Synthesis and Biological Activity of Some Derivatives of Rifamycin P

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A series of derivatives of rifamycin P, an antibiotic produced by fermentation of a mutant strain of *Nocardia mediterranea* or by chemical modification of rifamycin S, have been prepared. The structures of these compounds were determined by ^{1}H NMR, IR, UV, and LC/MS. Their in vitro and in vivo antibacterial activities in comparison with rifampicin and two other rifamycins under investigation were evaluated. The derivatives were more active than rifamycin P against *Mycobacterium avium* complex and other slowly and rapidly growing nontuberculous mycobacteria which frequently cause systemic infection in patients with AIDS. 2′-(Diethylamino)rifamycin P (P/DEA) appears suitable for further investigation.

Nontuberculous mycobacteria frequently cause chronic lung infections in immunocompromised patients and disseminated diseases in patients with AIDS.[1-6] The most common organism isolated from these patients is *M. avium* complex (MAC), but other species (e.g., *Mycobacterium xenopi, Mycobacterium fortuitum, Mycobacterium chelonae, Mycobacterium kansasii*) may be involved.[5-8]

The treatment of these infections is particularly difficult in AIDS patients; complex regimens including specific antimycobacterial agents such as rifampicin (RAMP; **2b**, Chart I), clofazimine, ethambutol, cycloserine, pyrazinamide, and other antibacterials (amikacin, quinolones, etc.) are used, but the outcome is often disappointing.[4,6]

Among rifamycins currently under investigation, rifabutin (ansamycin, LM 427, **3**, Chart I) is not always effective,[3,9] in spite of its good in vitro activity.[10-13] Rifapentine (**2c**, Chart I), a long-lasting rifamycin with good in vitro activity against these mycobacteria, is also under study.[13-15] Other rifamycins have been recently synthesized and tested in vitro against MAC.[15-18]

Rifamycin P (**1**, Chart I) was first isolated from the fermentation broth of a mutant strain of *Nocardia mediterranea*[19] and later obtained by synthesis.[19,20] It is quite active in vitro and in vivo against Gram-positive and Gram-negative bacteria but less active against MAC. We have prepared a series of derivatives with the aim of increasing the activity against MAC.

Chemistry

A series of compounds with different substituents in position 2′ of the thiazole ring have been described in the literature: (i) 2′-carboxamido and 2′-(*N,N*-dimethylhydrazido)rifamycins P, obtained[21] by treatment of 2′-(carbomethoxy)rifamycin P (**1a**, Chart I)[19] with the appropriate amines or *N,N*-dimethylhydrazine; (ii) 2′-alkyl- and 2′-(methylimino)rifamycins P, prepared[21] by condensation of rifamycin S (**2**) with suitable thioamino acids, or cysteine plus the appropriate hydrazines, followed by cyclization to the thiazole ring; (iii) 2′-*N*-monosubstituted rifamycin P derivatives obtained[22] by treatment of 3-bromorifamycin S (**2a**)[23,24] with N-monosubstituted thioureas (Scheme I). This reaction seems to be an extension of the well-known synthesis of 2-amino-substituted-6-hydroxynaphtho- or 5-hydroxybenzothiazoles, which were obtained by treating thiourea or N-monosubstituted or *N,N′*-disubstituted thioureas with an excess of 1,4-naphtho- or benzoquinone in ethanolic HCl;[25,26] although, it was shown that 2,5-dichlorobenzoquinone does not react with thiourea.[25]

We have applied the reaction shown in Scheme I to *N,N*-dialkylthioureas, such as *N,N*-diethyl- and *N,N*-dibutylthiourea obtaining the corresponding 2′-*N,N*-disubstituted rifamycin P derivatives (**6**, **7**) with good yield. In an alternative approach, 2′-*N,N*-diethylrifamycin P (**6**) was obtained, with low yield, by treating rifamycin P (**1**) with diethylamine in ethyl acetate (Scheme II). Since rifamycin P (**1**) behaves in solution as an internal salt,[19] position 2′ remains susceptible to nucleophilic attack.[27] This method

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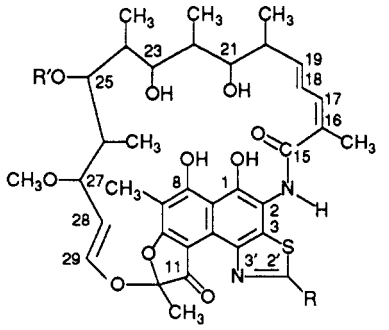
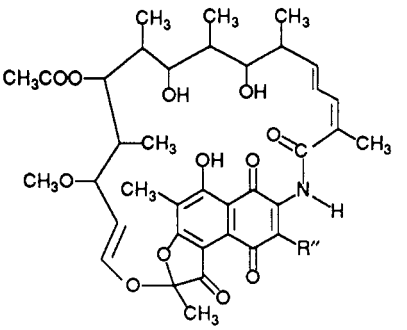
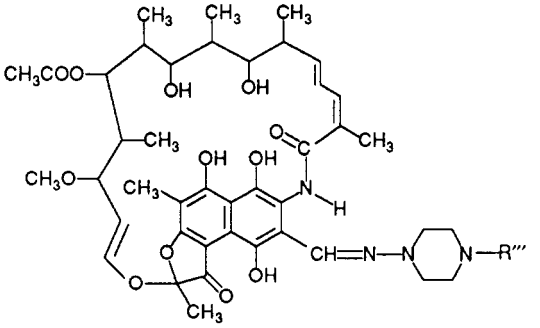
Chart I



