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Evaluation of Differential Hypoxic Cytotoxicity and Electrochemical Studies of Nitro 5-Deazaflavins

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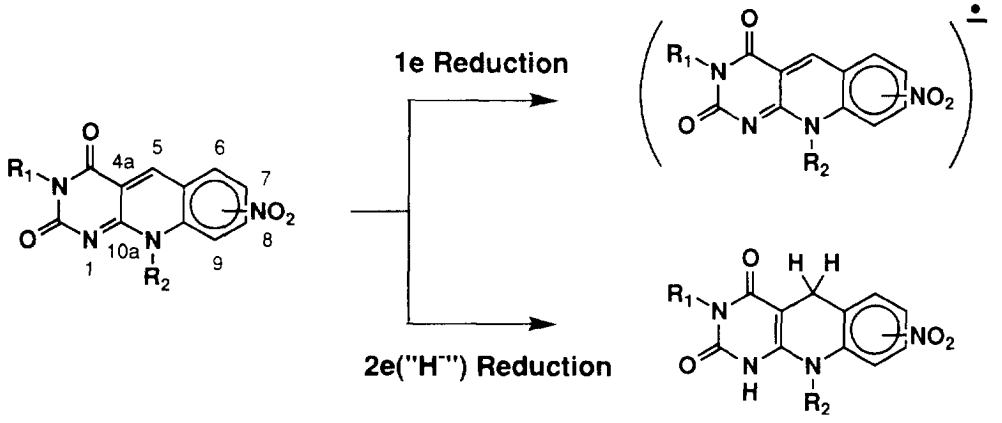
Abstract: Cytotoxicities of nitro 5-deazaflavins were evaluated in vitro towards hypoxic and oxic Chinese hamster cells (V79). 6-Nitro and 8-nitro derivatives were generally more toxic towards hypoxic cells than oxic cells, showing marked hypoxic selectivity. In contrast, 7-nitro and 9-nitro derivatives showed no significant hypoxic selectivity. Electrochemical study using cyclic voltammetry (CV) revealed that 6-nitro and 8-nitro derivatives generate a stable two electrons reduction product as well as a stable one electron reduction product and that 7-nitro and 9-nitro derivatives afford an unstable one electron reduction product. These results strongly support that not solely electron affinity but also stability of the one electron reduction products is crucial for the differential hypoxic cytotoxicities of nitro 5-deazaflavins.

In many solid tumors there is a proportion of hypoxic cells, which can be a direct cause of therapeutic resistance.[1] Because some drugs and radiation require oxygen to be maximally cytotoxic, hypoxic cells represent a potential clinical problem in the chemotherapy of solid tumors. Bioreductive drugs[2] have attracted considerable attention as hypoxic selective cytotoxins, because they undergo bioreduction to be more cytotoxic in hypoxic regions than in oxic regions. Among bioreductive drugs, nitrohetero-aromatic compounds[3] have been studied most extensively and their clinical use in combination with radiation[4] for cancer therapy is under evaluation.

As an extention of bioreductive drug approach, we have designed nitro 5-deazaflavins[5] (Scheme 1) as a novel type of nitrohetero-aromatic compounds that contain a redox coenzyme ring system. It should be of notice that nitro 5-deazaflavins have two redox centers, one is C(5)-C(4a)-C(10a)-N(1) redox system and the

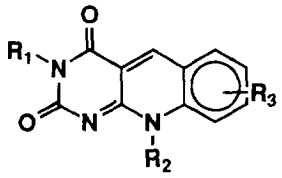


Scheme 1.

other is nitro group, both of which would enhance their redox reactivities reciprocally. As was described in the preceding paper,[5] they undergo one electron reduction to generate the corresponding nitro anion radicals (Scheme 1) which show potent antitumor activities, while they also undergo two eletrons or "(net) hydride" reduction to be transformed into nitro 1,5-dihydro-5-deazaflavins (Scheme 1) which may be less toxic than their oxidized forms. These interesting redox properties will affect significantly their biological actions under either unaerobic or aerobic condition. In the present paper, we wish to report differential hypoxic cytotoxicities of nitro 5-deazaflavins and their redox properties involving the stability of their reduction products through an electrochemical study.

