine (**1**), MAP-412 (**6d**), MAP-610 (**7c**), and PMAP-H10 (**10**).[a]

Compounds	Dose [mg kg^{-1}]	Survival ratio[b] [%]	Inhibition ratio[c] [%]	ED_{50} [mg kg^{-1}]
	1	125	14	
Chloroquine (**1**)	5	252	94	1.8
	15	344	98	
	1	106	0	
MAP-412 (**6d**)	5	106	16	8.2
	15	192	76	
	1	94	0	
MAP-610 (**7c**)	5	100	6	12.8
	15	158	67	
	1	104	0	
PMAP-H10 (**10**)	5	110	8	(ED_{30}=13.0)
	15	124	36	
control	–	100	0	–

[a] In vivo antimalarial activity was determined as described in the Experimental Section. [b] Survival ratio = (mean number of days treated mice survived)/(mean number of days controls survived). [c] Inhibition ratio (%) = [infection ratio (%) in controls—infection ratio (%) in treated mice]/[infection ratio (%) in controls] 100.

These results indicated that changing positions of amide bonds and pyridinium cations of PAM-86 (**5**) to those of MAP series **6**–**10** did not affect their antimalarial activities.

For the compounds bearing long alkyl chains in both ends such as PAM-86 (**5**), the effects of the *N*-alkyl chain length on the amide moieties (*m*) or linking moieties (*n*) on antimalarial activities were studied. It was found that IC_{50} values against *P. falciparum* reached a minimum at $m=6$ and $n=8$ or 10, and the activities were more potent than those of chloroquine (**1**) and PAM-86 (**5**).

Their toxicities increased with an increase in the side alkyl chain length (*m*). All compounds with the side alkyl chain length of $m=8$ showed obvious cytotoxicities. The length of alkyl chains in the linking moiety was recognized to be independent of cytotoxicities. MAP-610 (**7c**), whose linking alkyl chain length (*n*) is 10, exhibited the most potent in vitro antimalarial activity. Thus, fixing the linking alkyl chain length at $n=10$, we created compounds bearing various substituents, from alkyl chains to cyclic hydrocarbons or aromatic rings, on the amide groups. The antimalarial activity of the anilino derivative PMAP-H10 (**10**) was potent, whereas the antimalarial activities of compounds possessing morpholine or pyrrolidine rings were very weak. PMAP-H10 (**10**) showed cytotoxicity as low as that of the other compounds bearing the linking alkyl chain length of $n=10$. These results suggested that antimalarial activities of these bis-cation-type compounds are dependent on the structure of the side-chain moieties.

The most potent antimalarial compounds in each group, whose side alkyl chain length is 4 or 6, MAP-412 (**6d**), MAP-610 (**7c**), and PMAP-H10 (**10**) were tested for their in vivo antimalarial activities against *P. berghei*-infected mice. Table 2 shows the survival ratio and ED_{50} measured by a 4-day suppressive test.[20,21] MAP-412 (**6d**) and MAP-610 (**7c**), but not PMAP-H10 (**10**), showed significant suppression of antimalarial infection. However, their activities were less than that of chloroquine (**1**). The reason for their survival effects being less than that of chloroquine (**1**) may be the high level of excretion resulting from high water solubility caused by the cationic character. Surprisingly, the in vivo antimalarial potency of MAP-412 (**6d**) was greater than that of MAP-610 (**7c**), whereas the in vitro potency of **7c**, in contrast, was 20 times greater than that of **6d**. The activity mechanism is under investigation in an effort to explain why.

Conclusions

Our investigation into the development of new antimalarials that can be supplied at low price, which were designed from PAM-86 (**5**), commenced with the synthesis of the MAP series **6**–**10**. It was found that positions of the amide bonds and pyridinium cations had little effect on antimalarial activity. In addition, we succeeded in expanding the structure variation in the side-chain moieties of bis-cationic-type antimalarials synthesized from an inexpensive starting material, isonicotinic acid (**11**). Moreover, MAP-412 (**6d**) showed apparent in vivo antimalarial effects that are less than those of chloroquine (**1**) in vivo. Being prepared by just two steps and at low cost, our bis-cation-type antimalarial compounds may be useful as a lead compound for inexpensive antimalarials. Additionally, MAP compounds may show potency against resistant *P. falciparum* strains because they have a bis-cationic structure similar to that of G25 (**2**).[9]

Experimental Section

Chemistry. Melting points were determined with a Yanagimoto hot-stage melting point apparatus and are uncorrected. IR were recorded on JASCO FT/IR350 (KBr). ^{1}H NMR spectra were recorded on a VarianVXR-300 (300 MHz) or VarianVXR-500 (500 MHz) spectrometer. Elemental analysis was carried out with a Yanagimoto MT-5 CHN recorder elemental analyzer. FAB-MS was carried out with a VG70-SE.

