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Evaluation of Cytotoxicity of Some Mannich Bases of Various Aryl and Arylidene Ketones and Their Corresponding Arylhydrazones

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Abstract □ Mannich bases were synthesized and converted to the corresponding arylhydrazones. X-ray analysis of a ketone (**1a**) and a hydrazone (**4d**) revealed structural features of interest. All of the compounds showed cytotoxicity toward murine lymphocytic leukemia L1210 cells in the 4.9–25.0-μM range. The correlation coefficients generated by plotting the IC_{50} values (the concentrations of compounds that inhibit the growth of tumors by 50%) of some hydrazones against certain electronic, hydrophobic, and steric constants of the aryl substituents indicated only weak correlations. A few ketones and hydrazones displayed significant cytotoxicity to the WiDr human colon cancer cells, and these derivatives, especially the ketones, may serve as prototypes for future drug development. The KB tumor (a human epidermoid carcinoma of the nasopharynx) was somewhat refractory to selected compounds. In an in vitro assay conducted by the National Cancer Institute and involving ~53 tumor cell lines originating from eight neoplastic diseases, 65% of the compounds showed some selectivity toward one or more groups of cancers, principally leukemia, melanoma, and colon cancer. The bioevaluation of the ketones and hydrazones against the L1210, WiDr, and KB tumors, as well as evidence from proton nuclear magnetic resonance studies did not support the suggestion that hydrazones may be prodrugs of the corresponding ketones.

Various Mannich bases prepared from aryl and arylidene ketones designed as thiol alkylators have antineoplastic and cytotoxic properties either per se or after decomposition to the corresponding α,β-unsaturated ketones.[1–3] This work was undertaken because nucleic acids do not contain thiols, and hence, some of the important side effects displayed by traditional clinically used alkylating agents in cancer chemotherapy, such as carcinogenicity and mutagenicity due to reaction with hydroxyl and amino groups, may be absent in these Mannich bases. However, whereas some of these Mannich bases gave negative results in the Ames test,[4] murine toxicity was often pronounced.[2,5] One way to ameliorate toxicity is to prepare prodrugs of the Mannich bases that would gradually release the ketones. To examine the general validity of this approach, in vitro experiments to evaluate the cytotoxicities of the Mannich bases and the potential prodrugs were conducted. In choosing the carrier group, the facts that a number of tumors have lower pH than the corresponding normal tissues[6] and that hydrazones are capable of regenerating the corresponding ketones, especially under acidic conditions,[7] were considered. Therefore, the arylaminoazo (ArNHN=) group was chosen as the carrier group because it could enable selective release of the ketone in malignant tissue. The aims of the present investigation were twofold: (1) to prepare some model compounds as candidate prodrugs of several Mannich bases and to compare the cytotoxicities of these compounds with those of the ketones and (2) to evaluate the likelihood of the arylhydrazones acting as prodrugs for various ketones. The results from this study may shed light on whether these principles could be used subsequently in the design of compounds for in vivo evaluation. These considerations led to the preparation of compounds in series 1–4 (PHZ in structures 1 and 2 refers to the phenylhydrazono group).

In series 1, the ketones **1a** and **1c** may undergo deamination in the tumor cells to the corresponding acrylophenones, a class of compounds with considerable avidity for thiol groups.[8] The conjugated styryl ketone **2a** could react with thiols per se, as well as undergo deamination to the corresponding α, α', β, β'-diolefinic ketone to yield another site of interaction with cellular nucleophiles. Compound **2c** is incapable of such deamination and, hence, may be less cytotoxic than **2a**. The phenylhydrazones of these four ketones are **1b**, **1d**, **2b**, and **2d**. For series 3 and 4, variation of the substituents in the aryl ring of the arylaminoazo group should alter the rates of release of **1a** and **2a**, respectively.

Results

The β-aminoketones **1a**, **1c**, **2a**, and **2c** were prepared by a Mannich reaction with the appropriate ketone. However, attempts to prepare 2,2-dimethyl-3-dimethylamino-1-phenyl-1-propanone hydrochloride led to a product whose proton nuclear magnetic resonance (^{1}H NMR) spectrum was consistent with this structure but whose elemental analysis was unsatisfactory. Hence, this product was not submitted for cytotoxic evaluation but was converted satisfactorily to the corresponding hydrazone, **1e**. The arylhydrazones **1b**, **1d**, **2b**, **2d**, **3a–3g**, and **4a–4d** were prepared from the appropriate ketones and arylhydrazines or arylhydrazine hydrochlorides. The results of the evaluation of compounds in series 1–4 against L1210, WiDr, and KB cells and various human tumors are presented in Table I.

Discussion

The compounds were evaluated against L1210 lymphoid leukemia cells. A comparison of the cytoxicity of **1a** and **1c** reveals that the additional methyl group causes little change in bioactivity. By contrast, **2a** had approximately twice the activity of both the analogues containing two additional methyl groups, namely, **2c** and **1a**, in which the olefinic linkage is absent. Thus among the ketones **1a**, **1c**, **2a**, and **2c**, the presence of an olefinic group and the absence of an α-methyl function, as displayed in **2a**, gave the compound with the highest cytotoxicity. The hydrazones **1b** and **1d** were clearly more active than **1e**, whereas in series 2, the hydrazones **2b** and **2d** had similar effects on L1210 cells. Whereas the cytotoxicities of **1b** and **2b** were comparable, for the

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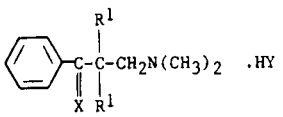
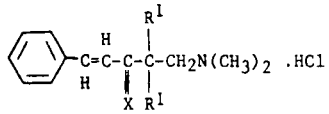


