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Synthesis and cytotoxic evaluation of some styryl ketones and related compounds

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Summary — A number of 1-aryl-4-methyl-1-penten-3-ones **1** were converted to the corresponding Mannich bases **2** and analogues **3**. Attempts to form the azines **4** from several members in series **1** led to the isolation of the corresponding pyrazolines **5** or aryl aldehyde azines **6**. Replacement of the isopropyl group of a compound in series **1** by methyl and ethyl functions led to ketones that reacted with hydrazine producing the corresponding azines. The Mannich bases displayed greater activity than the precursor ketones towards murine P388 and L1210 leukemia cells as well as to a panel of human tumour cell lines. Certain of the Mannich bases had selective toxicity towards some human tumour cell lines and others to L1210 cells (in contrast to human T lymphocytes). Several drug-resistant cell lines were shown to be free from cross resistance to a number of the Mannich bases.

α,β-unsaturated ketone / Mannich base / X-ray crystallography / cytotoxicity screening

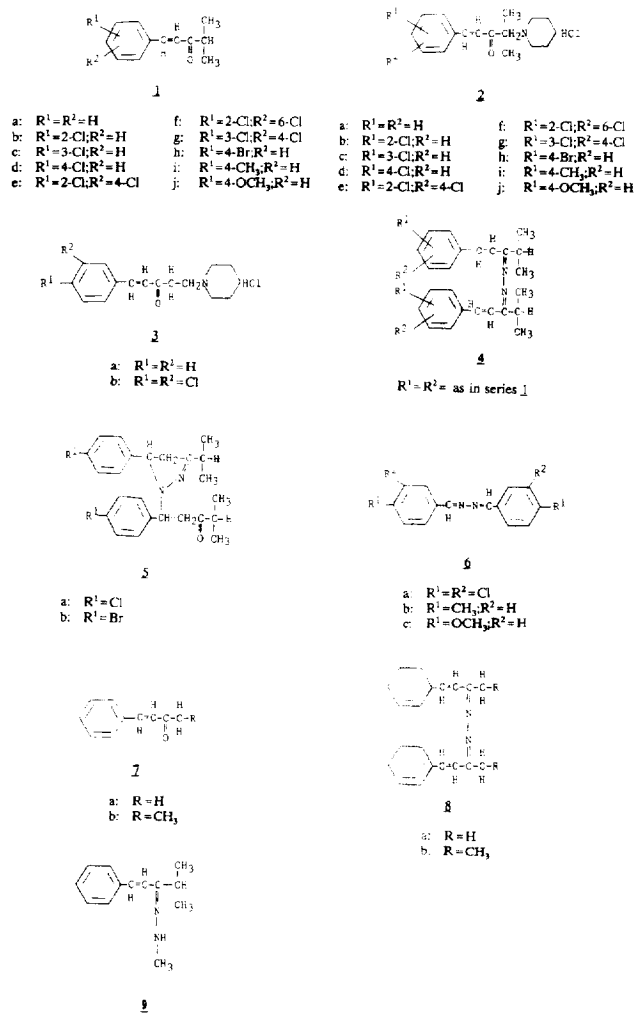
Introduction

A number of studies have described the cytotoxic activities of a variety of α,β-unsaturated ketones designed as thiol-alkylators [1–3]. The aims of the present investigation were as follows. Firstly, the preparation of a series of substituted styryl ketones **1** and related Mannich bases **2** was contemplated. A geminal dimethyl group was present so that in the case of series **2**, deamination would be prevented and bioactivity would be attributed to the intact molecules. Secondly, series **3** was suggested, which could deaminate to the corresponding α,α',β,β'-diolefinic ketone. Hence a comparison of the cytotoxicity of **3** with the analogues **2a**, **g** could be made. Thirdly, the extracellular and intracellular pH values of a number of tumours are lower than the pH of the corresponding normal tissues [4, 5] and since compounds containing the azomethine group are acid labile [6], azines derived from series **1** may regenerate the ketones preferentially in malignant tissues. Hence the synthesis of series **4** was contemplated.

Thus the objective was to prepare the compounds in series **1**–**4** and evaluate their cytotoxicity against different murine and human tumours. Today a major problem in cancer chemotherapy is drug resistance and hence examination of certain of these compounds against drug-resistant cell lines would indicate whether they act by a different mechanism than some current medications. In view of the antiviral potential of various compounds with structures similar to antitumour agents [7], the assessment of the activities of different compounds against certain pathogenic viruses was planned.

Chemistry

The compounds in series **1** were prepared by the Claisen–Schmidt condensation between the appropriate aryl aldehyde and 3-methyl-2-butanone. Reaction of *N*-methylenepiperidinium chloride with the α,β-unsaturated ketones led to the formation of the compounds in series **2**. Series **3** was synthesized as



follows. The appropriate aryl aldehyde was condensed with acetone leading to the formation of the related 4-aryl-3-buten-2-ones which, on treatment with *N*-methylenepiperidinium chloride, gave the desired compounds **3**. Reaction of **1a**, **d**, **g**, **h–j** with hydrazine hydrate gave rise to a mixture of products. From the reactions using **1d** and **1h**, the pyrazolines **5a** and **5b** were isolated while from **1g**, **i**, **j** the corresponding azines **6a–c** were obtained. No compound was isolated from the reaction between **1a** and hydrazine hydrate. An Ortep [8] diagram of the crystal structure of **5a** is given in figure 1. Reaction of the ketones **7a** and **7b** with hydrazine hydrate gave rise to the azines **8a** and **8b**. Methylhydrazine reacted with **1a** to form the corresponding substituted azomethine **9**. While **2g** was stable in a mixture of 90% dimethylsulphoxide-d_6 and 10% deuterated phosphate-buffered saline (PBS-*d*) for 48 h incubation at 37°C, the analogue **3b** decomposed by approximately 20% under these conditions. Using a solvent of 95% dimethylsulphoxide-d_6 and 5% PBS-*d*, solutions of the azines **8a** and **8b** decomposed by 3 and 55% respectively after 48 h at a temperature of 37°C.

