Synthesis and Biological Activity of 3′-Hydroxy-5′-aminobenzoxazinorifamycin Derivatives[1)]

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As a part of our studies on the syntheses of benzoxazinorifamycin derivatives, 3′-hydroxy-5′-aminobenzoxazinorifamycin derivatives were synthesized, and tested for their antimicrobial activities. The antimicrobial activities of these compounds against gram-positive and gram-negative bacteria were almost identical to those of rifampicin (RFP) and rifabutain (RFB), however, antimicrobial activities against *Mycobacterium tuberculosis* were superior to RFP, while being similar to RFB. 3′-Hydroxy-5′-(4-alkyl-1-piperazinyl)benzoxazinorifamycin derivatives also had *in vitro* potent activities against *Mycobacterium avium* complex (MAC). Their minimal inhibitory concentration values against MAC were 2—256 times greater than RFP and RFB. Their *in vivo* efficacies against *M. tuberculosis* and MAC, after oral administration to mice, were superior to RFP and RFB, except for RFB against *M. tuberculosis* activity *in vivo*. Although they were absorbed from the gastrointestinal tract, their plasma levels were lower than that of RFP. Among these 5′-(4-alkyl-1-piperazinyl) derivatives, 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin,[2)] compound 19 (KRM-1648), was selected as the most promising and its preliminary pharmacokinetic characteristics in mice were investigated. Compound 19 was distributed much more in tissues, especially in spleen and lung, than in plasma and had a long elimination time from tissues.

Keywords rifamycin; 3′-hydroxy-5′-aminobenzoxazinorifamycin; KRM-1648; antimicrobial activity; *Mycobacterium avium* complex; *Mycobacterium tuberculosis*

In a previous paper[3)] we reported the synthesis and biological activities of 5′-aminobenzoxazinorifamycin derivatives, among which 5′-piperidinylbenzoxazinorifamycin was selected as the most potent compound. However, its *in vivo* efficacy against *Mycobacterium tuberculosis* after oral administration to mice was inferior to that of rifampicin (RFP).[4)] To search for derivatives with potent *in vivo* efficacies against *M. tuberculosis* and *Mycobacterium avium* complex (MAC), many 5′-aminobenzoxazinorifamycin derivatives having hydroxyl, alkyl, or other functional groups substituted at the 3′, 4′, and 6′-positions in the benzoxazine ring were synthesized.[1,5)] Some of the compounds in the series showed excellent antimicrobial activities against *M. tuberculosis* and MAC *in vitro* and *in vivo*. Both bacteria cause infections in human, especially in lung with predisposing factors, as well as in immunocompromised-hosts such as human immunodeficiency virus infected patients. There are few effective drugs for infections caused by MAC. Therefore it is mandatory to develop new drugs active against MAC.

In this paper, we report the synthesis and biological activities of 3′-hydroxy-5′-aminobenzoxazinorifamycin derivatives. After screening, 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin **19** (KRM-1648) was selected as a promising compound. Its plasma and tissue concentrations were measured in mice after a single oral

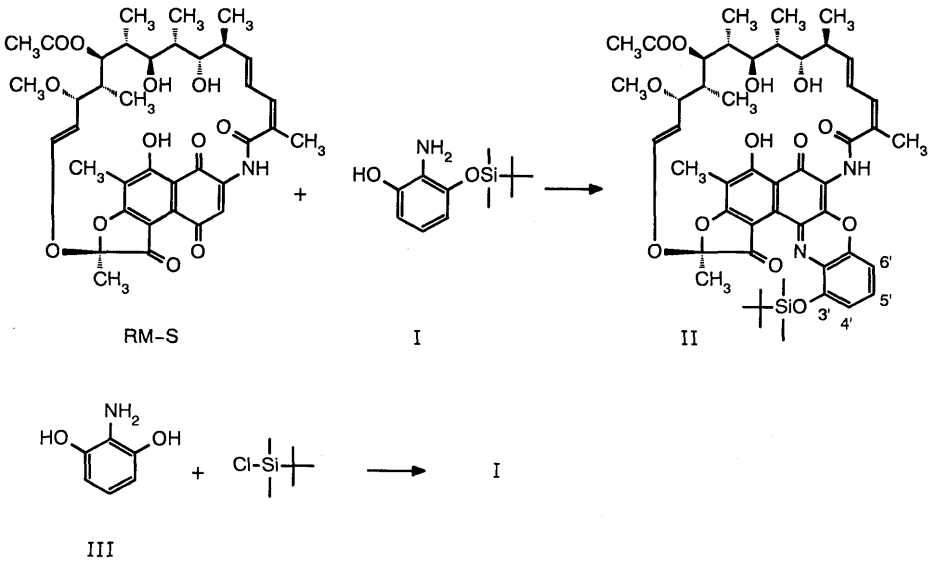


Chart 1

dose using high-performance liquid chromatography (HPLC).

Chemistry Among 3′, 4′, and 6′-hydroxy-5′-aminobenzoxazinorifamycin derivatives, only 3′-hydroxy derivatives were synthesized, because the 4′ and 6′-hydroxy derivatives were not stable at reaction conditions.

Rifamycin S (RM-S) reacted with 2-amino-3-*tert*-butyldimethylsilyloxyphenol (I) to yield 3′-*tert*-butyldimethylsilyloxybenzoxazinorifamycin (II, Chart 1). I was synthesized by mono-*O*-silylation of 2-aminoresorcinol (III, Chart 1). The *N*-silylated product of III was not observed in the silylation reaction. In the synthesis of II, the use of III instead of I furnished a reduction of RM-S (*i.e.* rifamycin SV) predominantly. Thus a redox reaction between RM-S and III occurred preferentially to a condensation reaction. When two hydroxyl groups in III were protected by *tert*-butyldimethylsilyl group, neither of the above reactions occurred and RM-S was recovered. Therefore, one hydroxyl group had to be protected by *tert*-butyldimethylsilyl group.

3′-Hydroxy-5′-aminobenzoxazinorifamycin derivatives (**1**—**26**) were synthesized from II and various secondary amines (Chart 2, Tables I and II). This addition reaction was reported previously[3] and *tert*-butyldimethylsilyl group was removed in the same reaction system. Among the secondary amines, 1-alkylpiperazines (**28**—**37**), except 1-methyl, 1-ethyl, and 1-*tert*-butylpiperazine, were synthesized by alkylation of piperazine with the corresponding alkyl bromide[6] (Table III). 1-*tert*-Butylpiperazine was synthesized according to the literature,[7] because the bulky *tert*-butyl group prevented the alkylation of piperazine. The other amines were commercially available.

