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Synthesis and Antimycobacterial Activity of Capuramycin Analogues. Part 1: Substitution of the Azepan-2-one Moiety of Capuramycin

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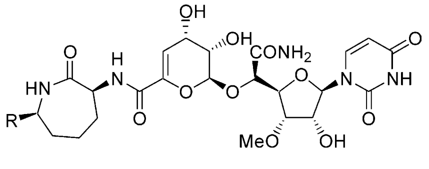
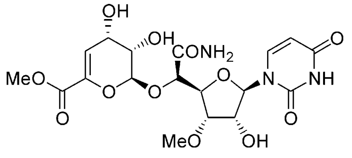
Abstract—Capuramycin analogues with a variety of substituents in place of the azepan-2-one moiety were synthesized from A-500359E and were tested for their translocase I inhibitory activity and in vitro antimycobacterial activity. Phenyl-type moieties were found to be effective substituents for capuramycin analogues.

Although standard medication for tuberculosis (TB) has already been established,[1] TB is still a major cause of death especially in the developing countries. Moreover, TB is on the rise once more even in the developed countries in part due to the spread of HIV infections and the emergence of multi-drug-resistant *Mycobacterium tuberculosis*.[2] Consequently, the development of a novel anti-TB drug with a new type of mechanism is being desired.

Translocase I (Mra Y) is one of the enzymes involved in the biosynthesis of peptidoglycan[3] and is a possible target for developing antibiotics.[4] In the course of screening for new antibiotics with translocase I inhibitory activity, we isolated capuramycin, which had originally been isolated from the culture broth of *Streptomyces griseus* 446-S3,[5,6] and its methylated derivative, A-500359A.[7] A-500359A inhibits reversibly and in a mixed and non-competitive manner with UDP-MurNAc-(N^{ε}-Dns)pentapeptide ($K_i = 7.9\,\text{nM}$) and undecaprenylphosphate, respectively.[8] A-500359E lacking the azepan-2-one moiety of capuramycin was also isolated.[9]



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



A-500359E

Capuramycin and A-500359A were proven to have selective antibacterial activity against mycobacteria.[8] The attempt to extend the antibacterial spectrum by chemical modifications was not successful, but it led to the discovery of new capuramycin analogues with weak

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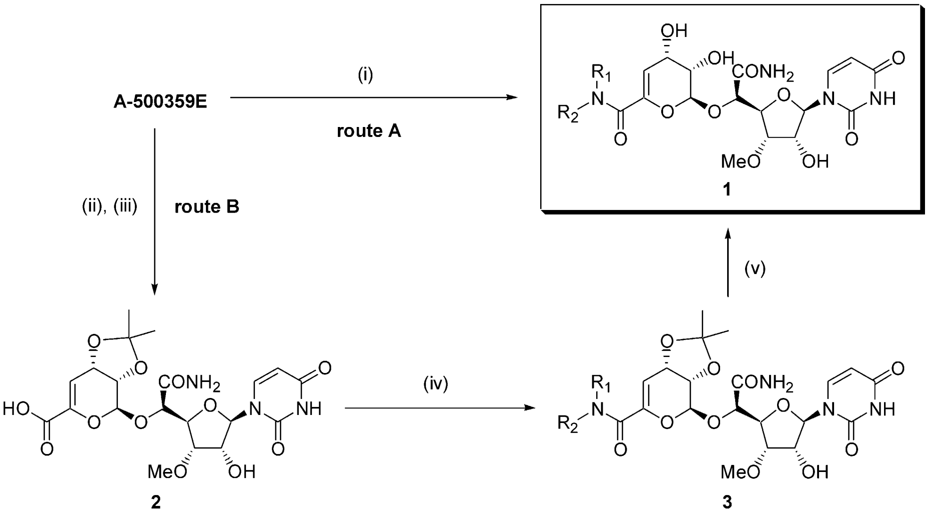


Scheme 1. (i) R_1R_2NH (excess), MeOH, rt to 60 °C; (ii) $Me_2C(OMe)_2$, Amberlyst 15 (H^+), Me_2CO, 79%; (iii) 0.04N NaOH, MeOH–H_2O, then Dowex 50W×8 (H^+), 92%; (iv) R_1R_2NH, DIPC, HOBT, DMF; (v) Amberlyst 15 (H^+), MeOH.

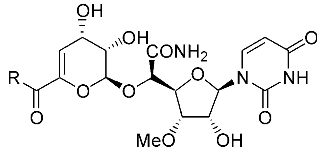
antimicrobial activity against several Gram-positive bacteria.[10] In order to develop a novel anti-TB drug, capuramycin analogues having a variety of substituents in place of the azepan-2-one moiety were synthesized and their antimycobacterial activities were tested.