Differential hypoxic cytotoxicities of nitro 5-deazaflavins were evaluated towards Chinese hamster cells (V79)[6] using the MTT assay[7] and nitrofurazone was employed as a reference drug. The results are shown in Table 1.

Table 1. Differential Hypoxic Cytotoxicities of (Nitro) 5-Deazaflavins towards V79 Cells (IC_{50}[8]) (μM))



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

	R1	R2	R3	IC50 (μM)		IC50(Air)
				IC50(Air)	IC50(N_2)	IC50(N_2)
1	H	n-C12H25	H	>1000	>1000	-
2	H	n-C12H25	6-NO2	4.3	1.1	**3.9**
3	H	n-C12H25	7-NO2	16	22	1 / 1.4
4	H	n-C12H25	8-NO2	300	190	**1.6**
5	H	n-C12H25	9-NO2	1.9	3.6	1 / 1.9
6	CH3	n-C12H25	H	>1000	>1000	-
7	CH3	n-C12H25	6-NO2	7.2	2.9	**2.5**
8	CH3	n-C12H25	7-NO2	>1000[9]	>1000[9]	-
9	CH3	n-C12H25	8-NO2	130	61	**2.1**
10	CH3	n-C12H25	9-NO2	>1000[9]	>1000[9]	-
11	C6H5	n-C8H17	H	>1000	>1000	-
12	C6H5	n-C8H17	6-NO2	3.6	0.56	**6.4**
13	C6H5	n-C8H17	7-NO2	27	19	1 / 1.4
14	C6H5	n-C8H17	8-NO2	6.1	1.2	**5.1**
15	C6H5	n-C8H17	9-NO2	1.7	3.7	1 / 2.2
Nitrofurazone				740	270	**2.7**[10]

As Table 1 shows, 6-nitro- and 8-nitro-5-deazaflavins were generally more cytotoxic under anaerobic condition than under aerobic condition. Marked hypoxic selectivity values (IC50(Air)/IC50(N_2)) of 1.6-6.4 were found in these compounds. In the 5-deazaflavins possessing a nitro group at C(6) or C(8) position, those

with phenyl group at N(3) position and octyl group at N(10) position, **12** and **14**, showed the highest hypoxic selectivity as well as the most potent antitumor activity under both aerobic and anaerobic conditions. In contrast to these results, 7-nitro- and 9-nitro-5-deazaflavins were generally more toxic under aerobic condition than anaerobic condition, showing no significant hypoxic selectivity. 5-Deazaflavins bearing no nitro group[11] showed no significant cytotoxicities under both aerobic and anaerobic conditions. These results indicate that the hypoxic selectivity of nitro 5-deazaflavins is mainly dependent upon the position of a nitro group, though the substituent at N(3) position (R1) and that at N(10) position (R2) also give some effects on the degree of hypoxic selectivity.

It is considerable that stability of one electron reduction product[12] as well as electron affinity of the compounds affect their hypoxic selectivity. To have a clue for mechanism of the hypoxic selectivity, redox properties of nitro 5-deazaflavins were investigated electrochemically by means of cyclic voltammetry (CV). The reduction potentials of the compounds **2-5**,**7-10**, and **12-15** were measured (Table 2) and cyclic voltammograms of the compounds **7** and **8** are examplified in Figure 1.

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

	R1	R2	R3	Ep (V)	
2	H	n-C12H25	6-NO2	–0.542	–0.880
3	H	n-C12H25	7-NO2	–0.782	
4	H	n-C12H25	8-NO2	–0.522	–0.838
5	H	n-C12H25	9-NO2	–0.702	
7	CH3	n-C12H25	6-NO2	–0.560	–0.920
8	CH3	n-C12H25	7-NO2	–0.804	
9	CH3	n-C12H25	8-NO2	–0.540	–0.850
10	CH3	n-C12H25	9-NO2	–0.682	
12	C6H5	n-C8H17	6-NO2	–0.524	–0.860
13	C6H5	n-C8H17	7-NO2	–0.760	
14	C6H5	n-C8H17	8-NO2	–0.498	–0.820
15	C6H5	n-C8H17	9-NO2	–0.694	
Nitrofurazone				–0.834	

