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Synthesis and Evaluation of Nitro 5-Deazaflavins as Novel Bioreductive Antitumor Agents

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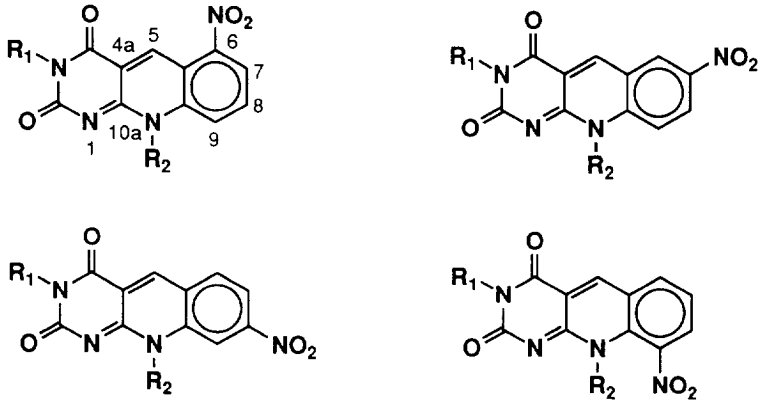
Abstract : A series of nitro 5-deazaflavins, 5-deazaflavins possessing a nitro group at C(6)-C(9) position, has been designed and synthesized as a novel class of bioreductive nitrohetero-aromatic compounds and their cytotoxicities towards L1210 and KB cells were evaluated. It has been found that the nitro 5-deazaflavins undergo one electron reduction on the nitro group and undergo two electrons or "(net) hydride" reduction on the C(5)-C(4a)-C(10a)-N(1) redox system. They showed much more potent antitumor activities than the other 5-deazaflavins bearing no nitro group. These results suggest that an activation of nitro group by biological one electron reduction is crucial for an expression of cytotoxicity.

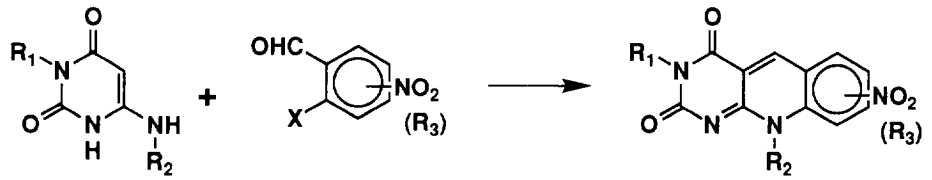
Nitrohetero-aromatic compounds[1] have attracted considerable attention as bioreductively activated cytotoxins.[2] These compounds have extensively been used clinically in the treatment of anaerobic infections and in the cancer therapy, especially as selective cytotoxins against hypoxic cells[3] and as radiosensitizers.[4] The critical step in cytotoxicity is enzymatic one electron reductive activation of nitro group of a drug, which subsequently results in the DNA damage due to the strand breaks and helix destabilization[5]. It is conceivable that the extent of the damage for DNA is dependent upon the capacity of the drug to act as a substrate for intracellular reductases,[6] the electron affinity of the drug[7] which affects the life time[8] of the activated drug, and the interaction of the activated drug with DNA.[9]



Scheme 1.

