determined, and apparent affinities were calculated as pA_2 values by the method of Arunlakshana and Schild.[22] Linear-regression analysis gave not only the pA_2 values but also the slopes of the Schild plots.

Drug-Discrimination Procedures. Eighteen 120-day-old Sprague–Dawley rats (Flow Laboratories, Dublin, VA) were used in this study. The animals' weights were reduced to 80% of their free-feeding weights by partial food deprivation. Animals had free access to water. Discrimination training was begun by initially training each rat to lever press for food (sweetened condensed milk diluted 2:1 with water) reinforcement using a two-lever operant chamber. After the rats were shaped to press both levers, each daily session was preceded by an ip injection of either the drug diluted in normal saline or a 1 mL/kg dose of normal saline. Pressing on one of the levers was reinforced after the administration of drug (5-OMe-DMT, 1.5 mg/kg), while responses on the opposite lever were reinforced following saline; all conditions were counterbalanced.

Discrimination training began with eight preliminary training sessions of 15-min duration; 5-OMe-DMT was administered on the first 4 days, followed by 4 days of saline. Each correct lever press resulted in reinforcement. Subsequent daily training sessions, also of 15-min duration, were composed of an initial 2.5-min extinction period, while lever pressing during the remainder of the session was reinforced according to a variable-interval schedule of 15 s (VI-15 s). The order of drug and saline training sessions consisted of a double alternation presentation, which was used throughout the remainder of the study.

After 40 training sessions, discrimination performance was stable (80–90%), and the ability of the 5-OMe-DMT stimulus to generalize to challenge compounds was studied during the 2.5-min extinction sessions interspersed between two to four training sessions. Discrimination performance was maintained by continuing training between test sessions using the same double alternation sequence described above. Data were collected only during 2.5-min test sessions and were recorded as percent correct responding on the 5-OMe-DMT drug lever. Compounds **9a–d**, **11**, and **12** were dissolved in saline and administered ip 15 min prior to a test session. In these studies, groups of three to six animals were each administered different doses of any given compound. In situations where generalization with 5-OMe-DMT did not occur (<70% correct drug-lever responding) or where a compound exhibited only partial generalization, the dose of administered compound was increased until behavior was disrupted; where generalization occurred, the results are reported as ED_{50} values.

Acknowledgment. This work was supported, in part, by U.S. Public Health Service Grant DA-01642. We thank Ms. D. Leming for her assistance in obtaining the affinity data and R. Young for his assistance in the discriminative stimulus studies. Compound **10** was a gift from Dr. A. Manian, NIMH.

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Fluorinated Analogues of 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea: An Attempt to Control Metabolism[1]

Allan B. Foster,* Michael Jarman,

Mass Spectrometry–Drug Metabolism Group, Division of Chemistry, Institute of Cancer Research, London, SW3 6JB, England

Paul L. Coe, John Sleigh, and J. Colin Tatlow

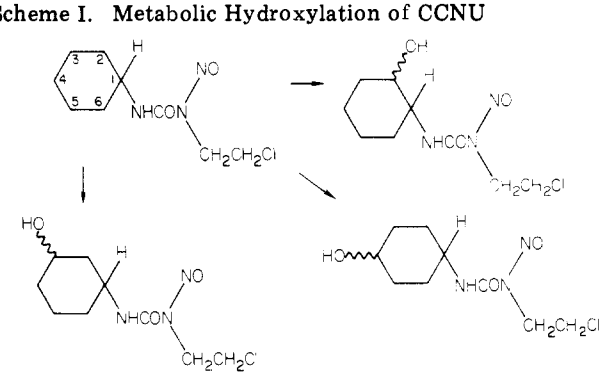
Chemistry Department, The University, Birmingham, B15 2TT, England. *Received February 19, 1980*

In seeking to block and thereby determine the role of the rapid in vivo hydroxylation of the cyclohexyl moiety of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in relation to antitumor activity and tissue distribution, the 3-(1*H*-decafluorocyclohexyl) analogue (FCCNU) was synthesized. FCCNU showed marked toxicity and little activity against the intracerebral L1210 leukemia in mice. At pH 7 in phosphate buffer at room temperature FCCNU rapidly decomposed to give 1-(1*H*-decafluorocyclohexyl)-3-nitrosoimidazolidin-2-one (**3**) and thence, by loss of HF, the 1-(nonafluorocyclohexenyl) derivative (**4**); CCNU did not follow this decomposition pathway to any significant extent. Both **3** and **4** were unstable in the buffer, but each was isolated crystalline and characterized. The formation of **3** and **4** account for the biological properties of FCCNU.

