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Synthesis and Antimycobacterial Activity of Capuramycin Analogues. Part 2: Acylated Derivatives of Capuramycin-Related Compounds

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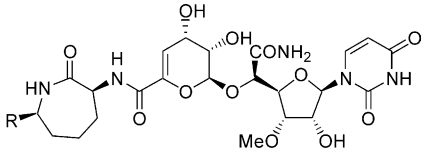
Abstract—Acylated derivatives of capuramycin and A-500359A were synthesized and tested for antimycobacterial activity. Compound **20** having a decanoyl group showed very potent activity.

Translocase I (Mra Y) is one of the enzymes involved in the early stage of biosynthesis of peptidoglycan,[1] which is a component of the bacterial cell wall. Therefore, translocase I can be a unique and selective target for developing antibiotics having a new mechanism of action.[2–8]

Capuramycin, a complex nucleoside antibiotic consisting of a nucleoside, sugar and lactam, was originally isolated by Yamaguchi et al.[9,10] from the culture broth of *Streptomyces griseus* 446-S3. In the course of screening for new antibiotics with translocase I inhibitory activity, we also isolated capuramycin and its derivative, designated as A-500359A, from the culture broth of *Streptomyces griseus* SANK 60196.[11]



Capuramycin (R = H)
A-500359A (R = Me)

A-500359A inhibits reversibly and in a mixed and non-competitive manner with UDP-MurNAc-(N^{ε}-Dns)pentapeptide ($K_i = 7.9$ nM) and undecaprenylphosphate, respectively.[12] Capuramycin and A-500359A inhibit translocase I with IC_{50} values of 10 ng/mL (18 nM) and 10 ng/mL (17 nM), respectively. The MIC values for capuramycin and A-500359A against *Mycobacterium smegmatis* SANK75075 are 12.5 and 6.25 μg/mL, respectively, demonstrating relatively weak antibacterial activity compared to their potent translocase I inhibitory activity. Moreover, mycobacteria is one of the very few microorganisms that are susceptible to these compounds.[12] We presumed that this might be partially attributable to the hydrophilic nature of these compounds, which prevents them from reaching the target molecule, translocase I, that is located in the cell membrane. Similar speculations have been published by Dini et al.[3] and Lin et al.[7] for liposidomycin and muraymycin, respectively. Therefore, we decided to synthesize and examine acylated derivatives of capuramycin and A-500359A in order to make them lipophilic enough to penetrate the cell membrane.

Acylated derivatives were synthesized as shown in Scheme 1 (route A). First, capuramycin or A-500359A was acetonized to give **1**. Compound **1** was subjected to acylation, followed by deprotection under acidic

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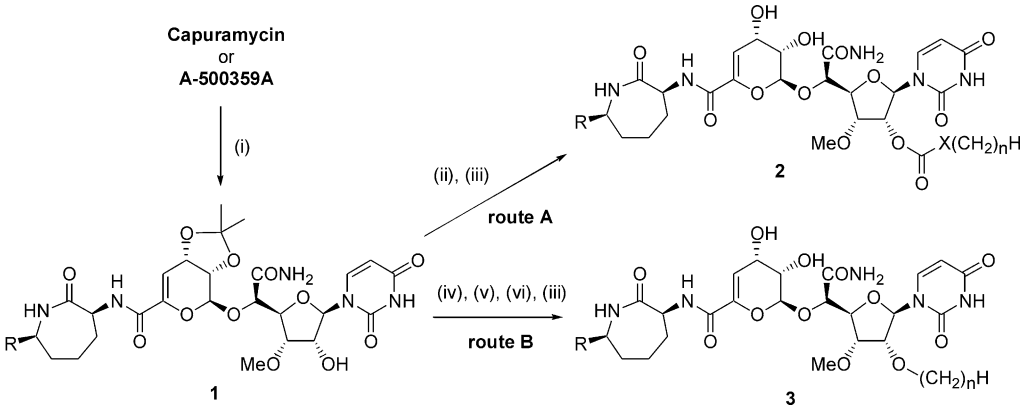
conditions to give desired product **2** (X = CH_2). Alkoxycarbonyl-type compounds (**2**, X = O) were synthesized in a similar manner by using alkyl chloroformate. Experimental details with spectroscopic data are provided in the patent.[13]

Table 1 shows the translocase I inhibitory activity and antimycobacterial activity for acyl/alkoxycarbonyl-type derivatives. Compound **20**[14] having a decanoyl group was found to exhibit ca. 50-fold less potency than the parent compound, A-500359A, with respect to translocase I inhibitory activity; however, **20** showed far superior antimycobacterial activity against *Mycobacterium avium* NIHJ1605, *Mycobacterium intracellulare* ATCC1954 E-3, and *Mycobacterium kansasii* ATCC12478 than A-500359A. Translocase I inhibitory



Scheme 1. (i) $Me_2C(OMe)_2$, Amberlyst 15 (H^+), Me_2CO; (ii) $[H(CH_2)_nXCO]_2O$, DMAP, pyridine, or $H(CH_2)_nXCO_2H$, DMAP, WSC (water-soluble carbodiimide), THF; (iii) Amberlyst 15 (H^+), MeOH; (iv) $PMBOCH_2Cl$, DBU, MeCN; (v) $H(CH_2)_nI$, DMF; (vi) DDQ, CH_2Cl_2–H_2O.