General procedure for synthesis of amide intermediates 12–16. A solution of isonicotinic acid (**11**) (4 mmol) in thionyl chloride (5 mL) was refluxed for 1–2 h. After the reaction was completed, thionyl chloride was removed under reduced pressure. For further removal of thionly chloride, toluene was added and then the remaining solution was removed under reduced pressure. THF (6 mL), potassium carbonate (12 mmol), and a corresponding amine (4 mmol) were added to the residue. After stirring for 30 min to 17 h, the mixture was poured into water (50 mL). The mixture was extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography or recrystallization to give a corresponding amide intermediate **12–16**.

***N*-Butyl-4-pyridinecarboxamide** (**12**). Colorless needles from CH_2Cl_2/*n*-hexane. Mp: 41.0–43.0 °C; Yield 73%; ^{1}H NMR($CDCl_3$, 500 MHz): δ = 8.72 (2H, dd, *J* = 4.5, 1.5 Hz), 7.59 (2H, dd, *J* = 4.5, 1.5 Hz), 6.27 (1H, br s), 3.47 (2H, q, *J* = 7.5 Hz), 1.61 (2H, quin, *J* = 7.5 Hz), 1.42 (2H, sex, *J* = 7.5 Hz), 0.96 ppm (3H, t, *J* = 7.5 Hz); IR (KBr): $\tilde{\nu}$ = 3276, 1651 cm^{-1}; FAB-MS *m/z*; 179 [*M*+H]$^+$.

***N*-Hexyl-4-pyridinecarboxamide** (**13**). Colorless needles from CH_2Cl_2/*n*-hexane. Mp: 59.5–61.5 °C; Yield 49%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.73 (2H, dd, *J* = 4.4, 1.5 Hz), 7.59 (2H, dd, *J* = 4.4, 1.5 Hz), 6.22 (1H, br s), 3.45 (2H, m), 1.62 (2H, m), 1.39 (2H, m), 1.34 (4H, m), 0.90 ppm (3H, t, *J* = 7.3 Hz); IR (KBr): $\tilde{\nu}$ = 3311, 1636 cm^{-1}. FAB-MS *m/z*; 207 [*M*+H]$^+$.

***N*-Octyl-4-pyridinecarboxamide** (**14**). Colorless needles from CH_2Cl_2/*n*-hexane. Mp: 67.5–68.0 °C; Yield 39%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.73 (2H, dd, *J* = 4.4, 1.7 Hz), 7.59 (2H, dd, *J* = 4.4, 1.7 Hz), 6.17 (1H, br s), 3.46 (2H, m), 1.62 (2H, quin, *J* = 7.0 Hz,), 1.38–1.27 (10H, m), 0.86 ppm (3H, t, *J* = 7.0 Hz); IR (KBr): $\tilde{\nu}$ = 3303, 1633 cm^{-1}; FAB-MS *m/z*; 235 [*M*+H]$^+$.

4-(4-pyridinylcarbonyl)morpholine (**15a**). Pale yellow oil. Yield 22%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (2H, d, *J* = 5.5 Hz), 7.41 (2H, d, *J* = 5.5 Hz), 3.81 (4H, br s), 3.65 (2H, br s), 3.38 ppm (2H, br s); IR (KBr): $\tilde{\nu}$ = 1634 cm^{-1}; FAB-MS *m/z*; 193 [*M*+H]$^+$.