Cytotoxicity

The cytotoxicity of various compounds against P388 D1 cells, human tumour cell lines, L1210 neoplasms and human T lymphocytes is presented in table I. The data in table II reveals the effect of some Mannich bases against wild type and drug-resistant tumour cell lines. Compounds **2e**, **f**, **3a**, **b**, **5a**, **7a**, **8a**, **b** and hydrazine dihydrochloride were evaluated for antiviral activity using Coxsackie B4, vesicular stomatitis and polio-1 virus in cultured HeLa cells; herpes simplex-1 (KOS), herpes simplex-1 (TK$^-$ B2006), herpes simplex-1 (TK$^-$ VMW 1837), herpes simplex-2 (G), vesicular stomatitis and vaccinia virus in E_6SM cells, parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4 and Semliki forest virus in Vero cells. Human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) were examined in human T-lymphocytes (CEM). The IC_{50} values for **2e** and **2f** against herpes simplex-1 (KOS) and herpes simplex-1 (TK$^-$ B2006) were 1.86 μM and for hydrazine dihydrochloride against vesicular stomatitis, Coxsackie B4 and Semliki forest virus the figures were 2.86, 2.86 and 1.91 mM, respectively. In all other instances no inhibition of virus-induced cytopathogenicity was noted with any of the compounds at the highest concentration utilized. The concentration of **2e** and **2f** required to cause

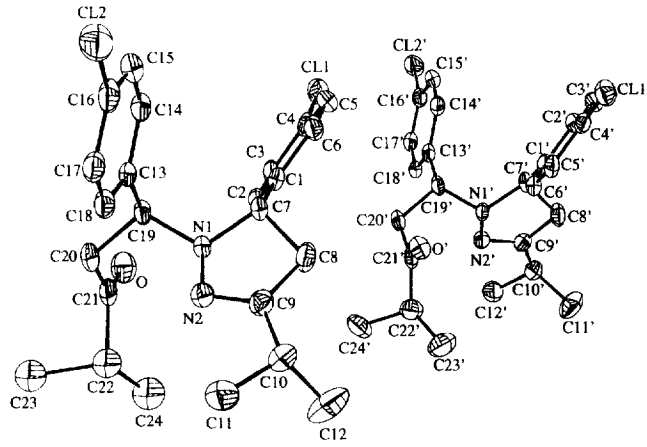


Fig 1. Ortep diagram of **5a** (50% probability ellipsoids). The hydrogen atoms and the disordered atoms with lower occupancies (C22d, C23d, C24d) are omitted for clarity).

Table I. Cytotoxic evaluations using murine P388 cells, human tumour cell lines, murine L1210 cells and human T lymphocytes.

Compound	*P388 cells IC_{50} (μM)*[a]	*Human tumours log GI_{50} MG MID values (M)*[b]	*L1210 cells IC_{50}(μM)*[a]	*Molt 4/C8 cells IC_{50} (μM)*[a]	*TI*[c]	*CEM cells IC_{50} (μM)*[a]	*TI*[c]
1a	11.3	–4.14	–	–	–	–	–
1b	11.9	–	60.38 ± 10.54	24.24 ± 16.63	0.40	12.56 ± 2.88	0.21
1c	10.7	–	70.92 ± 5.27	12.22 ± 5.03	0.17	5.80 ± 1.87	0.08
1d	11.2	–4.91	–	–	–	–	–
1e	8.0	–4.98	–	–	–	–	–
1f	2.9	–5.34	–	–	–	–	–
1g	10.4	–4.78	–	–	–	–	–
1h	17.9	–4.84	–	–	–	–	–
1i	14.4	–4.29	–	–	–	–	–
1j	36.3	–4.38	–	–	–	–	–
2a	2.2	–4.84	10.33 ± 1.30	7.73 ± 2.73	0.75	4.68 ± 2.01	0.45
2b	10.0	–	2.51 ± 0.82	2.28 ± 0.70	0.79	1.61 ± 0.20	0.64
2c	6.1	–	2.05 ± 0.29	1.75 ± 0.32	0.86	1.17 ± 0.53	0.57
2d	0.24	–5.42	2.16 ± 0.56	2.16 ± 0.35	1.00	1.67 ± 0.44	0.77
2e	0.14	–	2.02 ± 0.27	1.73 ± 0.08	0.86	0.40 ± 0.08	0.67
2f	0.79	–5.74	2.02 ± 0.21	1.51 ± 0.11	0.75	1.25 ± 0.11	0.62
2g	0.3	–5.72	1.78 ± 0.13	1.97 ± 0.29	1.10	1.27 ± 0.53	0.72
2h	0.3	–5.53	2.15 ± 0.72	1.71 ± 0.1	0.80	1.01 ± 0.08	0.47
2i	2.4	–5.20	8.73 ± 2.1	7.15 ± 0.87	0.82	3.42 ± 2.02	0.39
2j	4.2	–4.72	8.55 ± 5.95	8.88 ± 0.59	1.04	7.99 ± 1.66	0.93
3a	1.1	–4.41	8.93 ± 3.11	10.86 ± 0.50	1.22	10.26 ± 0.29	1.15
3b	0.31	–	30.69 ± 20.65	57.93 ± 4.01	1.89	46.75 ± 8.6	1.52
5a	23.1	–4.29	331.49 ± 157.63	49.61 ± 5.56	0.15	49.38 ± 4.17	0.15
5b	> 50	–4.23	–	–	–	–	–
7a	10.6	–	95.09 ± 33.52	42.76 ± 14.43	0.45	23.88 ± 3.76	0.25
7b	40.9	–4.25	–	–	–	–	–
8a	> 50	–4.01	> 652.74	> 652.74	–	> 652.74	–
8b	> 50	–4.00	> 636.07	> 636.07	–	> 636.07	–
9	> 50	–4.00	–	–	–	–	–
Melphalan	0.2	–4.64	1310.62 ± 566.84	576.671 ± 91.74	0.44	747.05 ± 206.42	0.57
Hydrazine dihydrochloride	> 50	–	≥ 1905.13	> 1905.13	–	> 1905.13	–
Methotrexate	–	–	0.22 ± 0.02	0.37 ± 0.07	1.70	0.26 ± 0.2	1.20

