NOVEL RIFAMYCINS. III[1]

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 3-AMIDINO- AND OF 4-AMINOIMIDAZOLO[4,5-c]RIFAMYCIN DERIVATIVES

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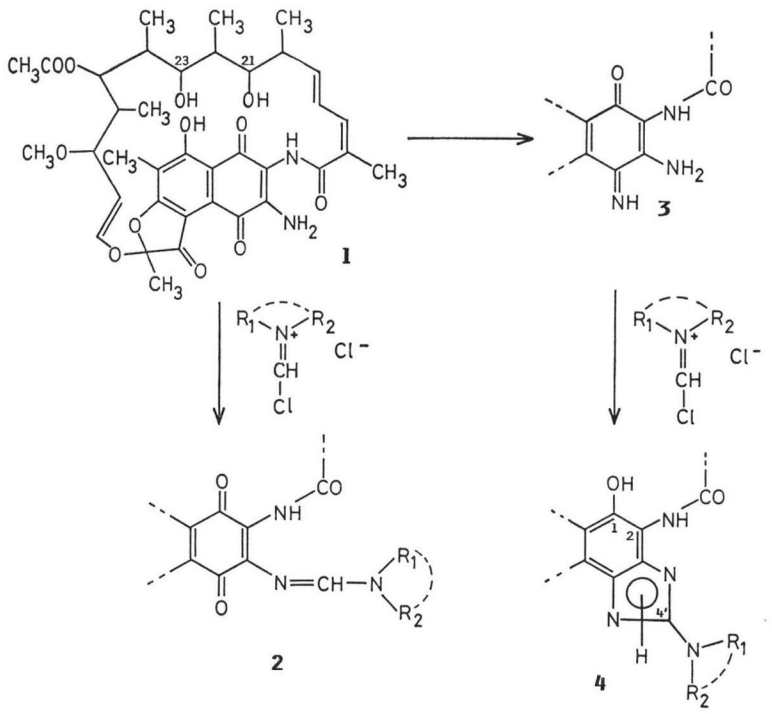
A number of semisynthetic rifamycin derivatives modified at position 3 and/or 4, belonging to general structures **2** and **4** (see Scheme 1), have been obtained. The synthesis and the biological activities of the new compounds are described. Compounds **4p** and **4q** display very good antimycobacterial activity in mice.

A broad program of synthesis, aimed at obtaining new rifamycin derivatives endowed with antibacterial activity, is currently under way in our laboratories and some of the previous results have been published.[1~8] As a continuation of the project, we describe here the synthesis and the biological

Scheme 1.



properties of two new classes of rifamycin derivatives, prepared from 3-aminorifamycin S (**1**)[4] and 3-amino-4-deoxo-4-iminorifamycin S (**3**).[5] The structures of the compounds and the synthetic pathways are shown in Scheme 1.

One example of each class, for derivatives of general formulae **2** and **4**, is described in the experimental section. Relevant data are reported in Table 1 for representative compounds. Treatment of **1** with the appropriate chloroformiminium chlorides in an aprotic solvent in the presence of triethylamine gives compounds **2**,[6] directly obtained in the quinone form (Q). Some of the derivatives listed in Table 1 have been transformed into the hydroquinone form (H_2Q) for characterization purposes. Compounds **2** display the ^1H NMR signal of the exocyclic –CH=N– group at *ca.* 8.15 and 8.7 ppm for the quinone and hydroquinone forms, respectively (see Table 1), and are easily converted by mild hydrolysis to the starting 3-amino derivative, **1**.

For the synthesis of derivatives of type **4**,[7] compound **3** is treated, parallel to the preparation of **2**,

Table 1. Examples of rifamycin derivatives*.