1-(4-pyridinylcarbonyl)pyrrolidine (**15b**). Pale yellow oil. Yield 30%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.72 (2H, d, *J* = 4.9 Hz), 7.47 (2H, d, *J* = 4.9 Hz), 3.66 (2H, t, *J* = 6.5 Hz), 3.39 (2H, t, *J* = 6.5 Hz), 2.05–1.88 ppm (4H, m); IR (KBr): $\tilde{\nu}$ = 1624 cm^{-1}; FAB-MS *m/z*; 177 [*M*+H]$^+$.

***N*-Phenyl-4-pyridinecarboxamide** (**16**). Colorless needles from EtOH/*n*-hexane. Mp: 171.0–173.0 °C; Yield 64%; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 10.49 (1H, br s), 8.79 (2H, dd, *J* = 4.3, 1.7 Hz), 7.86 (2H, dd, *J* = 4.3, 1.7 Hz), 7.77 (2H, d, *J* = 7.6, 1.5 Hz), 7.38 (2H, td, *J* = 7.6, 1.5 Hz), 7.14 ppm (1H, tt, *J* = 7.6, 1.5 Hz); IR (KBr): $\tilde{\nu}$ = 3349, 1647 cm^{-1}; FAB-MS *m/z*; 199 [*M*+H]$^+$.

General procedure for synthesis of 1,1′-alkylenebis[4-[(substituted amino)carbonyl]pyridinium bromide]s 6–11. A solution of the amide intermediate (0.5 mmol) and dibromoalkane (0.2 mmol) in dry DMF (1 mL) was heated at 100°C for 7–40 h.[24] After the reaction was completed, EtOAc or toluene was added to crystallize the target compounds **6–10** except for MAP-Mor10 (**9a**) and MAP-Pyr10 (**9b**). For further purification, recrystallization was performed from EtOAc/MeOH. Compounds **9a** and **9b** were purified by decantation with acetone and tetrahydrofuran as these compounds were not crystallized.

1,1′-(1,6-Hexanediyl)bis[4-[(butylamino)carbonyl]pyridinium bromide] (**6a**): **MAP-46**. Colorless needles from MeOH/EtOAc. Mp: 204.5–207.5 °C; Yield 48%; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.27 (4H, d, *J* = 6.8 Hz), 9.25 (2H, m), 8.45 (4H, d, *J* = 6.8 Hz), 4.65 (4H, t, *J* = 7.5 Hz), 3.33 (4H, m), 1.93 (4H, m), 1.55 (4H, quin, *J* = 7.4 Hz), 1.33 (8H, m), 0.91 ppm (6H, t, *J* = 7.4 Hz). IR (KBr): $\tilde{\nu}$ = 3273, 1661 cm^{-1}; FAB-MS *m/z*; 519, 521 [*M*+H−HBr]$^+$; Anal. Calcd for $C_{26}H_{40}Br_2N_4O_2$: C, 52.01; H, 6.71; N, 9.33. Found: C, 51.73; H, 6.47; N, 9.28.

1,1′-(1,8-Octanediyl)bis[4-[(butylamino)carbonyl]pyridinium bromide] (**6b**): **MAP-48**. Colorless needles from MeOH/EtOAc. Mp: 231.0–233.0 °C; Yield 80%; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.25 (4H, d, *J* = 6.9 Hz), 9.22 (2H, t, *J* = 5.4 Hz), 8.44 (4H, d, *J* = 6.9 Hz), 4.63 (4H, t, *J* = 7.3 Hz,), 1.91 (4H, m), 1.55 (4H, quin, *J* = 7.3 Hz), 1.35 (4H, m), 1.28 (12H, m), 0.91 ppm (6H, t, *J* = 7.4 Hz). IR (KBr): $\tilde{\nu}$ = 3206, 1659 cm^{-1}; FAB-MS *m/z*; 547, 549 [*M*+H−HBr]$^+$; Anal. Calcd for $C_{28}H_{44}Br_2N_4O_2 \cdot 2/5H_2O$: C, 52.90; H, 7.10; N, 8.81. Found: C, 52.58; H, 6.71; N, 8.62.