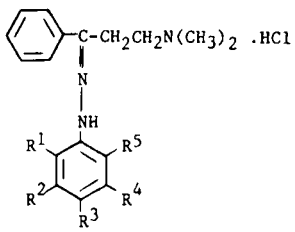
1

a: X = O, $R^1 = R^2 = H$, Y = Cl
b: X = PHZ, $R^1 = R^2 = H$, Y = Cl
c: X = O, $R^1 = CH_3$, $R^2 = H$, Y = Br
d: X = PHZ, $R^1 = CH_3$, $R^2 = H$, Y = Br
e: X = PHZ, $R^1 = R^2 = CH_3$, Y = Cl



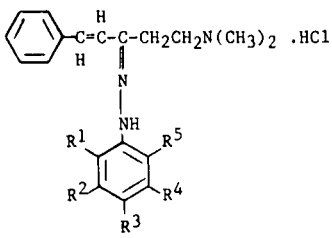
2

a: X = O, $R^1 = H$
b: X = PHZ, $R^1 = H$
c: X = O, $R^1 = CH_3$
d: X = PHZ, $R^1 = CH_3$



3

a: $R^1 = R^3 = Cl$, $R^2 = R^4 = R^5 = H$
b: $R^1 = R^5 = Cl$, $R^2 = R^3 = R^4 = H$
c: $R^1 = R^4 = R^5 = H$, $R^2 = R^3 = Cl$
d: $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Cl$
e: $R^1 = R^2 = R^3 = R^4 = R^5 = F$
f: $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = NO_2$
g: $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = OCH_3$



4

a: $R^1 = R^3 = Cl$, $R^2 = R^4 = R^5 = H$
b: $R^1 = R^4 = R^5 = H$, $R^2 = R^3 = Cl$
c: $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Cl$
d: $R^1 = R^2 = R^3 = R^4 = R^5 = F$

compounds containing geminal dimethyl groups, **2d** was 4.5 times more potent than **1e**. Hence, the presence or absence of methyl and olefinic groups in the phenylhydrazones influences bioactivity.

A comparison of the cytotoxicities of the arylhydrazones of **1a** and **2a**, in which the hydrazino aryl substitution patterns are similar, reveals higher cytotoxicity for derivatives of **1a**. The average concentration that inhibits the growth of the tumors by 50% (IC_{50}) for **1b**, **3a**, **3c**, **3d**, and **3e** was 7.3, whereas for **2b** and **4a–4d**, the value was 10.0. If **1b** and **3a–3g**, and **2b** and **4a–4d** are prodrugs of **1a** and **2a**, respectively, then the rates of release of the ketones may be reflected by different cytotoxicities. This release would be influenced by the electronic nature of the substituents in the hydrazino aryl ring. In addition, if the formation of the ketones is accelerated by enzymes, the sizes of the aryl substituents may influence alignment on the enzyme. Also, the transport of the molecules to a site of action in leukemia cells will be affected, in part at least, by the hydrophobicity of the aryl substituents. Hence, linear and logarithmic plots were made of the IC_{50} values of **1b** and **3a–3g** and of **2b** and **4a–4d** against electronic (σ and σ^* constants), steric (MR constants), and hydrophobic (π constants) characteristics of the hydrazino aryl substituents. A test for zero correlation[9] indicated correlations at $p < 0.10$, but not at $p < 0.05$, between the IC_{50} values and the σ and σ^* constants for both linear and logarithmic plots and between the IC_{50} values and the π constants for a logarithmic plot. No correlations were noted between MR constants and cytotoxicity.

To gain some insight about the effect on cytotoxicity of converting ketones into the corresponding arylhydrazones, various compounds were evaluated against the WiDr human colon tumor and a nasal carcinoma of the nasopharynx (KB screen; Table I). Compounds **1d** and **1e** at 10 μg/mL (viz. 27.6 and 30.1 μM, respectively) inhibited the growth of the cells in the KB screen by 30 and 11%, respectively. Against the WiDr and KB tumors, the ketones had lower IC_{50} values than the corresponding phenylhydrazones, except for **1a** and **1b**.

As the data in Table I indicate, the compounds were appreciably less active than the reference compounds melphalan and adriamycin in the L1210 and KB screens. However, **1a–1d** and **2a–2d** were 0.2–1.0 times as active as the reference drug 5-fluorouracil against WiDr cells. Because this observation was of interest in subsequent drug design, X-ray analysis of a representative compound, **1a**, was undertaken to discern any molecular features that may account for the marked activity toward this tumor. The ORTEP diagram of **1a** (Figure 1) indicates that **1a** is an extended molecule that is almost planar. Thus, the angle between the aryl ring (C1–C6) and the plane containing the C6, C7, O, and C8 atoms is 3.67 (4) ° (values in parentheses are the standard deviations of the last decimal places). Also in examining changes from planarity of the entire length of the molecule (excluding C10), the largest deviation from planarity is 0.122 (1) Å at C9. The internal aromatic angle C1–C6–C5 is 118.7 (2) °, which was attributed to the attached electron-withdrawing substituent.[10,11] The N–Cl distance [3.034 (2) Å] and the N–H · · · · Cl angle [176 (2) °] indicate a hydrogen bond between the nitrogen and chlorine atoms. Thus, it would be of interest to evaluate compounds with a marked lack of planarity, which could be achieved inter alia by *ortho* substitution in the aryl ring and replacement of the methylene protons by other functional groups.