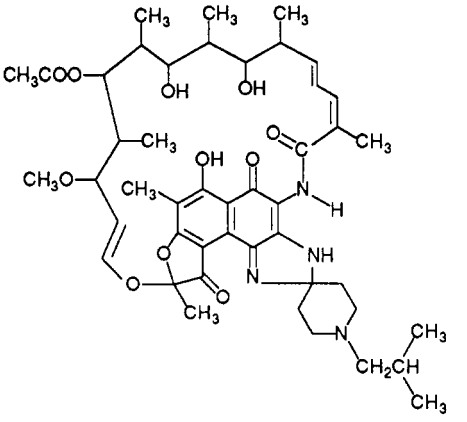
1: rifamycin P; R = H; R′ = $COCH_3$
1a: 2′-carbomethoxyrifamycin P; R = $COOCH_3$, R′ = $COCH_3$
1b: 25-desacetylrifamycin P; R = R′ = H



2: rifamycin S; R″ = H
2a: 3-Br-rifamycin S; R″ = Br

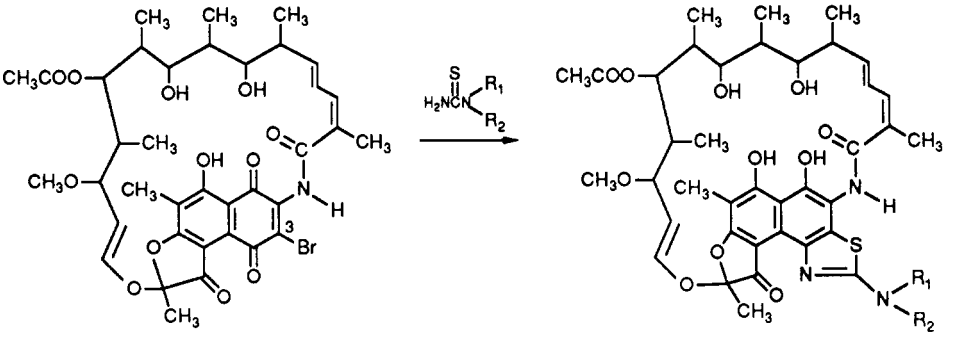


2b: rifampicin (RAMP); R‴ = CH_3
2c: rifapentine; R‴ = 



3: rifabutin

Scheme I



2a

4: R_1 = H; R_2 = C_2H_5
5: R_1 = H; R_2 = n-C_4H_9
6: R_1, R_2 = C_2H_5
7: R_1, R_2 = n-C_4H_9

was also applied to a series of heterocyclic amines (particularly N-substituted piperazines) in an inert solvent (morpholine does not require any solvent) to obtain the corresponding 2′-derivatives listed in Table I.

This reaction failed when applied to piperazine. Therefore, suitable derivatives of piperazine, i.e., *N*-carbethoxy- and *N*-[(phenylmethoxy)carbonyl]-piperazine, were allowed to react with **1**, giving compounds **17** and **18**. Decarbethoxylation of **17** with concentrated HCl in acetic acid led to the opening of the ansa chain. *N*-Piperazinylrifamycin P (**19**) was eventually obtained by hydrogenolysis of intermediate **18** under conditions that prevent the hydrogenation of the ansa double bonds.

The activity of compound **11** was compared with its 25-desacetylated derivative **11a**.[28] Hydrolysis of 2′-(carbomethoxy)rifamycin P (**1a**) under conditions stronger than those described[19] for obtaining rifamycin P led to 25-desacetylrifamycin P (**1b**, Chart I), which was subsequently allowed to react with *N*-methylpiperazine to give **11a**.

Attempts to condense rifamycin P with other nucleophiles, e.g., mercaptans, were unsuccessful, probably because the reducing medium prevents an oxidative step.

Some physicochemical characteristics of the rifamycin P derivatives are listed in Table I. The UV spectra (MeOH) showed absorption maxima corresponding to those found for **1**. The IR spectra ($CDCl_3$) showed the

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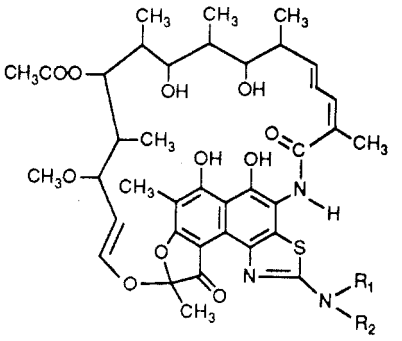
(28) 25-Desacetylrifampicin is the main metabolite of rifampicin.