A recent emphasis of our continuing program on studies of the metabolism of anticancer agents[2-4] has been metabolism-directed design,[5] and in this context we have synthesized and studied some analogues of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU).

CCNU is one of the most effective nitrosoureas against intraperitoneal (ip) and intracerebral (ic) leukemia L1210 in mice[6] and is used in the treatment of various human malignancies, particularly those of the brain.[7] In aqueous

Scheme I. Metabolic Hydroxylation of CCNU



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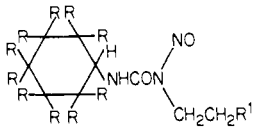
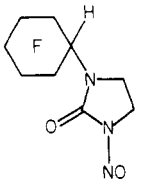
solution, CCNU decomposes to give carbamylating and alkylating species.[8] However, rapid and extensive metabolism of CCNU occurs in vivo, presumably cytochrome P-450 mediated, to give various hydroxycyclohexyl derivatives (Scheme I). Seven such metabolites are possible theoretically, namely, the 1-hydroxy and the cis and trans forms of the 2-, 3-, and 4-hydroxy derivatives. The cis and trans forms of 2-, 3-, and 4-HO-CCNU have been synthesized[9-11] and, although the half-lives for the chemical breakdown of these isomers were found to be similar, each HO-CCNU had a therapeutic index (LD_{10}/ED_{50}) greater than that of CCNU against ip and ic L1210 leukemia in mice.[11] Also, the carbamylating and alkylating activities of the various HO-CCNU derivatives showed significant differences from those of CCNU. The remaining possible metabolite, 1-HO-CCNU, would be expected to be unstable and to decompose to yield, inter alia, cyclohexanone; we have been unable to demonstrate that cyclohexanone is a product of the metabolism of CCNU by rat liver microsomes.[12]

Thus, it is probable[11] that the biological activity of CCNU is really that of the 2-, 3-, and 4-hydroxycyclohexyl metabolites. The role of hydroxylation in the biological activity of CCNU and its effect on tissue distribution might be assessed if this metabolic reaction could be retarded or blocked, and it was with this objective in mind that the effect of polydeuteration of the cyclohexyl group in CCNU was investigated. Surprisingly, the rate of hydroxylation of the cyclohexyl ring was not significantly affected by polydeuteration, and the activities of CCNU and the decadeuterated CCNU derivative 1 (CCNU-



1, R = ^{2}H; R^1 = Cl
2, R = F; R^1 = Cl
5, R = H; R^1 = CF_3



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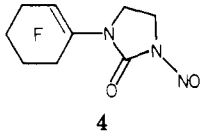

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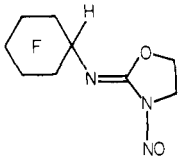

6

2,2',3,3',4,4',5,5',6,6'-d_{10}, NSC-301740, DCCNU) against the TLX-5 lymphoma in mice were not markedly different.[13]

Where the H/D isotope effect (k_H/k_D) for a metabolic reaction such as >C–H(D) → >C–OH is low (<2) and, hence, the rate of metabolism of the deuterated derivative is not significantly reduced, it may be appropriate to consider the corresponding fluorinated derivative and to evaluate the deuterium/fluorine gambit.[5] Attention was therefore turned to the decafluorinated CCNU derivative 2 (FCCNU) in which the metabolic hydroxylation of the cyclohexyl moiety should be blocked.