The compounds in series 1–4 were also examined in an in vitro screen conducted by the National Cancer Institute (NCI).[12] This evaluation generally uses 50–60 tumor cell lines from different diseases, namely, leukemia, melanoma, colon, non-small-cell lung, small-cell lung, central nervous system, ovarian, and renal cancers. The principal goal was to find compounds with selective toxicity for one or more types of tumor (e.g., to reveal molecules with specificity for leukemia or solid tumors). The data generated are presented in various ways: (1) GI_{50}, the concentration of compound that causes a

Table I—In Vitro Evaluation of Compounds against the L1210, WIDr, and KB Neoplasms and Various Human Tumors

Compound	IC_{50}, μM[a]			$\log_{10} GI_{50}$[b]		$\log_{10} TGI$[b]		$\log_{10} LC_{50}$[b]	
	L1210	WiDr	KB	MG MID	Selectivity[c]	MG MID	Selectivity	MG MID	Selectivity
1a	11.7	2.2	37.3	−4.24	—	−4.11	—	−4.04	—
1b	7.2	0.9	30.0	−4.93	—	−4.39	—	−4.13	—
1c	9.0	1.7	22.2	−4.43	leu	−4.15	leu	−4.05	mel, col, ren
1d	10.2	4.2	>27.6	−6.07	leu, col	−4.82	leu, ren, cns	−4.23	mel
1e	25.0	—	>30.1	−4.42	—	−4.08	—	−4.01	—
2a	5.1	1.4	12.8	−4.74	leu	−4.42	mel, col, ren	−4.15	mel, scl
2b	6.8	3.4	20.4	−5.82	leu, col	−5.14	leu	−4.61	mel
2c	10.0	0.8	9.8	−4.86	leu	−4.45	mel, col, ren	−4.15	mel, col, cns
2d	5.6	2.4	16.9	−5.19	col	−4.96	ren	−4.36	mel, col, ren
3a	8.8	—	—	−4.89	leu	−4.56	—	−4.25	mel, ren, cns
3b	21.0	—	—	−4.74	—	−4.38	—	−4.13	—
3c	5.7	—	—	−5.68	—	−5.32	—	−4.89	—
3d	7.9	—	—	−6.02	leu, col	−5.48	leu, mel, cns	−4.93	mel
3e	7.0	—	—	−4.53	col, ova	−4.11	leu	−4.06	leu, mel
3f	4.9	—	—	−5.03	leu, ren, cns	−4.63	ren	−4.27	—
3g	9.2	—	—	−5.45	leu, mel, col	−4.85	mel	−4.33	mel, col, ren
4a	10.5	—	—	−4.91	leu	−4.58	—	−4.26	—
4b	12.0	—	—	−5.59	—	−5.15	—	−4.64	—
4c	6.9	—	—	−5.49	—	−5.02	—	−4.52	—
4d	13.6	—	—	−4.25	leu	−4.02	leu, mel	−4.01	leu, mel

[a] The reference compounds (IC_{50} values in parentheses) used in the L1210, WiDr, and KB screens were melphalan (0.39 μM), 5-fluorouracil (0.8 μM), and adriamycin (0.14 μM), respectively. [b] The $\log_{10} GI_{50}$, $\log_{10}$ TGI, and $\log_{10} LC_{50}$ values are the mean graph midpoint (MG MID) values of the concentrations of the compounds that cause the growth of the cells to be 50, 0, and −50%, respectively, of the growth of the tumors in the absence of the compound (see *Discussion* and *Experimental Section* for further details); ~53 human tumor cell lines originating from eight neoplastic diseases were used in this assay; a reference compound, melphalan, had MG MID values of −4.58, −4.00, and −3.70 for the $\log_{10} GI_{50}$, $\log_{10}$ TGI, and $\log_{10} LC_{50}$ parameters, respectively. [c] Selectivity means the increased sensitivity of various compounds to tumors from one or more neoplastic diseases relative to other cell lines; leu, mel, col, ren, cns, ova, and scl refer to leukemia, melanoma, colon, renal, central nervous system, ovarian, and small-cell lung cancers, respectively.

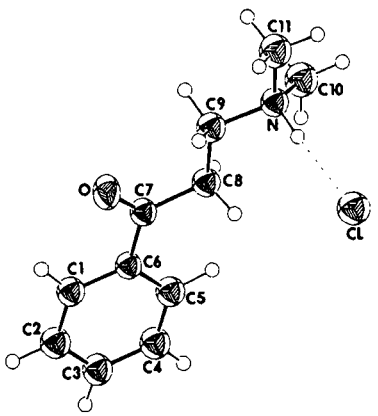


Figure 1—ORTEP diagram of the structure of **1a**.

50% reduction in the normal growth of cells; (2) TGI, the concentration of compound that causes a cytostatic effect (i.e., the concentration resulting in a 0% growth of cells compared with growth of untreated cells); and (3) LC_{50}, the concentration of compound that causes a cytocidal effect (i.e., the concentration resulting in a −50% growth of cells compared with growth of untreated cells. The data are presented in Table I as average $\log_{10} GI_{50}$, $\log_{10}$ TGI, and $\log_{10} LC_{50}$ values for compounds evaluated against ~53 cell lines. Compounds are examined usually in the concentration range of 10^{-8} to 10^{-4} M. Sometimes the effect of the compound on the growth of one or more tumors at the maximum concentration used is small or absent; in this case, the $\log_{10} GI_{50}$, $\log_{10}$ TGI, and $\log_{10} LC_{50}$ values are presented as >-4.00. (The value of >-4.00 was used to calculate the average $\log_{10} GI_{50}$, $\log_{10}$ TGI, and $\log_{10} LC_{50}$ values for each compound against all the cell lines examined; hence, the values in Table I are referred to as mean graph midpoint (MG MID) values rather than true mean values.) In general, the compounds were more active than the reference drug melphalan. Compounds **2b**, **3d**, **3f**, **4a**, and **4c** had $\log_{10} GI_{50}$, $\log_{10}$ TGI, or $\log_{10} LC_{50}$ values of $< 1.00 \times 10^{-4}$ against all the cell lines.