Alkaline hydrolysis of the 3′-hydroxy-5′-aminobenzoxazinorifamycins, for example, compound **19**, gave the corresponding 25-deacetyl derivative, 25-deacetyl-3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin **27** (Chart 3).

Tables I and II summarize the introduced amino component, reaction time, isolated yield, melting point, crystallized solvent, elementary analyses, and *Rf* values by thin-layer chromatography (TLC) of the 3′-hydroxy-5′-aminobenzoxazinorifamycin derivatives (**1**—**26**). Table III summarizes the introduced alkyl group, reaction time, isolated yield, boiling point, and the proton nuclear magnetic resonance (^{1}H-NMR) spectrum signal which derive from the *N*-alkyl group of the synthesized 1-alkylpiperazines (**28**—**37**).

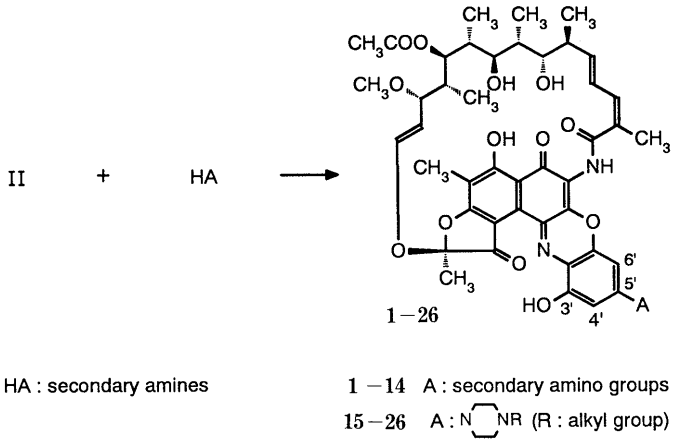


Chart 2

Biological Results and Discussion

The minimal inhibitory concentrations (MICs) against 9 bacteria[8] and the minimal effective dose (MED) after oral administration in mice against *Staphylococcus aureus* SMITH infection of newly synthesized 3′-hydroxy-5′-aminobenzoxazinorifamycin derivatives (compounds **1**—**27**) were determined. The MIC values of these compounds against gram-positive bacteria, gram-negative bacteria and mycobacteria, and MED values were compared with those of II (Chart 1), RFP and rifabutin (RFB, Chart 4). The results are summarized in Table IV.

Compounds **1**—**14** (Table I), which were synthesized from II and commercially available acyclic or cyclic amines were tested. All the compounds had potent activity against gram-positive bacteria (*i.e.*, *M. luteus*, *B. subtilis* and 2 strains of *S. aureus*), and their potencies were comparable to RFP and RFB. Compounds **1**—**6**, which have an acyclic amino group, showed slightly weaker activity than **7**—**14**, which have a cyclic amino group. Compound **3**, which has a phenyl group, reduced antimicrobial activity, however the pyridyl group in **5** retained antimicrobial activity. Compound **11**, having a 5′-(4-methyl-1-piperazinyl) group, had the most potent activity against gram-positive bacteria. However, **1**—**14** did not show potent activity against gram-negative bacteria (*i.e.*, *E. coli* and *K. pneumoniae*), as

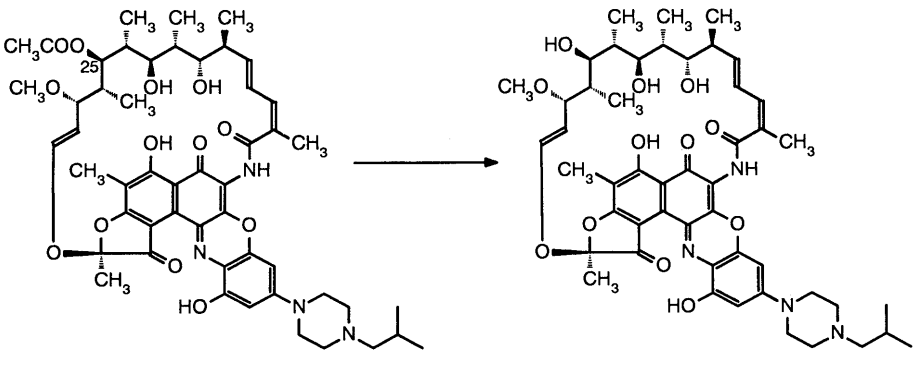


Chart 3

TABLE I. 3′-Hydroxy-5′-aminobenzoxazinorifamycin Derivatives

Compd. No.	5′-Amino group	Reaction time (h)	Yield[a] (%)	mp (°C) (Cryst. solv.)[b]	Formula	Analysis (%) Calcd (Found) C	H	N	TLC[c] *Rf*
1	$N(CH_3)_2$	89	31	220—225 (dec.) (E)	$C_{45}H_{53}N_3O_{13}\cdot H_2O$	62.71 (62.92)	6.43 6.35	4.87 4.74)	0.42
2	$N(CH_3)CH_2CH(CH_3)_2$	20	65	184—189 (dec.) (E, H)	$C_{48}H_{59}N_3O_{13}$	65.07 (65.07)	6.71 6.99	4.74 4.45)	0.54
3	$N(CH_3)CH_2CH_2Ph$	70	44	180—184 (dec.) (E, H)	$C_{52}H_{59}N_3O_{13}$	66.87 (66.57)	6.37 6.38	4.50 4.41)	0.55
4	$N(CH_3)CH_2CH_2N(CH_3)_2$	6	63	184—188 (dec.) (E)	$C_{48}H_{60}N_4O_{13}\cdot H_2O$	62.73 (62.70)	6.80 6.71	6.01 5.95)	0.16
5	$N(CH_3)CH_2CH_2$-(N-heterocycle)	72	51	174—180 (dec.) (C, H)	$C_{51}H_{58}N_4O_{13}\cdot H_2O$	64.27 (64.63)	6.35 6.11	5.88 5.88)	0.35
6	$N(CH_2CH_2OCH_3)_2$	168	35	178—182 (dec.) (E, H)	$C_{49}H_{61}N_3O_{15}$	63.15 (62.80)	6.60 6.68	4.51 4.25)	0.49
7	$N(CH_2)_3$	72	22	211—215 (dec.) (E)	$C_{46}H_{53}N_3O_{13}\cdot C_4H_8O_2$	63.61 (63.60)	6.51 6.23	4.45 4.72)	0.38
8	$N(CH_2)_4$	97	16	218—222 (dec.) (E)	$C_{47}H_{55}N_3O_{13}\cdot H_2O$	63.57 (63.60)	6.47 6.33	4.73 4.71)	0.43
9	$N(CH_2)_5$	44	57	206—209 (dec.) (E)	$C_{48}H_{57}N_3O_{13}\cdot 1.5H_2O$	63.28 (63.10)	6.64 6.54	4.61 4.53)	0.51
10	$N(CH_2)_2NH(CH_2)_2$	28	20	196—200 (dec.) (E)	$C_{47}H_{56}N_4O_{13}\cdot H_2O$	62.51 (62.13)	6.47 6.34	6.20 5.87)	0.06
11	$N(CH_2)_2NCH_3(CH_2)_2$	44	71	211—215 (dec.) (E)	$C_{48}H_{58}N_4O_{13}\cdot H_2O$	62.87 (63.06)	6.59 6.46	6.11 6.09)	0.21
12	$N(CH_2)_2NCHO(CH_2)_2$	24	48	214—219 (dec.) (C, H)	$C_{48}H_{56}N_4O_{14}\cdot 1.5H_2O$	61.33 (61.28)	6.33 6.14	5.96 5.90)	0.21
13	$N(CH_2)_2NCH_3(CH_2)_3$	44	33	195—200 (dec.) (E, H)	$C_{49}H_{60}N_4O_{13}\cdot C_4H_8O_2$	63.59 (63.42)	6.85 6.81	5.60 5.70)	0.16
14	$N(CH_2)_2O(CH_2)_2$	24	38	216—221 (dec.) (C, H)	$C_{47}H_{55}N_3O_{14}\cdot 2H_2O$	61.23 (61.55	6.44 6.11	4.56 4.52)	0.49