These new capuramycin analogues were synthesized from A-500359E combined with a variety of amines. In the case of amine with enough nucleophilicity, A-500359E was reacted with excess amounts of the amine in methanol, in a sealed test tube. The reaction took place via route A as shown in Scheme 1. Twenty test tubes containing different amines in each tube were placed in the 20-hole aluminum heat block and 20 reactions took place simultaneously. On the other hand, in the case of amine without enough nucleophilicity like aniline, A-500359E was acetonized, saponified, and then condensed with the amine using a condensing reagent via route B (Scheme 1). The desired product **1** was obtained after deprotection under acidic conditions. Experimental details with spectroscopic data are provided in the patent.[11]

Some of the primary screening results for new capuramycin analogues are described hereinafter. The MIC and IC_{50} values were determined according to previously reported methods.[8] Capuramycin and A-500359A inhibit translocase I with IC_{50} values of 10 ng/mL (18 nM) and 10 ng/mL (17 nM), respectively (Table 1).[8] The MIC values for capuramycin and A-500359A against *Mycobacterium smegmatis* SANK75075 are 12.5 and 6.25 µg/mL, respectively. Compound **5** having an *n*Bu moiety in place of the azepan-2-one moiety of capuramycin retained moderate translocase I inhibitory activity and showed the same MIC value as capuramycin. On the other hand, compound **6** having an NMe group in place of the NH group of **5** showed no inhibitory activity against both translocase I and *M. smegmatis*, demonstrating the importance of the NH group of the amide moiety of capuramycin and its analogues. Compounds **9** and **10** having a simple cyclohexyl and cycloheptyl moiety in place of the azepan-2-one moiety retained potent activity. Terminal modification of the alkyl chain substituents with functional groups reduced the activity of capuramycin analogues (**11**–**15**). Substitutions with medium-size alkyl chain moieties drastically reduced translocase I inhibitory activity (**16** and **17**); however, the analogues retained moderate inhibitory activity against *M. smegmatis*. Compound **18** having a long-chain alkyl moiety showed no activity.

Compounds **19**–**33** having phenethyl-type substituents had moderate activity (Table 2). Compounds **34**–**46** having benzyl-type substituents were less potent than phenethyl-type analogues (Table 3).

Table 1. Translocase I inhibitory activity and antibacterial activity (*M. smegmatis* SANK75075) of capuramycin analogues with aliphatic substituents



Compd	R	Translocase I IC_{50} (ng/mL)	*M. smegmatis* MIC (µg/mL)
Capuramycin		10 (18 nM)	12.5
A-500359A		10 (17 nM)	6.25
A-500359E	MeO–	27	> 100
4	MeNH–	180	> 100
5	$Me(CH_2)_3NH$–	280	12.5
6	$Me(CH_2)_3N(Me)$–	> 2500	> 100
7	Pyrrolidin-1-yl	420	> 100
8	$Me(CH_2)_5NH$–	30	12.5
9	Cyclohexyl-NH–	30	12.5
10	Cycloheptyl-NH–	27	25
11	$EtO(CH_2)_3NH$–	140	100
12	$MeS(CH_2)_3NH$–	70	50
13	$HO(CH_2)_2NH$–	90	> 100
14	$H_2N(CH_2)_2NH$–	600	100
15	$HO(CH_2)_4NH$–	170	> 100
16	$Me(CH_2)_9NH$–	260	25
17	$Me(CH_2)_{11}NH$–	390	25
18	$Me(CH_2)_{13}NH$–	> 2500	> 50

Table 2. Translocase I inhibitory activity and antibacterial activity (*M. smegmatis* SANK75075) of capuramycin analogues with phenethyl-type substituents[a]

Compd	R	Translocase I IC$_{50}$ (ng/mL)	*M. smegmatis* MIC (μg/mL)
19	Ph(CH$_2$)$_2$NH–	21	12.5
20	4-Me–Ph(CH$_2$)$_2$NH–	34	50
21	2-MeO–Ph(CH$_2$)$_2$NH–	32	50
22	3-MeO–Ph(CH$_2$)$_2$NH–	19	50
23	4-MeO–Ph(CH$_2$)$_2$NH–	40	25
24	3,4-(MeO)$_2$–Ph(CH$_2$)$_2$NH–	25	50
25	4-F–Ph(CH$_2$)$_2$NH–	22	12.5
26	2-Cl–Ph(CH$_2$)$_2$NH–	28	25
27	3-Cl–Ph(CH$_2$)$_2$NH–	20	12.5
28	4-Cl–Ph(CH$_2$)$_2$NH–	20	25
29	2,4-Cl$_2$–Ph(CH$_2$)$_2$NH–	19	50
30	4-HO–Ph(CH$_2$)$_2$NH–	60	50
31	Ph$_2$CHCH$_2$NH–	130	> 100
32	Pyridin-2-yl-(CH$_2$)$_2$NH–	110	25
33	Thiophen-2-yl-(CH$_2$)$_2$NH–	13	25

[a] Refer to the template structure in Table 1.