1,1′-(1,10-Decanediy)bis[4-[(butylamino)carbonyl]pyridinium bromide] (**6c**): **MAP-410.** Colorless powder from MeOH/EtOAc. Mp: 155.5–157.5 °C; Yield 89%; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.26 (4H, d, *J* = 6.7 Hz), 9.25 (2H, m), 8.44 (4H, d, *J* = 6.7 Hz), 4.63 (4H, t, *J* = 7.3 Hz), 3.34 (4H, m), 1.91 (4H, m,), 1.55 (4H, quin, *J* = 7.3 Hz), 1.41–1.26 (16H, m), 0.91 ppm (6H, t, *J* = 7.3 Hz); IR (KBr): $\tilde{\nu}$ = 3208, 1660 cm^{-1}. FAB-MS *m/z*; 575, 577 [*M*+H−HBr]$^+$; Anal. Calcd for $C_{30}H_{48}Br_2N_4O_2 \cdot 1/2H_2O$: C, 54.14; H, 7.42; N, 8.42. Found: C, 54.21; H, 7.07; N, 8.41.

1,1′-(1,12-Dodecanediyl)bis[4-[(butylamino)carbonyl]pyridinium bromide] (**6d**): **MAP-412.** Colorless powder from MeOH/EtOAc. Mp: 184.0–185.0 °C; Yield 72%; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.25 (4H, d, *J* = 6.8 Hz), 9.23 (2H, m), 8.44 (4H, d, *J* = 6.8 Hz), 4.63 (4H, t, *J* = 7.4 Hz), 3.33 (4H, m), 1.91 (4H, m), 1.55 (4H, quin, *J* = 7.3 Hz), 1.28 (20H, m), 0.91 ppm (6H, t, *J* = 7.3 Hz); IR (KBr): $\tilde{\nu}$ = 3177, 1661 cm^{-1}; FAB-MS *m/z*; 603, 605 [*M*+H−HBr]$^+$; Anal. Calcd for $C_{32}H_{52}Br_2N_4O_2 \cdot H_2O$: C, 54.70; H, 7.75; N, 7.97. Found: C, 54.70; H, 7.50; N, 7.80.

1,1′-(1,6-Hexanediyl)bis[4-[(hexylamino)carbonyl]pyridinium bromide] (**7a**): **MAP-66.** Colorless needles from MeOH/EtOAc. Mp: 203.5–205.0 °C; Yield 52%; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.25 (4H, d, *J* = 6.7 Hz), 9.24 (2H, m), 8.44 (4H, d, *J* = 6.7 Hz), 4.64 (4H, t, *J* = 7.4 Hz), 3.30 (4H, m), 1.92 (4H, m), 1.55 (4H, m), 1.29 (16H, m), 0.87 ppm (6H, t, *J* = 6.8 Hz); IR (KBr): $\tilde{\nu}$ = 3268, 1658 cm^{-1}; FAB-MS *m/z*; 575, 577 [*M*+H−HBr]$^+$; Anal. Calcd for $C_{30}H_{48}Br_2N_4O_2 \cdot 2/5H_2O$: C, 54.29; H, 7.41; N, 8.44. Found: C, 54.46; H, 7.18; N, 8.56.

1,1′-(1,8-Octanediyl)bis[4-[(hexylamino)carbonyl]pyridinium bromide] (**7b**): **MAP-68.** Colorless powder from MeOH/EtOAc. Mp: 217.0–218.5 °C; Yield 67%; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.26 (4H, d, *J* = 6.4 Hz), 9.25 (2H, m), 8.45 (4H, d, *J* = 6.4 Hz), 4.64 (4H, t, *J* = 7.4 Hz), 3.33 (4H, m), 1.92 (4H, m), 1.55 (4H, m), 1.29 (20H, m), 0.87 ppm (6H, t, *J* = 6.8 Hz); IR (KBr): $\tilde{\nu}$ = 3267, 1665 cm^{-1}; FAB-MS *m/z*; 603, 605 [*M*+H−HBr]$^+$; Anal. Calcd for $C_{32}H_{52}Br_2N_4O_2$: C, 56.14; H, 7.66; N, 8.18. Found: C, 56.15; H, 7.38; N, 8.14.