a) The yield was not optimized. *b*) C=chloroform, E=ethyl acetate, H=hexane. *c*) Chloroform–methanol (95:5, v/v).

TABLE II. 3′-Hydroxy-5′-(4-alkyl-1-piperazinyl)benzoxazinorifamycin Derivatives

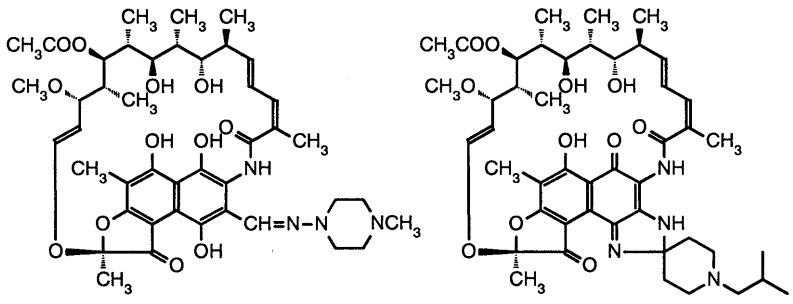
Compd. No.	4-Alkyl group	Reaction time (h)	Yield[a] (%)	mp (°C) (Cryst. solv.)[b]	Formula	Analysis (%) Calcd (Found) C	H	N	TLC[c] *Rf*
15	CH_2CH_3	24	72	208—212 (dec.) (C, H)	$C_{49}H_{60}N_4O_{13}\cdot 2H_2O$	62.01 (62.02)	6.80 6.48	5.90 5.87)	0.30
16	$(CH_2)_2CH_3$	21	58	203—207 (dec.) (D, H)	$C_{50}H_{62}N_4O_{13}$	64.78 (64.50)	6.74 6.81	6.04 6.25)	0.37
17	$CH(CH_3)_2$	25	70	205—210 (dec.) (E, H)	$C_{50}H_{62}N_4O_{13}\cdot H_2O$	63.54 (63.88)	6.83 6.90	5.93 5.86)	0.33
18	$(CH_2)_3CH_3$	42	38	199—204 (dec.) (E, C, H)	$C_{51}H_{64}N_4O_{13}$	65.09 (64.70)	6.85 6.89	5.95 5.86)	0.34
19	$CH_2CH(CH_3)_2$	24	53	195—200 (dec.) (C, H)	$C_{51}H_{64}N_4O_{13}\cdot H_2O$	63.87 (64.02)	6.94 6.81	5.84 5.81)	0.47
20	$CH(CH_3)CH_2CH_3$	22	64	198—203 (dec.) (E, H)	$C_{51}H_{64}N_4O_{13}\cdot 0.5C_4H_8O_2$	64.62 (64.62	6.96 7.03	5.69 5.91)	0.41
21	$C(CH_3)_3$	48	79	211—216 (dec.) (E, H)	$C_{51}H_{64}N_4O_{13}\cdot H_2O$	63.87 (64.19	6.94 6.95	5.84 5.72)	0.39
22	$(CH_2)_2CH(CH_3)_2$	40	35	198—202 (dec.) (C, H)	$C_{52}H_{66}N_4O_{13}\cdot 0.5H_2O$	64.78 (64.71	7.00 7.00	5.81 5.69)	0.48
23	$(CH_2)_2CH=CH_2$	24	65	191—196 (dec.) (C, H)	$C_{51}H_{62}N_4O_{13}\cdot H_2O$	64.00 (63.85	6.74 6.66	5.85 5.80)	0.40
24	$CH_2CH=C(CH_3)_2$	29	36	202—207 (dec.) (E, H)	$C_{52}H_{64}N_4O_{13}\cdot H_2O$	64.31 (64.67	6.85 6.83	5.77 5.76)	0.39
25	$CH_2CH_2OCH_3$	51	24	190—196 (dec.) (C, H)	$C_{50}H_{62}N_4O_{14}\cdot H_2O$	62.49 (62.65	6.71 6.56	5.83 5.83)	0.30
26	$CH_2CH_2OCH_2CH_3$	72	47	192—194 (dec.) (E, H)	$C_{51}H_{64}N_4O_{14}\cdot 2H_2O$	61.68 (61.94	6.90 6.71	5.64 5.50)	0.33

a) The yield was not optimized. *b*) C=chloroform, D=dichloromethane, E=ethyl acetate, H=hexane. *c*) Chloroform–methanol (95:5, v/v).

