NOTES

QUINONE MODIFIED DERIVATIVES OF CYANONAPHTHYRIDINOMYCIN

MILTON J. ZMIJEWSKI, Jr.*
and MARCIA J. MIKOLAJCZAK

University of Utah, College of Pharmacy,
Department of Medicinal Chemistry,
Salt Lake City, Utah 84112, U.S.A.

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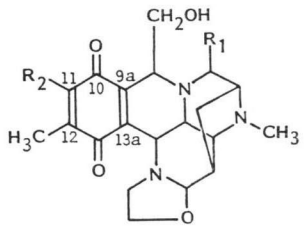
Naphthyridinomycin (**I**) is a novel antitumor antibiotic produced by *Streptomyces lusitanus*[1]. A semi-synthetic derivative of this antibiotic cyanonaphthyridinomycin (**II**), can be prepared by stirring an aqueous solution of naphthyridinomycin in the presence of sodium cyanide.

Cyanonaphthyridinomycin is a stable crystalline compound which has slightly better antitumor activity than the parent antibiotic[2]. Recently, cyanonaphthyridinomycin, also called cyanocycline A, has been isolated as a natural product of *S. flavogriseus*[3]. Our mechanism of action studies have implicated a role for the quinone substituent in the binding of these antibiotics to DNA[4~6]. We were interested in determining what effect changes in the substituent at C-11 had on the antimicrobial and antitumor activity of cyanonaphthyridinomycin. As with mitomycin A[7], cyanonaphthyridinomycin reacts with amines which replace the methoxy function at C-11 of the quinone. This report describes two of those amine derivatives along with their biological activity.



I R_1=OH, R_2=OCH_3
II R_1=CN, R_2=OCH_3
III R_1=CN, R_2=NH_2
IV R_1=CN, R_2=$NHCH_3$

In a typical reaction, the amine gas was liquefied by cooling and 30 ml of the solution added to 50 mg of cyanonaphthyridinomycin in a cooled pressure bottle. The bottle was capped and the solution stirred at 0°C for forty-five minutes. After evaporation of the amine, the residue was purified on an alumina (activity V) column developed in benzene - chloroform (6: 4). The purified derivatives were then precipitated from acetone - petroleum ether. The characteristics of cyanonaphthyridinomycin (**II**) and the amino (**III**) and methyl amino (**IV**) derivatives are compared in Table 1.

As expected for compounds with a quinoine functionality, the most visible change in the derivatives was their color. The new compounds took on different shades of purple. The water solubility of the amine compounds was also greater than cyanonaphthyridinomycin. The ultraviolet absorption spectra for all these compounds had one absorption maximum which increased in wavelength and decreased in extinction coefficient in going from methoxyl to amino to methyl amino (Table 1). The ^{13}C NMR spectra of the compounds were consistent with a change in the substituent a C-11. The methoxyl group at 61.1 ppm was absent in the spectra of both compounds. The methylamino compound demonstrated a new signal at 32.8 with the most dramatic change occurring in the resonance signal for C-11, which was shifted upfield by more than 40 ppm. The presence of cyanide functionality in the new compounds was confirmed by retention of the signal at 117 ppm. Taken together, the characteristics of these new derivatives are consistent with a change in the quinoine functionality at C-11 of cyanonaphthyridinomycin. In antimicrobial tests (Table 2), the new compounds were more active that the parent antibiotic with the amino compound being the most active of the three. This increase in activity was more pronounced in the case of the Gram-negative organisms tested. The antitumor activity comparisons were less spectacular (Table 3). Cyanonaphthyridinomycin and the amino compound

* Current address: The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285, U.S.A.

Table 1. Comparison of cyanonaphthyridinomycin (**II**) with compounds **III** and **IV**.

Characteristics	CH_3O– (II)	NH_2– (III)	CH_3NH– (IV)
Color	Bright orange	Red-purple	Dark blue-purple
UV max in MeOH (nm) (molar extinction coefficient)	269 (9,439)	276 (7,267)	278 (5,990)
Elemental analysis	$C_{22}H_{26}N_4O_5$	$C_{21}H_{23}N_5O_4 \cdot H_2O$	$C_{22}H_{27}N_5O_4 \cdot H_2O$
Calcd	C 61.97, H 6.10, N 13.15	C 58.74, H 6.39, N 16.31	C 59.76, H 6.49, N 15.60
Found	C 61.72, H 6.07, N 13.00	C 58.81, H 6.57, N 15.74	C 59.59 H 6.54, N 15.80
Yield	—	50%	80%
^{13}C NMR (ppm from TMS)			
CH_3O	61.1	—	—
CH_3NH-at C-11	—	—	32.8
C-13a	142.7	144.7	145.5
C-12	128.2	138.1	137.8
C-11	155.8	109.5	108.1
C-10	181.1	181.6	182.7
C-9a	141.0	143.9	145.3
C≡N	117.2	117.4	117.5
Solubility			
+++	Chloroform	Water, acetone, chloroform	Water, acetone, chloroform
±	Water, acetone, ethyl ether	Ethyl ether	Ethyl ether

Table 2. Minimum inhibitory concentration (μg/ml) of **II**, **III** and **IV** against bacteria.

Organism	II	III	IV
Staphylococcus aureus	0.012	0.006	0.012
S. epidermiditis	0.94	0.015	0.12
Streptococcus faecalis	5.00	0.08	0.16
Proteus vulgaris	0.47	0.06	0.23
P. mirabilis	7.50	0.23	0.94
Enterobacter aerogenes	1.90	0.06	0.23
Escherichia coli	0.50	0.06	0.23
Pseudomonas aeruginosa	3.80	0.94	1.90
Klebsiella pneumoniae	0.94	0.06	0.16

Table 3. Antitumor activity of **II** and **III** against P388 leukemia grown in mice.

Compound	Concentration tested (mg/kg)	T/C (%)
II	3.20	Toxic
	1.60	Toxic
	0.80	154
	0.40	152
III	4.00	Toxic
	2.00	Toxic
	1.00	148
	0.50	135
	0.25	118

were tested against P388 leukemia in mice. Although the amino compound protected mice at a lower dose than the parent compound, it was no better at prolonging the life span of the mice. This result is very different from that found in the mitomycins. Mitomycin C (amino) was much better than mitomycin A (methoxy) in prolonging the life of P388 infected mice[8]. The monomethylamino compound (**IV**) is currently being tested.

Recently, ITOH *et al.*[9] demonstrated that the methoxyl group at C-11 of naphthyridinomycin is susceptible to acid hydrolysis, generating a new compound SF-1739 HP. This semi-synthetic derivative has a hydroxyl group in place of the methoxyl functionality. This change markedly enhanced the antitumor activity against P388 in mice. Our results have also confirmed that changes in the substituent at C-11 has a pronounced effect on antimicrobial activity but little effect on antitumor activity against P388 was observed. Whether this change in activity is due to a change in the reduction potential of the quinone or the increased water solubility of the derivatives remains to be determined.

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