Table I. Activity of CCNU and FCCNU against TLX-5 Lymphoma in Mice

		% increase in survival time					
compd	dose, mg/kg:	5	10	20	40	80	100
CCNU		21.2	53.1	95.9	189.3	-25.6	-36.2
FCCNU		4.2	6.3	-2.2	11.6	-38.3	-57.5

Table II. Activity of CCNU, DCCNU, and FCCNU against Intracerebrally Implanted[a] Leukemia L1210

compd	dose,[b] mg/kg	median day of death	% ILS[c]	45-day survivors/ total no. of mice
control		8.0		
CCNU	80	16.5	+135	
	60	26.5	+278	8/10
	50	31.5	+350	8/10
	40			10/10
	30	37.5	+435	8/10
DCCNU	80	10.0	+42	3/8
	60	20.0	+185	9/10
	50	7.0		8/9
	40	25.0	+257	8/9
	30	15.0	+114	1/9
FCCNU	140-30		toxic	
	35	7.0	-13	
	30	8.0	0	
	25	8.0	0	
	10	8.0	0	
	15	8.0	0	
	10	8.0	0	

[a] Inoculum of 10^5 cells. [b] Intraperitoneally on day 1 only. [c] Of animals dying.

In contrast to the effect of replacing one or two hydrogens in a drug molecule by deuterium, the introduction of one or more fluorine substituents may alter significantly the physicochemical characteristics and, irrespective of the influence on metabolism profile, markedly change the biological activity.[14,15]

The route used[6] in the conventional synthesis of CCNU was employed to obtain FCCNU by reaction of 1*H*-decafluorocyclohexylamine[16] with 2-chloroethyl isocyanate, followed by nitrosation of the product.

The data in Table I show that the activity of FCCNU is greatly inferior to that of CCNU against the TLX-5 lymphoma in mice. The activities of DCCNU and FCCNU against the ic implanted L1210 leukemia in mice in comparison with that of CCNU are included in Table II. Whereas the antitumor activities of CCNU and DCCNU were not very different, with that of the latter possibly being slightly lower, the toxicity of FCCNU was striking. When the dosage of FCCNU was reduced to a nontoxic level, then no antitumor activity could be detected.

In seeking an explanation for the unexpectedly high toxicity of FCCNU, the decomposition of the compound

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was investigated. In aqueous ethanolic phosphate buffer at pH 7 and room temperature, FCCNU decomposed rapidly and, after 10 min, the two major, UV-absorbing products could be isolated as a crystalline, near-equimolar, two-component mixture (as indicated by TLC and ^1H and ^{19}F NMR data). The components were separated by chromatography and isolated as relatively stable crystalline compounds, although each decomposed at a significant rate when dissolved in the above phosphate buffer. On the basis of the following data and argument, these products were assigned the structures 1-(1*H*-decafluorocyclohexyl)-3-nitrosoimidazolidin-2-one (**3**) and 1-(nonafluorocyclohex-1-enyl)-3-nitrosoimidazolidin-2-one (**4**).

The formulas of **3** and **4** were indicated by elemental analysis and mass spectrometry to be $C_9H_5F_{10}N_3O_2$ and $C_9H_4F_9N_3O_2$, respectively. The ^{19}F NMR spectrum of **3** contained signals for the $C_6F_{10}H$ ring, which were similar to those for FCCNU. Two possibilities were considered for **3**, namely, the imidazolidin-2-one structure **3** (formed from FCCNU by displacement of Cl by N-1) and the 2-iminooxazolidine structure **6** (formed by displacement of Cl by the carbonyl oxygen). Montgomery et al.[17] proposed an oxazolidine analogous to **6** as an unstable intermediate in the decomposition of BCNU in aqueous media. However, even if the oxazolidine **6** were stabilized by the $C_6F_{10}H$ group, the IR and NMR data for the product obtained from FCCNU did not accord with expectations for such a structure.

Thus, the strong IR absorption at 1770 cm^{-1} accords with the carbonyl group in the imidazolidine-2-one structure **3**. An IR absorption in the range 1620–1695 cm^{-1} would be expected[18] for the exocyclic C=N group in the oxazolidine structure **6**, whereas that for the carbonyl group in the cyclic urea structure **3** would be expected[19] at substantially higher frequency than the value (1715 cm^{-1}) of the acyclic precursor **2**. Moreover, the chemical shifts of the methylene protons in the oxazolidine structure **6** would be expected to be very different (τ ~6.1 for CH_2N and ~5.6 for CH_2O) in contrast to the 4-proton singlet at τ 6.05 found and expected for **3**. Similar arguments apply to **4** [ν_{max} 1780 cm^{-1}; τ 6.05 (s, 4 H)].

