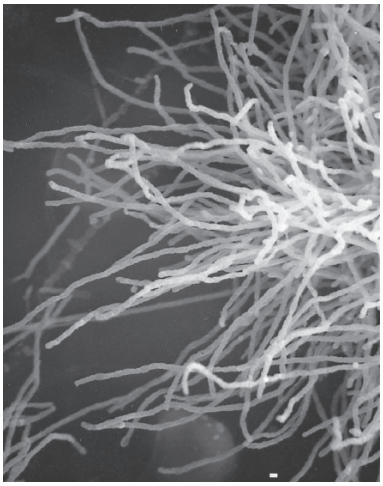
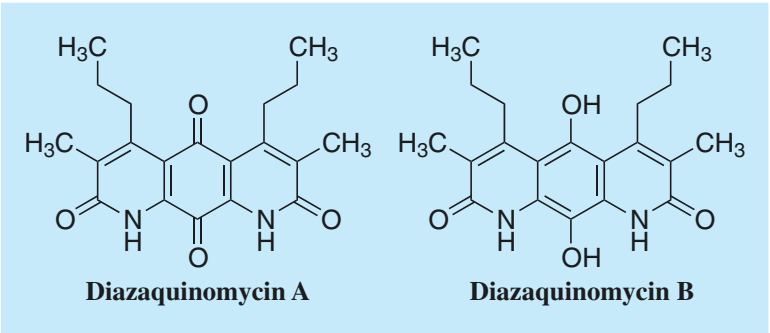
Diazaquinomycin

1. Discovery, producing organism and structures[1,2)]

Diazaquinomycins were isolated from *Streptomyces* fungal strain OM-704 while screening for antifolate substances in microorganisms. Diazaquinomycin A inhibited the growth of Gram-positive bacteria. 11,18-Diacetoxydiazaquinomycin A exhibited antitumor activity against Meth-A fibrosarcoma. The total synthesis of diazaquinomycin A has been reported by two groups (See Appendix-I). The first total synthesis was achieved by Kelly *et al*.[3)]



Streptomyces sp. OM-704

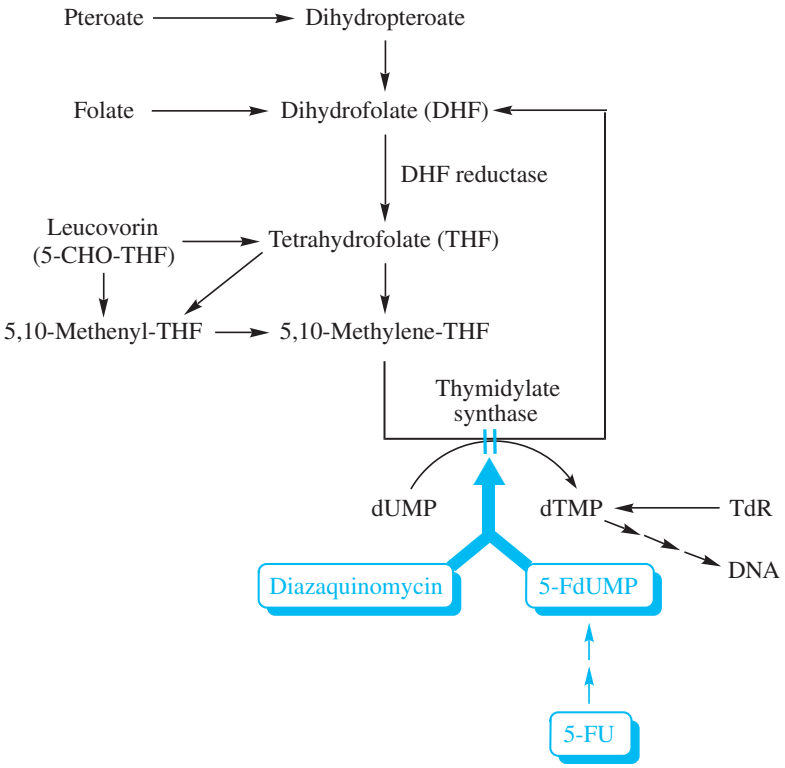


2. Physical data (Diazaquinomycin A)

Red crystals. $C_{20}H_{22}N_2O_4$; mol wt 354.41. Slightly sol. in DMSO, MeOH, acetone, $CHCl_3$. Insol. in H_2O, hexane.

3. Screening method[4)]

Most general microorganisms cannot incorporate folate-related compounds, but some special microorganisms such as *Streptococcus* sp. and *Lactobacillus* sp. require folate-related compounds and thus can incorporate them. Antifolates are used clinically as anticancer and antibacterial drugs. To screen the antifolate compounds, we selected a culture broth of soil isolates showing inhibitory activity against a *Streptococcus* sp. grown in a medium containing a limited amount of pteroate, enough amino acids, bases, and nucleosides, (except thymine and thymidine (TdR)), but lacking inhibitory activity against organisms grown in the same medium supplemented with a sufficient amount of TdR.



