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Cytotoxic and anticancer properties of some 4-aryl-3-arylcarbonyl-1-ethyl-4-piperidinols and related compounds

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A previous investigation revealed that various 4-aryl-3-arylcarbonyl-1-ethyl-4-piperidinols and related vinylogs were cytotoxic to both murine and human tumour cell lines. In particular, **1a** and **2a** were identified as useful prototypic molecules. Structural modifications of **1a** and **2a** were accomplished leading to **1b**–**e** and **2b**–**d** which displayed cytotoxicity towards murine P388 and L1210 leukemic cells as well as human Molt 4/C8 and CEM T-lymphocytes. Among the new compounds, the greatest average potencies against these four cell lines were displayed by **1b** and **2b,** having approximately one quarter and one half of the potency of the reference drug melphalan, respectively. The synthesis and bioevaluation of three open chain analogues of **1b**–**d**, namely **3a**–**c**, did not reveal unequivocally whether this molecular modification led to increases in cytotoxicity or not. Compounds **2a**–**d** were substantially more active than melphalan using a panel of human tumour cell lines. In addition, several compounds displayed selective toxicity to both colon and leukemic cancer cells. The 4-piperidinol **2d** was active in the *in vivo* hollow fibre assay. This study revealed compounds with greater potency than **1a** and **2a** and it has confirmed that 1,3,4-trisubstituted-4-piperidinols and related compounds are novel groups of candidate antineoplastic and anticancer agents.

1. Introduction

One of the major interests of this laboratory is the design, synthesis and cytotoxic evaluation of Mannich bases [1]. In general the compounds studied are acyclic Mannich bases which are capable of assuming a wide range of conformations. Very recently two series of novel cytotoxic agents **1** and **2** were disclosed [2] which may be regarded as semicyclic Mannich bases. In these compounds the dimethylene *N*-ethylamino group has been incorporated into a piperidine ring thus restricting the flexibility of the molecules. From this investigation, **1a** and **2a** evolved as promising prototypic molecules. In the first case, **1a** had the highest cytotoxicity in the series against a range of human tumours having a potency of 1.4 times that of the reference drug melphalan. Secondly, **2a** not only possessed marked cytotoxicity towards a number of murine and human neoplastic cell lines, but reduced the sizes of two colon tumours passaged in athymic mice [2]. This compound also induced apoptosis in human Jurkat leukemic cells [3].

The principal objective of the present investigation was to prepare further analogues of **1a** and **2a** with a view to confirming their noteworthy antineoplastic activity. The aryl substituents chosen were influenced by the Hammett σ and Hansch π values of the groups in **1a** and **2a,** which were $+\sigma/+\pi$ and $-\sigma/-\pi$, respectively, with the exception of **1e** in which the nitro group has a negative π constant.

2. Investigations, results and discussion

The compounds were prepared by the route outlined in the Scheme. The structures of the 1,3,4-trisubstituted piperidones in series **1** and **2** were established unequivocally by ^{1}H NMR spectroscopy, X-ray crystallography and literature precedents [2]. The groups containing the aryl rings at the 3 and 4 positions of the heterocycle have the *trans* arrangement and the olefinic double bonds adopted the *E* configuration.

Most of the compounds in series **1** and **2** were evaluated against murine P388 and L1210 cells which are claimed to be good predictors of clinically useful drugs [4]. In addition, use was made of the human Molt 4/C8 and CEM T-lymphocyte screens which provides an insight as to the likelihood of cytotoxicity being displayed towards human tumours. The results are summarized in Table 1.

With regard to series **1**, the following generalizations regarding structures and cytotoxicity may be made. All five compounds were cytotoxic towards P388 cells and maximum activity was displayed by **1b**. Taking into consideration the overall potencies of **1b**–**e** towards P388, L1210, Molt 4/C8 and CEM cells, the average potency (AP) values indicated that cytotoxicity was in the order of **1b > 1d > 1e > 1c**. There was no correlation between the electronic nature of the aryl substituents and bioactivity. However, hydrophobicity, as revealed by the aryl π values, was in the order **1b > 1d > 1c > 1e**, revealing that the two most cytotoxic compounds had the highest lipophilicity. A noteworthy feature of the AP results is the observation that **1b** has over one quarter of the potency of melphalan and is clearly a useful lead molecule.

The results in series **2** followed a similar pattern. All of the compounds evaluated in the P388 screen were markedly cytotoxic. The overall cytotoxicity as evidenced from the AP values was **2b > 2a > 2d > 2c**. There was no apparent correlation with σ constants and cytotoxicity. Hydrophobicity would be predicted to be as follows: **2d > 2c > 2a**, **b**. In series **2,** therefore, the two most active compounds had the lowest lipophilicity. The most potent compound in series **2**, namely **2b**, approached the activity of melphalan.