Selective toxicity for certain groups of tumors was displayed by 13 of 20 compounds. Specificity for various tumors was found in three of four ketones (i.e., **1c**, **2a**, and **2c**) and 10 of 16 hydrazones (i.e., **1d**, **2b**, **2d**, **3a**, **3d–3g**, **4a** and **4d**. In series **1**, only compounds containing an α-methyl group displayed selectivity for different groups of neoplasms. All compounds in series **2** displayed some preference for various groups of tumors. In series **3**, compound **3a**, which has a 2,4-dichloro substitution pattern in the aryl ring, showed selectivity for certain specific diseases, whereas the structural isomers containing chlorine atoms at positions 2 and 6 (**3b**) and 3 and 4 (**3c**) did not show this property. The remaining compounds in series **3** had some partiality for certain groups of tumors. The 2,4-dichloro and pentafluoro derivatives **4a** and **4d**, respectively, displayed selectivity, in contrast to the compounds containing 3,4-dichloro and 4-chloro substituents. Because activity had been demonstrated previously against L1210 leukemia cells and the WiDr colon tumor, of particular interest was whether the compounds would be selective toward leukemias and colon tumors. Compounds **1c**, **1d**, **2a–2c**, **3a**, **3d–3g**, **4a**, and **4d** showed selective toxicity to a panel of leukemia cell lines, and **1c**, **1d**, **2a–2d**, **3d**, **3e**, and **3g** showed specificity to colon tumors. The following compounds showed selectivity for melanoma: **1c**, **1d**, **2a–2d**, **3a**, **3d**, **3e**, **3g**, and **4d**. Compounds **1c**, **1d**, **2a**, **2c**, **2d**, **3a**, **3f**, and **3g** were selective for renal tumors, and **1d**, **2c**, **3a**, **3d**, and **3f** were selective for central nervous system cell lines. Compounds **2a** and **3e** showed selective toxicity for small-cell lung and ovarian cancers, respectively. Thus, of the 20 compounds in series **1–4**, 60% showed selective toxicity toward leukemia, 55% toward melanoma, and 45% toward colon tumors. Specificity for certain tumors was displayed by 75% of the compounds in series **2** and **4** and by 58% of the compounds in series

1 and 3; hence, the insertion of an olefinic bond between the phenyl ring and the keto group should be considered in future molecular modification studies.

The validity of the suggestion that the hydrazones give rise to ketones in vitro was considered first by evaluating the screening results for the L1210, WiDr, and KB tumors and second by considering ^{1}H NMR studies under simulated physiological conditions.

If the arylhydrazones have no cytotoxicity and decompose completely and exclusively to the corresponding ketones and arylhydrazines, bioactivity will be due to either the ketones alone or to a combination of the ketones and the carrier groups, namely, the relevant arylhydrazines. Thus, the ratios of the cytotoxicities of the hydrazones with similar carrier groups should be similar to the ratios of the potencies of the ketones released. Examination of the data for the L1210 screen (Table I) suggests that the hydrazones do not behave in this manner. Thus, the ratio of the potencies of ketones **1a** and **2a** was 2.29, which was not similar to the ratios of the cytotoxicities of the following pairs of hydrazones: **1b** and **2b**, **3a** and **4a**, **3c** and **4b**, **3d** and **4c**, and **3e** and **4d**, which were 1.06, 0.84, 0.48, 1.14, and 0.51, respectively. By using the same approach, the ratios of the potencies of **1a** and **2a** for the WiDr and KB tumors were found to be 1.57 and 2.91, respectively, whereas the ratios for the corresponding hydrazones **1b** and **2b** for these two neoplasms were 0.26 and 1.47, respectively. Therefore, it is likely that the hydrazones are either active per se, or if noncytotoxic, they do not break down completely to the ketones and arylhydrazines alone. In addition, if the arylhydrazones themselves are inactive and decompose to the ketones that are solely responsible for cytotoxicity, then the rates of release of the ketones should correlate with the IC_{50} values. The fact that plots of certain physicochemical constants of the aryl substituents in series 3 and 4 did not correlate with the IC_{50} values vide supra suggests, once again, that conversion of the hydrazones solely to the corresponding ketones does not take place.

High-resolution ^{1}H NMR spectroscopy was used to examine the fate of representative hydrazones under quasi physiological conditions. Because hydrazones in solution often exist as a mixture of geometrical isomers,[13,14] X-ray crystallography of a representative hydrazone was undertaken to obtain information about the shape of the molecule and especially the stereochemistry of the carbimino (C=N) group. This configuration would be expected to be present in the sole or major isomer on dissolution of the compound, and such information would aid the interpretation of the NMR spectrum, especially if it changed with time. Figure 2 is an ORTEP diagram of **4d**, and the following molecular features are of interest. The carbimino group has the *Z* configuration. The C6–C7–C8, C7–C8–C9, and C8–C9–N2 angles are >120 °, and hence, this part of the central linkage in the molecule tends more toward linearity than might be expected. The bond distances and angles indicate that the conjugated system of two aryl rings plus the central linkage (C7=C8–C9=N2–N3) is virtually planar, and the interplanar angle between the two aromatic rings is 12.8 (2) °. For the aryl ring containing fluorine atoms, the internal angle at C14 is <<120 °, whereas the adjacent angles at C15 and C19 are >120 ° to compensate for the narrowing at C14. No inter- or intramolecular hydrogen bonding was found in **4d**, although a very short nonbonded intermolecular interaction between N2 and F3 (1−x,y,1/2−z) was noted. [The interatomic distance is 3.010 (4) Å, and the van der Waals radii of nitrogen and fluorine atoms are 1.60 and 1.38 Å, respectively.][15]

Solutions of **1a**, **2a**, **3e**, and **4d** were prepared and examined by ^{1}H NMR spectroscopy initially and after 48 h (the incubation period of compounds in series 1–4 with both L1210 cells and the tumors used in the NCI screen). Compounds **1a** and

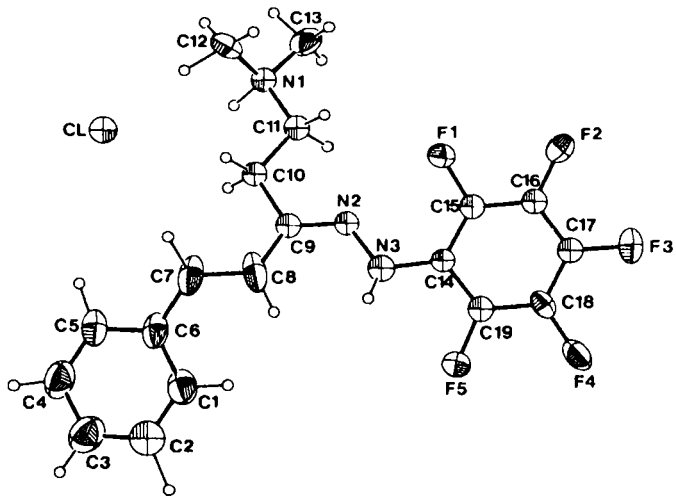


Figure 2—ORTEP diagram of the structure of **4d**.