[a]The IC_{50} figures refer to the concentrations of compounds required to inhibit the growth of the cells by 50%. [b]The log GI_{50} meangraph midpoint (MG MID) values are the concentrations of the compounds required to inhibit the growth of approximately 53 human tumour cell lines by 50%. [c]The letters TI refer therapeutic indices, *ie* the IC_{50} figures for the Molt 4/C8 or CEM lymphocytes/IC_{50} figures for the L1210 cells. [d]The line – throughout this table means that the compound was not evaluated in this test.

Table II. Evaluation of some Mannich bases against wild type and drug-resistant tumour cell lines.

Compound	*H69*	*H69/AR*	*FR*[a]	*MCF-7*	*MCF-7/AR*	*FR*[a]	*MCF-7/ML*	*FR*[a]	*MatB*	*MatB/AR*	*FR*[a]	*MatB/ML*	*FR*[a]
	IC_{50} (μM)	IC_{50} (μM)		IC_{50} (μM)	IC_{50} (μM)		IC_{50} (μM)		IC_{50} (μM)	IC_{50} (μM)		IC_{50} (μM)	
2a	4.10 ± 0.21	7.27 ± 2.75	1.77	5.63 ± 1.67	7.06 ± 1.55	1.25	7.79 ± 3.13	1.38	5.60 ± 2.0	4.89 ± 1.49	0.87	4.37 ± 0.46	0.78
2d	5.20 ± 0.72	4.27 ± 2.37	0.82	1.92 ± 0.58	2.24 ± 1.53	1.17	5.15 ± 1.02	2.68	2.50 ± 1.12	2.40 ± 1.19	0.96	4.06 ± 1.49	1.62
2g	4.47 ± 1.72	4.90 ± 1.56	1.10	0.56 ± 0.07	0.73 ± 0.21	1.30	0.77 ± 0.01	1.38	0.50 ± 0.32	0.40 ± 0.16	0.80	0.84 ± 0.14	1.68
2h	–	–	–	3.56 ± 0.05	5.83 ± 0.08	1.64	3.40 ± 3.15	0.96	5.73 ± 1.60	3.35 ± 1.77	0.58	3.99 ± 0.97	0.70
2i	5.67 ± 0.58	8.60 ± 2.40	1.52	5.85 ± 0.23	8.14 ± 2.34	1.39	5.79 ± 1.65	0.99	6.63 ± 1.94	4.38 ± 2.02	0.66	5.13 ± 0.21	0.77
2j	8.40 ± 1.15	17.00 ± 1.73	2.02	4.60 ± 0.20	6.86 ± 3.34	1.49	7.63 ± 0.61	1.66	> 10	> 10	–	6.57 ± 2.25	< 0.66
Adria-mycin[b]	0.074 ± 0.045	4.00 ± 2.31	54.1	0.011 ± 0.001	2.88 ± 0.29	261.8	0.089 ± 0.013	8.09	0.007 ± 0.003	1.20 ± 1.13	171.4	0.023 ± 0.02	3.29
Mel-phalan[b]	7.28 ± 3.08	14.54 ± 5.99	2.00	11.44 ± 3.89	29.97 ± 8.19	2.62	69.37 ± 5.25	6.06	0.43 ± 0.43	0.93 ± 0.10	2.16	13.21 ± 2.22	30.7

[a]The letters FR indicate fold resistance. [b]The figures against H69 and H69/AR cell lines are taken from reference [32] and reproduced with the permission of the copyright owner.

a microscopically detectable alteration of the normal cell morphology of E_6SM cells was 10.62 μM, while the minimum cytotoxic concentration for HeLa and Vero cells was 1.06 and > 1.06 mM, respectively.

Results and discussion

The initial cytotoxic screen used murine P388D1 lymphocytic leukemia cells. In the case of series **1** and **2**, linear plots were made between the Hammett σ and/or Taft σ* values, the Hansch hydrophobic contants (π) and molar refractivity (MR) figures of the aryl substituents with the IC_{50} data. Using the test for zero correlation [9], a statistically valid relationship between cytotoxicity and the σ* and σ values ($p < 0.05$) and π constants ($p < 0.1$) in series **1** was noted. No other correlations were observed ($p > 0.1$). Compound **1a** had similar activity to **7a** and was nearly four times as cytotoxic as the ethyl analogue **7b**. Conversion of the styryl ketones into the corresponding Mannich bases increased cytotoxicity fivefold; the average IC_{50} figures for **1** and **2** are 13.5 and 2.7 μM, respectively. Of particular note are the potencies of **2d**, **e**, **g**, **h**, which are comparable with the reference drug melphalan.