TABLE III. 1-Alkylpiperazines

Compd. No.	1-Alkyl group	Reaction time (h)	Yield[a] (%)	bp (°C) Pressure (mmHg)	^{1}H-NMR chemical shifts[b] in $CDCl_3$ δ (J=Hz)
28	$(CH_2)_2CH_3$	21	45	176—183 760	0.89 (3H, t, J=7, CH_3), 1.50 (2H, m, CH_2), 2.30 (2H, t, J=7, NCH_2)
29	$CH(CH_3)_2$	70	31	34—47 10	1.03 (6H, d, J=7, CH_3), 2.63 (1H, m, CH)
30	$(CH_2)_3CH_3$	14	61	48—60 10	0.93 (3H, br, CH_3), 1.76 (4H, br, CH_2CH_2)
31	$CH_2CH(CH_3)_2$	72	44	52—58 10	0.91 (6H, d, J=7, CH_3), 1.80 (1H, m, CH), 2.07 (2H, d, J=7, NCH_2)
32	$CH(CH_3)CH_2CH_3$	66	43	43—47 10	0.89 (3H, t, J=7, CH_3), 0.98 (3H, d, J=7, CH_3), 1.63 (2H, m, CH_2), 2.40 (1H, m, NCH)
33	$(CH_2)_2CH(CH_3)_2$	17	62	57—65 10	0.90 (6H, d, J=7, CH_3), 1.45 (3H, m, CH_2CH)
34	$(CH_2)_2CH=CH_2$	23	38	42—47 10	5.03 (2H, m, $=CH_2$), 5.73 (1H, m, $-CH=$)
35	$CH_2CH=C(CH_3)_2$	17	32	66—74 10	1.63 (6H, d, J=7, CH_3), 5.27 (1H, m, $-CH=$)
36	$CH_2CH_2OCH_3$	17	15	72—85 10	3.36 (3H, s, CH_3), 3.53 (2H, t, J=6, CH_2O)
37	$CH_2CH_2OCH_2CH_3$	19	14	67—82 10	1.18 (3H, t, J=7, CH_3), 3.56 (4H, m, CH_2O)

a) The yield was not optimized. *b*) The signals which derive from *N*-alkyl group are shown.



rifampicin (RFP) rifabutin (RFB)

Chart 4

did RFP and RFB. The activity of **1**—**14** except **3** against *M. smegmatis* was more potent than that of RFP, and similar to RFB. With the exception of **10**, the compounds had potencies against *M. tuberculosis* which were stronger than that of RFP and similar or superior to RFB, except **12** and **13**. All compounds (**1**—**14**) showed a greater activity against MAC than RFP, and most were more active than RFB. However, the antimicrobial activity against MAC of II was equal to that of RFP. It is noteworthy that the introduction of an amino component into the benzoxazine ring, especially a 1-methylpiperazinyl group (**11**), enhanced anti-MAC activity. MED values of these compounds (**1**—**14**) were less than 2.5 mg/kg, indicating that they were absorbed from the gastrointestinal tract. Compound **11**, which has a 5′-(4-methyl-1-piperazinyl) group, showed especially excellent MED value.

Next, we focused on the 3′-hydroxy-5′-(4-alkyl-1-piperazinyl)benzoxazinorifamycin derivatives, and various of these (**15**—**26**) were synthesized (Table II) and tested (Table IV). They also showed potent activity against gram-positive bacteria, but did not against gram-negative bacteria, as true of RFP and RFB. Compounds **15**—**26** showed potencies against *M. smegmatis* and *M. tuberculosis* which were stronger than RFP, and slightly stronger or identical to RFB. All compounds (**15**—**26**) showed excellent activity against MAC. The MIC values were 8—256 times superior to RFP, and 2—64 times superior to RFB. Their *in vivo* efficacies (MED) against *S. aureus* SMITH were the same as or less than **11**, which has a 5′-(4-methyl-1-piperazinyl) group.

The 25-deacetylated **27** (Chart 3) had slightly stronger activity against gram-positive bacteria, but similar activity against gram-negative bacteria and mycobacteria as the parent **19**. Details of *in vitro* antimicrobial activities against *M. tuberculosis* and MAC were reported by Yamamoto *et al.*[9] and Saito *et al.*[10]

Based on the results of their *in vitro* antimicrobial activities and their *in vivo* efficacy against *S. aureus* SMITH, 12 of the 3′-hydroxy-5′-(4-alkyl-1-piperazinyl)benzoxazinorifamycin derivatives (**11**, **15**—**24** and **26**) and the 5′-morpholinyl derivative (**14**) were tested for their therapeutic effect on *M. tuberculosis* H37Rv in mice. The results are shown in Table V. The tests were done 4 times using RFP as a control compound. Compounds **16**—**24**,

TABLE IV. Antimicrobial Activities of 3′-Hydroxy-5′-aminobenzoxazinorifamycin Derivatives

Compd. No.	Minimal inhibitory concentration (μg/ml)									MED[a] (mg/kg)
	Gram-positive				Gram-negative		Mycobacteria			
	Ml[b] IFO 12708	*Bs*[c] IFO 3134	*Sa*[d] IFO 12732	*Sa*[d] SMITH	*Ec*[e] IFO 12734	*Kp*[f] IFO 3512	*Ms*[g] ATCC 607	*Mt*[h] H37Rv	MAC[i] 31F093T	*Sa*[d] SMITH
1	0.01	0.01	0.005	0.005	>10	>10	2.5	0.008	0.3	0.5
2	0.01	0.04	0.01	0.005	>10	>10	5	0.008	0.6	2.5
3	0.04	0.16	0.04	NT	>10	>10	>10	NT	NT	NT
4	0.02	0.02	0.01	0.01	5	5	5	0.07	1.25	0.5
5	0.02	0.02	0.01	0.01	>10	>10	2.5	0.017	1.25	0.1
6	0.02	0.02	0.02	0.02	>10	>10	2.5	0.004	0.6	0.25
7	0.0013	0.005	0.0013	0.0013	>10	>10	2.5	0.008	0.15	0.5
8	0.005	0.02	0.0025	0.0025	>10	>10	0.63	0.017	0.15	1.0
9	0.005	0.02	0.005	NT	>10	>10	1.25	0.035	0.15	NT
10	0.02≧	0.08	0.02≧	NT	2.5	2.5	5	0.6	0.6	2.5
11	0.0013	0.0025	0.0006	0.0013	5	5	1.25	0.017	0.035	0.05
12	0.0025	0.02	0.0013	0.0025	10	10	5	0.3	1.25	1.0
13	0.005	0.02	0.0006	0.0013	5	2.5	2.5	0.15	0.6	0.5
14	0.0025	0.01	0.0013	0.0025	>10	>10	2.5	0.008	0.3	0.1
15	0.01	0.01	0.005	0.005	10	>10	0.31≧	0.002	0.07	0.25
16	0.01	0.02	0.005	0.01	>10	>10	1.25	0.07	0.035	0.1
17	0.01	0.02	0.005	0.01	10	>10	1.25	0.017	0.07	0.1
18	0.005	0.04	0.0025	0.005	>10	>10	5	0.035	0.07	0.25
19	0.005	0.02	0.0025	0.0025	>10	>10	2.5	0.017	0.07	1.0
20	0.005	0.01	0.0013	0.0025	>10	>10	2.5	0.017	0.035	0.25
21	0.02≧	0.02≧	0.02≧	NT	>10	>10	1.25	0.008	0.017	0.5
22	0.01	0.04	0.0025	0.005	>10	>10	5	0.07	0.15	2.5
23	0.02≧	0.02≧	0.02≧	NT	>10	>10	2.5	0.017	0.07	1.0
24	0.005	0.04	0.0025	0.0025	>10	>10	2.5	0.035	0.035	0.5
25	0.0025	0.005	0.0006	0.0013	>10	>10	2.5	0.035	0.15	0.1
26	0.01	0.02	0.005	0.005	>10	>10	2.5	0.07	0.6	0.05
27	0.0025	0.005	0.0013	0.0025	>10	>10	2.5	0.017	0.07	0.25
II[j]	0.01	0.02	0.005	0.005	>10	>10	0.63	0.035	5	1.0
RFP[k]	0.005	0.01	0.005	0.005	10	5	10	0.6	5	0.25
RFB[l]	0.01	0.02	0.01	0.01	10	5	2.5	0.035	1.25	1.0