As a novel class of bioreductively activated nitrohetero-aromatic compounds, we designed a series of nitro 5-deazaflavins, namely 5-deazaflavins (5-carba-5-deazaisoalloxazines) possessing a nitro group at C(6)-C(9) position (Scheme 1). 5-Deazaflavin[10] is one of the redox coenzymes which play important roles in biological systems. It is known that 5-deazariboflavin acts as a flavin antagonist and shows anticoccidial[11] and antiprotozoal activities.[12] 5-Deazaflavins have higher redox potentials and undergo quick two electrons or "(net) hydride" reduction on the C(5)-C(4a)-C(10a)-N(1) redox system and slow one electron reduction.[13] Recently, using 5-deazaflavin linked, modified oligo nucleotide, we have demonstrated that there are significant interactions between the 5-deazaflavin molecule and DNA.[14] From these points of view, nitro 5-deazaflavins would be anticipated to undergo enzymatic one electron reduction on the nitro group much faster than the C(5)-C(4a)-C(10a)-N(1) redox system. The reduced intermediates would be stabilized by the conjugation with electrophilic 5-deazaflavin ring system and allowed to have enough life time to interact with DNA and cause DNA damage, resulting in the high cytotoxicity. In the present paper, we wish to describe syntheses and antitumor activities of nitro 5-deazaflavins as well as the redox properties of these novel and unique molecules.

The nitro 5-deazaflavins were synthesized according to Yoneda's method[15] (Scheme 2). Condensation reactions of 6-dodecylaminouracil with 2-fluoro-6-nitrobenzaldehyde, 2-methoxy-5-nitrobenzaldehyde, 2-chloro-4-nitrobenzaldehyde, and 2-methoxy-3-nitrobenzaldehyde gave 6-nitro- **2**, 7-nitro- **3**, 8-nitro- **4**, and 9-nitro-5-deazaflavin **5** (Table 3) respectively in 70-85 % . Similarly compounds **9-12** and **16-19** (Table 3) were obtained by the condensations of 3-methyl-6-dodecylaminouracil or 3-phenyl-6-octylaminouracil with the respective nitrobenzaldehydes described above in 65-90 %. Compounds **22-26** (Table 3) were prepared by treatment of the corresponding 5-deazaflavins with ethyl bromoacetate in DMF in the presence of potassium carbonate in 85-90 % .



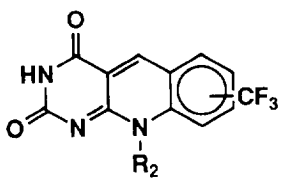
R_1 = H, CH_3, C_6H_5
R_2 = n-C_8H_{17}, n-$C_{12}H_{25}$
X = F, Cl, OCH_3

2-5, 9-12, 16-19

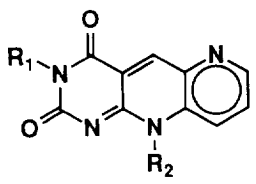
Scheme 2.

5-Deazaflavins possessing no nitro group[15] were prepared as reference compounds to evaluate antitumor activities. 5-Deazaflavins with electron withdrawing trifluoromethyl group **6** and **7** (Scheme 3) were also synthesized utilizing 2-fluoro-5-trifluoromethyl or 2-fluoro-4-trifluoromethyl benzaldehyde in 75-80 %. Furthermore, 6-aza-5-deazaflavins **13** and **20** (Scheme 3), and 9-aza-5-deazaflavins **14** and **21** (Scheme 3) were prepared by condensations of the respective 6-aminouracils with 3-methoxy-2-pyridinecarboxaldehyde or 2-chloro-3-pyridinecarboxaldehyde in 76-85 %. Although these 5-deazaflavins do not possess a nitro group, they will have high redox potentials due to the the presence of the electron withdrawing trifluoromethyl group or the ring nitrogen atom at N(6) or N(9) position. Thus, these compounds may be suitable for the comparison with the corresponding nitro compounds in terms of antitumor activities.

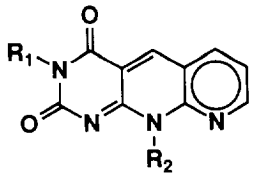
The nitro 5-deazaflavins appear to have two different redox centers; one is C(5)-C(4a)-C(10a)-N(1) redox system and the other is nitro group, and redox properties of these compounds have entirely been unknown. To investigate the redox properties of nitro 5-deazaflavins and to have a clue for mechanism of their



6; 7-CF_3, R_2 = n-$C_{12}H_{25}$
7; 8-CF_3, R_2 = n-$C_{12}H_{25}$



13; R_1 = CH_3, R_2 = n-$C_{12}H_{25}$
20; R_1 = C_6H_5, R_2 = n-C_8H_{17}



14; R_1 = CH_3, R_2 = n-$C_{12}H_{25}$
21; R_1 = C_6H_5, R_2 = n-C_8H_{17}

Scheme 3.