Compound		Yield (%)	Formula	FD-MS	^1H NMR ($CDCl_3$, δ values, multiplicities**)		Mp (dec.) (°C)
2a (H_2Q)		12.5	$C_{42}H_{55}N_3O_{12}$	793 $M^+\cdot$	CH=N	8.74 (s)	155~158
2b (H_2Q)		25.0	$C_{44}H_{59}N_3O_{12}$	821 $M^+\cdot$	CH=N	8.65 (s)	158~160
2c (Q)		25.0	$C_{43}H_{55}N_3O_{12}$	805 $M^+\cdot$	CH=N	8.15 (s)	—
2d (Q)		9.4	$C_{44}H_{57}N_3O_{12}$	819 $M^+\cdot$	CH=N	8.17 (s)	155~158
4a*		12.5	$C_{40}N_{52}N_4O_{11}$	764 $M^+\cdot$	$N(CH_3)_2$	3.21 (s)	—
4d		33.8	$C_{44}H_{60}N_4O_{11}$	820 $M^+\cdot$	$(CH_3)_2CH$	1.52 (d)	—
4f		33.4	$C_{46}H_{64}N_4O_{11}$	848 $M^+\cdot$	CH_3–CH CH_3–CH_2	1.41 (d) 0.92 (t)	—
4o		43.8	$C_{42}H_{54}N_4O_{11}$	790 $M^+\cdot$	$(CH_2)_2N$	3.4~3.7 (m)	190~192
4p*		12.4	$C_{43}H_{56}N_4O_{11}$	804 $M^+\cdot$	$(CH_2)_2N$	3.3~3.8 (m)	173~175
4r		5.0	$C_{42}H_{54}N_4O_{12}$	806 $M^+\cdot$	$(CH_2)_2N$ and $(CH_2)_2O$	3.4~3.7 and 3.4~4.0 (m)	190~191
4s		27.5	$C_{43}H_{57}N_5O_{11}$	819 $M^+\cdot$	$(CH_2)_2N$ N-CH_3	2.4~2.7 and 3.4~3.7 (m) 2.38 (s)	192~195

* All the compounds gave elemental analyses agreeing with the calculated values within ±0.7%.

** s=singlet: d=doublet; t=triplet; m=multiplet.

*** In the ^{13}C NMR spectra the signal of the imidazole C-4′ is at 146.3 ppm (**4a**) and 145.5 ppm (**4p**), the signal of C-5 is at 109.1 ppm (**4a**) and 109.3 ppm (**4p**) in $CDCl_3$ solution.

with a suitable chloroformiminium chloride in an aprotic solvent in the presence of a tertiary amine. Derivatives **4** are characterized in their ^{13}C NMR spectra by the signal of the quaternary imidazole carbon atom C-4′ bearing the basic nitrogen atom which characteristically resonates at about 146 ppm (see Table 1).

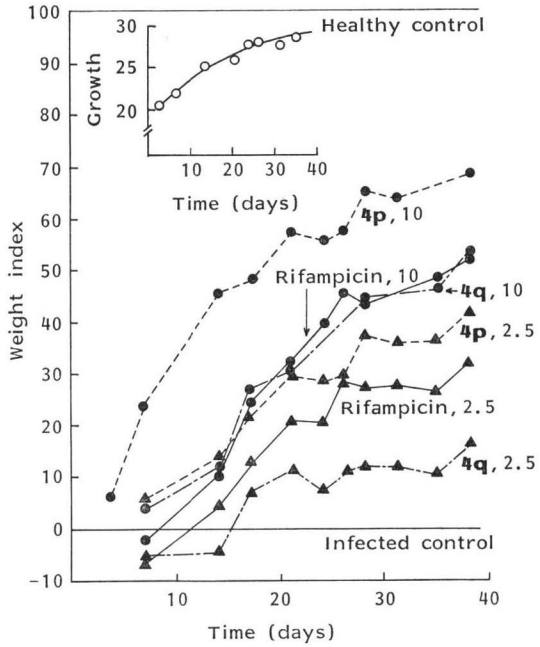
The derivatives **2a**~**d** (Table 2) show high antibacterial activity *in vitro* against Gram-positive strains and *Mycobacterium tuberculosis*, but display a rather low-efficacy on Gram-negative bacteria, particularly as far as compound **2a** is concerned. These compounds were not tested *in vivo* because of their tendency to undergo hydrolytic cleavage to **1** (see above).