Table I



compd	R_1	R_2	reaction conditions: Rifamycin P,[a] g/mL of solvent	reagent,[b] mL	time,[b] h	purification[c]	% yield	TLC,[d] R_f	mp,[e] °C	formula[f]	MW (LC/MS)	UV[g] (MeOH) λ_{max}, nm	log ε
4	H	C_2H_5				e	50[h]		178–180	$C_{40}H_{51}N_3O_{11}S$	781.9	229	4.60
												307	4.42
												430	4.13
5	H	nC_4H_9				f	30[h]		173–178	$C_{42}H_{55}N_3O_{11}S$	809.9	229	4.57
												308	4.38
												430	4.10
6	C_2H_5	C_2H_5	0.5/25 EtOAc	5	120	a,d,e	6.0; 49[h]	0.39	180–183	$C_{42}H_{55}N_3O_{11}S$	809.9	229	4.61
												309	4.46
												432	4.12
7	nC_4H_9	nC_4H_9				e	30[h]		>165	$C_{46}N_{63}N_3O_{11}S$	866.1	229	4.61
												310	4.46
												431	4.10
8	$-(CH_2)_4-$		1/150 THF		5	a,c,f	18.3	0.36	180–184	$C_{42}H_{53}N_3O_{11}S$	807.9 $[M + H]^+$ (m/z 808)	228	4.62
												308	4.46
												432	4.11
9	$-(CH_2)_5-$		1/	15	30	a,c,f	13.6	0.40	172–175	$C_{43}H_{55}N_3O_{11}S$	821.9 $[M]^-$ (m/z 821) $[M + H]^+$ (m/z 822)	228	4.76
												308	4.31
												430	4.25
10	$-(CH_2)_2O(CH_2)_2-$		1/100 THF	5	5	a,c,e	20.7	0.41	198–200	$C_{42}H_{53}N_3O_{12}S$	823.9	228	4.62
												308	4.47
												432	4.08
11	$-(CH_2)_2N(CH_3)-(CH_2)_2-$		2/300 EtOAc	10+10	1.5; 3.5	b,e	57.6	0.57	183–185	$C_{43}H_{56}N_4O_{11}S$	837.0 $[M]^-$ (m/z 837)	227	4.62
												308	4.47
												430	4.08
11a	$-(CH_2)_2N(CH_3)-(CH_2)_2-$ 25-desacetyl		0.8/100 EtOAc	4+4	1.5; 3.5	b,c,e	32.8[i]	0.51	190–192	$C_{41}H_{54}N_4O_{10}S$	794.9	228	4.64
												308	4.50
												432	4.12
12	$-(CH_2)_2N((CH_2)_2OH)(CH_2)_2-$		1/50 EtOAc	5+4	4; 24	b,d,f	17.1	0.53	183–185	$C_{44}H_{58}N_4O_{12}S$	867.0	227	4.61
												308	4.46
												430	4.07
13	$-(CH_2)_2N(C_6H_5)-(CH_2)_2-$		1/50 EtOAc	5+5	2; 18	a,d,g	30.5	0.38	179–180	$C_{48}H_{58}N_4O_{11}S$	899.1	230	sh
												242	4.68
												308	4.51
												430	4.08
14	$-(CH_2)_2N(p\text{-}Cl\text{-}C_6H_4)(CH_2)_2-$		1/200 EtOAc	2 g	12	b,c,f	30.2	0.39	182–183	$C_{48}H_{57}ClN_4O_{11}S$	933.5	228	4.67
												252	4.62
												307	4.54
												431	4.11
15	$-(CH_2)_2N(p\text{-}CH_3O\text{-}C_6H_4)(CH_2)_2-$		2/400 EtOAc	4	12	b,c,f	9.6	0.41	176–179	$C_{49}H_{60}N_4O_{12}S$	929.1	237	sh
												242	4.69
												309	4.51
												432	4.08
16	$-(CH_2)_2N(CH_2C_6H_5)(CH_2)_2-$		1/50 EtOAc	5	15	a,c,f	20.0	0.46	171–174	$C_{49}H_{60}N_4O_{11}S$	913.1	nd[j]	
17	$-(CH_2)_2N(COOC_2H_5)(CH_2)_2-$		0.6/40 EtOAc	4	15	a,c,f	34.5	0.38	165–167	$C_{45}H_{58}N_4O_{13}S$	895.0 $[M - H]^+$ (m/z 894)	nd	
18	$-(CH_2)_2N(COOCH_2C_6H_5)(CH_2)_2-$		1.5/100 EtOAc	2	18	a,c,f	17.6	0.75	163–166	$C_{50}H_{60}N_4O_{13}S$	957.1	229	4.59
												310	4.44
												432	3.99
19	$-(CH_2)_2NH(CH_2)_2-$						17.9[i]	0.30	200–201	$C_{42}H_{54}N_4O_{11}S$	822.9	230	4.55
												310	4.44
												436	3.97

[a] 25-Desacetylrifamycin P (**1b**) was the starting material for compound **11a**. [b] When two quantities are reported the reagent was added in two portions at the times indicated. [c] See the Experimental Section: (a) acid treatment as described for compound **8**; (b) acid/base treatment as described for compound **11**; (c) preparative TLC, eluent mixture CH_2Cl_2–MeOH 95:5; (d) flash chromatography, eluent mixture CH_2Cl_2–MeOH; (e) crystallized from EtOAc; (f) precipitated from EtOAc with petroleum ether; (g) precipitated from CH_2Cl_2 with petroleum ether. [d] Eluent mixture $CHCl_3$/MeOH 90:10; rifamycin P (1) R_f = 0.42. [e] Determined in glass capillary tubes (uncorrected). [f] All compounds were within 0.4% of the calculated values for C, H, N, Cl (compound **14**), S (compounds **4**, **6**, and **14**) analysis for the formulas listed above. The analyses were done after drying the samples at 150 °C under a nitrogen atmosphere. [g] Rifamycin P (1) shows the following values: λ_{max}^{MeOH} (log ε) 225 (4.63), 260 (4.45), 305 (4.35), 352 (3.91), 412 nm (4.19). [h] From 3-bromorifamycin S (**2a**). [i] From **18**. [j] Nd = not determined.

