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Synthesis and Cytotoxicity of 5-Deazaflavins Containing *o*- and *p*-Quinone Moieties

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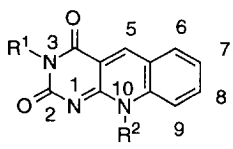
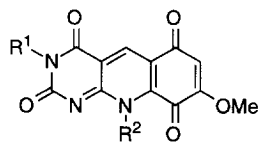
Abstract: A series of 5-deazaflavo-10,11-quinones having *o*-quinone structure in the molecule were synthesized. The cytotoxicity of 5-deazaflavo-6,9-quinones (*p*-quinone derivatives) and 5-deazaflavo-10,11-quinones (*o*-quinones) was evaluated *in vitro* against L1210 and KB cells. Some of the synthesized compounds exhibited cytotoxic activity comparable to that of mytomycin C.

The redox-active quinones such as coenzyme PQQ (methoxazin) which oxidizes alcohols, amines, etc., and coenzyme Q (ubiquinone) which transports electrons in the respiratory chain, are widespread in biological systems. Naturally occurring quinones and their derivatives exhibiting several biological activities including antibacterial[1] and antitumor activities[2] have been reported. As for antitumor agents, a number of chemical modifications have been undertaken in order to overcome undesirable side effects as well as occurrence of natural and acquired resistance to these compounds in tumor cells. Some of the analogues classified into this category have been series of heterocyclic quinones[3,4,5,6].

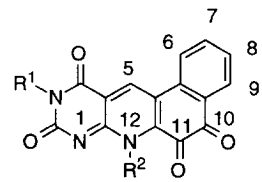
Chart 1



1
5-deazaflavin



2a ~ 2i
5-deazaflavo-6,9-quinones



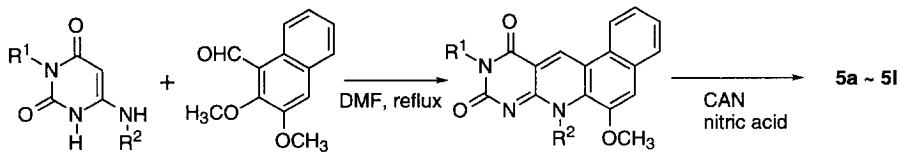
5a ~ 5l
5-deazaflavo-10,11-orthoquinones

In the meantime, the synthesis and catalytic redox properties of 5-deazaflavins (5-carba-5-deazaisoalloxazines) **1** as model compounds of flavin and NAD have already been reported[7,8,9] (Chart 1). We have also reported the synthesis and amine oxidizing abilities of 5-deazaflavo-6,9-quinones **2a-2i** as a new type of 5-deazaflavins having *p*-quinone function in the molecule[10]. These 5-deazaflavo-6,9-quinones behaves not only as catalysts for autorecycling benzylamine oxidation to benzaldehyde which could be

regarded as a model system of amine oxidase, but also as electron transporting agents in mitochondria such as flavin and coenzyme Q[11]. Most recently antitumor activity of some of 5-deazaflavins have been reported by this group[12], and the multifunctionality of 5-deazaflavin derivatives encouraged us to synthesize and evaluate cytotoxicity of 5-deazaflavoquinones. We wish to report a convenient synthesis and cytotoxicity of new 5-deazaflavo-10,11-quinones which have an *o*-quinone group in the molecule and might be regarded as a hybrid of 5-deazaflavin and coenzyme PQQ[13]. Cytotoxic evaluation of 5-deazaflavo-6,9-quinones which have already been synthesized as a hybrid model compound of 5-deazaflavin and coenzyme Q is also detailed in this report.

According to the method reported by Yoneda *et al.*[14], condensation of 3-substituted-6-alkylaminouracils and 2,3-dimethoxynaphthaldehyde (**3**) (**Scheme 1, Table 1**) in DMF gave 11-methoxy-5-deazaflavin intermediates **4a-4l** in moderate yields. The aldehyde **3** was prepared from 2,3-dihydroxynaphthalene by Vilsmeier formylation ($POCl_3$, DMF, 90°C, 8h, quant.) and subsequent methylation (CH_3I, K_2CO_3, acetone, reflux, 8h, 72.5 %). The 11-methoxy-5-deazaflavins were oxidized to the desired *o*-quinone derivatives using CAN in the presence of nitric acid[15] (**Table 1**).

