Correlation of Antitumor Activity and Electron Deficiency of Nitrofuranylhydrazone Compounds

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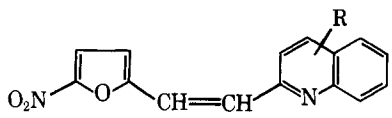
Abstract □ Phenylhydrazone derivatives of 5-nitro-2-furaldehyde were tested for antitumor activity against L-1210 murine leukemia *in vitro*. A substantial increase in antitumor activity was observed in these series of compounds as the electron deficiency of the six-membered aromatic ring was increased. A possible mechanism of action involving drug reduction as well as antibacterial and antitumor efficacy is discussed.

Keyphrases □ Nitrofuranylhydrazones—correlation, antitumor activity, electron deficiency □ Electron deficiency—correlation of antitumor activity, nitrofuranylhydrazone compounds □ Antitumor activity—electron deficiency, nitrofuranylhydrazone compounds

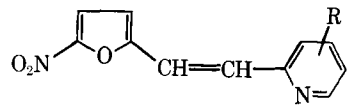
Nitrofuran compounds exhibit a wide range of useful pharmacologic activity. Nitrofurazone, for example, is a broad spectrum topical antibacterial agent used primarily in the treatment of mixed infections of wounds and diseases of the skin. Nitrofurantoin, a chemically related compound, is a urinary tract antiseptic quite active against many strains of common urinary pathogens. In addition to their current use as antibiotics, nitrofuran compounds have in the past been considered useful against selected neoplasias. *cis*-Dichlorodiammine platinum and bleomycin are currently considered two of the drugs of choice for the treatment of testicular carcinomas (1). However, the use of nitrofurazone and nitrofurantoin in testicular tumor therapy has been reported frequently during the past three decades (2–6).

Although there are a few reports of structure–activity relationships for nitrofurans as antitumor agents (7–10), this area has not been fully explored. This is especially true for simple phenylhydrazone derivatives of 5-nitro-2-furaldehyde. There still exists, therefore, potential for the development of new drugs in this class for the treatment of testicular or other tumor types.

The most thorough synthetic and pharmacologic studies on new types of antineoplastic nitrofuran derivatives are those of Ujiie and Miura *et al.* (7, 8, 10). These authors have prepared a series of compounds like Compounds I, II, and III, and have screened these in a variety of experimental tumor systems. Certain derivatives of Compounds I and II were found to completely cure Ehrlich ascites carcinoma in mice. The compounds are relatively nontoxic and doses of up to 1000 mg/kg did not cause any drug-related deaths. Moderate antitumor activity was also seen with Schiff base derivatives like Compound III.



I



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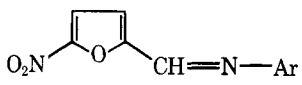


III

The present study describes the *in vitro* antitumor activity of a new series of phenylhydrazone derivatives of 5-nitro-2-furaldehyde. An examination was made of the correlation of electron withdrawing potential of each compound as a contributing factor to the possible mechanism of antibacterial and antitumor activity of this series of drugs.

EXPERIMENTAL

Cell Culture—Mouse leukemic L-1210 cells[1] were maintained in medium containing 10% fetal calf serum[2]. Stock cultures of cells were diluted with the medium to give final concentrations of 5×10^5 cells/ml. The compounds were dissolved in dimethyl sulfoxide and diluted to a concentration 500 times greater than the experimental level desired, so the 10 μl of the compound solution could be added to 5 ml of the inoculated growth medium. No precipitation occurred during this addition. This amount of compound in solution yielded a final dimethyl sulfoxide concentration of 0.2%, which itself had no effect on cell growth.

Each ID_{50} experiment (i.e., the determination of that dose which results in 50% inhibition of L-1210 growth) was run four times. Cell cultures were incubated at 37° in a 95% O_2–5% CO_2 humidified atmosphere and were counted after 96 hr. All cell counts were done by diluting the cell culture 1:40 and then counting with an electronic cell counter. The concentration of cells in the control tubes of L-1210 after 96 hr was ~2.5×10^6/ml.

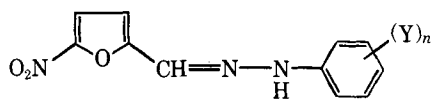
Hydrazone Syntheses—The hydrazones were prepared from commercially available hydrazines and 5-nitrofurancarboxaldehyde by heating an ethanolic solution of these precursors containing a small portion of glacial acetic acid. The hydrazones that precipitated were recrystallized from ethanol and all had satisfactory elemental analyses. Elemental analyses and melting point data are listed in Table I.

RESULTS AND DISCUSSION

The series of nitrofuranylhydrazones like Compound IV clearly show a substantial and significant increase in antitumor activity as the electron deficiency of the six-membered aromatic ring increases.

The *in vitro* L-1210 screening data for the various compounds, the sum of σ values for the six-membered ring substituents, as well as pertinent ^{1}H-NMR shifts, are represented in Table II.

The metabolic reaction first occurring with most nitrofurans in animals involves reduction of the furanyl nitro group to a hydroxylamine (12). This is followed by further reduction to the amine and finally ring opening and fragmentation (13). Hydrolysis of the azomethine linkage can also



IV

[1] Sloan Kettering Institute, Rye, N.Y.
[2] McCoy's 5a, Grand Island Biological Co., Grand Island, N.Y.