Table 1. [a] Translocase I inhibitory activity and antibacterial activity for acyl/alkoxycarbonyl-type prodrugs of capuramycin or A-500359A

Compd	R	X	*n*	IC_{50} (ng/mL)	MIC (μg/mL)			
				Translocase I	*M. smegmatis* SANK75075	*M. avium* NIHJ1605	*M. intracellulare* ATCC1954 E-3	*M. kansasii* ATCC12478
Capuramycin	H			10 (18 nM)	12.5	8	8	8
A-500359A	Me			10 (17 nM)	6.25	8	4	16
4	H	CH_2	5	40	25	n.d.[b]	n.d.	n.d.
5	H	CH_2	7	180	12.5	n.d.	n.d.	n.d.
6	H	CH_2	8	300	12.5	0.5	0.25	1
7	H	CH_2	10	n.d.	3.13	<0.063	0.125	0.125
8	H	CH_2	12	n.d.	1.56	2	1	4
9	H	CH_2	13	n.d.	3.13	2	1	4
10	H	CH_2	14	n.d.	1.56	1	2	4
11	H	CH_2	15	n.d.	50	n.d.	n.d.	n.d.
12	H	CH_2	16	n.d.	50	n.d.	n.d.	n.d.
13	H	CH_2	18	n.d.	50	n.d.	n.d.	n.d.
14	H	CH_2	20	n.d.	100	n.d.	n.d.	n.d.
15	Me	CH_2	3	24	100	32	2	16
16	Me	CH_2	4	43	50	16	2	2
17	Me	CH_2	5	45	50	4	1	2
18	Me	CH_2	6	190	25	2	1	1
19	Me	CH_2	7	170	25	n.d.	n.d.	n.d.
20	Me	CH_2	8	550	6.25	<0.063	<0.063	<0.063
21	Me	CH_2	10	2200	1.56	<0.063	<0.063	0.5
22	Me	CH_2	12	4500	3.13	<0.063	0.125	4
23	Me	CH_2	13	2400	6.25	0.125	0.25	4
24	Me	CH_2	14	430	6.25	<0.063	0.25	4
25	Me	CH_2	15	n.d.	>100	2	8	4
26	Me	CH_2	16	8000	>100	n.d.	n.d.	n.d.
27	Me	CH_2	18	30000	>100	n.d.	n.d.	n.d.
28	Me	CH_2	20	15000	>100	n.d.	n.d.	n.d.
29	Me	O	6	130	50	n.d.	n.d.	n.d.
30	Me	O	7	200	50	0.25	1	2
31	Me	O	8	500	25	0.25	0.5	2
32	Me	O	9	1400	12.5	n.d.	n.d.	n.d.
33	Me	O	16	>2500	>100	0.5	2	8
Rifampicin				n.d.	n.d.	0.125	0.125	0.25
Isoniazid				n.d.	n.d.	1	8	2

[a]Refer to the template structure of compound **2** in Scheme 1.
[b]Not determined.

Table 2. [a] Translocase I inhibitory activity and antibacterial activity for 2′-*O*-alkylated derivatives of A-500359A

Compd	R	*n*	IC_{50} (ng/mL)	MIC (μg/mL)			
			Translocase I	*M. smegmatis* SANK75075	*M. avium* NIHJ1605	*M. intracellulare* ATCC1954 E-3	*M. kansasii* ATCC12478
34	Me	5	50	100	>32	4	>32
35	Me	6	40	50	32	4	32
36	Me	7	55	100	32	4	32
37	Me	8	150	50	16	2	>32
38	Me	10	640	25	1	4	>32

[a]Refer to the template structure of compound **3** in Scheme 1.

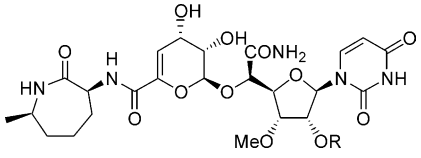
activity weakened relative to an increase in the length of the acyl side chain. On the other hand, acyl side chain of optimal lengths with respect to antimycobacterial activity, that is dodecanoyl (**7**) for capuramycin and decanoyl (**20**) for A-500359A, were identified. This may be due to the suitable lipophilicity conferred to these compounds, which enabled them to penetrate the cell membrane of mycobacteria more effectively. As a further speculation explaining very potent antimycobacterial activities of **7** and **20**, these compounds may be prodrugs, which revert to more potent parent compound, A-500359A, in the bacteria. The alkoxycarbonyl-type derivatives (**29**–**33**) seemed to be less potent than the acyl-type derivatives.