The compounds **4a**~**n** (Table 3) are often less active than rifampicin; this trend reaches the maximum with derivatives **4e** and **4n**. *In vivo* all these compounds are less effective than rifampicin.

The other compounds listed in Table 3 (**4o**~**s**) present a good antibacterial activity *in vitro* especially against *M. tuberculosis*. The protective activity *in vivo* (see Table 4) is always lower than rifampicin on Gram-positive and Gram-negative sepsis. It is noteworthy that two members of this group (**4p** and **4q**) show very good antimycobacterial activity in mice. (Fig. 1). This efficacy is related to the pharmacokinetic properties: high area under the curve (AUC) and peak values, mainly in the lung (Table 5).

Fig. 1. Weight index (WI) against time (days) of compounds **4p**, **4q** and rifampicin at 2.5 and 10 mg/kg.

Up, on the left, the growth curve of the healthy animals.



Experimental

^1H NMR spectra were recorded on Varian XL-200 (200 MHz), CFT-20 (80 MHz), or A-60/A (60 MHz) spectrometers in $CDCl_3$ solution. ^{13}C NMR spectra were recorded on a Varian XL-200 (50 MHz) spectrometer in $CDCl_3$ solution. The signals are listed as δ values (TMS, δ 0.0 ppm). Mass spectra were recorded on a Finnigan MAT 311A spectrometer equipped with a combined FI/FD/EI ion source. Thin-layer chromatography was performed on silica gel 60 F 254 Merck plates. Melting points are uncorrected.

Minimal inhibitory concentrations (MIC) were determined by the serial twofold dilution technique in Difco Antibiotic Medium No. 3 with 15‰ of Difco Agar for Gram-positive and Gram-negative bacteria, and in Difco Bacto-Dubos Albumin Broth for *M. tuberculosis* H37Rv. The MICs were the lowest concentrations of antibiotic which prevented any visible growth after 1 day or 7 days (*M. tuberculosis*) of incubation at 37°C.

In Vivo Activity

Protection studies were done in mice experimentally infected with: (A) *Staphylococcus aureus* PV_3, *Streptococcus pyogenes* ATCC 12384, *Salmonella abortivoequina* F. I. and (B) *Mycobacterium tuberculosis* H37Rv.

Table 2. *In vitro* antibacterial activity of derivatives **2**.

Compound	MIC (μg/ml)											
	Klebsiella pneumoniae	*Proteus vulgaris*	*Escherichia coli*	*E. coli*, ginetta	*E. coli* rifa. res. (ginetta)	*Pseudomonas aeruginosa*	*Salmonella abortivo-equina*	*Staphylococcus aureus*	*S. aureus* rifa. res.	*Streptococcus pyogenes*	*S. faecalis*	*M. tuberculosis* H37Rv
2a (H_2Q)	>2	2.5	>20	>20	>200	>20	>20	0.0011	>200	1.25	1.25	0.0012
2b (H_2Q)	5	1.25	10	20	>200	10	5	0.0005	—	0.3	0.6	0.0025
2c (Q)	5	1.25	10	10	>200	20	10	≤0.0005	—	—	0.075	0.0006
2d (Q)	10	20	10	>20	200	10	—	0.0005	200	—	0.15	0.0012
Rifampicin	5	2.5	5	10	>200	10	2.5	0.0022	>200	1.25	0.3	0.005

rifa. res.: Rifampicin resistant strain.

Table 3. *In vitro* antibacterial activity of derivatives **4**.