1,1′-(1,10-Decanediyl)bis[4-[(hexylamino)carbonyl]pyridinium bromide] (**7c**): **MAP-610.** Colorless powder from MeOH/EtOAc. Mp: 176.0–177.5 °C; Yield 35%; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.25 (4H, d, *J* = 6.8 Hz), 9.22 (2H, t, *J* = 5.8 Hz), 8.43 (4H, d, *J* = 6.8 Hz), 4.63 (4H, t, *J* = 7.3 Hz), 3.31 (4H, m) 1.91 (4H, m), 1.55 (4H, m), 1.27 (24H, m), 0.87 ppm (6H, t, *J* = 6.8 Hz); IR (KBr): $\tilde{\nu}$ = 3189, 1660 cm^{-1}; FAB-MS *m/z*; 631, 633 [*M*+H−HBr]$^+$; Anal. Calcd for $C_{34}H_{56}Br_2N_4O_2 \cdot 1/2H_2O$: C, 56.59; H, 7.96; N, 7.76. Found: C, 56.66; H, 7.73; N, 7.74.

1,1′-(1,12-Dodecanediyl)bis[4-[(hexylamino)carbonyl]pyridinium bromide] (**7d**): **MAP-612.** Colorless powder from MeOH/EtOAc. Mp: 151.0–152.5 °C; Yield 72%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.25 (4H, d, J = 6.4 Hz), 9.23 (2H, m), 8.44 (4H, d, J = 6.4 Hz), 4.63 (4H, t, J = 7.3 Hz), 3.33 (4H, m), 1.91 (4H, m), 1.55 (4H, m), 1.28 (28H, m), 0.87 ppm (6H, t, J = 6.8 Hz); IR (KBr): $\tilde{\nu}$ = 3206, 1661 cm^{-1}; FAB-MS m/z; 659, 661 [M+H−HBr]$^+$; Anal. Calcd for $C_{30}H_{44}Br_2N_4O_2\cdot 2/3\,H_2O$: C, 57.67; H, 8.20; N, 7.47. Found: C, 57.27; H, 7.80; N, 7.36.

1,1′-(1,6-Hexanediyl)bis[4-[(octylamino)carbonyl]pyridinium bromide] (**8a**): **MAP-86.** Colorless powder from MeOH/EtOAc. Mp: 205.0–207.0 °C; Yield 41%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.27 (4H, d, J = 6.6 Hz), 9.24 (2H, m), 8.45 (4H, d, J = 6.6 Hz), 4.65 (4H, t, J = 7.1 Hz), 3.31 (4H, m), 1.93 (4H, m), 1.55 (4H, m), 1.29 (20H, m), 0.86 ppm (6H, t, J = 6.7 Hz); IR (KBr): $\tilde{\nu}$ = 3239, 1665 cm^{-1}; FAB-MS m/z; 631, 633 [M+H−HBr]$^+$; Anal. Calcd for $C_{34}H_{56}Br_2N_4O_2\cdot 1/2\,H_2O$: C, 56.59; H, 7.96; N, 7.76. Found: C, 56.71; H, 7.84; N, 7.74.

1,1′-(1,8-Octanediyl)bis[4-[(octylamino)carbonyl]pyridinium bromide] (**8b**): **MAP-88.** Colorless powder from MeOH/EtOAc. Mp 234.5–235.5 °C; Yield 51%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.25 (4H, d, J = 6.6 Hz), 9.22 (2H, t, J = 5.4 Hz), 8.44 (4H, d, J = 6.6 Hz), 4.63 (4H, t, J = 7.6 Hz), 1.90 (4H, m), 1.55 (4H, m), 1.27 (28H, m), 0.86 ppm (6H, t, J = 6.8 Hz); IR (KBr): $\tilde{\nu}$ = 3239, 1665 cm^{-1}; FAB-MS m/z; 659, 661 [M+H−HBr]$^+$; Anal. Calcd for $C_{36}H_{60}Br_2N_4O_2$: C, 58.38; H, 8.16; N, 7.56. Found: C, 58.09; H, 7.84; N, 7.46.

1,1′-(1,10-Decanediyl)bis[4-[(octylamino)carbonyl]pyridinium bromide] (**8c**): **MAP-810.** Colorless powder from MeOH/EtOAc. Mp: 134.5–136.0 °C; Yield 81%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.24 (4H, d, J = 6.8 Hz), 9.23 (2H, m), 8.43 (4H, d, J = 6.8 Hz), 4.63 (4H, t, J = 7.6 Hz), 3.32 (4H, m), 1.91 (4H, m), 1.55 (4H, m), 1.28 (33H, m), 0.86 ppm (6H, t, J = 6.6 Hz); IR (KBr): $\tilde{\nu}$ = 3180, 1664 cm^{-1}; FAB-MS m/z; 687, 689 [M+H−HBr]$^+$. Anal. Calcd for $C_{34}H_{40}Br_2N_4O_2\cdot 1/2H_2O$: C, 58.34; H, 8.44; N, 7.16. Found: C, 58.41; H, 8.04; N, 6.78.