a) Minimal effective dose. *b*) *Micrococcus luteus*. *c*) *Bacillus subtilis*. *d*) *Staphylococcus aureus*. *e*) *Escherichia coli*. *f*) *Klebsiella pneumoniae*. *g*) *Mycobacterium smegmatis*. *h*) *Mycobacterium tuberculosis*. *i*) *Mycobacterium avium* complex. *j*) 3′-*tert*-Butyldimethylsilyloxybenzoxazinorifamycin. *k*) Rifampicin. *l*) Rifabutin. NT: not tested.

TABLE V. Therapeutic Effect of 3′-Hydroxy-5′-aminobenzoxazinorifamycin Derivatives on *Mycobacterium tuberculosis* H37Rv in ddY Mice[a]

Compd. No.	Run 1[b]	Run 2[c]	Run 3[d]	Run 4[d]
11	20%			
14			0%	
15		93%		
16			100%	
17			100%	
18			100%	
19	100%	100%		100%
20			100%	
21			100%	
22				100%
23			100%	
24				100%
26				90%
RFP[e]	30%	67%	40%	70%
RFB[f]		100%		

a) Male ddY mice were inoculated *via* tail vein with approximately 1×10^9 colony forming unit of *M. tuberculosis* H37Rv. Treatment of the mice with each drug (daily oral administration of 10 mg/kg except Sunday) was started 24 h after the inoculation, and continued until the 40th day of infection. Each result shows the survival ratio of the tested mice after the 40th day. *b*) 20 ddY mice were tested for each drug. *c*) 15 ddY mice were tested for each drug. *d*) 10 ddY mice were tested for each drug. *e*) Rifampicin. *f*) Rifabutin.

TABLE VI. Therapeutic Effect of 3′-Hydroxy-5′-(4-alkyl-1-piperazinyl)-benzoxazinorifamycin Derivatives on *Mycobacterium avium* Complex 31F093T in Beige Mice[a]

Compd. No.	logCFU from lung at 6 and 12 weeks after infection		
	0	6	12 week
Control	4.35	7.13	8.18
11		5.15 (1.98)[b]	6.19 (1.99)
17		4.11 (3.02)	6.19 (1.99)
19		3.27 (3.86)	5.51 (2.67)
RFP[c]		6.49 (0.64)	7.74 (0.44)
RFB[d]		6.27 (0.86)	7.54 (0.64)
Control	4.83	7.36	8.68
16		5.30 (2.06)	6.95 (1.73)
20		5.24 (2.12)	6.86 (1.82)
21		5.13 (2.23)	6.58 (2.10)
RFP[c]		7.19 (0.17)	8.11 (0.57)

a) Female beige mice were inoculated *via* tail vein with approximately 1×10^8 colony forming unit (CFU) of *M. avium* complex 31F093T, a mouse-virulent strain. Treatment of the mice with each drug (daily oral administration of 20 mg/kg except Sunday) was started 24 h after the inoculation, and continued throughout 12 weeks of infection. For evaluation of the therapeutic effect of the drug, CFUs of the infecting organisms recovered from the lungs of 3 mice were determined following the course of infection. *b*) The values in parentheses show the difference of logCFU between untreated mice and treated mice in each test. *c*) Rifampicin. *d*) Rifabutin.

TABLE VII. Plasma Levels of 3′-Hydroxy-5′-(4-alkyl-1-piperazinyl)benzoxazinorifamycin Derivatives after Oral Administration to Mice[a]

Compd. No.	Plasma level (μg/ml)[b]				AUC[c] (μg·h/ml)
	1	5	8	24 h	
11	0.23	0.23	0.10	ND	2.3
16	0.13	0.18	0.10	0.01	2.0
17	ND	0.05	0.15	ND	1.6
19	0.04	0.16	0.51	0.21	7.2
20	0.05	0.19	0.07	0.07	2.0
21	0.03	0.24	0.08	0.03	1.9
RFP[d]	11.13	14.84	12.57	0.80	205.6
RFB[e]	1.27	0.90	0.51	0.04	11.5

a) Dose: 20 mg/kg. *b*) Means of 2 animals. *c*) Area under the plasma concentration curve within 24 h, and values are calculated by trapezoidal rule using the data of mean concentration. *d*) Rifampicin. *e*) Rifabutin. ND: not detected.

which have a 5′-(4-C_3-C_5-alkyl-1-piperazinyl) group, and RFB showed excellent therapeutic effects. Death was not observed until the 40th day of infection. The survival ratio of RFP was 30—70%.

Table VI shows the therapeutic effects of 6 compounds (**11**, **16**, **17**, **19**—**21**) on MAC 31F093T infection. The tests were done twice. The test compounds, 3′-hydroxy-5′-(4-alkyl-1-piperazinyl)benzoxazinorifamycin derivatives, caused a 1.73—3.86 logarithm of colony forming unit (CFU) decrease at week 6 and 12, compared to those for untreated mice, while RFP and RFB failed to show such an effect; differences of their logCFUs were 0.17—0.86. Details of the *in vivo* antimicrobial activities against *M. tuberculosis* and MAC were reported by Kuze *et al.*[11] and Tomioka *et al.*[10b]

Table VII shows plasma levels at 1, 5, 8 and 24 h, and the area under the plasma concentration curve (*AUC*) in **11**, **16**, **17**, **19**—**21** after oral administration to mice. The plasma levels were determined by HPLC. Plasma levels of the test compounds were lower than RFP levels but only moderately lower than RFB levels, whereas therapeutic effects against *M. tuberculosis* and MAC were excellent. This suggests these compounds have good levels in tissue.

