NOVEL RIFAMYCINS

IV[1]. 3-AMINOMETHYLAZINOMETHYLRIFAMYCINS, A NEW CLASS OF RIFAMYCINS, ENDOWED WITH REMARKABLE ANTIBACTERIAL ACTIVITY

LEONARDO MARSILI, GIOVANNI FRANCESCHI, MARZIA BALLABIO, SERGIO VIOGLIO, ARISTIDE VIGEVANI

Farmitalia Carlo Erba SpA, Ricerca & Sviluppo Chimico
Via dei Gracchi, 35 20146 Milan, Italy

DOMENICO UNGHERI, COSTANTINO DELLA BRUNA and AURORA SANFILIPPO

Farmitalia Carlo Erba SpA, Ricerca & Sviluppo Biologico
Via Giovanni XXIII, 24 20014 Nerviano, Milan, Italy

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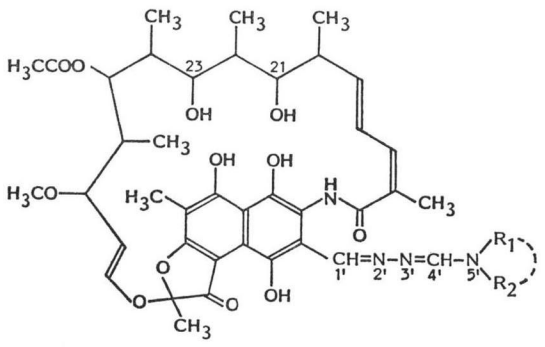
The synthesis and the biological activities of the new compounds **1**, endowed with favorable pharmacokinetic behavior, are described. In particular, compound **1a** has been chosen for further investigations.

Following our previous work on 3-amidinorifamycins[1] we contrived the novel class of compounds **1** in order to create a chemical fit distantly reminiscent of rifampicin (**2**). Compounds **1** are obtained by treatment of 3-hydrazinomethylrifamycin SV[2] with a suitable chloroformiminium chloride (in tetrahydrofuran, $0° \sim +20°C$)[1]. Relevant data are reported in Table 1 for representative compounds. The ^1H and ^{13}C NMR assignments are based on the observation that the signals of 4′-H and 4′-C are less affected than the 1′-H and 1′-C signals by oxidation to the quinone form (quinone of **1a**, δ: H-1′, 8.61; H-4′, 7.81; C-1′, 146.4; C-4′, 161.2; quinone of **1d**, δ: H-1′, 8.66; H-4′, 7.86; C-1′, 148.1; C-4′, 160.6 ppm). Carbon and hydrogen signals were correlated by heteronuclear 2D experiments.[3]. The ^1H and ^{13}C NMR data deserve a few comments. The "hydrazonic" 1′-CH group (formally rifampicin-like) shows a remarkable chemical shift difference with respect to rifampicin both for the C-1′ atom (δ 149.8 ~ 151.6 ppm vs. 134.4 ppm for rifampicin[4]) and for the corresponding proton (δ 8.99 ~ 9.08



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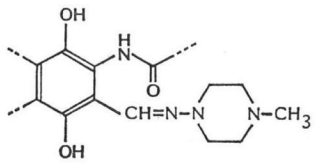

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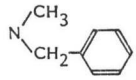
Table 1. Examples of 3-aminomethylazinomethylrifamycins.

Compound No.		Formula	FD-MS	MP (°C, dec)	Yields	NMR ($CDCl_3$, δ values)			
						H-1′	H-4′	C-1′	C-4′
1a		$C_{44}H_{58}N_4O_{12}$	834	270	70.0	9.02	7.72	149.9	160.6
1b		$C_{43}H_{56}N_4O_{12}$	820	260	43.5	9.08	7.95	150.2	158.0
1c		$C_{45}H_{60}N_4O_{12}$	848	258~260	51.5	9.06	7.82	149.9	161.3
1d		$C_{43}H_{56}N_4O_{13}$	836	265	60.7	9.02	7.74	151.6	160.1
1e	$N(CH_3)_2$	$C_{41}H_{54}N_4O_{12}$	794	268	55.0	9.07	7.76	150.0	161.4
1f	$N(C_2H_5)_2$	$C_{43}H_{58}N_4O_{12}$	822	255	45.0	9.01	7.73	149.8	160.2
1g	$N(nC_3H_7)_2$	$C_{45}H_{62}N_4O_{12}$	850	173~175	43.0	9.00	7.78	150.2	160.7
1h		$C_{46}H_{62}N_4O_{12}$	862	227~231	50.5	9.03	7.87	149.8	160.0
1i		$C_{47}H_{58}N_4O_{12}$	870	168~170	50.0	9.03	7.94	151.5	160.6
1j		$C_{44}H_{60}N_4O_{12}$	836	218~220	35.0	9.01	7.78	*	*
1k	$N(secC_4H_9)_2$	$C_{47}H_{66}N_4O_{12}$	878	178~180	41.5	8.99	7.89	*	*

* Value not determined.