That the reaction **2** → **3** was a function of the decafluorocyclohexyl group was indicated by the observation that, in aqueous ethanolic phosphate buffer at pH 7 and room temperature, CCNU had afforded no UV-absorbing product after 2 h, indicating that none of its decomposition products retained the nitroso function, a result consistent with a previous report[20] on the course of decomposition of CCNU in buffered aqueous solution.

The imidazolidine mixture **3** + **4** was lethal toward mice at a dose of 40 mg/kg but not at 20 mg/kg, a result which accords with the toxicity of FCCNU noted above.

The formation of **3** and **4** must involve the displacement of the chlorine substituent in FCCNU by N-1. The mechanism of this reaction has not been elucidated, but it may be noted that the vicinal effect on the amino group of 1*H*-decafluorocyclohexylamine by the fluorine substituents at positions 2 and 6 did not significantly impede the reaction with 2-chloroethyl isocyanate used in the synthesis of FCCNU. This, coupled with the fact that CCNU apparently did not yield an imidazolidine derivative, suggests the intermediacy of a nitrogen anion in the conversion **2** → **3**. Further investigation of the decomposition of FCCNU revealed that **3** and **4** were formed sequentially not simultaneously.

The possibility was then considered of blocking the cyclization FCCNU → **3** by replacing Cl by CF_3. Whereas the pK_a values (1.04×10^{-4} and 7×10^{-5}, respectively)[21] of $ClCH_2CH_2COOH$ and $CF_3CH_2CH_2COOH$ indicate the inductive effects of Cl and CF_3 to be not greatly different, the latter group should resist nucleophilic displacement. However, this possibility was not pursued since, when this structural change was applied to CCNU, the product **5**, as might be expected,[22] had insignificant growth inhibitory activity against the TLX-5 lymphoma in mice.

Experimental Section

Where analyses are indicated only by symbols of the elements, they are within ±0.4% of the theoretical values. IR spectra in Nujol were obtained with a Perkin-Elmer 257 spectrophotometer. NMR spectra (^1H, 100.1 MHz, Me_4Si; ^{19}F, 94.1 MHz, CCl_3F) were obtained with a Varian XL100 spectrometer. TLC was performed on Kieselgel G_{254} (Merck) and column chromatography on Kieselgel 60 (Merck). Melting points were determined using an Electrothermal apparatus and are uncorrected. Mass spectra were determined with an A.E.I. MS-12 instrument.

1-(2-Chloroethyl)-3-(1*H*-decafluorocyclohexyl)-1-nitrosourea (2, FCCNU, NSC-301741). 2-Chloroethyl isocyanate (1.27 g, 0.012 mol) was added dropwise to a stirred solution at 0–5 °C of 1*H*-decafluorocyclohexylamine[16] (3.35 g, 0.012 mol) in dry ether (15 mL). The mixture was then warmed to room temperature and stirred overnight. Removal of the solvent in vacuo and recrystallization of the residue from $CHCl_3$ afforded 1-(2-chloroethyl)-3-(1*H*-decafluorocyclohexyl)urea (2.14 g): mp 149–150; IR ν_{max} 3380, 3300 (NH), 1640 (C=O), 1565 cm^{-1} (CNH). The ^1H and ^{19}F NMR data [$(CD_3)_2CO$] were consistent with the assigned structure. Anal. ($C_9H_7ClF_{10}N_2O$) C, H, Cl, F, N.