Table 1. Reduction of Nitro 5-Deazflavins with Three Reaction Systems*

	R1	R2	R3	NaBH4[a]		BNAH[b]		Na2S2O4[c]	
				27 (%)	**28** (%)	**27** (%)	**28** (%)	**27** (%)	**28** (%)
9	CH3	n-C12H25	6-NO2	98	0	96	0	0	92
10	CH3	n-C12H25	7-NO2	95	0	95	0	0	90
11	CH3	n-C12H25	8-NO2	90	0	90	0	0	88
12	CH3	n-C12H25	9-NO2	96	0	98	0	0	90

*All the reactions were carried out under a condition where [5-dFl] = 2.0 x 10^{-2}(M), [Reagent] = 1.0 x 10^{-1}(M) under argon.

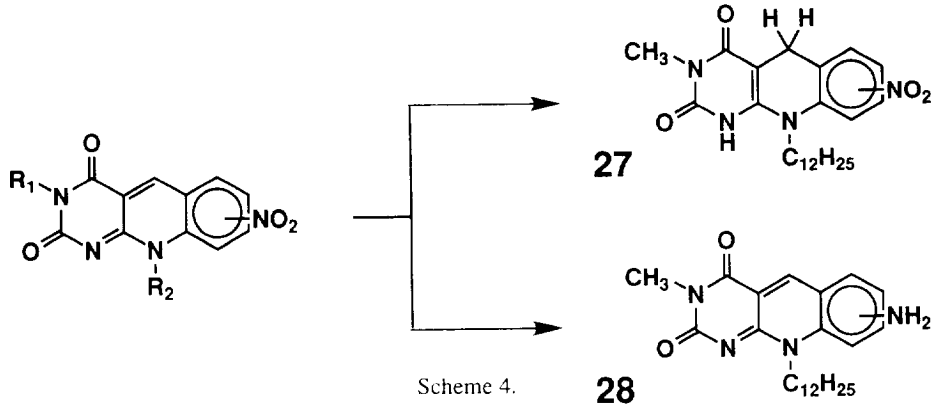
a) At 298K, in ethanol. The reactions proceeded instantaneously.

b) BNAH represents 1-benzyl-1,4-dihydronicotinamide. At 298K, in acetonitrile. The reactions proceeded instantaneously.

c) At 298K, in methanol containig water (90%). The reactions proceeded instantaneously.

biological action, the reduction reactions of the compounds have been carried out. Thus, compounds **9-12** were reduced with three kinds of reaction systems; [1] NaBH4, [2] NAD(P)H model compound, and [3] Na2S2O4. The reduced products were identified (Scheme 4) and the results are shown in Table 1.

As Table 1 shows, the treatment of nitro 5-deazaflavins with sodium borohydride or 1-benzyl-1,4-dihydronicotinamide (BNAH) afforded nitro 1,5-dihydro-5-deazaflavins **27** (Scheme 4) almost quantitatively as is the case with usual 5-deazaflavins bearing no nitro group. In contrast to these results, the treatment with sodium hydrosulfite gave instantaneously amino 5-deazaflavins **28** (Scheme 4) even at room temperature.[16] It is known that reduction of the 5-deazflavins possessing no nitro group with sodium hydrosulfite requires higher reaction temperature and proceeds sluggishly[17] to give the corresponding 1,5-dihydro-5-deazaflavins.



Scheme 4.