2a were readily soluble in deuterated phosphate buffered saline (PBS-d; pD 7.4) and determined as 10 mM solutions, whereas **3e** and **4d** were virtually insoluble in this solvent. To obtain satisfactory spectra, a 5 mM solution of **3e** and **4d** in PBS-d:dimethyl sulfoxide-d_6 (5:95) was used. The solutions were kept at 37 °C during the test period. The objective was to examine if the compounds were unstable under these quasi-physiological conditions and, if so, to obtain some idea of the nature of the breakdown products.

Compound **1a** underwent deamination, and the percentages of the methyl protons found as dimethylamine hydrochloride initially and after 48 h were 2 and 35, respectively. Examination of the integrals of the dimethylamino, methylene, and aryl protons of **1a** confirmed that 30–33% of this compound had decomposed during the 48-h period. Whereas two singlets at 6.23 and 6.10 ppm were present after 48 h, which may be attributed to the olefinic peaks of 1-phenyl-2-propen-1-one (the expected product of deamination), their integrals were 2 and 9% that of the predicted values based on the size of the dimethylamine hydrochloride resonances. This unsaturated ketone formed may undergo dimerization[16] and/or polymerization.[17] Alternatively, the ketone could have precipitated from solution, although none was apparent. This possibility of subsequent reactions occurring after deamination was strengthened by examining the ratio of the combined integrals of the dimethylamino protons (from **1a** and dimethylamine hydrochloride) with that of the internal standard, sodium 2,2-dimethyl-2-silapentane-5-sulfonate. This ratio should be the same after 0.5 and 48 h; however, a discrepancy of 18% between these ratios suggests that additional breakdown products were formed after deamination. Other unidentified peaks were found at 4.10 (0.16), 3.95 (0.22), 3.65 (0.46), and 3.40 (1.4) ppm (values in parentheses are numbers of protons).

Compound **2a** behaved like **1a**. It underwent deamination, and the ratios of the methyl protons of dimethylamine hydrochloride to those in **2a** were 4:96 (0.5 h) and 45:55 (48 h). Some unidentified peaks were present in the 48-h spectrum, including additional aryl absorptions in the 7.2–7.5-ppm region of the spectrum and two peaks at 6.1 and 6.4 ppm, which may have been due to the vinyl protons in the deaminated product, 1-phenyl-1,4-pentadien-3-one. The behavior of these two Mannich bases may be summarized by stating that, over a 48-h period, some of the compound is converted to other products by deamination and other reaction pathways.

The spectra of the hydrazones **3e** and **4d** did not change

during the 48-h period at 37 °C. Whether a lack of stability would have been demonstrated had PBS-d only been used is impossible to determine. However, under these conditions, the compounds were stable.

Conclusions

The activity of eight representative compounds, especially the ketones, against the human WiDr tumor is noteworthy; these compounds may be templates for future molecular modification. The fact that some compounds were selectively toxic to colon cancer in the NCI screen further supports the value of modifying the structures of various compounds to examine them for activity against colon tumors. Both ketones and hydrazones showed cytotoxicity toward L1210 leukemia cells, and 60% of these compounds demonstrated selective toxicity to leukemia in the NCI screen. Evidence from the cytotoxicity evaluations and ^{1}H NMR spectroscopic studies suggests that the arylhydrazones prepared in this study probably cannot be used as prodrugs of the corresponding ketones.

Experimental Section

Melting points are uncorrected. Elemental analyses (C,H,N; performed by Mr. K. Thoms, Department of Chemistry, University of Saskatchewan) were within 0.4% of the calculated values for series 1–4. Compound 5 could not be analyzed satisfactorily (calcd for $C_{13}H_{20}ClNO$: C, 67.43; H, 9.43; N, 6.55; found: C, 64.13; H, 8.23; N, 6.13). Methanol was dried over molecular sieves (5 Å, 4–8 mesh). The 60-MHz ^{1}H NMR spectra of compounds in series 1–4 supported the proposed structures. ^{1}H NMR spectra were determined on a Varian T-60 machine (60 MHz) and a Bruker AM 300 FT instrument (300 MHz). The latter instrument was used in studies of the stability of **1a**, **2a**, **3e**, and **4d**, and in these cases, chemical shift values were referenced to the methyl resonance of sodium 2,2-dimethyl-2-silapentane-5-sulfonate and are quoted in parts per million to ±0.01. Spectra were recorded at a probe temperature of 37 °C (310 K), and the midpoint acquisition time of the initial spectrum was 0.5 h. Crystallographic computations were performed with a VAX8650 computer at the University of Saskatchewan.

Chemistry—*Synthesis of Ketones—1a, 1c, 2a, and 2c*—Compound **1a** was prepared by a literature procedure[18] in 70% yield and recrystallized from acetone–95% ethanol; mp, 138 °C (literature value,[18] 155–156 °C).

The ketone **1c** was synthesized as follows. A solution of propiophenone (2.7 g, 0.022 mol) and *N,N*-dimethyl(methylene)ammonium bromide (3.0 g, 0.02 mol) in acetonitrile (75 mL) was stirred for 24 h at 45 °C. After removal of the solvent, the oily residue was triturated with anhydrous diethyl ether, and the crude product was recrystallized from diethyl ether–methanol to give **1c** in 92% yield; mp, 116 °C (literature value,[19] 132 °C).