Solutions of **2g** and **3b** in deuterated phosphate buffer (pD = 7.4) were incubated at 37°C for 48 h which were the temperature and time used in the P388 and human tumour cell line assays. While **2g** was stable, **3b** decomposed by approximately 20%. The breakdown products were not identified. A comparison of the cytotoxicity and **2a**, **g** with **3a**, **b** did not reveal unequivocally whether the presence or absence of a geminal dimethyl group favoured activity since **3a** had twice the potency of **2a**, whereas the dichloro compounds **2g** and **3b** had similar potencies.

The next phase was the attempted synthesis of the candidate prodrugs **4**. However ^{1}H-NMR spectroscopy, mass spectrometry and elemental analysis of the product obtained from the reaction of **1d** with hydrazine hydrate suggested that the pyrazoline **5a** had been formed; this deduction was confirmed by X-ray crystallography (fig 1). The aryl rings are aligned in a somewhat parallel fashion but not eclipsed which may be due to π stacking. The analogue **5b** was isolated from the reaction of **1h** with hydrazine hydrate. The synthesis of **5a**, **b** may have resulted from an initial reaction between **1d**, **h** and hydrazine hydrate leading to the formation of the intermediate 3-alkyl-5-aryl-2-pyrazolines [10, 11], which then reacted with excess of the α,β-unsaturated ketone to produce **5a**, **b**. These compounds are β-aminoketones or Mannich bases, which may be deaminated in a biological milieu. In order to explore this possibility, **5a**, which is virtually insoluble in water, was incubated at 37°C for 48 h in a mixture of dimethylsulphoxide and PBS pH 7.4 (9:1). The isolated product was shown by infrared spectroscopy to be **5a**, *ie* it was stable under these conditions. Table I indicated that **5a**, **b** showed little or no activity towards P388D1 cells.

The reaction with **1g**, **i**, **j** and hydrazine led to the azines **6a–c** respectively whose structures were confirmed by ^{1}H-NMR and infrared spectroscopy, elemental analyses and unambiguous syntheses. By analogy with a literature precedent [12], the unsaturated ketones may have undergone a *retro*-aldol reaction which liberated the aryl aldehyde, which subsequently reacted with hydrazine to give the azines **6**.

In order to explore possible reasons for the lack of formation of the azines **4**, the bulky isopropyl group of **1a** was replaced by methyl and ethyl moieties to give **7a**, **b**. Reaction of these ketones with hydrazine hydrate gave the azines **8a**, **b**. In addition, **1a** reacted with methylhydrazine to give the hydrazone **9** rather than a pyrazoline or azine. Hence the nature of both the unsaturated ketones and hydrazines influence the reaction pathway.

The structure of the azine **8b** was examined by X-ray crystallography and an Ortep diagram is portrayed in figure 2. The stereochemical features of interest were first that the olefinic and carbimino groups have the *E* configuration, second the molecule adopts an extended conformation and third the atoms lie in one plane with the exception of the ethyl groups which are orientated above and below this plane. Thus the angle between the planes C1-C2-C3-C4-C5-C6-C7-C8-C9-N and C9-C10-C11 was found to be 89.8 (0.2).

The possibility of the azines acting as prodrugs was examined by incubating **8a**, **b** in a mixture of dimethylsulphoxide and PBS-d for 48 h at 37°C. Under these conditions, **8a** decomposed by 3% and a new peak at 2.4 ppm did not correspond to the methyl absorption of **7a** which was at 2.31 ppm. The ethyl analogue **8b** decomposed by 55% under these conditions and new absorptions at 1.30 and 1.09 ppm did not correspond to the methyl absorption of **7b** which was at 1.01 ppm. Hence there is no indication that the azines act as prodrugs of unsaturated ketones. The difference in the extent of breakdown of the two azines is noteworthy and reinforces the observation made earlier that the groups adjacent to the unsaturated linkages influence reactivity considerably. Both azines along with the potential breakdown product hydrazine and the methylhydrazone **9** were devoid of activity towards P388 cells at the maximum concentration employed (50 μM).

The data in table I also indicate the results of two assays designed to detect compounds with selective toxicity towards one or more groups of tumours. First, representative compounds were evaluated against a

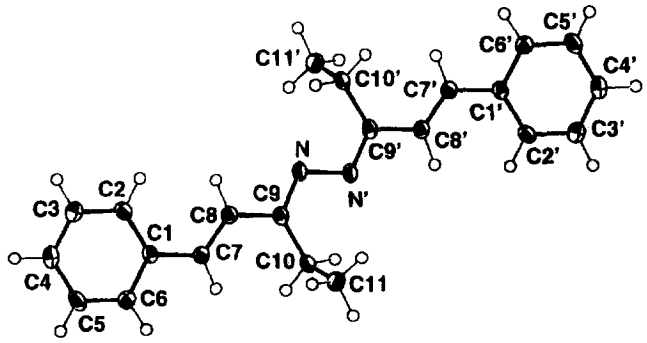


Fig 2. Ortep diagram of **8b**.

panel of human tumour cell lines [13]. Selective toxicity was noted with **1a** and **2i** whereby the average toxicity to leukemia cells was 2.0 and 2.3, respectively, times greater than the average log GI_{50} for all cell lines.