Table 3. Translocase I inhibitory activity and antibacterial activity (*M. smegmatis* SANK75075) of capuramycin analogues with benzyl-type substituents[a]

Compd	R	Translocase I IC$_{50}$ (ng/mL)	*M. smegmatis* MIC (μg/mL)
34	PhCH$_2$NH–	120	50
35	2-Me–PhCH$_2$NH–	500	12.5
36	4-Me–PhCH$_2$NH–	1200	50
37	4-F–PhCH$_2$NH–	200	50
38	2-MeO–PhCH$_2$NH–	280	50
39	4-MeO–PhCH$_2$NH–	700	100
40	3,4,5-(MeO)$_3$–PhCH$_2$NH–	420	> 100
41	3,4-(OCH$_2$O)–PhCH$_2$NH–	200	50
42	(*S*)-PhCH(Me)NH–	130	50
43	Naphthalen-2-yl-CH$_2$NH–	110	25
44	Furan-2-yl-CH$_2$NH–	280	100
45	Thiophen-2-yl-CH$_2$NH–	77	50
46	Pyridin-2-yl-CH$_2$NH–	360	100

[a] Refer to the template structure in Table 1.

Table 4. Translocase I inhibitory activity and antibacterial activity (*M. smegmatis* SANK75075) of capuramycin analogues with phenyl-type substituents[a]

Compd	R	Translocase I IC$_{50}$ (ng/mL)	*M. smegmatis* MIC (μg/mL)
47	PhNH–	6.5	6.25
48	PhN(Me)–	> 2500	> 100
49	3-Me–PhNH–	7.6	12.5
50	4-Me–PhNH–	13	12.5
51	2,4-Me$_2$–PhNH–	60	25
52	2-Et–PhNH–	54	50
53	4-Et–PhNH–	20	12.5
54	4-*n*Pr–PhNH–	31	12.5
55	4-*i*Pr–PhNH–	120	25
56	4-(Me(CH$_2$)$_5$)–PhNH–	160	12.5
57	4-CF$_3$–PhNH–	30	12.5
58	2-MeO–PhNH–	31	25
59	2-EtO–PhNH–	37	50
60	4-BnO–PhNH–	23	25
61	3-F–PhNH–	10	6.25
62	4-F–PhNH–	37	6.25
63	2,3-F$_2$–PhNH–	13	12.5
64	2,4-F$_2$–PhNH–	15	12.5
65	3,4-F$_2$–PhNH–	9	6.25
66	4-Cl–PhNH–	18	6.25
67	4-Br–PhNH–	20	6.25
68	2-NO$_2$–PhNH–	40	25
69	Thiazol-2-yl-NH–	18	25
70	4-*t*Bu-Thiazol-2-yl-NH–	15	100
71	[1,3,4]Thiaziazol-2-yl-NH–	105	> 100

[a] Refer to the template structure in Table 1.

Table 5. Antimycobacterial activity for capuramycin analogues

Compd	MIC (μg/mL)		
	M. avium NIHJ1605	*M. intracellulare* ATCC1954 E-3	*M. kansasii* ATCC12478
Capuramycin	8	8	8
A-500359A	8	4	16
47	16	4	8
49	4	1	8
61	2	2	8
62	4	2	2
65	2	0.5	1
66	4	2	16
67	8	0.5	8
Rifampicin	0.125	0.125	0.25
Isoniazid	1	8	2

Compared with the above-mentioned compounds, capuramycin analogues with phenyl-type substituents had relatively potent activity (Table 4). Compound **47** having a phenyl moiety proved to be a more potent inhibitor of translocase I (6.5 ng/mL) than natural compounds, capuramycin and A-500359A. Compound **48** lacking the NH group of the amide exhibited no activity as in the case of **6**. As compounds **47**, **49**, **61**–**62**, and **65**–**67** showed relatively potent antibacterial activities against *M. smegmatis*, these compounds along with capuramycin and A-500359A were further tested for their antibacterial activities against mycobacteria of clinically more significance, that is *Mycobacterium avium* NIHJ1605, *Mycobacterium intracellulare* ATCC1954 E-3, and *Mycobacterium kansasii* ATCC12478, and their activities were compared with those of two typical anti-TB drugs, rifampicin and isoniazid (Table 5). Capuramycin, A-500359A and **47**, which have a simple phenyl moiety, possessed almost similar MIC values ranging between 4 and 16 μg/mL. Among the capuramycin analogues, **65**,[12] which has a 3,4-difluorophenyl moiety, was found to have MIC values ranging between 0.5 and 2 μg/mL, demonstrating that **65** was less potent than rifampicin but equipotent to or more potent than isoniazid.

Among all the analogues mentioned so far, the antibacterial activities did not always parallel the translocase I inhibitory activities. This may be partially attributable to the difference in cell-permeability of the analogues as translocase I is located in the bacterial cell membrane. In conclusion, it was found that capuramycin analogues having phenyl-type substituents in place of the azepan-2-one moiety could be considered as candidates of novel anti-TB drugs based on their new mechanism of action. The in vivo antibacterial activity of these analogues will be published in a separate literature.[13]

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