1,1′-(1,12-Dodecanediyl)bis[4-[(octylamino)carbonyl]pyridinium bromide] (**8d**): **MAP-812.** Colorless powder from MeOH/EtOAc. Mp: 162.5–163 °C; Yield 65%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.24 (4H, d, J = 6.5 Hz), 9.23 (2H, m), 8.43 (4H, d, J = 6.5 Hz), 4.65 (4H, t, J = 7.3 Hz), 3.31 (4H, m), 1.91 (4H, m), 1.55 (4H, m), 1.22 (32H, m), 0.86 ppm (6H, t, J = 6.8 Hz); IR (KBr): $\tilde{\nu}$ = 3186, 1660 cm^{-1}; FAB-MS m/z; 715 , 717 [M+H−HBr]$^+$; Anal. Calcd for $C_{40}H_{68}Br_2N_4O_2$: C, 60.29; H, 8.60; N, 7.03. Found: C, 59.89; H, 8.13; N, 6.86.

1,1′-(1,10-Decanediyl)bis[4-[(morpholinyl)carbonyl]pyridinium bromide] (**9a**): **MAP-Mor10.** Pale yellow oil. Yield 31%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.20 (4H, d, J = 6.8 Hz), 8.22 (4H, d, J = 6.8 Hz), 4.59 (4H, t, J = 7.6 Hz), 3.68 (8H, m), 3.58 (4H, m), 3.31 (4H, m), 1.91 (4H, m), 1.29 ppm (12H, m); IR (KBr): $\tilde{\nu}$ = 1670 cm^{-1}. FAB-MS m/z; 603, 605 [M+H−HBr]$^+$; Anal. Calcd for $C_{30}H_{44}Br_2N_4O_4\cdot 7/2\,H_2O$: C, 48.20; H, 6.88; N, 7.49. Found: C, 48.26; H, 6.49; N, 7.50.

1,1′-(1,10-Decanediyl)bis[4-[(pyrrolidinyl)carbonyl]pyridinium bromide (**9b**): **MAP-Pyr10.** Pale yellow solid. Yield 33%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.19 (4H, d, J = 6.7 Hz), 8.25 (4H, d, J = 6.7 Hz), 4.61 (4H, t, J = 7.4 Hz), 3.51 (4H, t, J = 6.7 Hz), 3.30 (4H, m), 1.89 (12H, m), 1.27 ppm (16H, m); IR (KBr): $\tilde{\nu}$ = 1637 cm^{-1}. FAB-MS m/z; 571, 573 [M+H−HBr]$^+$; Anal. Calcd for $C_{30}H_{44}Br_2N_4O_2\cdot 2/3\,H_2O$: C, 54.22; H, 6.88; N, 8.43. Found: C, 54.23; H, 6.90; N, 8.49.

1,1′-(1,10-Decanediyl)bis[4-[(phenylamino)carbonyl]pyridinium bromide] (**10**): **PMAP-H10.** Yellow powder from MeOH/EtOAc. Mp: 209.0–210.0 °C; Yield 36%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.85 (s, 2H), 9.35 (4H, d, J = 6.7 Hz), 8.60 (4H, d, J = 6.7 Hz), 7.80 (4H, d, J = 7.6 Hz), 7.45 (4H, t, J = 7.6 Hz), 7.30 (2H, t, J = 7.6 Hz), 4.70 (4H, t, J = 7.6 Hz), 1.95 (4H, m), 1.30 ppm (12H, m); IR (KBr): 3415, 1673 cm^{-1}; FAB-MS m/z; 615, 617 [M+H−HBr]$^+$; Anal. Calcd for $C_{34}H_{40}Br_2N_4O_2\cdot 1/2\,H_2O$: C, 57.88; H, 5.86; N, 7.94. Found: C, 57.79; H, 5.72; N, 7.82.