Considering the results of *in vivo* efficacy tests, plasma levels, tissue levels, toxicological studies, stability and facility of chemical synthesis, **19** (Chart 3, KRM-1648) was selected as the representative compound and a pharmacokinetic study was carried out.

Plasma and tissues levels of **19**, RFP and RFB in male ddY mice after a single oral dose (20 mg/kg) are shown in Table VIII. Compound **19** and RFB showed high tissue levels and low plasma levels while RFP showed high levels in both plasma and tissues. The *AUC* values in tissues were higher in the following order: **19**, RFP, RFB in lung, RFP, **19**, RFB in liver and kidney, **19**, RFB, RFP in spleen. Although the peak concentrations of **19** in liver and kidney were lower than RFP and RFB concentrations, those in lung or spleen were equal to or greater than those of RFP or RFB levels. Furthermore, the concentrations of **19** in tissues 48 h after dosing were much higher than those of RFP and RFB, except RFP levels in liver, indicating that **19** has a long elimination time from tissues. Based on these observations, we conclude that the tissue-distribution properties of **19** are comparable to RFP, and superior to RFB. Compound **19** was more effective than RFP and RFB in experimental infections induced by *M. tuberculosis* and MAC in mice, except in the case of RFB against *M. tuberculosis* (Tables V and VI). The good tissue-distribution properties of **19** may be a contributive reason for its good efficacy *in vivo*.

TABLE VIII. Plasma and Tissue Levels of **19**, RFP[a] and RFB[b] after Oral Administration to Mice[c]

Plasma or Tissues	Time[d] (h)	Plasma, tissue levels[e] (μg/ml or g) and their AUC[f] values (μg·h/ml or g)		
		19	RFP	RFB
Plasma	1	0.18±0.10	7.92±2.01	1.13±0.14
	3	0.26±0.04	13.04±2.88	0.95±0.15
	5	0.30±0.06	12.33±3.12	0.96±0.18
	8	0.61±0.07	9.66±0.59	0.43±0.03
	12	0.17±0.02	7.54±0.82	0.08±0.04
	24	0.15±0.05	0.69±0.13	0.01±0.01
	48	0.11±0.03	0.03±0.02	ND
	AUC	9.1	175.7	8.3
Lung	1	1.11±0.24	5.29±1.18	7.83±0.71
	3	2.59±0.46	9.35±1.69	6.74±0.79
	5	4.84±0.72	8.70±1.50	5.28±1.17
	8	4.48±0.82	6.50±0.62	2.60±0.08
	12	6.71±0.22	4.89±0.70	0.84±0.24
	24	2.99±0.74	0.32±0.11	0.13±0.03
	48	0.67±0.11	0.07±0.06	0.06±0.02
	AUC	150.2	116.9	57.3
Liver	1	3.52±0.70	56.22±9.78	12.01±2.05
	3	5.03±0.77	63.19±12.93	6.58±0.60
	5	6.42±0.42	71.18±9.05	5.11±0.86
	8	5.79±0.44	64.06±8.29	2.99±0.26
	12	4.94±0.24	59.09±4.36	0.99±0.19
	24	1.78±0.81	12.87±2.77	0.03±0.02
	48	0.35±0.06	1.31±0.38	ND
	AUC	127.4	1333.0	62.9
Kidney	1	1.14±0.18	8.30±2.02	6.98±0.96
	3	2.38±0.22	15.51±2.36	5.22±0.58
	5	2.89±0.13	14.36±1.97	5.11±0.96
	8	3.61±1.17	11.36±0.23	2.29±0.05
	12	2.43±0.15	9.00±0.93	0.77±0.16
	24	1.07±0.24	0.67±0.22	0.08±0.05
	48	0.21±0.05	ND	ND
	AUC	67.6	203.2	49.3
Spleen	1	1.19±0.36	1.10±0.07	5.21±0.80
	3	3.35±1.23	2.29±0.90	4.00±0.38
	5	8.52±0.74	1.94±0.72	3.11±0.60
	8	9.56±2.20	1.04±0.09	1.66±0.15
	12	16.29±1.33	1.54±0.37	0.57±0.14
	24	5.23±2.46	0.03±0.02	0.03±0.03
	48	0.40±0.16	ND	ND
	AUC	292.5	27.6	34.5

a) Rifampicin. *b*) Rifabutin. *c*) Dose: 20 mg/kg. *d*) Time after administration. *e*) Means±standard error of 4 animals. *f*) Area under the plasma concentration curve within 48 h, and values are calculated by trapezoidal rule using the data of mean concentration. ND: not detected.

Compound **19** did not exhibit any significant toxicity in mice when administered orally once a day for 5 d at a dose of 600 mg/kg, while RFP exhibited toxicity in liver under the same conditions.[12]

In conclusion, 3′-hydroxy-5′-aminobenzoxazinorifamycins were synthesized *via* a two-step reaction from RM-S, and their *in vitro* and *in vivo* antimicrobial activity and plasma levels were tested. From among these compounds, 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin **19** (KRM-1648) was selected as the most potent and its pharmacokinetic properties were studied in mice using RFP and RFB as control compounds. Compound **19** demonstrated high tissue affinity and a long elimination

time from tissues.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points are uncorrected. Fast atom bombardment mass spectrum (FAB-MS) was measured with a VG ZAB-HF mass spectrometer. ^{1}H-NMR spectra were determined at 90 MHz on a Varian EM-390 NMR spectrometer. Chemical shifts are expressed in ppm values relative to tetramethylsilane as an internal standard. Significant ^{1}H-NMR data are described in the order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet) and coupling constants in Hz. Column chromatography was done with a Wakogel C-200 (Wako Pure Chemical Industries, Ltd.). General methods to prepare the compounds (**1**—**26**) are described using **19** as a typical example. ^{1}H-NMR spectral data of compounds (**1**—**27**) are shown in Table IX. General methods to prepare 1-alkylpiperazines (**28**—**37**) are described using **31** as a typical example. RFB was synthesized according to the literature.[13]

2-Amino-3-*tert*-butyldimethylsilyloxyphenol (I) To a suspension of 32.3 g (0.2 mol) of 2-aminoresorcinol hydrochloride and 69.7 ml (0.5 mol) of triethylamine in 2000 ml of *N*,*N*-dimethylformamide (DMF) at −15 °C was added dropwise 36.2 g (0.24 mol) of *tert*-butylchlorodimethylsilane in 1000 ml of DMF solution for 6 h. After the addition the suspension was stirred for 15 h at room temperature, and then insoluble materials were filtered. The filtrate was evaporated to dryness, and then the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with water and brine, dried and evaporated to dryness. The residue was crystallized from hexane, to yield 37.9 g (79%) of I, mp 95 °C. *Anal.* Calcd for $C_{12}H_{21}NO_2Si$: C, 60.21; H, 8.84; N, 5.85. Found: C, 60.01; H, 8.86; N, 5.90. ^{1}H-NMR ($CDCl_3$) δ: 0.23 (6H, s, $SiCH_3$), 1.02 (9H, s, $C(CH_3)_3$), 4.27 (3H, br, NH_2 and OH), 6.43 (3H, m, arom H).