Table 2. *In vitro* antibacterial activity of derivatives **1**.

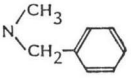
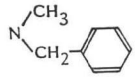
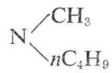
Compound No.		MIC (μg/ml)				
		Staphylococcus aureus FDA 209 P	*Streptococcus pyogenes* ATCC 12384	*Escherichia coli* B	*Salmonella abortus-equi* ATCC 9842	*Mycobacterium tuberculosis* H37 Rv
1a		0.037	1.25	10	5	0.01
1b		0.037	5	20	20	0.01
1c		0.009	1.25	20	10	0.04
1d		0.009	1.25	20	20	0.02
1e	$N(CH_3)_2$	0.018	2.5	10	10	0.02
1f	$N(C_2H_5)_2$	0.0045	1.25	10	5	0.02
1g	$N(nC_3H_7)_2$	0.0022	2.5	10	10	0.02
1h		0.075	10	10	10	0.01
1i		0.018	1.25	20	20	0.04
1j		0.018	1.25	10	5	0.04
1k	$N(secC_4H_9)_2$	0.037	10	20	10	0.04
Rifampicin		0.018	2.5	10	10	0.01

Table 3. Pharmacokinetic parameters in mice following oral administration of 10 mg/kg.

Compound No.		Plasma half-life (hours)	C_{max} (μg/ml)	t_{max} (hours)
1a		19	7	7
1b		*	*	*
1c		22	9	3
1d		4.5	3.5	4
1e	$N(CH_3)_2$	6	5.7	2.5
1f	$N(C_2H_5)_2$	25	11.5	7
1g	$N(nC_3H_7)_2$	25	8.8	4
1h		24	33.9	4
1i		26	10	4
1j		18	8	4
1k	$N(secC_4H_9)_2$	30	2.6	4
Rifampicin		6.1	10.6	1

* Discontinued for toxicity.

ppm, vs. 8.22 ppm for **2**[4]). This indicates that the additional amino-methine group conjugated with the hydrazono group (formally an amidrazono-moiety) induces a significant electronic distribution change with respect to rifampicin. On the other hand the basicity of the 5′ nitrogen does not affect the chemical shift values of the amidrazone 4′-CH group; in fact, no difference is noticed for the less basic morpholino residue **1d**.* One example of the synthetic procedure is described in the experimental section. The compounds belonging to this class have been tested for antibacterial activity *in vitro* and pharmacokinetics in mouse.

The results of Tables 2 and 3 show that the compounds of this class are potent antibacterial agents similarly to rifampicin. Noticeably, they are characterized by very long plasma half-life values in mice following oral dosage. One of the compounds of this class (compound **1a**) has been chosen for further investigations because of its very good acute tolerability (LD_{50} in mice by oral route >5 g/kg) together with activity and pharmacokinetic behavior.[6]

Experimental

^1H and ^{13}C NMR spectra were recorded on Varian XL-200 or CFT-20 spectrometers in $CDCl_3$ solutions. Signals are reported in ppm from zero TMS. Mass spectra were recorded on a Finnigan Mat 311A spectrometer equipped with a combined FI/FD/EI ion source. Melting points are un-

* *pKa* values[5] for the  amines (Table 1) are in the range 10.5 ~ 11.3, with the exception of morpholine and of *N*-methylbenzylamine, which have *pKa* 8.5 and 9.6, respectively.

corrected. All compounds gave elemental analyses in agreement with the calculated values within ±0.7%.

Minimal inhibitory concentrations (MIC) were determined by the serial two-fold 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 *Mycobacterium 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. The results are given in Table 2. Plasma levels were determined in CD1 Cobs albino mice.

Groups of 5 animals were treated orally with 10 mg/kg of the substances diluted in phosphate buffer pH 7 + 5% of dimethylformamide (0.1 ml/10 g body weight). After various times (between 1 and 36 hours) 3 mice/group were sacrificed, and plasma was collected for bioassay on *Micrococcus luteus* ATCC 9341 by the agar diffusion technique. Half-life values, peak concentrations (C_{max}) and time of peak (t_{max}) are reported in Table 3.

3-(Piperidinomethylazinomethyl)rifamycin SV (**1a**)

5 g of 3-formylrifamycin SV were dissolved in 250 ml of THF and added dropwise to a solution of 0.35 ml hydrazine hydrate in 50 ml of THF during 15 minutes under stirring at −20°C. The absence of the starting material was checked by TLC and 2 ml of triethylamine were added keeping the temperature at −20°C. 5 g of chloropiperidyl formiminium chloride were added portionwise and the mixture warmed gently to room temperature under stirring. EtOAc (350 ml) were added and the resulting solution washed with H_2O. After drying over anhydrous sodium sulfate, the solvent was evaporated and the crude product was crystallized from MeOH and then from acetone. 2.3 g of **1a** were obtained.

Anal Calcd for $C_{44}H_{58}N_4O_{12}$: C 63.02, H 7.02, N 6.68.
Found: C 62.59, H 7.13, N 6.61.

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References

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