4. Biological activity[1,5,6)]

1) Antimicrobial activities

Diazaquinomycin A inhibited the growth of Gram-positive bacteria (MIC: 3.13–50 μg/ml) with the exception of *Bacillus spp*.

Test organism	MIC (μg/ml)	Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	6.25	*Bacillus subtilis* ATCC 6633	>100
Staphylococcus aureus ATCC 6538P	6.25	*Bacillus cereus* IFO 3001	>100
Staphylococcus aureus KB 199 (erythromycin resistant)	6.25	*Mycobacterium smegmatis* ATCC 607	>100
		Escherichia coli NIHJ JC-2	>100
Staphylococcus aureus FS 1277 (penicillin resistant)	50	*Klebsiella pneumoniae* ATCC 10031	>100
		Proteus vulgaris IFO 3167	>100
Streptococcus faecium IFO 3181	6.25	*Serratia marcescens* ATCC 8100	>100
Streptococcus pyogenes C 203	100	*Pseudomonas aeruginosa* IFO 3080	>100
Micrococcus luteus ATCC 9341	3.13		

Minimal inhibitory concentrations (MIC) were determined by the agar dilution method using heart infusion agar (pH 7.0, 37°C, 20 hrs).

2) Cytotoxicity

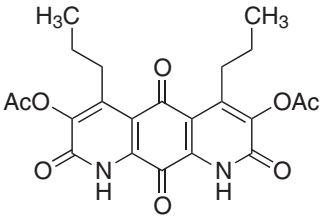
IC_{50} = 0.86 μg/ml (Vero cells), 0.23 μg/ml (Raji cells)

3) Acute toxicity (mice i.p.)

LD_{50} = 100 mg/kg

4) Antitumor activity

11,18-Diacetoxydiazaquinomycin A exhibited antitumor activity against Meth-A fibrosarcoma (10 mg/kg/day, day 1–4, T/C 141%; 100 mg/kg/day, days 1–4, T/C 175%).



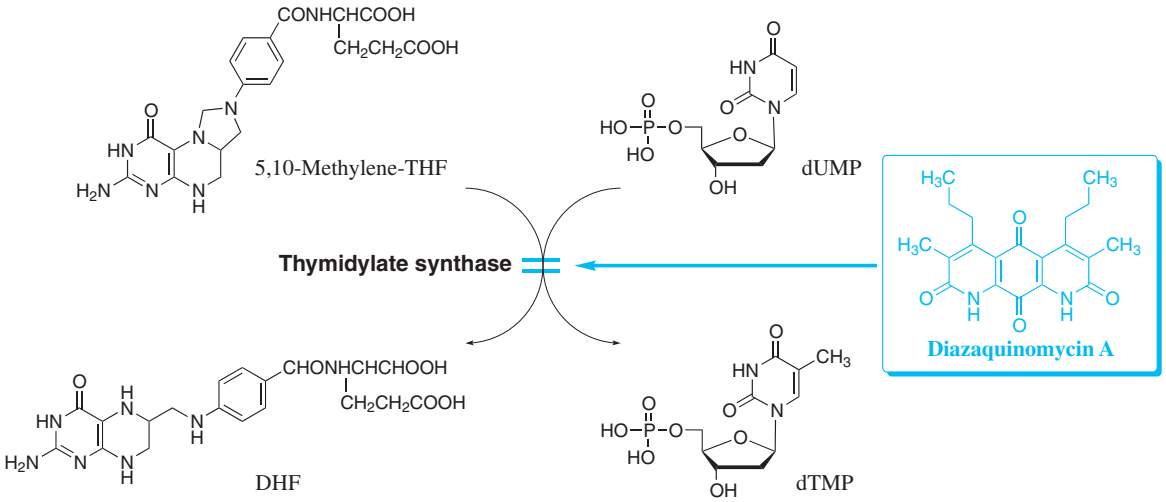
11,18-Diacetoxy-diazaquinomycin A

5. Mode of action[5)]

The inhibitory site of diazaquinomycin A was confirmed to be thymidylate synthase. It competitively inhibited bacterial and mammalian thymidylate synthases.

K*i* values of diazaquinomycin A against thymidylate synthases

Origin	K*i*
Enterococcus faecium	36 μM
Ehrlich ascites carcinoma	14 μM



6. References

1. [250] S. Ōmura *et al*., *J. Antibiot*. **35**, 1425-1429 (1982)
2. [267] S. Ōmura *et al*., *Tetrahedron Lett*. **24**, 3643-3646 (1983)
3. T. R. Kelly *et al*., *Tetrahedron Lett*. **29**, 3545-3546 (1988)
4. [326] S. Ōmura *et al*., *J. Antibiot*. **38**, 1016-1024 (1985)
5. [327] M. Murata *et al*., *J. Antibiot*. **38**, 1025-1033 (1985)
6. [412] K. Tsuzuki *et al*., *J. Antibiot*. **42**, 727-737 (1989)