Table II. In Vitro Antibacterial Activity (MIC, μg/mL) of Rifamycin P Derivatives in Comparison with Other Rifamycins

organism	1[a]	1b	4	5	6[b]	7	8	9	10	11	11a	12	13	14	15	16	17	18	19	2b[d]	2c[e]	3[f]
S. aureus Tour L 165	0.004	0.032	0.063	0.032	0.125	0.063	0.004	0.063	0.002	0.004	0.008	0.016	0.008	0.063	0.063	0.008	0.063	0.063	0.125	0.016	0.063	0.063
Staphylococcus epidermidis ATCC 12228	0.002	0.008	0.032	0.016	0.063	0.125	0.063	0.063	0.063	0.063	0.125	0.125	0.032	0.063	0.063	0.063	0.063	0.032	0.063	0.008	0.063	0.032
Streptococcus pneumoniae UC 41	0.008	0.125	0.063	0.063	0.063	0.063	0.032	0.125	0.016	0.032	0.063	0.008	0.004	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.032
S. pyogenes C 203 SKF 13400	0.032	0.125	0.063	0.063	0.125	0.125	0.008	0.125	0.032	0.032	0.063	0.016	0.032	0.125	0.125	0.063	0.063	0.125	0.125	0.063	0.063	0.032
Streptococcus faecalis ATCC 7080	1	4	1	1	2	4	2	2	1	2	1	4	1	4	4	1	4	2	2	1	2	2
Escherichia coli SKF 12140	16	16	16	16	32	>128	16	32	16	16	8	8	4	8	8	32	16	>128	8	8	16	16
Klebsiella pneumoniae ISM	32	16	32	64	32	>128	32	32	32	32	16	32	>128	>128	>128	64	32	>128	16	16	32	16
Proteus vulgaris X19H ATCC 881	1	4	8	16	32	>128	16	32	8	8	8	16	16	64	64	32	16	64	8	8	32	16
Pseudomonas aeruginosa ATCC 10145	8	8	4	8	8	>128	8	8	16	16	8	16	8	128	128	16	32	32	8	8	8	8
N. gonorrhoeae ISM 68/126	0.032	0.125	0.125	0.125	0.5	2	0.5	0.5	0.125	0.125	1	0.125	0.125	0.25	0.125	0.125	0.125	0.125	0.125	0.125	0.5	0.5
H. influenzae ATCC 19418	0.125	0.125	0.125	0.125	0.5	1	0.125	1	0.5	0.25	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.25	0.125	0.5	0.125
M. tuberculosis H37Rv ATCC 9660	0.5	0.5	0.125	nd[c]	0.125	nd	2	0.25	2	1	1	1	1	1	0.25	0.125	2	0.5	nd	0.25	0.063	0.008

[a] Rifamycin P. [b] P/DEA. [c] nd = not determined. [d] Rifampicin (RAMP). [e] Rifapentine. [f] Rifabutin.

Table III. Activity of Rifamycin P Derivatives in the Murine Model of *S. aureus* Tour Septicemia in Comparison with other Rifamycins

compd	ED_{50}, mg/kg per day os	ED_{50}, mg/kg per day sc	compd	ED_{50}, mg/kg per day os	ED_{50}, mg/kg per day sc
1	0.93	0.38	**12**	>20	3.3
6	0.82	0.21	**13**	2.5	0.63
9	0.63	0.16	**14**	3.3	3.3
10	0.21	0.21	**15**	1.9	0.63
11	0.36	0.16	**2b**[a]	0.28	0.09
11a	13	10	**2c**[b]	0.22	0.18

[a] Rifampicin (RAMP). [b] Rifapentine.

relevant absorption bands of **1**, including the bands of the thiazole ring[19] at 1460 cm^{-1} and in the interval 1445–1425 cm^{-1}. Other characteristic bands are at 1680–1620 cm^{-1} (νC=O, amide I); 1650–1640 cm^{-1} (νC=O, furanone), interpreted as the existence of an intramolecular H bond; and 1515–1500 cm^{-1} (δNH, amide II), which is accounted for by the usual "trans" conformation of the amide group.[29,30]

The ^1H NMR spectra ($CDCl_3$) are in accordance with the structures assigned. All the signals characteristic of **1** are present,[19] in particular (i) two singlets attributed to two mobile protons of (C-8)OH···O(C-1) and (C-11)=O···=NH^+(3′) systems (Scheme II) in the intervals δ 17.40–18.24 ppm and δ 15.72–16.90 ppm, respectively (for **1** at δ 17.33 and 15.73 ppm);[19] (ii) a doublet of doublets in the interval δ 6.34–6.49 ppm, attributed to H-18 (for **1** at δ 6.48 ppm), indicating the "transoid" conformation of the amide C=O with respect to the C(2)–C(3) bond;[29,30] and the absence of the singlet at δ 8.96 ppm (thiazole CH=N) for compounds **4–19**.

Desacetylrifamycin P (**1b**) and its derivative **11a** also showed signals at δ 3.98 ppm (OH-25) and δ 4.83 ppm (H-25) instead of the CH_3CO singlet at δ 2 ppm.

The mass spectra of a selected number of compounds were taken by LC/MS in both positive and negative chemical ionization. Rifamycin P exhibited the molecular ion at $[M]^-$ m/z 738, whereas the derivatives checked gave $[M]^-$, $[M + H]^+$, or $[M - H]^+$ ions. The chromophoric ion at m/z 316 was sometimes observed.