Compound **5a** was 2.8 times more toxic to small cell lung tumours than towards all cell lines. In addition, approximately half of the compounds were more cytotoxic than the reference drug melphalan. A comparison of the potencies of the Mannich bases **2a**, **d**, **f–j** with the corresponding unsaturated ketones **1a**, **d**, **f–j** revealed that in each case greater cytotoxicity was found with the Mannich bases. The most promising compound is **2i** with greater potency than melphalan and selective toxicity towards human leukemia cells (not shown). Second, a comparison was made between the cytotoxicity of various compounds towards murine L1210 cells and two types of human T lymphocytes designated Molt 4/C8 and CEM. Since the Mannich bases **2**, **3** had shown the greatest cytotoxicity towards both P388 cells and human tumour cell lines, these compounds were examined in this screen along with several other analogues. The most favourable therapeutic indices noted were **3a**, **b** which is an observation of value in subsequent molecular modification. However, most of the compounds, as well as melphalan, were more toxic to the lymphocytes than the L1210 cells resulting in therapeutic indices of less than 1. The Mannich bases displayed the greatest activity towards the L1210 cells and in particular **2b**, **c** were approximately 30 times more potent than the analogous ketones **1b**, **c**. The pyrazoline **5a** and azines **8a**, **b** had only low potency or were inactive at the highest concentrations utilized.

Finally the question of whether these novel compounds would be cytotoxic towards cells that had become resistant to such clinically useful drugs such as melphalan (ML) and adriamycin (AR) was addressed. Three sensitive tumour cell lines were employed, namely a human small cell lung cancer (H69) [14, 15], a human breast cancer (MCF-7) [16, 17] and a rat mammary carcinoma model (Mat B) [18, 19]. The corresponding drug-resistant cell lines were as follows. The H69/AR tumours are resistant to adriamycin and several heterocyclic compounds and weakly resistant to melphalan [14]. The MCF/AR and Mat B/AR cells are resistant to adriamycin and other drugs while MCF/ML and Mat B/ML cells are cross resistant to cisplatin, a nitrosourea (BCNU) and bifunctional alkylators [16–19]. The data in table II reveal that the Mannich bases did not show any marked cross resistance to the drug-resistant cells; in fact 43% of the fold resistance (FR) figures were less than 1.0. An important observation is the fact that while the MCF-7/ML and Mat B/ML cell lines were cross resistant to melphalan and many other alkylating agents, these Mannich bases were virtually free from this drawback. Thus the mode of action of the compounds in series **2** is presumably different from traditional alkylating agents used in cancer treatment and hence they have the potential of being developed as drug candidates. The greatest potency against MCF-7, Mat B and the corresponding resistant cell lines was demonstrated by **2g**.

Conclusions

The study has revealed the cytotoxic properties of a number of conjugated styryl ketones and their conversion into the related Mannich bases increased potencies against murine P388 and L1210 cells as well as human tumour cell lines. Compounds **1a**, **2i**, **5a** were selectively toxic to one group of tumour cell lines and two Mannich bases, namely **3a**, **b** had greater cytotoxicity to L1210 cells than human T lymphocytes. Of particular interest is the fact that the Mannich bases were not cross resistant to certain drug-resistant cell lines.

Experimental protocols

Chemistry

Melting points and boiling points are uncorrected. Elemental analyses were undertaken by K Thoms, Department of Chemistry, University of Saskatchewan on series **1** and **7** (C, H) and series **2**, **3**, **5**, **8** and **9** (C, H, N) and were within 0.4% of the calculated values. ^{1}H-NMR spectra were determined routinely on the compounds synthesized using a Varian T-60 spectrophotometer. The stability studies were carried out using a Bruker AM 300 FT instrument. Infrared spectra were determined using a Beckman Acculab 4 spectrophotometer. The mass spectrum was obtained with a Fisons VG 70-SQ instrument coupled to a DEC Micro PDP 11/73 data system. TLC was accomplished using silica-gel sheets. Silica-gel 60 (70–230 mesh) was used in column chromatography.

Synthesis of the α,β-unsaturated ketones ***1a–j***, ***7a*** *and* ***7b***

Compounds **1a**, **g**, **i**, **j** and **7b** were prepared by a literature procedure [20] and **1h** was synthesized by a reported methodology [21]. Compound **7a** was obtained from Janssen Chemica and recrystallized from petroleum ether (bp 40–60°C). The vapour pressures at elevated temperatures (torr/°C) or melting points (°C) and percentage yields of the unsaturated ketones were as follows: **1a**: 0.5/90, 59; **1b**: 0.2/140, 65; **1c**: 0.2/135, 72; **1d**: 0.1/145, 75; **1e**: 0.1/120, 70; **1f**: 0.3/138, 65; **1g**: 45–48, 80; **1h**: 0.25/112, 82; **1i**: 0.2/150, 65; **1j**: 0.3/136, 59; **7a**: 40–41, –; and **7b**: 0.2/82, 74.

Synthesis of the Mannich bases ***2a–j***, ***3a*** *and* ***3b***

The synthesis of **2a**, **d**, **g–j** has been described previously [22]. The general method for the preparation of **2b**, **c**, **e**, **f** was as follows. *N*-Methylenepiperidinium chloride was synthesized by a literature method [23]. A mixture of the appropriate styryl ketone **1** (0.036 mol) and *N*-methylenepiperidinium chloride (0.036 mol) in dry acetonitrile (80–100 ml) was heated at 70–80°C for 12 h. The mixture was cooled, concentrated *in vacuo* and refrigerated overnight (4°C). After 24 h, the precipitate was collected, washed with either acetonitrile or an ether/methanol mixture (1:3) and recrystallized from ether/methanol.

4-(3,4-Dichlorophenyl)-3-buten-2-one required for the synthesis of **3b** was prepared by a literature procedure [20]. Compounds **7a** and 4-(3,4-dichlorophenyl)-3-buten-2-one were converted into **3a** and **3b**, respectively, by the method used for synthesizing **2b**, **c**, **e**, **f** and were purified by recrystallization from ether/methanol.