In addition, 2′-*O*-alkylated derivatives were examined. The 2′-*O*-alkylation reaction was performed via protection at the 3-*N* position, which is the site most susceptible to alkylation, with a *p*-methoxybenzyloxymethyl group as shown in Scheme 1 (route B). These compounds retained weak activity (Table 2), which demonstrates that there is a spatial tolerance around the 2′-position to some extent with regard to the interaction with translocase I.

The unsaturated acyl-type derivatives possessed good antimycobacterial activity (**39**–**41**); however, the acyl-type derivatives which contained aromatic groups were not potent (**46**–**48**) as shown in Table 3.

In the preceding paper,[15] we described a capuramycin-analogue having a phenyl group in place of the azepan-2-one moiety, **49** (Table 4), that was a more potent translocase I inhibitor than capuramycin or A-500359A. The acylated derivatives of **49** were synthesized in the same manner as shown in Scheme 1 (route A). However, contrary to our expectations, these compounds (**50**–**54**) proved to be less potent than the corresponding derivatives for A-500359A.

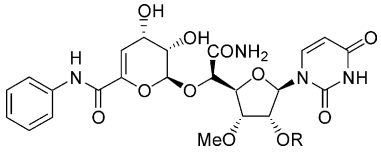
Table 3. Translocase I inhibitory activity and antibacterial activity (*M. smegmatis* SANK75071) for acylated A-500359A



Compd	R	Translocase I IC_{50} (ng/mL)	*M. smegmatis* MIC (μg/mL)
39	Octadec-9-enoyl[a]	n.d.[b]	6.25
40	Octadeca-9,12-dienoyl[a]	n.d.	6.25
41	Octadeca-9,12,15,-trienoyl[a]	n.d.	3.13
42	Eicos-11-enoyl[a]	n.d.	50
43	Docos-13-enoyl[a]	n.d.	>100
44	2-Methyldodecanoyl	600	25
45	2,2-Dimethyldodecanoyl	1000	6.25
46	Benzoyl	n.d.	25
47	3,3-Diphenylpropionyl	n.d.	25
48	2-(4-Nitrophenyl)propionyl	n.d.	25

[a]Double bonds are all *cis*.

Table 4. Translocase I inhibitory activity and antibacterial activity for 2′-*O*-acylated prodrugs of phenyl-type analogues of capuramycin



Compd	R	IC_{50} (ng/mL)	MIC (μg/mL)			
		Translocase I	*M. smegmatis* SANK75075	*M. avium* NIHJ1605	*M. intracellulare* ATCC1954 E-3	*M. kansasii* ATCC12478
49[a]	H	6.5	6.25	16	4	8
50	Heptanoyl	240	25	0.5	1	0.5
51	Nonanoyl	700	12.5	0.25	1	1
52	Decanoyl	1900	6.25	0.5	1	2
53	Dodecanoyl	>2500	6.25	1	4	8
54	Hexadecanoyl	>2500	12.5	2	16	32

[a]This compound is identical to compound **47** in the preceding paper.[15]

In conclusion, compound **20** having a decanoyl group showed very potent antimycobacterial activity. The acylated derivatives along with the phenyl-type capuramycin analogues in the preceding paper[15] have been further examined with regard to their antibacterial activity against *Mycobacterium tuberculosis* in vitro and in vivo, and the results will be published in a separate literature.[16]

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14. Analytical data for **20**: IR (KBr) ν 3390, 2927, 2855, 1689, 1510, 1459, 1430, 1384, 1336, 1269, 1151, 1109, 1062, 1022; ^{1}H NMR (270 MHz, CD_3OD) δ 7.95 (1H, d, $J=8.1$ Hz), 6.01 (1H, d, $J=3.8$ Hz), 5.97 (1H, d, $J=4.7$ Hz), 5.72 (1H, d, $J=8.1$ Hz), 5.44 (1H, t, $J=4.9$ Hz), 5.24 (1H, d, $J=5.4$ Hz), 4.68 (1H, d, $J=1.7$ Hz), 4.55 (2H, m), 4.41 (1H, t, $J=4.1$ Hz), 4.06 (1H, t, $J=4.8$ Hz), 3.97 (1H, t, $J=5.0$ Hz), 3.58 (1H, m), 3.38 (3H, s), 2.37 (2H, t, $J=7.4$ Hz), 2.05–1.75 (4H, m), 1.63–1.20 (19H, m), 0.90 (3H, t, $J=6.8$ Hz).
15. Part 1 of this series: Hotoda, H.; Furukawa, M.; Daigo, M.; Murayama, K.; Kaneko, M.; Muramatsu, Y.; Miyazawa Ishii, M.; Miyakoshi, S.; Takatsu, T.; Inukai, M.; Kakuta, M.; Abe, T.; Harasaki, T.; Fukuoka, T.; Utsui, Y.; Ohya, S. *Bioorg. Med. Chem. Lett*. See preceding paper. doi: 10.1016/S0960-894X(03)00596-1.
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