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Table I—Elemental Analysis and Melting Point Data of Nitrofuranylhydrazone Compounds

Compound	Formula	Theoretical, %	Found, %	Melting Point
IV*a*[a]	$C_{12}H_{11}N_3O_4$			same as lit. (11)
IV*b*	$C_{11}H_9N_5O_7$	C 57.14	56.90	
		H 3.92	3.88	181–185 dec.
		N 18.17	18.05	
IV*c*[a]	$C_{11}H_8N_3O_3Cl$			same as lit. (11)
IV*d*	$C_{12}H_9N_3O_5SF_2$	C 41.74	41.71	
		H 2.63	2.73	237–239 dec.
		N 12.17	12.05	
IV*e*[a]	$C_{11}H_8N_4O_5$			same as lit. (11)
IV*f*	$C_{11}H_8N_4O_5$	C 47.83	48.04	
		H 2.92	2.94	220–223 dec.
		N 20.28	20.15	
IV*g*	$C_{10}H_7N_5O_5$	C 43.33	43.32	
		H 2.55	2.60	238–241 dec.
		N 25.26	25.26	
IV*h*	$C_{11}H_7N_5O_7$	C 41.13	41.18	
		H 2.20	2.17	269–270 dec.
		N 21.80	21.60	
IV*i*	$C_{10}H_6N_6O_7$	C 37.28	37.54	
		H 1.88	2.02	242–245 dec.
		N 26.08	25.38	

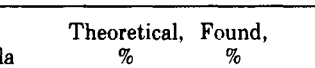
occur, followed by oxidation to 5-nitrofuroic acid. The latter is found in the urine of experimental animals (14).

It should be noted that nitrofurans are reduced in a number of bacterial enzyme systems, and a number of researchers have advanced the theory that in accepting electrons, the nitrofurans interfere with the oxidation–reduction linked metabolism of the organism. It has been suggested that nitrofurazone interferes with normal metabolism by virtue of its capacity to be reduced (15). In addition, previous authors have concluded from a study of nitrofurans, nitrothiophenes, nitrobenzenes, and related compounds, that ease of reducibility plays a major role in the antibacterial effectiveness of heterocyclic nitro compounds (16). These authors also proposed a relationship between the redox potential developed by the organism in culture and that required by the inhibitory nitrofuran, implying that interference with microbial reproduction was associated with formation of a reduction product (hydroxylamine) from the nitro compound (17). Other workers have measured these reduction potentials and confirmed these proposals (18).

It is reasonable to assume that the well-known antibacterial activity of nitrofuranyl hydrazones and the *in vitro* antitumor activity observed against mouse leukemia L-1210 may have similarities with regard to their mechanisms of action. The dramatic increase in cytotoxicity with increasing electron deficiency of the pi system in IV*i*, (see Table II) suggests that biological activity may well be associated with a reductive process. The furanyl nitro group in IV*i* should be much more readily reduced than that in IV*a*. The relationship between L-1210 cytotoxicity and electron deficiency can qualitatively be seen by examining measures of the latter, *i.e.*, the summation of the σ_p and σ_0 values for the six-membered ring substituents, and/or the methine ^1H chemical shifts (Table II). Surprisingly, the antibacterial activity of IV*i*, one of the more active compounds against L-1210, was negligible against 18 Gram-positive and Gram-negative organisms compared with nitrofurazone and nitrofurantoin. It possessed somewhat better activity against two staphylococci (64 μg/ml minimal inhibitory concentration) but not as much activity as nitrofurazone (8 μg/ml) or nitrofurantoin (16–32 μg/ml). In contrast, the cytotoxicity of IV*i* is still very high against both HeLa cells and P-388 cells. The differential activity of IV*i* in the antineoplastic and antibacterial screens could result from an inability of the compound to penetrate the bacterial cell wall. The compound is extremely insoluble and is completely inactive in an *in vivo* L-1210 screen.

Perhaps the most surprising result of this report is that 5-nitrofurancarboxaldehyde is more cytotoxic than any of the hydrazones studied (Table II). This introduces the interesting possibility that hydrazone activity may, in part, result from hydrolytic cleavage to the parent aldehyde. The variation in hydrazone cytotoxicity may, in fact, be partially related to the variable rates of hydrolytic cleavage.

Table II—Antitumor Activity and Electron Deficiency Determinations of Nitrofuranylhydrazone Compounds



R = Ar-(Y)n		L-1210 ID_{50}, μg/ml	$\Sigma\sigma$[a]	δ ppm[b] CH=N
IV*a*	*p*-OCH_3	>10	−.27	7.7
IV*b*	*p*-H	3	0	7.7
IV*c*	*p*-Cl	5	+.23	7.8
IV*d*	*p*-SO_2CHF_2	4	+.73	7.8
IV*e*	*p*-NO_2	3	+.78	7.9
IV*f*	*o*-NO_2	1.8	+.97	8.6
IV*g*	2-aza-6-nitro	1.0	+1.43	8.5
IV*h*	2,4-dinitro	0.8	+1.75	8.6
IV*i*	2-aza-4,6-dinitro	0.5	+2.21	9.1
5-nitrofurancarboxaldehyde		0.1	—	

[a] Substituent constants (σ) were obtained from Ref. 19. In calculating the sums ($\Sigma\sigma$), the assumption was made that $\sigma ortho = \sigma para$. [b] NMR spectra were obtained in dimethyl sulfoxide d_6 solution with tetramethylsilane as an internal reference. They were recorded on a JEOL MH-100 NMR spectrometer.

Toxicological studies of many nitroheterocycles have revealed a substantial mutagenic and carcinogenic liability. Since mutagenic and antibacterial modes of action are probably intimately related at the molecular level, it is unlikely that important nonmutagenic antibacterial nitroheterocycles will be found. Because most antineoplastic agents have substantial carcinogenic acitivity, a liability of this type associated with an antineoplastic nitrofuran is not severe, and the potential use of such compounds in cancer chemotherapy should not be overlooked.

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