The melting points (°C) and yields (%) were as follows: **2a**: 183–184, 28; **2b**: 171–172, 42; **2c**: 182–183, 46; **2d**: 186–187, 33; **2e**: 178–179, 38; **2f**: 175–179, 38; **2g**: 182–185, 30; **2h**: 179–182, 26; **2i**: 167–169, 38; **2j**: 180–182, 41; **3a**: 171–172, 30; and **3b**: 208, 32.

Reaction of ***1a***, ***d***, ***g–j*** *with hydrazine hydrate*

A solution of hydrazine hydrate (0.01 mol) in methanol (10 ml) was added slowly to a stirring solution of the unsaturated ketone (0.02 mol) in methanol (20 ml). The reaction was monitored by TLC using a solution of benzene and methanol (7:3). After stirring the solution at room temperature for 16–20 h, no hydrazine hydrate was detected by TLC and the reaction mixture was concentrated and refrigerated at 4°C. The precipitate was collected and recrystallized from methanol to give the pyrazolines **5a** and **5b** from **1d** and **1h**, respectively, and the azines **6a–c** from **1g**, **1i** and **1j**, respectively. In the case of the reaction of hydrazine hydrate with **1a** an oil was obtained, the TLC of which revealed the presence of at least four different compounds.

The data for the pyrazolines were as follows: **5a**: mp 99–100°C, 21% yield, IR (KBr): 1730 cm^{-1} (CO), ^1H NMR ($CDCl_3$, 300 MHz, numbering in figure 1 used): δ: 7.68–7.04 (m, 8H, aryl H), 4.07 (pseudo t, 1H, C19H, $J_{C19H/C20H}$ = 7.1 Hz), 3.58 (dd, 1H, C7H, $J_{C7H/C8H}$ = 9.8 Hz, J_{gem} = 14.2 Hz), 3.41 (dd, 1H, C20H, J_{gem} = 16.6 Hz), 2.97 (dd, 1H, C20H), 2.59 (dd, 1H, C8H, J_{gem} = 16.1 Hz), 2.45 (q, 1H, $CH(CH_3)_2$, J = 6.9 Hz), 2.40 (q, 1H, $CH(CH_3)_2$), 2.30 (poorly resolved pseudo-t, 1H, C8H, J = 15 Hz approx), 1.00 (d, 3H, CH_3CH CH_3), 0.99 (d, 3H, H_3CCHCH_3), 0.96 (d, 3H, CH_3CHCH_3), 0.89 (d, 3H, CH_3CHCH_3). A COSY experiment revealed or confirmed the coupling pattern. Mass: 430.1590 (calcd for $C_{24}H_{28}Cl_2N_2O$: 430.1579); **5b**: mp 99–101°C, 18% yield, IR (KBr): 1720 cm^{-1} (CO). The 60 MHz ^1H-NMR spectrum of **5b** was similar to that of **5a**. The data for the azines were as follows: **6a**: mp 171°C, 29% yield; **6b**: mp 144°C (lit [24] mp 154–155°C), 4% yield; and **6c**: mp 161–162°C (lit [24] mp 163–165°C), 8% yield.

The azines **6a–c** were also prepared by the following route. A solution of hydrazine hydrate (0.005 mol) in methanol (10 ml) was added to a stirred solution of the appropriate aryl aldehyde (0.01 mol) in methanol (20 ml). The reaction mixture stood at room temperature for 24 h and the precipitates were collected and washed with methanol. Recrystallization from methanol gave the following azines (mp, % yield): **6a**: 175°C, 85; **6b**: 147–148°C, 82; and **6c**: 165–166°C, 86. The infrared spectra (KBr) of the samples of **6a–c** obtained by these two independent routes were identical.

Synthesis of the azines ***8a*** *and* ***8b***

A solution of hydrazine hydrate (0.01 mol) in methanol (10 ml) was added slowly to a stirred solution of **7a** or **7b** (0.02 mol) in methanol (30 ml). After standing at room temperature for 24 h, the reaction mixture was concentrated and refrigerated (4°C) for 24 h. The precipitates were collected, washed with methanol and recrystallized from methanol to give the azines **8a**, mp 156–158°C and **8b**, mp 138–140°C in yields of 21 and 32%, respectively. The ^1H-NMR (300 MHz) spectra of solutions of **8a** and **8b** in a mixture of dimethylsulphoxide-d_6 and PBS-*d* (pD = 7.4) (95:5) were as follows: **8a**: δ: 7.70 (d, 4, *meta* aryl H), 7.48 (m, 6, *ortho* and *para* aryl H), 7.30 (d, 2, CH=, $J_{olefinic}$ = 16.8 Hz), 7.09 (d, 2, CH=), 2.18 (s, 6, CH_3); **8b**: δ: 7.71 (d, 4, *meta* aryl H), 7.48 (m, 6, *ortho* and *para* aryl H), 7.31 (d, 2, CH=, $J_{olefinic}$ = 16.6 Hz), 6.99 (d, 2, CH=), 2.73 (q, 4, CH_2), 1.11 (t, 6, CH_3).

Synthesis of the N-methylhydrazone ***9a***

Methylhydrazine (0.01 mol) in ethanol (20 mL) was added to a stirred solution of **1a** (0.01 mol) in ethanol (20 ml) and the mixture was left to stand at room temperature for 24 h. Evaporation of the solvent gave the crude reaction product which was purified by flash chromatography using silica gel and an eluting solvent of benzene and methanol (9:1) to give **9a** as an oil in 28% yield. δ ($CDCl_3$, 60 MHz): 1.10–1.38 (q, 6, $CH(CH_3)_2$), 2.5–2.93 (m, 3, $NHCH_3$), 3.53–3.98 (m, 1, $CH(CH_3)_2$), 6.00–6.20 (d, 1, olefinic H), 7.00–7.38 (m, 6, aryl and one olefinic H), 7.50–7.72 (br, 1, NH).