A disubstituted nitrogen atom in position 2′ of the thiazole ring of rifamycin P (**1**) characterizes compounds **6–19**. Spectroscopic and ionization properties of two of them, namely 2′-(1″-morpholinyl)- and 2′-(4″-methyl-1″-piperazinyl)rifamycin P (**10** and **11**) were studied in detail in comparison with those of rifamycin P (**1**). These rifamycin P derivatives show an unusual basicity of the nitrogen on the thiazole ring ($pK_a \cong 7.5$) in comparison with rifamycin P (pK_a 4.2), thiazole ($pK_a \cong 2.52$), and 2-aminobenzothiazole ($pK_a \cong 4.3$). This increased basicity may be due to a lowering of energy in the chromophoric system caused by a strong intramolecular H bond between the C(11)–O and the protonated thiazole nitrogen as shown in Scheme II.

Microbiological Activity

The in vitro antibacterial activity of the compounds against several bacterial species is compared in Table II with those of rifamycin P (**1**), rifampicin (**2b**, RAMP), rifapentine (**2c**), and rifabutin (**3**).

No improvement was found in their activity against Gram-positive or Gram-negative bacteria. Against *My-*

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(30) Malabarba, A.; Ferrari, P.; Depaoli, A.; Gallo, G. G.; Cavalleri, B. *Farmaco, Ed. Sci.* **1986**, *41*, 131–150.

Scheme II

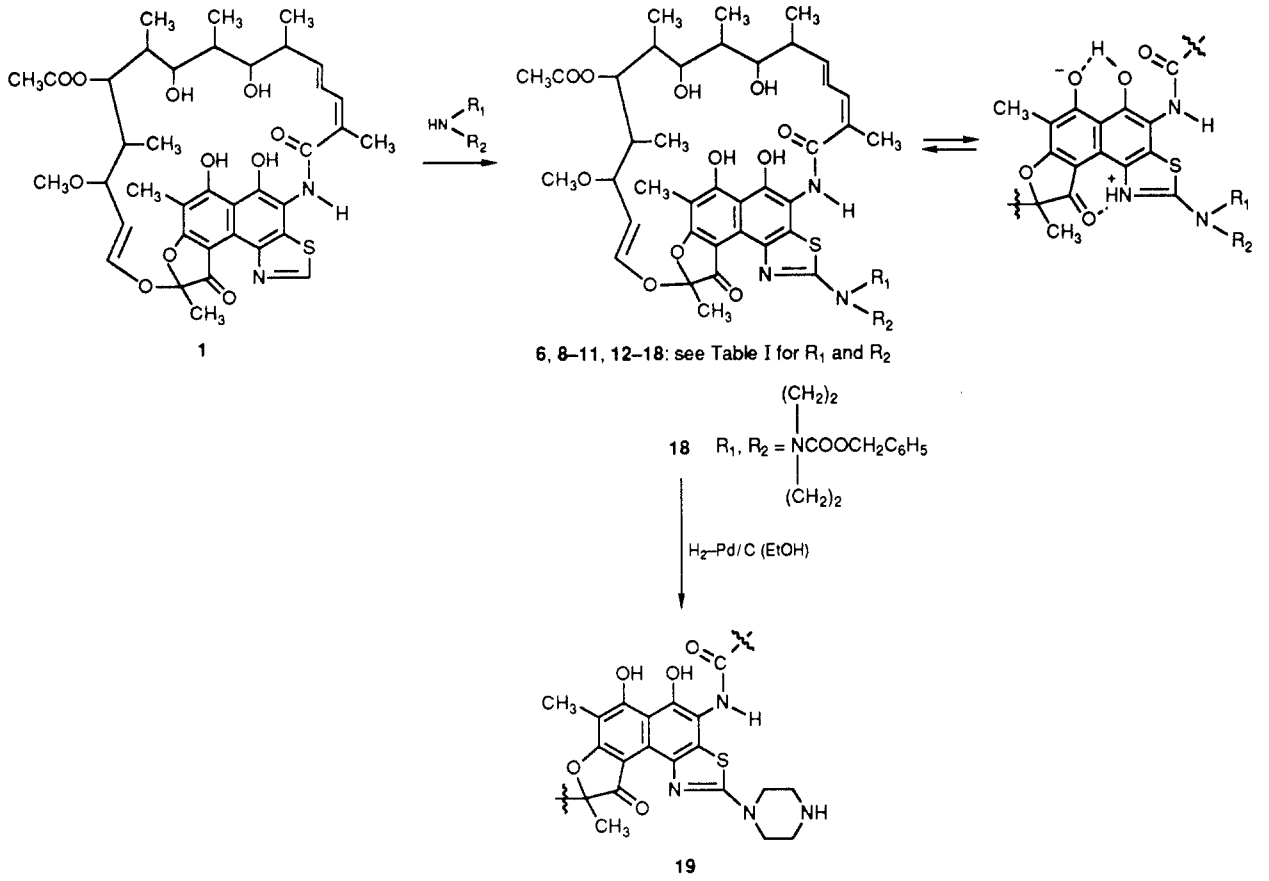

Table IV. Activity of 2′-*N*,*N*-Dialkylrifamycin P Derivatives and the Corresponding 2′-*N*-Alkylrifamycin P Derivatives in Experimental *S. pyogenes* Septicemia in the Mouse

compd	MIC, μg/mL, vs *S. pyogenes* C203	ED_{50}, mg/kg os	ED_{50}, mg/kg sc
4	0.063	>4	nd[b]
5	0.063	>10	6.6
6	0.135	1.23	1.62
7	0.125	5	3.3
2b[a]	0.063	0.47	0.35

[a] Rifampicin (RAMP). [b] Nd = not determined.

cobacterium tuberculosis some of the derivatives were less active than the comparison rifamycins.