TABLE IX. ^{1}H-NMR Data of 3′-Hydroxy-5′-aminobenzoxazinorifamycin Derivatives[a]

Compd. No.	Chemical shifts in $CDCl_3$ δ ppm J (Hz)
1	3.06 (6H, s, NCH_3)
2	0.97 (6H, d, J=7, CH_3), 3.16 (3H, s, NCH_3), 3.28 (2H, br, NCH_2)
3	3.06 (3H, s, NCH_3), 3.76 (2H, br, NCH_2), 7.29 (5H, m, phenyl H)
4	2.29 (6H, s, NCH_3), 3.17 (3H, s, NCH_3), 3.60 (2H, br, NCH_2)
5	3.06 (3H, s, NCH_3), 3.96 (2H, br, NCH_2), 7.22 (2H, m, pyridyl H), 7.67 (1H, m, pyridyl H), 8.63 (1H, d, J=4, pyridyl H)
6	3.36 (6H, s, OCH_3), 3.70 (8H, br, NCH_2CH_2O)
7	4.17 (4H. br, NCH_2)
8	2.10 (4H, br, CH_2), 3.51 (4H, br, NCH_2)
9	1.73 (6H, br, CH_2), 3.63 (4H, br, NCH_2)
10	3.64 (4H, br, NCH_2)
11	2.59, 3.65 (each 4H, br, NCH_2)
12	3.70 (8H, br, NCH_2), 8.06 (1H, s, CHO)
13	3.73 (4H, br, NCH_2)
14	3.67 (4H, br, NCH_2), 3.86 (4H, br, OCH_2)
15	2.60, 3.70 (each 4H, br, NCH_2)
16	2.61, 3.57 (each 4H, br, NCH_2)
17	1.08 (6H, d, J=7, CH_3), 2.73, 3.73 (each 4H, br, NCH_2)
18	2.70, 3.78 (each 4H, br, NCH_2)
19	0.86 (6H, d, J=7, CH_3), 2.54, 3.67 (each 4H, br, NCH_2)
20	2.68, 3.67 (each 4H, br, NCH_2)
21	1.06 (9H, s, CH_3), 2.74, 3.69 (each 4H, br, NCH_2)
22	0.90 (6H, d, J=6, CH_3), 2.62, 3.73 (each 4H, br, NCH_2)
23	3.83 (4H, br, NCH_2), 5.17 (2H, m, $=CH_2$), 5.93 (1H, m, $-CH=$)
24	2.03 (6H, s, CH_3), 2.63, 3.68 (each 4H, br, NCH_2)
25	2.67 (6H, br, NCH_2), 3.39 (3H, s, OCH_3), 3.52 (2H, t, J=4, OCH_2), 3.69 (4H, br, NCH_2)
26	1.20 (3H, t, J=7, CH_3), 3.57 (4H, br, NCH_2)
27	0.90 (6H, d, J=7, CH_3)

a) Signals which derive from the introduced amino group are shown.

3′-*tert*-Butyldimethylsilyloxybenzoxazinorifamycin (II) To a solution of 54.1 g (78 mmol) of RM-S in 900 ml of toluene at room temperature was added 18.6 g (78 mmol) of I. Afrer the addition the solution was stirred for 17 h at room temperature, and then evaporated to dryness. The residue was dissolved in 700 ml of ethanol, and 25.0 g (280 mmol) of manganese dioxide was added to the ethanol solution. The suspension was stirred for 30 min at room temperature. After filtration of insoluble materials, the filtrate was evaporated to dryness. The residue was purified by silica-gel column chromatography using ethyl acetate–hexane (1 : 1, v/v) as an eluent, to yield 37.0 g (52%) of II, mp 175—178 °C (dec.). *Anal.* Calcd for $C_{49}H_{62}N_2O_{13}Si$: C, 64.31; H, 6.83; N, 3.06. Found: C, 64.13; H, 6.81; N, 3.06. ^{1}H-NMR ($CDCl_3$) δ: 0.26 (6H, s, $SiCH_3$), 0.58, 0.83 (each 3H, d, J=7, $CHC\underline{H}_3$), 1.03 (9H, s, $C(CH_3)_3$), 1.78, 2.03, 2.11, 2.30 (each 3H, s, CH_3), 3.05 (3H, s, OCH_3), 6.85—7.43 (3H, m, arom H), 7.51 (1H, br, NH), 14.18 (1H, s, phenol OH).

3′-Hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin (19) To a solution of 80.0 g (87.4 mmol) of II in 800 ml of dimethyl sulfoxide at room temperature was added 24.9 g (174.8 mmol) of 1-isobutylpiperazine and 80.0 g (920 mmol) of manganese dioxide. After the addition the suspension was stirred for 24 h at room temperature, and then ethyl acetate was added and insoluble materials were filtered. The filtrate was washed with water and brine, dried and evaporated to dryness. The residue was purified by silica-gel column chromatography using chloroform–methanol (98 : 2, v/v) as an eluent, and then crystallized from chloroform–hexane, to yield 43.3 g (53%) of **19**, mp 195—200 °C (dec.). FAB-MS *m/z*: 941 (M^++H).

1-Isobutylpiperazine (31) To a solution of 430.7 g (5 mol) of piperazine and 348.6 ml (2.5 mol) of triethylamine in 3000 ml of ethanol was added dropwise 342.5 g (2.5 mol) of 1-bromo-2-methylpropane at room temperature. The solution was refluxed for 72 h, and then the ethanol was evaporated. After filtration of insoluble materials, the filtrate was evaporated to dryness. The residue was dissolved in water and extracted 3 times with dichloromethane. The combined dichloromethane layer was evaporated to dryness, and then the residue was distilled under reduced pressure (bp 52—58 °C (10 mmHg)), to yield 156.7 g (44%) of 1-isobutylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (6H, d, J=7, CH_3), 1.40 (1H, s, NH), 1.76 (1H, m, CH), 2.07 (2H, d, J=7, $C\underline{H}_2CH$), 2.33, 2.55 (each 4H, t, J=5, NCH_2).