Compound	MIC (μg/ml)											
	K. pneumoniae	*P. vulgaris*	*E. coli*	*E. coli*, ginetta	*E. coli* rifa. res. (ginetta)	*P. aeruginosa*	*S. abortivo-equina*	*S. aureus*	*S. aureus* rifa. res.	*S. pyogenes*	*S. faecalis*	*M. tuberculosis* H37Rv
4a	10	5	5	5	>200	10	5	0.0025	>200	2.5	0.3	0.0025
4b	10	2.5	5	10	>200	10	5	0.0045	>200	2.5	0.3	0.005
4c	10	2.5	10	10	200	10	2.5	0.018	>200	10	0.6	0.02
4d	10	2.5	10	10	200	10	2.5	0.0045	>200	—	0.6	0.04

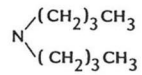
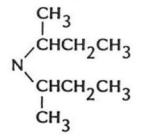
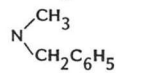
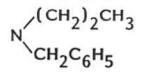
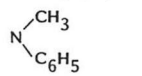
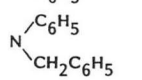
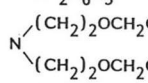
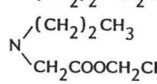
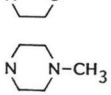
4e		>200	200	>200	>200	>200	200	200	0.037	>200	200	0.6	0.01
4f		10	20	10	10	>200	10	1.25	≤0.0005	200	1.25	0.3	0.01
4g		10	2.5	5	10	>200	10	1.25	≤0.0005	200	1.25	0.3	0.02
4h		10	2.5	5	10	>200	10	1.25	≤0.0005	200	1.25	0.3	0.02
4i		10	2.5	2.5	20	>200	10	2.5	≤0.0005	100	1.25	0.15	0.02
4l		20	5	10	20	>200	20	5	0.0022	10	1.25	0.6	0.04
4m		10	5	5	10	>200	20	5	0.0022	>200	2.5	0.3	0.01
4n		>200	>200	>200	>200	>200	200	200	0.0011	>200	2.5	0.6	0.04
4o		10	5	5	10	>200	20	2.5	0.0022	>200	0.6	0.15	0.01
4p		10	5	10	5	200	5	2.5	0.0022	—	0.6	0.15	0.005
4q		5	1.25	5	20	>200	10	5	0.0045	—	0.6	0.15	0.01
4r		10	10	10	10	>200	20	5	0.0022	—	0.6	0.15	0.005
4s		10	5	5	10	>200	10	5	0.0022	>200	2.5	0.6	0.005
Rifampicin		5	2.5	5	10	>200	10	2.5	0.0022	>200	1.25	0.3	0.005

Table 4. *In vivo* efficacy after oral (p.o.) and subcutaneous (s.c.) treatment in experimentally infected mice.

Compound	ED_{50} (mg/kg)				
	S. aureus PV_3		*S. pyogenes*	*S. abortivoequina*	*M. tuberculosis*
	p.o.	s.c.	s.c.	s.c.	H37Rv, p.o.
4a	0.37	0.28	20.0	31.8	>15.0
4b	0.47	0.31	—	>50.0	—
4c	0.74	0.48	—	25.0	—
4d	0.55	0.23	—	43.9	—
4e	>0.60	1.00	—	—	—
4f	2.10	0.50	—	>50.0	—
4g	0.70	0.50	14.6	13.4	—
4h	>0.90	>0.72	—	>50.0	—
4i	0.86	0.54	—	>50.0	—
4l	1.40	0.36	—	>50.0	—
4m	1.10	0.36	—	>50.0	—
4n	1.40	1.29	—	—	—
4o	0.20	0.23	17.0	54.5	>10.0
4p	0.66	0.29	20.0	30.8	3.2
4q	0.31	0.28	12.6	32.0	4.0
4r	0.86	0.56	>30.0	29.9	—
4s	>0.70	>0.40	>30.0	44.2	>10.0
Rifampicin	0.14	0.09	2.3	13.4	3.2

Table 5. Kinetic parameters (area under the curve=AUC, and maximum concentration=C max) in plasma and tissues of mice after oral administration of 10 mg/kg.