Some of the compounds were tested in murine experimental septicemia caused by *Staphylococcus aureus* Tour (Table III). Compounds **10** and **11** were more active than rifamycin P (**1**) and had similar activity to RAMP when administered orally. The sc activity of rifamycin P derivatives **6**, **9**, **10**, and **11** was comparable to that of RAMP and rifapentine and slightly better than that of rifamycin P (**1**). The 25-desacetyl derivative **11a** was much less active than the parent compound **11**.

Table IV compares the activities of the monoalkyl derivatives **4** and **5** with those of the corresponding dialkyl derivatives **6** and **7** in the experimental *Streptococcus pyogenes* septicemia. The monoalkyl derivatives were less active than the dialkyl derivatives; compound **6** was the most effective of the series.

Several derivatives, selected on the basis of their in vivo activity, were tested for in vitro activity against 20 MAC clinical isolates (Table V). The dialkylamino derivatives **6** and **7** were more active (MIC_{90} = 2 μg/mL) than the corresponding monoalkylamino derivatives, RAMP and rifapentine and approximately as active as rifabutin (**3**).

Table V. In Vitro Activity of Selected Rifamycin P Derivatives against 20 MAC Clinical Isolates in Comparison with Other Rifamycins

compd	MIC range, μg/mL	MIC_{50}, μg/mL	MIC_{90}, μg/mL
1[a]	32–>128	>128	
4	8–128	32	64
5	2–64	8	32
6[b]	0.5–4	2	2
7	0.25–8	1	2
9	4–64	8	16
10	16–>128	32	128
11	16–128	64	64
2b[c]	2–128	16	64
2c[d]	0.5–32	8	16
3[e]	0.25–8	1	4

[a] Rifamycin P. [b] P/DEA. [c] Rifampicin (RAMP). [d] Rifapentine. [e] Rifabutin.

Compound **6** (P/DEA) had an MIC comparable to that of RAMP but higher than that of rifapentine and rifabutin for most species of slowly growing mycobacteria (Table VI).

Most isolates of rapidly growing mycobacteria (Table VII) are only slightly or moderately susceptible to rifamycins. Compound **6** was somewhat more active than RAMP against these species.

Other Animal Studies. Pharmacokinetic studies in the rat showed compound **6** to be rapidly absorbed orally, with plasma concentrations ranging from 2.65 to 4.51 μg/mL between 1 and 4 h. The levels then declined with a mean terminal half-life of 2.76 h. The mean value of the area under the curve extrapolated to infinity was 30.46 μg h/mL. Urinary excretion in 48 h accounted for 5.89% of the administered dose.

In the mouse, the LD_{50} of compound **6** was 336.1 (95% confidence limits 240.7–419.4) mg/kg upon intraperitoneal administration and >2000 mg/kg orally.

Table VI. In Vitro Activity (MIC, μg/mL) of Compound **6** against Slowly Growing Species of *Mycobacterium* in Comparison with Other Rifamycins

mycobacteria	strain[a]	6[b]	2b[c]	2c[d]	3[e]
M. kansasii	1291	2	16	4	1
M. kansasii	1298	0.25	0.125	0.063	0.063
M. kansasii	1299	2	8	2	0.5
M. marinum	1388	2	1	0.125	0.25
M. marinum	1390	4	2	0.5	1
M. marinum	1391	1	0.5	0.125	0.25
M. marinum	1392	1	0.5	0.125	0.25
M. marinum	1393	1	0.5	0.125	0.25
M. marinum	1394	1	0.5	0.125	0.25
M. marinum	1447	1	1	0.125	0.25
M. gordonae	1294	0.5	0.5	0.5	0.5
M. gordonae	1441	2	4	1	1
M. scrofulaceum	1292	0.032	0.063	0.016	0.032
M. scrofulaceum	1293	0.125	0.063	0.032	0.032
M. scrofulaceum	1297	0.063	0.125	0.063	0.063
M. scrofulaceum	1301	2	8	4	2
M. xenopi	554	2	1	0.25	0.125
M. xenopi	555	4	1	0.25	0.125
M. xenopi	556	4	1	0.5	0.125
M. xenopi	1446	4	1	0.25	0.125

[a] Clinical isolates from different hospitals. [b] P/DEA. [c] Rifampicin. [d] Rifapentine. [e] Rifabutin.

Table VII. In Vitro Activity (MIC, μg/mL) of Compound **6** against Rapidly Growing Species of *Mycobacterium* in Comparison with Other Rifamycins

mycobacteria	strain[a]	6[b]	2b[c]	2c[d]	3[e]
M. fortuitum	645	2	4	2	1
M. fortuitum	646	32	128	32	8
M. fortuitum	1287	2	2	1	1
M. fortuitum	1302	2	1	0.5	1
M. fortuitum	1440	16	64	32	16
M. fortuitum	726	32	32	16	16
M. fortuitum (Minetti)	114	16	128	32	8
M. fortuitum (peregrinum)	1285	16	4	4	4
M. fortuitum (ranae)	140	16	64	32	4
M. chelonae	1443	>128	>128	>128	32
M. flavescens	1284	32	128	32	8
M. flavescens	1286	0.25	0.25	0.125	0.063
M. phlei	141	0.032	0.032	0.016	0.016
M. phlei	1289	2	8	2	2
M. phlei (timoteus)	1283	0.125	0.25	0.063	0.016
M. smegmatis	31	8	64	16	4
M. smegmatis	139	8	64	16	4
M. smegmatis	1442	8	64	16	4

[a] Clinical isolates from different hospitals. [b] P/DEA. [c] Rifampicin. [d] Rifapentine. [e] Rifabutin.