25-Deacetyl-3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin (27) To a solution of 0.96 g (24 mmol) of sodium hydroxide in 18 ml of 50% (v/v) aqueous ethanol at room temperature was added 1.05 g (1.2 mmol) of **19**. After the addition the solution was stirred for 2 h at room temperature, and then the solution was neutralized with dilute hydrochloric acid and extracted 3 times with chloroform. The chloroform extracts were evaporated to dryness. The residue was purified by silica-gel column chromatography using chloroform–methanol (98 : 2, v/v) as an eluent, and then crystallized from ethyl acetate–hexane, to yield 0.70 g (69%) of **27**, mp 190—195 °C. *Anal.* Calcd for $C_{49}H_{62}N_4O_{12}\cdot 1/2C_4H_8O_2$: C, 64.95; H, 7.05; N, 5.94. Found: C, 64.91; H, 6.95; N, 5.99.

Antimicrobial Activity 1) *In vitro* antimicrobial activities (MIC) of the synthesized compounds were tested as previously described.[3,8,9] MICs were defined as the lowest concentration of the compounds that inhibited growth of bacteria after incubation at 37 °C for 17 h for gram-positive and gram-negative bacteria, 41 h for *M. smegmatis*, 28 d for *M. tuberculosis* and 14 d for MAC. 2) *In vivo* antimicrobial activity against *S. aureus* SMITH was evaluated as follows. Female ICR mice (5 per dose) were inoculated intraperitoneally with 10 times the median lethal dose of *S. aureus* (approximately 5×10^4 CFU/mouse). Each compound was administered orally to mice 1 h after infection. Surviving mice were checked at 3 d after infection. The MED (mg/kg) means the dose required for protecting more than 50% of infected mice from death. 3) *In vivo* antimicrobial activities against *M. tuberculosis* H37Rv and MAC 31F093T were tested as previously described.[11] The anti-*M. tuberculosis* activity of each compound was evaluated by the survival ratio of the mice on the 40 d after infection. The anti-MAC activity of each compound was evaluated by the logarithm of the average CFUs in the lungs of 3 mice at 6 and 12 weeks after infection.

Determination of Plasma and Tissue Levels Compound **11**, **16**, **17**, **19**—**21**, RFP or RFB suspended in 2.5% gum arabic containing 0.2% polyoxyethylene (20) sorubitan monooleate (Tween 80) was administered orally to male ddY mice (35—45 g) at the dose of 20 mg/kg. At different intervals, 2 or 4 mice were sacrificed, and plasma and tissue samples were obtained. Plasma was purified with a Bond Elute (Analytichem International Co.) C8 cartridge (for **16** and **19**) or C18 cartridge (for **11**, **17**, **20**, **21**, RFP and RFB). Tissue samples were homogenized in 0.2 M acetate buffer, pH 4.0 (for **19**) or 2% sodium ascorbate plus

phosphate-buffered saline (PBS), pH 7.4 (for RFP) or PBS, pH 7.4 (for RFB) and then extracted with acetone (for **19** and RFB) or methanol (for RFP). The extracts were evaporated to dryness. The residues were dissolved in methanol and diluted with PBS (for **19** and RFP) or chloroform (for RFB). The solution of tissue extracts was purified with a Bond Elute C18 cartridge (for **19** and RFP) or SI cartridge (for RFB). Drug concentrations were determined by HPLC.

References and Notes

1) A part of this paper was presented at the 110th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, August 1990.
2) Chemical name: 5,12-Dihydroxy-2,4-dimethyl-10-[4-(2-methylpropyl)-1-piperazinyl]-2,7-[oxy(5-acetoxy-7,9-dihydroxy-3-methoxy-4,6,8,10,14-pentamethyl-15-oxo-1,11,13-pentadecatriene-1,15-diyl)imino]-6*H*-benzofuro[4,5-*a*]phenoxazine-1(2*H*), 6-dione.
3) T. Yamane, T. Hashizume, K. Yamashita, K. Hosoe, T. Hidaka, K. Watanabe, H. Kawaharada and S. Kudoh, *Chem. Pharm. Bull.*, **40**, 2707 (1992).
4) T. Yamane, T. Hashizume, K. Yamashita, K. Hosoe, K. Kuze and K. Watanabe, Eur. Patent 336914 (1990) [*Chem. Abstr.*, **113**, 171779*s* (1990)].
5) T. Yamane, F. Kano, H. Kondo, K. Yamashita, K. Hosoe, K. Watanabe, S. Kudoh and S. Hosojima, The 107th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1987; T. Yamane, K. Yamashita, K. Hosoe, K. Watanabe and S. Kudoh, Program and Abstracts of the 27th ICAAC, New York, Oct. 1987, Abstract No. 279, p. 145.
6) D. K. Yung, L. G. Chatten and D. P. Macleod, *J. Pharm. Sci.*, **57**, 2073 (1968).
7) M. J. Cook, R. A. Y. Jones, A. R. Katritzky, M. M. Mañas, A. C. Richards, A. J. Sparrow and D. L. Trepanier, *J. Chem. Soc., Perkin Trans. 2*, **1973**, 325.
8) MIC Committee of the Japan Society of Chemotherapy, *Chemotherapy* (Tokyo), **29**, 76 (1981).
9) T. Yamamoto, R. Amitani, F. Kuze and K. Suzuki, *Kekkaku*, **65**, 805 (1990).
10) *a*) H. Saito, H. Tomioka, K. Sato, M. Emori, T. Yamane, K. Yamashita, K. Hosoe and T. Hidaka, *Antimicrob. Agents Chemother.*, **35**, 542 (1991); *b*) H. Tomioka, H. Saito, K. Sato, T. Yamane, K. Yamashita, K. Hosoe, K. Fujii and T. Hidaka, *ibid.*, **36**, 387 (1992).
11) F. Kuze, T. Yamamoto, R. Amitani and K. Suzuki, *Kekkaku*, **66**, 7 (1991).
12) Unpublished results.
13) A. Sanfilippo, C. Della Bruna, L. Marsili, E. Morvillo, C. R. Pasqualucci, G. Schioppacassi and D. Ungheri, *J. Antibiot.*, **33**, 1193 (1980).