Stability studies on ***2g***, ***3b***, *and* ***8a***, ***b***

The ^1H-NMR spectra of 10 mM solutions were recorded at 37°C as soon as possible after dissolution and after 48 h incubation at 37°C. Compounds **2g** and **3b** were dissolved in a mixture of 90% dimethylsulphoxide-d_6 and 10% PBS-*d* (pD = 7.4). The spectrum of **3b** obtained after 48 h revealed a decline in the integral of all parts of the spectrum except in the region between 7.2 and 7.8 ppm. In this region a corresponding rise in the integral was observed.

Compounds **8a** and **8b** were dissolved in a mixture of 95% dimethylsulphoxide-d_6 and 5% PBS-*d* (pD = 7.4). The percentage decomposition was measured using the methyl resonances. After 48 h, the spectrum of a solution of **8a** revealed a new peak at 2.4 ppm while in the case of **8b** several new absorptions, including two triplets at 1.30 and 1.09 ppm, were detected.

X-Ray crystallography of ***5a*** *and* ***8b***

Compound **5a** was crystallized by the vapour diffusion method. Diethyl ether vapour was diffused into a saturated solution of **5a** in a mixture of butanol, isopropanol, ethanol and methanol (1:1:1:1). The data are as follows: $C_{24}H_{28}Cl_2N_2O$, M_r = 431.4. The crystals were colourless rods, triclinic, $P\bar{1}$, a = 8.0911(8),

b = 14.5490(10), c = 19.8410(20) Å, α = 77.99(1), β = 87.427(9), γ = 89.55(1)°, V = 2282.2(3) Å^3, Z = 4, D_m (by flotation) = 1.256, D_x = 1.210 Mg m^{-3}, λ (Cu K_α) = 1.5418 Å, μ = 2.72 mm^{-1}, F (000) = 912, T = 123 K. A crystal with dimensions 0.70 x 0.15 x 0.15 mm was used for data collection on an Enraf-Nonius CAD-4 diffractometer. Cell dimensions were determined from setting angles for 25 reflections having 24.74 ≤ θ ≤ 42.5°. During data collection, three reflections monitored after every 5000 s of X-ray exposure indicated no significant decay over the entire data collection. No absorption correction was applied. A total of 9538 reflections were measured with a θ range of 0–75°; $-17 \le h \le 17$, $-9 \le k \le 9$, $0 \le l \le 23$; 8632 were unique reflections, $(\sin\theta/\lambda)_{max}$ = 0.6180 Å^{-1}, merging R was 0.015 for 906 replicates. The structure was solved by direct methods using NRCVAX [25]. All of the non-hydrogen atoms were found on the E map and refined anisotropically except for three atoms (C22, C23 and C24) which were refined isotropically. Atoms C22, C23 and C24 were disordered and occupied two different positions with occupancies of 0.65 (C22, C23, C24) and 0.35 (C22d, C23d, C24d). All of the hydrogen atoms were calculated and not refined. A total of 5148 reflections with $I > 2.5\ \sigma(I)$ were used in the least-squares refinement. The final reliability index R was 0.065, wR was 0.071 [$w = 1/\sigma^2(F) + 0.0002\ F^2$], and the goodness of fit parameter S was 3.45. A total of 514 variables were refined. In the final least-squares cycle, the maximum shift/sigma ratio was 0.010. In the final difference map $\Delta\rho_{max}$ = 1.11 eÅ^{-3} and $\Delta\rho_{min}$ = –0.72 eÅ^{-3}. Atomic scattering factors were taken from the literature [26].

Compound **8b** was crystallized from ethanol/isopropanol by the vapour diffusion method. The data are as follows: $C_{22}H_{24}N_2$, M_r = 316.45, monoclinic, $P2_1/c$, brown plates, 0.30 x 0.30 x 0.20 mm, a = 4.3918(7), b = 9.434(1), c = 21.271(2) Å, β = 92.75(1)°, V = 880.3(2) Å^3, Z = 2, D_m (by flotation) = 1.213, Mg mm^{-3}, D_x = 1.194 Mg mm^{-3}, λ (MoK_α) = 0.70930 Å, μ = 0.756 cm^{-1}, F(000) = 339.94, T = 123 K. The estimated standard deviations of the unit cell dimensions were obtained by least-squares refinement using 25 reflections in the $18 < 2\theta < 28$ range; 1784 reflections were measured of which 1544 were unique, and 1183 observed reflections [$I \ge 2\sigma(I)$] were used in the refinement, $(\sin\theta/\lambda)_{max}$ = 0.595 Å^{-1}, $-5 \le h \le 5$, $0 \le k \le 11$, $-1 \le l \le 25$. No absorption correction was applied. The structure was solved using XTAL 3.2 system [8]. All of the non-hydrogen atoms were found in an E-map and refined anisotropically. All hydrogen atoms were then found in a Fourier difference map and were refined isotropically. Merging R was based on intensities 0.016 for 240 reflections, $R(F)$ = 0.044, R_w = 0.030, S = 3.183 for 1183 observed reflections. Parameters refined = 157 [$w = 1.0/\sigma^2(F)$], final $(\Delta/\sigma)_{max}$ = 0.001. In the final difference map $\Delta\sigma_{max}$ = 0.31 eÅ^{-3} and $\Delta\rho_{min}$ = –0.290 eÅ^{-3}. All the atomic scattering factors and anomalous dispersion corrections were taken from the literature [26].

Cytotoxicity screening

Evaluation using P388D1 and L1210 cells and human T-lymphocytes

The procedure using P388 cells has been described in detail previously [27]. Briefly, the compounds were dissolved in a suitable solvent and aliquots of the solution were incubated with the cells at 37°C for 48 h. Each compound was examined in triplicate using at least three different concentrations and the IC_{50} values were determined graphically. The compounds examined for activity using L1210 cells and T-lymphocytes were assayed by a literature procedure [28].