Dry sodium nitrite (0.57 g, 8.25 mmol) was added in small portions to a stirred solution of the foregoing compound (1.06 g, 2.75 mmol) in formic acid (98–100%, 20 mL) at 5 °C. The mixture was stirred at 5 °C for 45 min and then H_2O (20 mL) was added. The yellow precipitate was collected, washed with ice–water, dried, and eluted from a column (30 × 1 cm) of Kieselgel with CH_2Cl_2 to afford **2** (0.79 g): mp 66.5–67 °C; IR ν_{max} 3325 (NH), 1715 cm^{-1} (C=O). The ^1H and ^{19}F NMR data ($CDCl_3$) indicated **2** to be a single isomer, and comparison of the ^1H NMR data with those published[5] for the 1- and 3-nitroso derivatives of 1-(2-chloroethyl)-3-cyclohexylurea clearly indicated that **2** had the assigned structure. Anal. ($C_9H_6ClF_{10}N_3O_2$) C, H, Cl, F, N.

Decomposition of FCCNU (2) in Phosphate Buffer (pH 7). To a solution of **2** (1 g) in EtOH (100 mL), 0.1 M phosphate buffer (100 mL, pH 7) was added. The reaction was monitored by TLC (benzene). After 10 min, concentration of the solution gave pale yellow crystals, which were collected, washed with water, and dried. The product (0.2 g) had mp 133–144 °C and on TLC (C_6H_6) was found to contain two UV-absorbing components: R_f 0.23 and 0.29. Mass spectral data: component of R_f 0.23, m/z 357 (M^+, 11% of base peak at m/z 272); component of R_f 0.29, m/z 377 (M^+, 7% of base peak at m/z 272). ^{19}F NMR data ($CDCl_3$) indicated a mixture of **3** and **4** in the ratio 57:43. Anal. (equimolar mixture of $C_9H_5F_{10}N_3O_2$ and $C_9H_4F_9N_3O_2$) C, H, F, N. Under the above conditions CCNU afforded no UV-absorbing product.

Fractionation of the foregoing mixture (0.16 g) by column chromatography (CCl_4–$CHCl_3$, 3:7) gave first 1-(1*H*-decafluorocyclohexyl)-3-nitrosoimidazolidin-2-one (**3**, 0.08 g): mp 153–154.5 °C; IR ν_{max} 1770 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) τ 4.7 (t, $J_{H,F}$ = 24 Hz) and 6.05 (s) in the integral ratio 1:4; ^{19}F NMR 3 AB quartets (integral ratio 4:4:2) centered at 122.4, 134.4, and 133.2 ppm with all J_{AB} = 280 Hz (cf. J_{AB} = 280 Hz for **2**) consistent

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with structure 3. Anal. ($C_9H_5F_{10}N_3O_2$) C, H, F, N.

Eluted second was 1-(nonafluorocyclohex-1-enyl)-3-nitrosoimidazolidin-2-one (4, 0.06 g): mp 96–97 °C; IR ν_{max} 1780 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) τ 6.05 (s); ^{19}F NMR, 3 collapsed AB signals (integral ratio 2:2:4) centered at 111.0, 119.3, and 133.5 ppm, and a signal at 120.1 ppm (integral ratio 1) consistent with structure 4. Anal. ($C_9H_4F_9N_3O_2$) C, H, F, N.

In a subsequent decomposition of FCCNU (1 g), when the reaction mixture was worked up almost immediately, 50% of the material (0.7 g) recovered was unreacted FCCNU and the remainder was a mixture of 3 and 4.

1-(3,3,3-Trifluoropropyl)-3-cyclohexyl-1-nitrosourea (5). Cyclohexyl isocyanate (0.63 g, 5 mmol) was added dropwise to a stirred solution of 3,3,3-trifluoropropylamine[23] (0.57 g, 5 mmol) in dry ether (8 mL) at 0 °C. The mixture was then warmed to room temperature and stirred overnight. Removal of the solvent in vacuo and recrystallization of the residue from acetone–water afforded 1-(3,3,3-trifluoropropyl)-3-cyclohexylurea (0.77 g): mp 125–126 °C; IR ν_{max} 3330 (NH), 1625 (C=O), 1575 cm^{-1} (CNH); ^{19}F NMR [$(CD_3)CO$] showed a triplet centered at 64.8 ppm (J = 11.3 Hz); ^{1}H NMR data was consistent with the assigned structure. Anal. ($C_{10}H_{17}F_3N_2O$) C, H, N.