Compound **2a** was prepared by a previously described procedure[20] in 45% yield and recrystallized from 95% ethanol; mp, 148 °C (literature value,[20] 157–157.5 °C).

A literature method[21] was used to prepare **2c** which was purified by recrystallization from diethyl ether–methanol, in a yield of 32%; mp, 182 °C. An attempt was made to prepare 2,2-dimethyl-3-dimethylamino-1-phenyl-1-propanone hydrochloride (5) from isobutyrophenone and *N,N*-dimethyl(methylene)ammonium chloride via the method used for synthesizing **1c**. The compound obtained was recrystallized from ether–methanol and did not give satisfactory combustion analyses; mp, 146 °C (literature value,[22] 137–140 °C). However, the ^{1}H NMR spectrum (60 MHz, $CDCl_3$) was in accord with the proposed structure, and the material was converted satisfactorily to **1e** vide infra.

Synthesis of Arylhydrazones 1b, 1d, 1e, 2b, 2d, 3a–3g, and 4a–4d—The arylhydrazones were prepared from the Mannich base hydrohalides with either arylhydrazines (method A) to form **1b**, **1d**, **1e**, **2b**, **2d**, **3d**, **3e**, and **4d** or arylhydrazine hydrochlorides (method B) to form **3a–3c**, **3f**, **3g**, and **4a–4c**. With method A, a solution of the Mannich base hydrohalide (10 mmol), arylhydrazine (10 mmol), and glacial acetic acid (5 mmol) in dry methanol (30 mL) was stirred at room temperature for various periods. In some cases, solids deposited while the reaction mixture was left at room temperature (**1b**, **2b**, **3d**, and **3e**) or on refrigeration (**2d**). For **1d** and **4d**, the solvent was removed from the reaction mixture, the residue was triturated with diethyl ether, and the resultant solid was purified by recrystallization. Compound **1e** was prepared by method A except that the free base rather than the Mannich base hydrochloride was used. After removal of the solvent, the residue was dissolved in diethyl ether, hydrogen chloride was passed into the ethereal solution, and the precipitate was collected and recrystallized. The melting points (°C), yields (%), times of stirring the reactants at room temperature (h), and recrystallization solvents for these compounds were as follows: **1b**: 143 (literature value,[23] 172 °C), 50, 2, 95% ethanol; **1d**: 120, 68, 26, petroleum ether (bp, 40–60 °C)–acetone; **1e**: 147–148, 17, 26, petroleum ether (bp, 40–60 °C)–acetone; **2b**: 162 (literature value,[24] 169 °C), 54, 0.5, methanol; **2d**: 170, 4, 3, methanol; **3d**: 158, 33, 24, ethanol; **3e**: 168–169, 5, 7–8, methanol; and **4d**: 138, 48, 8, diethyl ether–methanol.

With method B, a mixture of the arylhydrazine hydrochloride (18 mmol), sodium acetate (18 mmol), and dry methanol (50 mL) was added to a vigorously stirred solution of the Mannich base hydrochloride (18 mmol) in dry methanol (50 mL). The resultant mixture was stirred at room temperature for either 24 h (**3a**, **3b**, **4a–4c**) or 48 h (**3c**, **3f**, **3g**). For **3a**, a solid that deposited from the reaction mixture was purified by recrystallization. For the other compounds, the solvent was removed from the reaction mixture, the residue was triturated with diethyl ether, and the product was recrystallized. The melting points (°C), yields (%), and recrystallization solvents for these arylhydrazones were as follows: **3a**: 160, 35, diethyl ether–methanol; **3b**: 148, 69, water:methanol (9:1); **3c**: 164, 77, methanol; **3f**: 184, 44, *n*-butanol; **3g**: 151, 32, ethanol; **4a**: 180, 64, water:methanol (1:1); **4b**: 186, 5, ethanol; and **4c**: 190–192, 31, diethyl ether–methanol.

Cytotoxic Evaluation of Compounds—The procedure used for evaluation of the compounds in the L1210 screen has been described in detail previously.[25] In brief, the compounds were dissolved in ethanol or water; initially, three concentrations of these derivatives were used. Other quantities of the compounds were used if necessary to compute the IC_{50} values. Control determinations were made by adding the solvents to L1210 cells. All test and control experiments were carried out in triplicate at each concentration of the compound, and the percentage of cell survival was noted at the end of 48 h.

The cytotoxicities of **1a–1d** and **2a–2d** against the WiDr tumor in soft agar was evaluated according to a literature procedure,[26] which has been summarized recently.[1] The compounds were dissolved in ethanol, and initially, concentrations of 1, 0.1, and 0.01 μM were used. Other amounts of the derivative were generally required to obtain the IC_{50} values. All determinations were carried out in quadruplicate, with three or four concentrations of the compound. The number of surviving colonies in soft agar was noted at the end of 9 days.

The KB screen was conducted as follows. A stock solution of KB cells was prepared in Eagle's minimum essential medium supplemented with 10% calf serum and incubated at 37 °C in a humidified atmosphere containing 5% carbon dioxide. Cells were counted with a Neubauer hemocytometer, and the stock solution contained 3×10^4 cells/mL. A 100-μL aliquot of the cell stock solution was placed in wells and incubated for 24 h. The test compound was dissolved in dimethyl sulfoxide and further diluted in the cell culture medium, and 100 μL of this solution was added to the wells. The final concentrations of compounds in the well were 10, 5, 1, 0.5, and 0.1 μg/mL. Control wells were prepared in the same way except that the compounds were absent. All test and control experiments at each concentration of the compound were carried out in triplicate. The contents of the wells were cultured for 3 days, and the cells were fixed with glutaraldehyde solution (25%, w/v; 20 μL) for 15 min, washed with water, dried, stained with crystal violet solution (0.05%, w/v; 100 μL) for 15 min, washed with water, and dried. The wells were eluted with 100 μL of sodium dihydrogen phosphate (0.05 M):ethanol (1:1), and absorbances were read on a multiscan spectrophotometer at 540 nm. The IC_{50} values were calculated as (A − 50%)/(A − B), where A is the percent inhibition >50% and B is that <50%. This procedure allowed interpolations between two dilutions; the interpolated values in micrograms per milliliter were converted to values in micromolar.