Evaluation using human tumours

Details of this experimental technique have been described previously [13, 29]. The compounds were evaluated on average against 53 cell lines. The number of cell lines with log GI_{50} figures less than –4.00 M/no of cell lines examined were as follows: **1a**: 28/51; **1d**: 49/51; **1e**: 59/60; **1f**: 59/60; **1g**: 51/52; **1h**: 54/54; **1i**: 37/46; **1j**: 51/56; **2a**: 56/56; **2d**: 54/54; **2f**: 60/60; **2g**: 55/57; **2h**: 55/55; **2i**: 56/56; **2j**: 58/58; **3a**: 49/60; **5a**: 31/43; **5b**: 29/47; **7b**: 43/59; **8a**: 1/50; **8b**: 0/54; **9**: 2/56; and melphalan: 60/60.

*Evaluation of **2a**, **d**, **g–j** against sensitive and drug-resistant tumour cell lines*

The methodology for evaluating compounds using sensitive (H69) and multidrug resistant (H69AR) cell lines has been described previously [14, 30]. An incubation time of 4 d was utilized. The screening of certain Mannich bases against sensitive and drug-resistant MCF-7 and MatB cell lines utilized reported procedures [16–19].

Antiviral assays. Examination of various compounds against viruses

The antiviral evaluations using infected E_6SM, HeLa and Vero cells were carried out using a literature methodology [31].

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References

1 Cheng CC (1988) *Progress in Medicinal Chemistry, Volume 25* (Ellis GP, West GB, eds) Elsevier Science Publishers, Amsterdam,The Netherlands, 35–83

2 Dimmock JR, Erciyas E, Sidhu KK *et al* (1990) *Drug Design and Delivery* 7, 45–49

3 Dimmock JR, Wong MCL (1976) *Can J Pharm Sci* 11, 35–53

4 Thistlethwaite AJ, Leeper DB, Moylan III MD, Nerlinger BS (1985) *Int J Radiat Oncol Biol Phys* 11, 1647–1652

5 Wike-Hooley JL, Havenman J, Reinhold JS (1984) *Radiother Oncol* 2, 343–366

6 Loewenthal HJE (1973) *Protective Groups in Organic Chemistry* (McOmie JFW, ed) Plenum Press, London, UK, 340–343

7 Korolkovas A (1988) *Essentials of Medicinal Chemistry*, John Wiley and Sons, New York, USA, 2nd edn, p 739

8 XTAL 3.2 (1989) *The XTAL System of Crystallographic Programs* (Hall SR, Stewart JM, eds) Universities of Western Australia and Maryland

9 Bolton S (1984) *Pharmaceutical Statistics*, Marcel Dekker, NY, USA, 207–209

10 Schmidbaur H, Bublak W, Schier A, Reber G, Mueller G (1988) *Chem Ber* 121, 1373–1374
11 El-Maghraby MA, Khalafalla AK, Hassan ME, Soleiman HA (1986) *J Indian Chem Soc* 63, 910–913
12 Jurkowska-Kowalczyk E (1976) *Rocz Chem* 50, 489–497; *Chem Abstr* (1976) 85, 94272v
13 Boyd MR (1990) *Cancer: Principles and Practice of Oncology Update* (De Vita Jr VT, Hellman S, Rosenberg SA, eds) JB Lippincott, Philadelphia, PA, USA, Vol 3(10), 1–12
14 Cole SPC (1990) *Cancer Chemother Pharmacol* 26, 250–256
15 Cole SPC, Bhardwaj G, Gerlack JH *et al* (1992) *Science* 258, 1650–1654
16 Batist G, Myers CE, Sinha BK, Katke AG, Cowan K (1986) *J Biol Chem* 261, 15544–15549
17 Batist G, Torres-Garcia S, DeMuys M *et al* (1989) *Mol Phamacol* 36, 224–230
18 Schecter R, Woo A, Batist G (1991) *Cancer Res* 51, 1434–1442
19 Schecter R, Alaoui-Jamali M, Batist G (1993) *Cancer Res* 53, 4900–4911
20 Dimmock JR, Phillips OA, Wonko SL *et al* (1989) *Eur J Med Chem* 24, 217–226
21 Smith PJ, Dimmock JR, Taylor WG (1972) *Can J Chem* 50, 871–879
22 Dimmock JR, Kumar P, Manavathu EK, Obedeanu N, Grewel J (1995) *Pharmazie* 49, 909–912
23 Bohme H, Hartke K (1960) *Chem Ber* 93, 1305–1309
24 Rosini G, Soverini M, Ballini R (1983) *Synthesis* 11, 909–910
25 Gabe EJ, LePage Y, Charland JP, Lee FL, White PS (1989) *J Appl Cryst* 22, 384–387
26 *International Tables for X-ray crystallography*, vol IV (1974) Kynoch, Birmingham, UK
27 Phillips OA, Nelson LA, Knaus EE, Allen TM, Fathi-Afshar R (1989) Drug Design Delivery 4, 121–127
28 Balzarini J, De Clercq E, Mertes MP, Shugar D, Torrence PF (1982) *Biochem Pharmacol* 2, 3673–3682
29 Monks A, Scudiero D, Skeham P *et al* (1991) *J Natl Cancer Inst* 83, 757–766
30 Cole SPC (1986) *Cancer Chemother Pharmacol* 17, 259–263
31 DeClercq E, Balzarini J, Torrence PF *et al* (1980) *Mol Pharmacol* 19, 321–330