These results clearly indicate that the two electrons or "(net) hydride" reduction takes place on the C(5)-C(4a)-C(10a)-N(1) redox system much faster than on the nitro group of nitro 5-deazaflavins. On the other hand, the one electron reduction takes place on the nitro group much faster than the C(5)-C(4a)-C(10a)-N(1) redox system.

Furthermore, to compare the electron affinity of the compounds, reduction potentials of nitro 5-deazaflavins and other 5-deazaflavins bearing no nitro group were measured by means of cyclic voltammetry (CV) and the reduction potentials[18] of compounds **6-14** are shown in Table 2. It has been found that nitro 5-deazaflavins have higher reduction potentials than other electrophilic 5-deazaflavins, indicating that one electron reduction proceed more readily in nitro 5-deazaflavins. The results of model redox reactions and the reduction

Table 2. Reduction Potentials (Ep(V)) of (Nitro) 5-Deazaflavins**

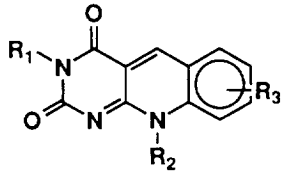
	R1	R2	R3	Ep(V)	
8	CH_3	$C_{12}H_{25}$	H	–1.098	
9	CH_3	$C_{12}H_{25}$	6-NO_2	–0.560	–0.920
10	CH_3	$C_{12}H_{25}$	7-NO_2	–0.804	
11	CH_3	$C_{12}H_{25}$	8-NO_2	–0.540	–0.850
12	CH_3	$C_{12}H_{25}$	9-NO_2	–0.682	
13	CH_3	$C_{12}H_{25}$	6-Aza	–0.888	
14	CH_3	$C_{12}H_{25}$	9-Aza	–0.882	
6	H	$C_{12}H_{25}$	7-CF_3	–0.982	
7	H	$C_{12}H_{25}$	8-CF_3	–0.900	

**All the potentials were measured at 298K in DMF, [5-dFl] = 1.0×10^{-3} (M), [Bu_4NClO_4] = 1.0×10^{-1} (M) versus an aqueous Ag/AgCl reference electrode under N_2.

potentials of nitro 5-deazaflavins suggest that they easily undergo biological one electron reduction to generate a nitro anion radical[19] which is believed to induce significant cytotoxicity by damaging DNA. Otherwise they will undergo two electrons or "(net) hydride" reduction to be transformed into nitro 1,5-dihydro-5-deazaflavins which may be less toxic than their oxidized forms because of their lower redox potentials.[20] Therefore it would be the capacity of nitro 5-deazaflavins to act as substrates for one electron reductase (NADPH cytochrome P450 reductase, xanthine oxidase, etc.) or two electrons reductase (DT diaphorase) that determines their cytotoxic potencies .

To evaluate cytotoxicities of (nitro) 5-deazaflavins, compounds **1-26** were tested for antitumor activity towards murine lymphoma L1210 cells and human epidermoid carcinoma KB cells in vitro by using an MTT assay developed by Carmichael.[21] The IC_{50} values (the concentration (μM) required for 50% inhibition of cell growth) for these compounds are shown in Table 3. As Table 3 shows, significant cytotoxicities (IC_{50} = 0.5-20 μM) towards these tumor cells were generally observed in a series of nitro 5-deazaflavins, though the cytotoxic potencies are varied depending upon R1 and R2 groups.[22] 5-Deazaflavins possessing no nitro group, and those with electron withdrawing trifluoromethyl group **6** and **7**, and electrophilic aza analogues **13**, **14**, **20** and **21** showed no significant cytotoxicities (IC_{50} > 200 μM). Although the reduction potentials of nitro 5-deazaflavins are higher than those of other 5-deazaflavins bearing no nitro group, only the reduction potentials could not account for their cytotoxic effects. The results of antitumor activities and redox properties of these compounds suggest that activation of the nitro group of nitro 5-deazaflavins through biological one electron reduction would be essential for an expression of significant cytotoxicities. And only the inherent