Culture of *Plasmodium falciparum*. Antimalarial activities of compounds against *Plasmodium falciparum* (FCR-3 strain) were determined. *P. falciparum* was cultivated by a modification of the method of Trager and Jensen using a 5% hematocrit of type A human red blood cells (RBC) suspended in RPMI 1640 medium supplemented with 44 mM HEPES, 24 mM $NaHCO_3$, 25 μg mL^{-1} gentamicin, and heat-inactivated 10% type A human serum. The plates were placed under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2 at 37 °C. The medium was changed daily until 5% parasitemia (which means five parasite-infected RBC in every 100 RBC).

Culture of mammalian cells. Cytotoxicity of the test compounds against mouse mammary tumor FM3A cells (wild-type, subclone F28-7) was determined. FM3A cells were maintained in suspension culture at 37 °C in a 5% CO_2 atmosphere in plastic bottles containing ES medium supplemented with 2% heat-inactivated fetal bovine serum. FM3A cells grew with a doubling time of about 12 h.

In vitro antimalarial activity assay.[25] Five μL of each solution dissolved in DMSO at several concentrations was added to individual wells of a 24-well plate. RBC with 0.3% parasitemia were added to each well containing 995 μL of culture medium to give a final hematocrit level of 3%. Plates were incubated at 37 °C for 72 h under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. To evaluate the antimalarial activities of the test compounds, thin blood films from each culture were prepared and stained with Diff-Quik stain. Test compounds were tested in duplicate at each concentration. Compound-free control cultures were run simultaneously. All data points represent the mean of two experiments (Parasitemia in control reached between 4% and 5% at 72 h). The 50% inhibitory concentration (IC_{50}), which is the concentration of 50% parasite growth inhibition compared to the control, was calculated from the growth inhibition curve for each compound.

Cytotoxicity against mammalian cell line.[25] Prior to exposure to the test compounds, cell density was adjusted to 5×10^4 cells mL^{-1}. A cell suspension of 995 μL was dispensed to the test plate, and each test compound at various concentrations suspended in DMSO (5 μL) was added to individual wells of a 24-well plate. The plate was incubated at 37 °C for 48 h under an atmosphere of 5% CO_2. All of the test compounds were assayed in duplicate at each concentration. Cell numbers were determined by using a cell counter CC-108 (Toa Medical Electrics). All data points represent the mean of two experiments. The IC_{50} value refers to the concentration of the compound necessary to inhibit the increase in cell density at 48 h by 50% of the control.

In vivo antimalarial activity assay.[25,26] The study was conducted according to internationally accepted principles of laboratory animal use. In vivo antimalarial activities were determined in mice infected with *P. berghei* (NK 65 strain). Five-week-old ICR male mice obtained in sterile containers from Charles River Co. Ltd. (weighing 28 ± 3.5 g) were used. The mice were randomly assigned to treatment groups and housed in cages each containing five individuals. Parasites were collected by cardiac puncture in a heparinized syringe from a donor mouse harboring about 15% parasitemia. The blood was diluted with PBS(−) to a final concentration of 1×10^6 infected RBC (infecting suspension in 0.2 mL of PBS(−)). Test compounds were prepared at doses of 1, 5, and 15 mg kg^{-1} in PBS(−) (1% DMSO). Five mice were treated with each dose. Initially, each mouse was inoculated intravenously in the tail vein with 1×10^6 parasitized RBC (infecting suspension in 0.2 mL of PBS(−)). The compounds were administrated interperitoneally (i.p.) once a day starting on day 0 and continued on days 1, 2, and 3. The first administration of test compounds started 2 h after parasite inocula-

tion. Parasitemia levels were determined on the day following the last treatment (on day four). To evaluate the antimalarial activity of the compounds, we prepared tail blood smears and stained them with Diff-Quik stain. The suppression of parasitemia for the doses of MAP-412 (**5 d**), MAP-610 (**6 c**), and PMAP-H10 (**10**) was calculated by the formula [(average % of parasitemia in controls - average % of parasitemia in treated mice)/average % of parasitemia in controls] 100. Five infected mice treated with PBS(−) (1% DMSO) were used as a control. The data shown are the mean values of five mice.

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