Scheme 1



3 **4a ~ 4l**

R^1 = H, CH_3, C_6H_5
R^2 = *n*-C_nH_{2n+1}
(n = 2,3,4,6,8,12)

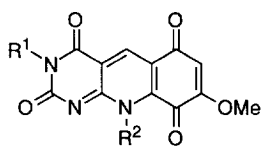
Table 1

Compounds	R^1	R^2	Yield % (M.p. °C) **4**	**5**
a	H	C_4H_9	24 (>300)	41 (260)
b	H	C_8H_{17}	27 (256)	50 (224)
c	H	$C_{12}H_{25}$	39 (>300)	44 (>300)
d	CH_3	C_2H_5	61 (>300)	21 (>300)
e	CH_3	C_3H_7	61 (>300)	40 (262)
f	CH_3	C_4H_9	55 (262)	22 (>300)
g	CH_3	C_6H_{13}	53 (261)	65 (210)
h	CH_3	C_8H_{17}	62 (210)	9 (192)
i	CH_3	$C_{12}H_{25}$	74 (145)	12 (127)
j	C_6H_5	C_4H_9	56 (245)	26 (185)
k	C_6H_5	C_8H_{17}	61 (218)	17 (180)
l	C_6H_5	$C_{12}H_{25}$	44 (161)	29 (125)

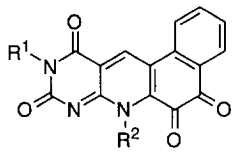
Table 2 summarizes the *in vitro* inhibitory concentration of these compounds against murine leukemia L1210 and human epidermoid carcinoma KB cells using mitomycin C (MMC) as a standard. As

shown in **Table 2**, most of 5-deazaflavoquinones exhibited cytotoxicity comparable to that of MMC. It is evident that introduction of *o*- or *p*- quinone structure into the 5-deazaflavin skeleton is crucial for the activity, because the precursors of these quinone derivatives were impotent to cancer cells (entry 1, entry 12). It was shown that compounds **2a, 2b, 2c, 5a, 5b,** and **5c** having no substituent at R^1, showed generally low activity against cancer cells (entry 2-4, 13-15).

Table 2. *In vitro* cytotoxicity of 5-deazaflavoquinones against murine leukemia L1210 and human epidermoid carcinoma KB cells.



2a ~ 2i



5a ~ 5l

				IC_{50} (μM)[a]	
entry	compound	R^1	R^2	L1210	KB
1	**1**	CH_3	$C_{12}H_{25}$	>50	>50
2	**2a**	H	C_6H_{13}	4.8	4.5
3	**2b**	H	C_8H_{17}	4.4	1.5
4	**2c**	H	$C_{12}H_{25}$	2.5	12.7
5	**2d**	CH_3	C_4H_9	1.2	0.9
6	**2e**	CH_3	C_6H_{13}	0.5	0.6
7	**2f**	CH_3	C_8H_{17}	0.4	5.8
8	**2g**	CH_3	$C_{12}H_{25}$	0.1	1.8
9	**2h**	C_6H_5	C_6H_{13}	1.3	0.4
10	**2i**	C_6H_5	C_8H_{17}	1.8	1.0
11	**2j**	C_6H_5	$C_{12}H_{25}$	2.7	0.3
12	**4a**	CH_3	C_2H_5	>50	>50
13	**5a**	H	C_4H_9	2.1	5.2
14	**5b**	H	C_8H_{17}	4.2	3.2
15	**5c**	H	$C_{12}H_{25}$	3.0	6.9
16	**5d**	CH_3	C_2H_5	0.1	0.3
17	**5e**	CH_3	C_3H_7	0.7	1.2
18	**5f**	CH_3	C_4H_9	0.4	0.2
19	**5g**	CH_3	C_6H_{13}	0.1	0.8
20	**5h**	CH_3	C_8H_{17}	0.4	1.7
21	**5i**	CH_3	$C_{12}H_{25}$	0.3	0.2
22	**5j**	C_6H_5	C_4H_9	4.9	28.2
23	**5k**	C_6H_5	C_8H_{17}	3.1	22.9
24	**5l**	C_6H_5	$C_{12}H_{25}$	2.8	17.9
		mytomycin C		0.6	0.4

[a] IC_{50} = Concentration of the compound (μM) required to inhibit the cellular growth by 50% as determined by the MTT assay.

p-Quinone derivatives **2h**, **2i**, and **2j** having a phenyl substituent at the R^1 position were potent to KB cells, whereas the corresponding *o*-quinone derivatives **5j**, **5k**, and **5l** were less active (entry 9-11, 22-24). It is noteworthy that significant *in vitro* cytotoxicity against L1210 and KB cells was displayed when R^1 of the compound was substituted with a methyl group. Particularly, many of the *o*-quinone derivatives showed stronger activity against L1210 and KB cells than MMC, however there seemed no correlation between the length of the side chain at R^2 and antitumor activity (entry 16-21). Further investigations to identify these structural relationships to the cytotoxic potency and to clarify the mechanism of cytotoxicity are currently in progress.

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15. All the new compounds in this article were identified by ^{1}H-NMR, IR, and elemental analyses.

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