Conclusions

In addition to the reaction of the N,N-disubstituted thioureas with 3-bromorifamycin S, direct reaction of rifamycin P with the appropriate amines is a suitable way to introduce N-disubstituted moieties in position 2′ of rifamycin P.

The 2′-*N*,*N*-dialkyl derivatives showed good activity against MAC, better than that of the corresponding 2′-*N*-monoalkyl derivatives and of established and experimental rifamycins. In experimental Gram-positive infections the *N*,*N*′-dialkyl derivatives were more active than the corresponding *N*-monoalkyl compounds. Other studies on compound **6** (P/DEA) have shown it to be more bactericidal than RAMP, rifapentine, or rifabutin for MAC both at neutral pH and at pH 5.2 (L, Heifets, personal communication), suggesting that bacilli surviving within microphage phagolysosomes (pH 5) might be eliminated by P/DEA. We plan further evaluation of P/DEA as a potential drug for treatment of MAC infections.

Experimental Section

The organic extracts were dried on anhydrous Na_2SO_4. Evaporation was with a rotary evaporator at 45 °C under vacuum. TLC was done on silica gel 60 F 254 plates (Merck). The R_f values were calculated with solvent run to 10 cm. Preparative TLC was on silica gel 60 PF 254 plates (Merck). Flash chromatography was on silica gel 60 (0.04–0.06 mm) (Merck).

UV–vis spectra were recorded with a Unicam SP 800 apparatus in MeOH solutions. IR spectra were registered with a Perkin Elmer Model 580 spectrophotometer in $CDCl_3$ solution. ^1H NMR spectra were recorded at 270 MHz in $CDCl_3$ solution on a Bruker WH 270 cryospectrometer (TMS δ = 0.00 ppm as internal reference). LC-MS was done on a HP 5985 B instrument in both positive and negative chemical ionization. The instrument was equipped with a 25-cm RP-8 column eluted with CH_3CN–H_2O, 75:25 (v:v). The eluent was used as reactant gas for ionization.

The analytical results for C, H, N, S (and Cl) were in accordance with the theoretical values.

2′-(*N*,*N*-Diethylamino)rifamycin P (6). (a) From Rifamycin P (1). Diethylamine (5 mL) was added to a solution of 1 (0.5 g, 0.67 mmol) in 25 mL of EtOAc and the reaction mixture was allowed to stand at room temperature for 5 days, then it was poured into ice-water and acidified with dilute HCl. The organic layer was separated, washed with water to neutrality, and evaporated to dryness. The residue was dissolved with 2 mL of CH_2Cl_2 and subjected to flash chromatography on silica gel 60 (35 g) which was eluted with CH_2Cl_2 (250 mL), CH_2Cl_2 containing 0.5% (v/v) of MeOH (500 mL), CH_2Cl_2 containing 1% (v/v) of MeOH (500 mL), and CH_2Cl_2 containing 1.8% (v/v) of MeOH (1 L). This last mixture elutes the product. After evaporation to dryness the residue was crystallized from EtOAc to give 33 mg of **6**.

(b) From 3-Bromorifamycin S (2a). 1,1-Diethylthiourea (1.85 g, 14 mmol) and **2a** (10 g, 13 mmol) were stirred in 300 mL of MeOH for 90 min at room temperature. The solvent was distilled off and the residue was dissolved in EtOAc (300 mL). After washing with 0.1 N HCl and then with water to pH 7 and drying, the solvet was evaporated to dryness.

The residue was purified by flash chromatography (silica gel/compound 80:1 w/w). Elution with 2.5% MeOH in CH_2Cl_2 gave pure material that was crystallized from EtOAc to give 5.1 g of pure **6** (49% yield).

Compounds **4**, **5**, and **7** were prepared from **2a** with the appropriate thioureas by the same procedure.

2′-(1″-Pyrrolidinyl)rifamycin P (8). Pyrrolidine (10 mL) was added to a solution of 1.0 g (1.35 mmol) of **1** in 150 mL of THF at room temperature. The reaction mixture was stirred for 5 h, poured into ice-water, acidified, and extracted with EtOAc (2 × 100 mL). The combined extracts were washed with water to neutrality and concentrated to a small volume. The addition of petroleum ether gave a precipitate that was purified by preparative TLC.

2′-(1″-Piperidinyl)rifamycin P (9). A solution of **1** (1.0 g, 1.35 mmol) in 15 mL of piperidine was stirred for 30 h at room temperature. The reaction mixture was poured into ice/water and worked up as described for compound **8**.

2′-(4″-Methyl-1″-piperazinyl)rifamycin P (11). 1-Methylpiperazine (10 mL) was added to a solution of **1** (2.0 g, 2.7 mmol) in 300 mL of EtOAc. The reaction mixture was stirred for 90 min at room temperature, and then an additional 10 mL of 1-methylpiperazine was added. Stirring was continued for 3.5 h, then the reaction mixture was poured into ice-water and acidified (HCl) to pH 4–5. The organic layer was discarded and the aqueous phase was brought to pH 7 with $NaHCO_3$ and extracted with EtOAc. The extract was dried and concentrated to a small volume. Upon cooling, crystalline **11** was obtained.