Table 3. IC50 Values of Nitro 5-Deazaflavins and Other 5-Deazaflavin Derivatives on L1210 and KB Cells Growth in vitro[21)]



R_1 = H, CH_3, C_6H_5, $CH_2COOC_2H_5$
R_2 = n-C_8H_{17}, n-$C_{12}H_{25}$

	R1	R2	R3	IC50 (μM)	
				L1210 cell	KB cell
1[15)]	H	n-C12H25	H	>200	>200
2	H	n-C12H25	6-NO2	0.4	3.2
3	H	n-C12H25	7-NO2	6.8	26.4
4	H	n-C12H25	8-NO2	2.5	12.8
5	H	n-C12H25	9-NO2	1.8	2.6
6	H	n-C12H25	7-CF3	>200	>200
7	H	n-C12H25	8-CF3	>200	>200
8[15)]	CH3	n-C12H25	H	>200	>200
9	CH3	n-C12H25	6-NO2	1.6	6.8
10	CH3	n-C12H25	7-NO2	21.6	15.8
11	CH3	n-C12H25	8-NO2	5.2	8.0
12	CH3	n-C12H25	9-NO2	7.8	20.0
13	CH3	n-C12H25	6-Aza	>200	>200
14	CH3	n-C12H25	9-Aza	>200	>200
15[15)]	C6H5	n-C8H17	H	>200	>200
16	C6H5	n-C8H17	6-NO2	2.6	8.4
17	C6H5	n-C8H17	7-NO2	9.6	7.6
18	C6H5	n-C8H17	8-NO2	2.6	12.4
19	C6H5	n-C8H17	9-NO2	1.5	6.0
20	C6H5	n-C8H17	6-Aza	>200	>200
21	C6H5	n-C8H17	9-Aza	>200	>200
22	CH2CO2C2H5	n-C8H17	H	>200	>200
23	CH2CO2C2H5	n-C8H17	6-NO2	3.0	7.6
24	CH2CO2C2H5	n-C8H17	7-NO2	12.0	30.0
25	CH2CO2C2H5	n-C8H17	8-NO2	8.4	7.6
26	CH2CO2C2H5	n-C8H17	9-NO2	4.0	3.6
	Mitomycin C			0.6	0.4

capacity of 5-deazaflavin ring system to accept two electrons or "(net) hydride" does not lead to significant cytotoxicities even the compounds are electrophilic enough owing to an introduction of a ring nitrogen atom or an electron withdrawing substituent other than nitro group. Also the position of a nitro group has been found to influence their cytotoxic effects. There is a tendency of the cytotoxicities in the following order 6-NO_2 > 9-NO_2, 8-NO_2 > 7-NO_2 in the nitro 5-deazaflavins bearing the same R_1 and R_2. This could be accounted by the electron affinities[20] and the stability[23] of their one electron reduction intermediates.

In conclusion, we first synthesized a series of nitro 5-deazaflavins as a novel class of bioreductive nitrohetero-aromatic compounds and evaluated their antitumor activities. The differential hypoxic cytotoxicities as well as the redox properties of these compounds are described in the following paper.[23]

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16. Amino 1,5-dihydro-5-deazaflavins were obtained by refluxing the reaction solutions of nitro 5-deazaflavins **9-12**. Reduction of nitro 5-deazaflavins **2-5**, **9-12**, **16-19**, **23-25**, and **26** at room temperarure gave only amino 5-deazaflavins.
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18. Reduction potentials of compounds **1-5** and **15-26** are similar to those of the corresponding 5-deazaflavins shown in Table 2. See also ref. 23. The stability of one electron reduction products of nitro 5-deazaflavins is described in ref. 23.
19. The importance of nitro anion radical as a DNA damaging species have been well discussed (ref 8.) and the interaction of reduced nitrohetero-aromatic compounds with DNA have been studied (ref 9).
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