Evaluation of 1–4 in NCI In Vitro Screen—This assay was undertaken by the NCI, Bethesda, Md, with their protocols[12] whereby each compound was evaluated against 53 (44–56) human tumors. For **1d**, **2a–2d**, **3d**, **3f**, **3g**, and **4a–4c**, the $\log_{10}$ GI_{50} values were < -4.00 for all cell lines, and hence, these values are true mean values. For

the remaining compounds, the number of cell lines for which the $\log_{10}$ GI_{50} value was > -4.00 was 1–6, except for **1a**, **1c**, and **4d**, which gave $\log_{10}$ GI_{50} values of > -4.00 for 26 of 48, 19 of 54, and 22 of 53 tumors, respectively. The $\log_{10}$ TGI values (Table I) are true mean values for **2b**, **3d**, **3f**, **4a–4c**. We noted $\log_{10}$ TGI values of > -4.00 for 1–8 tumors for eight of the compounds and for 18 of 45 cell lines for **1b**, whereas for **1a**, **1c**, **1e**, **3e**, and **4d**, the $\log_{10}$ TGI values were > -4.00 for over half of the tumors. The $\log_{10}$ LC_{50} values (Table I) are true mean values for **2b**, **3d**, **3f**, **4a**, and **4c**. For **2a**, **2c**, **2d**, **3a–3c**, **3g**, and **4b**, $\log_{10}$ LC_{50} values were > -4.00 for 3–20 cell lines. The remaining compounds had $\log_{10}$ LC_{50} values of > -4.00 for over half of the cell lines.

Plots of IC_{50} Values of 1b, 2b, 3, and 4 versus σ, σ^*, π, and MR Constants of Aryl Substituents—The σ and σ^* constants were taken from the literature,[27] and the π and MR constants were obtained from a reference work.[28] The following plots (type, correlation coefficient) were made: IC_{50} versus σ and σ^* (linear, 0.548), IC_{50} versus σ and σ^* (logarithmic, 0.533), IC_{50} versus π (linear, 0.475), IC_{50} versus π (logarithmic, 0.505), IC_{50} versus MR (linear, 0.384), and IC_{50} versus MR (logarithmic, 0.369).

X-ray Crystallographic Determinations of 1a and 4d—The crystal of **1a** was grown by vapor diffusion of diethyl ether into a solution of the compound in methanol at 14 °C in 1 week. Density was measured by flotation in a mixture of hexane and 2-iodopropane. The relevant parameters are as follows: formula, $C_{11}H_{15}NO \cdot HCl$; dimensions, 0.03 × 0.02 × 0.38 mm; M_r = 213.71; triclinic; $P\bar{1}$,a = 6.0175 (6); b = 7.3509 (8); c = 13.9006 (12) Å; α = 94.986 (8); β = 92.470 (7); γ = 112.4 (1) °; V = 564.27 Å^3; Z = 2; D_m = 1.27 mg · m^{-3}; D_x = 1.258 mg · m^{-3}; T = 14 °C; λ(CuKα) = 1.5418 Å; μ = 27.3 cm^{-1}; and F(000) = 228. The cell parameters were determined by least-squares analysis of 25 reflections, with $26.52 < \Theta < 43.08$ °. A CAD-4 diffractometer was used for data collection, with 2329 unique reflections: $-7 < h < 7$, $-9 < k < 9$, $0 < l < 17$, $[(\sin \Theta)/\lambda]_{max}$ = 0.62653 Å^{-1}. There were three monitoring reflections, with no significant intensity variation and no absorption or extinction correction. The merging R value was based on intensities of 0.0112 for 214 replicate reflections.

The structure was solved by direct methods with XTAL-2.6.[27]. All nonhydrogen atoms were found on an E map and refined anisotropically, and hydrogen atoms were found on ΔF maps and refined isotropically. A total of 2141 reflections with $I > 2\sigma(I)$ were used in refinement. A total of 191 parameters were refined, and F magnitudes were used in least-squares refinement to give the following values: R = 0.046, wR = 0.048[$w = 1/\sigma^2(F)$], S = 3.02, final $(\Delta/\sigma)_{av}$ = 0.025, and $(\Delta/\sigma)_{max}$ = 0.23. The $\Delta\rho$ values in the final ΔF map were between +0.50 and −0.42 eÅ^{-3}. The atomic scattering factors were taken from the literature.[28]

Crystals of **4d** were grown by vapor diffusion of diethyl ether into a solution of the compound in methanol at 11 °C in 1 week. Density was measured by flotation in cesium chloride solution. The relevant parameters are as follows: formula, $C_{19}H_{18}F_5N_4 \cdot HCl$; M_r = 413.82; monoclinic; C2/c, a = 15.165 (2); b = 19.881 (3); c = 14.695 (3) Å; β = 61.88 (1) °; V = 3907.71 Å^3, Z = 8; D_m = 1.42 mg · m^{-3}; D_x = 1.427 mg · m^3; λ(CuKα) = 1.5418 Å; μ = 23.0 cm^{-1}; F(000) = 1728; T = 14 °C. The dimensions of the crystal were 0.05 × 0.15 × 0.20 mm, with crystal forms {010}, {101}, and {111}. The cell parameters were measured by least-squares analysis of 25 reflections, with $14.93 < \Theta < 22.03$ °. A CAD-4 diffractometer was used for data collection, with 3871 unique reflections: $-20 < h < 20$, $-20 < k < 20$, $-20 < l < 0$, $[(\sin)\Theta/\lambda]_{max}$ = 0.62653 Å^{-1}. There were three monitoring reflections, with an intensity variation within 2.93%. No absorption or extinction correction was made. The merging R value was based on intensities of 0.0125 for 339 replicate reflections.