25-Desacetylrifamycin P (1b). A solution of 4.5 g (5.5 mmol) of **1a** in 200 mL of Me_2CO and 100 mL of 50% aqueous NaOH was stirred at room temperature. The reaction was monitored by TLC ($CHCl_3$–MeOH, 95:5) until the starting compound disappeared (30 min) and spots of **1b** and 2′-carboxyrifamycin P appeared at R_f 0.35 and 0.00, respectively. The reaction mixture was poured into ice–water and extracted with EtOAc. The extracts were dried and then evaporated to dryness under vacuum for 30 min. The residue was dissolved in 300 mL of the same Me_2CO–

aqueous NaOH mixture and the solution was evaporated for 30 min. The residue was dissolved in $CHCl_3$ and chromatographed on silica gel (220 g), eluting with $CHCl_3$ containing increasing amounts of MeOH (from 1.5 to 2% in volume). Fractions containing **1b** were pooled, and the solvents were evaporated to dryness. By crystallization from EtOAc, 3.5 g (90%) of the desired compound was obtained: mp 172–174 °C; UV λ_{max}^{MeOH} (log ϵ) 224 (4.26), 260 (4.21) 298 (4.17), 405 nm (4.14). Anal. C, H, N, S.

Compounds **12, 14,** and **15** were prepared as described for **11**, and compounds **10, 13,** and **16–18** were prepared as described for **8**. Compound **11a** was prepared as described for **11**, starting from **1b**. The reaction conditions and the procedures for the purification are indicated in Table I.

2′-(1″-Piperazinyl)rifamycin P (19). A solution of 0.22 g (0.23 mmol) of **18** in 40 mL of absolute EtOH was stirred under a hydrogen stream at room temperature and atmospheric pressure in the presence of 50 mg of 10% Pd/C for 1 h. The reaction was monitored by TLC (R_f 0.4, relative to the starting compound). The reaction mixture was filtered and the solvent was evaporated. The residue was dissolved in MeOH and purified by preparative TLC (eluent mixture $CHCl_3$–MeOH, 9:1); the EtOAc extract was washed with an aqueous $NaHCO_3$ solution at pH 8.5.

Microbiological Activity. The MICs were determined by the 2-fold-dilution method (concentration range: 128–0.0001 μg/mL) in microtiter (Gram-positive and Gram-negative bacteria) and in tube (*M. tuberculosis*) or agar dilution (nontuberculous mycobacteria). The following media were used: Oxoid Iso-Sensitest broth (staphylococci, *Enterococcus faecalis*, and Gram-negative bacilli); Difco Todd-Hewitt broth (streptococci); Difco GC broth plus hemin (10 μg/mL) and 1% BBL Iso-VitaleX (*Neisseria gonorrhoeae*); Difco Brain Heart infusion broth plus 1% Difco Supplement C (*Hemophilus influenzae*); Kirchner broth plus 1% glycerol and 10% horse serum (*M. tuberculosis*); Difco Middlebrook 7H10 agar supplemented with 2% glycerol and 10% Difco OADC enrichment (nontuberculous mycobacteria).

Inocula were 10^4 cfu/mL for broth-dilution MICs. Mycobacteria from slants of Loevenstein–Jensen medium were grown in Difco 7H9 broth with 0.5% Tween and 10% OADC. For agar-dilution MIC, inoculation was with a multipoint inoculator (Dynatech); bacterial suspensions were adjusted to a no. 1 McFarland standard. The plates were sealed and incubated at 22, 30, or 37 °C for different times (from 4 to 20 days) according to the strains; *M. tuberculosis* was incubated at 37 °C for 7 days. Other species were incubated at 37 °C for 18–24 h; *Neisseria* and *Hemophilus* were incubated for 48 h in 5% CO_2 atmosphere.

S. aureus Tour or *S. pyogenes* C203 experimental septicemia was established by ip infection of mice (male and female CD1, Charles River, weight 18–22 g). The bacterial challenge was adjusted so that untreated animals died within 48 h. Each treatment group consisted of five mice. Animals were treated once daily for 3 days starting immediately after infection (*S. aureus*) or only once immediately after infection (*S. pyogenes*). On the 10th day the ED_{50} (50% effective dose) was calculated on the basis of the percentage of surviving animals at each dose.[31]

Pharmacokinetics. Twelve male CD rats (Charles River, weight 250–275 g) were fasted overnight and treated orally with a single 10 mg/kg dose of compound **6**; six were used for plasma pharmacokinetics and six for urinary recovery. Food was offered to the animals 4 h later; water was at libitum. Blood samples were collected from the caudal vein with heparinized capillaries at various times; blood was centrifuged to obtain plasma. Urine samples were collected in metabolic cages at various intervals. Samples were stored at −20 °C until assay. The concentration of compound **6** was determined by an agar-diffusion method using *Sarcina lutea* ATCC 9341 as the test organism. The noncompartmental analysis was applied to plasma data for each rat. The final disposition rate constant z was estimated by log–linear least squares fit of the apparent terminal phase. The area under the plasma concentration time curve was calculated by the trapezoidal rule[32] and extrapolated to infinity.

Toxicity. Groups of five male and five female mice received a single intraperitoneal or oral dose of compound **6** suspended in 0.5% Methocel K15 Premium (Dow Chemical). The LD_{50} with 95% confidence limits was calculated from the mortality data using probit analysis.

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