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

It has been found that the reduction potentials and the pattern of cyclic voltammograms are mainly dependent upon the position of the nitro group; the compounds possessing a nitro group at the same position show similar reduction potentials and similar pattern of cyclic voltammograms, though R1 and R2 groups give some effects to them. It has also been revealed that 6-nitro- and 8-nitro-5-deazaflavins gave similar cyclic voltammograms (Figure 1(a)) and they have higher reduction potentials (Table 2) than those of 7-nitro and 9-nitro derivatives which afforded similar cyclic voltammograms (Figure 1(b)). As Figure 1(a) shows, 6-nitro and 8-nitro derivatives reversibly generate not only a stable one electron reduction product but also a two electrons reduction product. On the other hand, as Figure 1(b) shows, 7-nitro- and 9-nitro-5-deazaflavins afford an unstable one electron reduction product which has very short life time. These results clearly indicate that the position of a nitro group of nitro 5-deazaflavins affect both the electron affinity of the compounds and the stability of the reduction products.

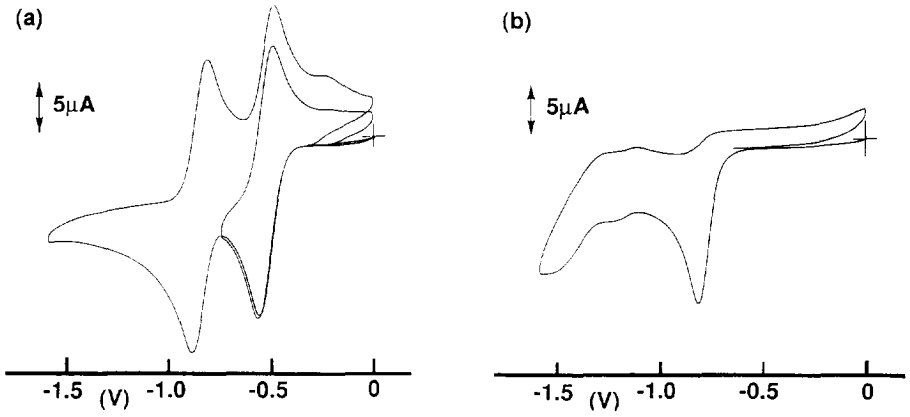


Figure 1. Cyclic Voltammograms (scan rate v = 100mVs^{-1}) of (a) 3-Methyl-10-Dodecyl-6-Nitro-5-Deazaflavin **7**, and (b) 3-Methyl-10-Dodecyl-7-Nitro-5-Deazaflavin **8**

From the results of electrochemical and biological studies, it has been revealed that electron affinity and stabilities of the reduction products of nitro 5-deazaflavins affect significantly their cytotoxic potencies and hypoxic selectivity. 6-Nitro- and 8-nitro-5-deazaflavins have higher electron affinity and give stable reduction products, which subsequently lead to marked hypoxic selectivity. On the other hand, 7-nitro- and 9-nitro-5-deazaflavins generate an unstable reduction product, showing no significant hypoxic selectivity.

In the present and the preceding[5] papers, we first described the design and syntheses of nitro 5-deazaflavins as a novel class of nitrohetero-aromatic compounds which show marked differential hypoxic cytotoxicities as well as potent antitumor activities. And we demonstrated that the unique and interesting redox properties of these compounds affect significantly their biological actions. In conclusion, the present studies have revealed that 6-nitro- and 8-nitro-5-deazaflavins can be candidates for a novel type of bioreductive drug which would potentially be useful for the chemotherapy of solid tumors.

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8. IC50 values represent the drug concentrations that reduce the optical density to 50% of control. Also see ref. 7.
9. The drug was partially soluble in the medium.
10. The hypoxic selectivity value (IC50(Air)/IC50(N2)) for nitrofurazone (2.7) observed in our experiments was smaller than that reported in ref 7 (8.5).
11. 6-Aza-, 9-aza-, 7-trifluoromethyl-, and 8-trifluoromethyl-5-deazaflavins (See ref. 5.) showed no significant cytotoxicity. (IC50 >1000μM). The details will be described elsewhere.
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