An investigation was made to determine if the mono-Mannich base portion of series **1**, as indicated in the Fig., was responsible for cytotoxicity and, if so whether or not the rigid nature of the piperidinols **1** was advantageous. A comparison was therefore made between the AP values of **1b**–**d** and **3a**–**c**. The results show that **1b > 3a**, **3b > 1c,** and **1d** and **3c** displayed similar potencies. One may conclude that the circled group in series **1** (Fig.) likely contributes significantly to the cytotoxicity and the remainder of the molecule in **1** has an additional role, which may increase or decrease cytotoxicity. This possibility is consistent with the observation noted earlier that lipophilicity

Scheme

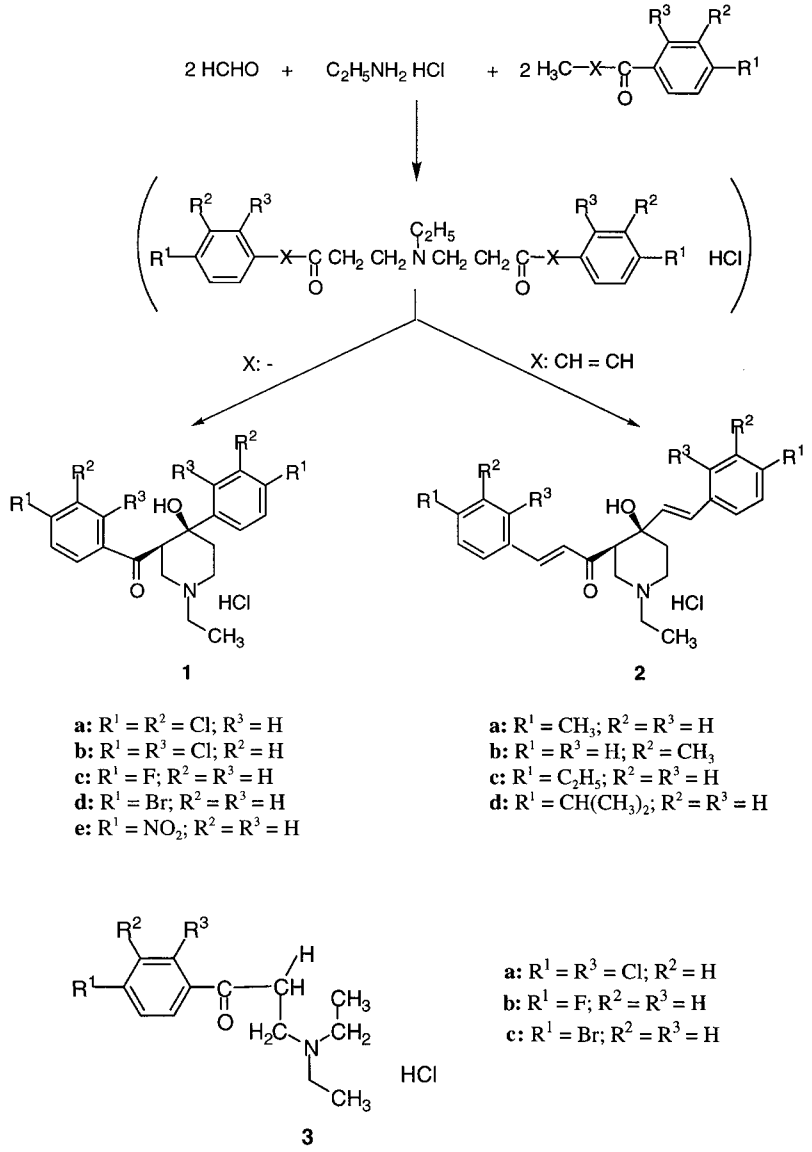

Table 1: Evaluation of 1a–e, 2a–d, 3a–c against P388, L1210, Molt 4/C8 and CEM cells

Compd.	IC_{50} (μM)				AP[b]
	P388	L1210	Molt 4/C8	CEM	
1a	3.11[a]	–	–	–	–
1b	2.93	8.25 ± 1.19	9.10 ± 1.13	8.65 ± 0.65	7.23
1c	5.62	90.8 ± 5.7	84.8 ± 14.1	52.3 ± 5.5	58.4
1d	6.59	35.5 ± 2.2	35.7 ± 21.8	41.7 ± 8.3	29.9
1e	27.3	45.4 ± 4.6	43.2 ± 2.5	41.8 ± 4.0	39.4
2a	2.10[a]	2.59 ± 1.05	11.5 ± 7.2	5.98 ± 2.72	5.54
2b	1.64	4.36 ± 2.36	7.34 ± 0.54	2.51 ± 0.68	3.96
2c	1.45	17.6 ± 4.1	13.1 ± 8.0	25.4 ± 10.4	14.4
2d	1.21	8.25 ± 0.83	8.23 ± 0.61	7.30 ± 0.15	6.25
3a	1.20	20.5 ± 16.6	26.4 ± 1.8	31.3 ± 5.6	19.9
3b	1.22	77.7 ± 60.0	32.5 ± 1.5	53.8 ± 4.2	41.3
3c	1.49	34.3 ± 10.0	44.1 ± 0.8	35.9 ± 4.9	29.0
Melphalan	0.22[a]	2.13 ± 0.03	3.24 ± 0.79	2.47 ± 0.30	2.02

[a] Reprinted in part from J. Med. Chem. **41**, 4012 (1998). Copyright 1998 American Chemical Society.
[b] The average potency (AP) value indicates the average figure of the IC_{50} values using all four cell lines.