Dry sodium nitrite (0.62 g, 8.98 mmol) was added in small portions to a stirred solution of the foregoing compound (0.71 g, 2.98 mmol) in formic acid (98–100%, 20 mL) at 5 °C. The mixture was stirred at 5 °C for 45 min and then H_2O (20 mL) was added. The yellow precipitate was collected, washed with ice–water, dried, and eluted from a column (30 × 1 cm) of Kieselgel with CH_2Cl_2 to afford 5 (0.63 g): mp 47–48 °C; IR ν_{max} 3390 (NH), 1710 cm^{-1} (C=O). ^{19}F NMR ($CDCl_3$) showed a triplet centered at 66.63 ppm; ^{1}H NMR data was consistent with the assigned structure. Anal. ($C_{10}H_{16}F_3N_3O_2$) C, H, F, N.

Assay of Antitumor Activities. Using the previously described protocol,[24] CCNU and FCCNU (2) were tested against the TLX-5 lymphoma in mice. The results are given in Table I.

The ic implanted leukemia L1210 tests were performed by the Cancer Screening Division of the Southern Research Institute (Birmingham, Ala.) under the direction of Dr. F. M. Schabel. The results are given in Table II.

Toxicity Assay. The mixture 3 + 4 described above was administered ip as a single injection in 10% ethanol/oil to female CBA/LAC mice (two animals in each group). Animals given 40 or 80 mg/kg died within 24 h of injection. A dose of 20 mg/kg elicited no toxic symptoms (death or weight loss) compared with untreated animals, within 10 days after injection.

Acknowledgment. This work was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research:Royal Cancer Hospital) from the Medical Research Council (G973/786) and to the University of Birmingham from the Cancer Research Campaign. We thank Mrs. P. M. Goddard for the TLX-5 and toxicity tests and M. H. Baker for technical assistance.

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Synthesis and Biological Activity of 5-Fluoro-2′-deoxyuridine 5′-Phosphorodiamidates[1]

Mary E. Phelps,

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706

Peter W. Woodman,

Division of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

and Peter V. Danenberg*

Department of Biochemistry and the Comprehensive Cancer Center, University of Southern California, School of Medicine, Los Angeles, California 90033. *Received May 1, 1980*

Three 5′-phosphorodiamidate derivatives of 5-fluoro-2′-deoxyuridine (FdUrd), 5-fluoro-2′-deoxyuridine 5′-phosphorodiamidate (**4a**), 5′-phosphorodiimidazolidate (**4b**), and 5′-phosphorodimorpholidate (**4c**), were synthesized by aminolysis of 5-fluoro-2′-deoxyuridine 5′-phosphorodichloridate with the respective amine. In culture, these 5′-phosphorodiamidates inhibited the growth of murine leukemia (L5178Y) cells. 5-Fluoro-2′-deoxyuridine 5′-phosphorodiamidate (**4a**) was the most active derivative and, on a molar basis, produced a cytostatic effect comparable to that of FdUrd and 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUrd-5′-P). Compounds **4b** and **4c** were less active than **4a**, with relative rates of activity **4a** > **4b** > **4c** that corresponded to their rates of hydrolysis to FdUrd-5′-P. None of the 5′-phosphorodiamidates inhibited thymidylate synthetase of concentrations up to 1 mM.

5-Fluorouracil (FUra) and 5-fluoro-2′-deoxyuridine (FdUrd) have been widely used to treat patients with disseminated cancers.[2] The fluoropyrimidines are converted to the active metabolite 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUrd-5′-P) through several different pathways.[2] FdUrd-5′-P inhibits thymidylate synthetase and thus the biosynthesis of DNA; this activity had been regarded for a long time as the principal mechanism by which these drugs exert their therapeutic cytotoxicity.[3]

(1) A preliminary account of portions of this work was presented by P. V. Danenberg and P. W. Woodman at the 61st Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, IL, Apr 1977. See P. V. Danenberg and P. W. Woodman, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **36**, 64 (1977).

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0022-2623/80/1823-1229$01.00/0