Compound	AUC (0~>7 hours) (mg×liter×hour)			C max (mg/liter)		
	Plasma	Liver	Lung	Plasma	Liver	Lung
4o	43.45	132.3	6.51	14.9	29.2	1.4
4p	116.20	233.04	51.68	31.4	52.3	12.5
4q	100.78	177.30	25.16	18.2	30.9	4.5
4r	4.35	81.90	2.43	2.5	25.0	1.3
4s	<0.60	49.91	0.60	0.05	9.0	1.0

(A) Groups of 8 female CD1 Cobs mice were challenged by the intraperitoneal route with the test organism. The challenge dose was three times the median lethal dose (LD_{50}). One or two hours after infection the animals were treated by oral or s.c. route with a solution or suspension of compounds to be tested in phosphate buffer, pH 7.2+5% of dimethylacetamide (0.1 ml/10 g body weight). The number of animals surviving at day 5 was used to calculate the median effective dose (ED_{50}).

(B) Groups of 10~12 mice, as above, were challenged by the i.v. route with $3LD_{50}$ of *M. tuberculosis* H37Rv. The animals were treated orally as described in (A), starting 3 days after infection. The treatment was repeated for 6 weeks (5 days treatment and 2 days interval weekly). The number of animals surviving at week 8 was used to calculate the ED_{50}. The weight increase was calculated according to the index: $WI=(W_t-W_i/W_h-W_i)\times 100$, where $W=\sum$ of the weights of single animals (dead animals were assigned an arbitrary weight of 9 g); h=healthy animals; i=infected animals; t=infected and treated animals.

Tissue and Plasma Level

Groups of female CD1 Cobs mice were treated orally with 10 mg/kg of the compounds prepared as described in (A); after 15, 30, 60, 120, 240, 420 minutes 3 mice/group were sacrificed, the heparinated blood was collected and centrifuged; lungs and livers were removed and homogenized in pH 7.2 phosphate buffer. Tissues and plasma were used for microbiological assay on *Micrococcus luteus* ATCC 9341.

3-[(Piperidin-1-yl)methylenamino]rifamycin S (**2c**)

8 g of 3-aminorifamycin S (**1**) was dissolved in 100 ml of dichloromethane; 7 ml of triethylamine was added and the solution was cooled to −40°C. A solution of 8 g of piperidylchloroformiminium chloride in 50 ml of dichloromethane was added dropwise and the temperature was kept at −40°C for 60 minutes. The solution was gently warmed to room temperature, washed with diluted acetic acid and then with water. After drying over sodium sulfate, the solution was evaporated under vacuum and the residue was extracted with 400 ml of ethyl ether. The ethereal solution was washed with a phosphate buffer solution at pH 7.5 and then with water. After treatment with anhydrous sodium sulfate the solution was diluted with 100 ml of petroleum ether and then concentrated to 50 ml under vacuum. The precipitate was filtered, and 2 g of **2c** was obtained.

4-(Piperidin)imidazolo[4,5-c]rifamycin SV (**4p**)

8 g of 3-amino-4-deoxo-4-iminorifamycin S was dissolved in 100 ml of dichloromethane; 7 ml of triethylamine was added and the solution was cooled to −40°C. A solution of 8 g of piperidylchloroformiminium chloride in 50 ml of dichloromethane was added dropwise and the temperature was kept at −40°C for one hour. The solution was gently warmed to room temperature, washed with dilute acetic acid and then with water. After drying over anhydrous sodium sulfate, the solution was concentrated to 30 ml. 10 ml of petroleum ether was added and the solution was allowed to crystallize, affording 1.0 g of compound **4p**.

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