The structure was solved by direct methods with XTAL-2.6.[29] All nonhydrogen atoms were found on an E map and refined anisotropically, and hydrogen atoms were found on ΔF maps and refined isotropically. A total of 2400 reflections with $I > 2\sigma(I)$ were used in refinement. Least-square refinement gave R = 0.077, wR = 0.073[$w = 1/\sigma^2(F)$], and S = 3.03; 326 parameters were refined, and F magnitudes were used in least-squares refinement. The final $(\Delta/\sigma)_{av}$ and $(\Delta/\sigma)_{max}$ values were 0.013 and 0.15, respectively. The $\Delta\rho$ value in the final ΔF map was between +0.46 and −0.46 eÅ^{-3}. The atomic scattering factors were taken from the literature.[30]

Tables of fractional coordinates and equivalent isotropic thermal parameters; certain bond distances and angles, fractional coordinates, and thermal parameters for hydrogen atoms; anisotropic thermal parameters for nonhydrogen atoms; and structure amplitude tables may be obtained from the authors on request.

References and Notes

1. Dimmock, J. R.; Phillips, O. A.; Wonko, S. L.; Hickie, R. A.; Tuer, R. G.; Ambrose, S. J.; Reid, R. S.; Mutus, B.; Talpas, C. J. *Eur. J. Med. Chem.* **1989**, *24*, 217–226.
2. Dimmock, J. R.; Patil, S. A.; Leek, D. M.; Warrington, R. C.; Fang, W. D. *Eur. J. Med. Chem.* **1987**, *22*, 545–551.
3. Dimmock, J. R.; Taylor, W. G. *J. Pharm. Sci.* **1975**, *64*, 241–249.
4. Dimmock, J. R.; Hamon, N. W.; Chow, E. W. K.; Kirkpatrick, D. L.; Smith, L. M.; Prior, M. G. *Can. J. Pharm. Sci.* **1980**, *15*, 84–88.
5. Rousseaux, C. G.; Townsend, H. G.; Phillips, O. A.; Dimmock, J. R. *Fund. Appl. Toxicol.* **1990**, *14*, 318–326.
6. Wike-Hooley, J. L.; Haveman, J.; Reinhold, H. S. *Radiother. Oncol.* **1984**, *2*, 343–366.
7. Loewenthal, H. J. E. In *Protective Groups in Organic Chemistry*; McOmie, J. F. W., Ed.; Plenum: London, 1973; pp 342–343.
8. Geiger, W. B.; Conn, J. E. *J. Am. Chem. Soc.* **1945**, *67*, 112–116.
9. Bolton, S. *Pharmaceutical Statistics*; Dekker: New York, 1984; p 207.
10. Hope, H. *Acta Crystallogr.* **1969**, *B25*, 78–87.
11. Jia, Z.; Quail, J. W.; Arora, V. K.; Dimmock, J. R. *Acta Crystallogr.* **1989**, *C45*, 285–289.
12. Boyd, M. R. In *Cancer: Principles and Practice of Oncology Update*; DeVita, V. T., Jr., Ed.; J. B. Lippincott: Philadelphia, 1989; Vol. 3 (10) pp 1–12.
13. Dimmock, J. R.; Jonnalagadda, S. S.; Hussein, S.; Tewari, S.; Quail, J. W.; Reid, R. S.; Delbaere, L. T. J.; Prasad, L. *Eur. J. Med. Chem.* **1990**, *25*, 581–588.
14. Karabatsos, G. J.; Vane, F. M.; Taller, R. A.; His, N. *J. Am. Chem. Soc.* **1964**, *86*, 3351–3357.
15. Nyburg, S. C.; Faerman, C. H. *Acta Crystallogr.* **1985**, *B41*, 274–279.
16. Karrer, P. *Organic Chemistry, 4th ed.*; Elsevier Publishing: Amsterdam, 1950; pp 651 and 652.
17. Selegny, E.; Merle-Aubry, L.; Thoai, N. *Bull. Soc. Chim. France* **1967**, 3166–3167.
18. Maxwell, C. E. *Org. Syn.* **1943**, *23*, 30–31.
19. Shyam, K., Ph.D. Thesis; University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1982,
20. Dimmock, J. R.; Qureshi, A. M.; Noble, L. M.; Smith, P. J.; Baker, H. A. *J. Pharm. Sci.* **1976**, *65*, 38–43.
21. Jacquier, R.; Mousseron, M.; Boyer, S. *Bull. Soc. Chim. France* **1956**, 1653–1666.
22. Atwal, M. S.; Bauer, L.; Dixit, S. N.; Gearien, J. E.; Megahy, M.; Morris, R.; Pokorny, C. *J. Med. Chem.* **1969**, *12*, 994–997.
23. Nisbet, H. B. *J. Chem. Soc.* **1945**, 126–129.
24. Nisbet, H. B. *J. Chem. Soc.* **1938**, 1237–1241.
25. Phillips, O. A.; Nelson, L. A.; Knaus, E. E.; Allen, T. M.; Fathi-Afshar, R. *Drug Des. Del.* **1989**, *4*, 121–127.
26. Hickie, R. A.; Klaassen, D. J.; Carl, G. Z.; Meyskens, F. L. Jr.; Kreutzfeld, K. L.; Thompson, S. P. In *Human Tumor Cloning*; Salmon, S. E.; Trent, J. M., Eds.; Grune and Stratton: Orlando, FL, 1984; pp 409–424.
27. Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pKa Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981; pp 109, 110, 112.
28. Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979; p 49.
29. *The XTAL System of Crystallographic Programs*; Hall, S. R.; Stewart, J. M., Eds.; Universities of Western Australia and Maryland: 1989.
30. *International Tables for X-ray Crystallography*, Kynoch: Birmingham, AL 1974; Vol. IV.

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