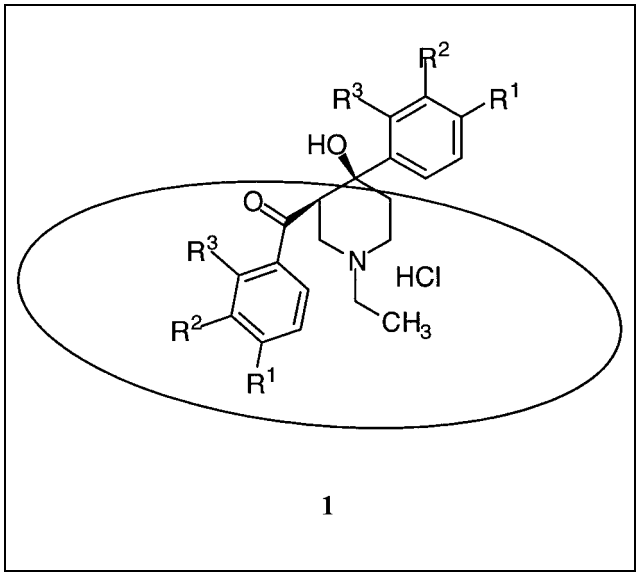

Fig.: The mono-Mannich base portion of series **1**

Table 2: Evaluation of 1a, e, 2a–d and reference compounds against human tumour cell lines

Compd.	All cell lines	Colon cancer cells		Leukemic cells	
	MG MID[a] (μM)	MG MID[a] (μM)	SI[b]	MG MID[a] (μM)	SI[b]
1a	16.6[c]	18.4	0.9	10.3	1.6
1e	23.5	20.0	1.2	18.4	1.3
2a	5.01[c]	2.18	2.3	2.30	2.2
2b	2.69	1.88	1.4	6.03	0.5
2c	3.63	2.13	1.7	2.11	1.7
2d	2.29	2.10	1.1	2.10	1.1
Melphalan	23.5	44.6	0.5	4.65	5.1
5-Fluorouracil	32.6	7.90	4.1	27.6	1.2

[a] The letters MG MID refer to the mean graph midpoint values. This term is explained in the text.
[b] The letters SI indicate the selectivity index. This figure was obtained by dividing the MG MID figures for all cell lines by the MG MID data for either the colon or leukemic cell lines.
[c] Reprinted in part from J. Med. Chem. **41**, 4012 (1998). Copyright 1998 American

likely influences cytotoxicity. Hence the group outside the circle in the Fig. could modulate bioactivity by hydrophobic considerations.

Certain compounds were selected by the National Cancer Institute, USA for evaluation against approximately 55 human tumour cell lines from eight or nine different neoplastic diseases including colon cancers and leukemia. The results are presented in Table 2. Cytotoxicity is expressed in terms of mean graph midpoint (MG MID) values [5] rather than IC_{50} figures for the following reason. The concentrations of compounds required to inhibit 50% of the growth of the cell lines are determined using a concentration range of 10^{-8} to 10^{-4} M. However, if a compound does not inhibit 50% of the growth of the tumour at the highest concentration used i.e. 10^{-4} M, then this figure of 10^{-4} M is still incorporated into the calculations of the MG MID values. A review of the data in Table 2 revealed that both **1a** and **1e** possessed greater or similar cytotoxicity than melphalan. In addition, the four compounds in series **2** had, on average, seven times the activity of the anticancer alkylator melphalan suggesting the importance of molecular modification of these prototypic molecules.

An examination of the mean graphs [6] was undertaken in order to detect compounds displaying selective toxicity towards one or more groups of tumours. The assumption has been made that these compounds may display preferential cytotoxicity towards various tumours relative to normal cells. In view of the interest in this laboratory in compounds active against colon tumours [7, 8] and leukemia [9, 10], selectivity index (SI) figures for these two groups of tumours were generated and the results are presented in Table 2. 5-Fluorouracil (5-FU) and melphalan were chosen as reference drugs since they are used clinically in the treatment of colon cancers [11] and leukemia [12], respectively. An increase of 50% in selectivity was arbitrarily chosen as an indication of preferential cytotoxicity to colon or leukemic cell lines, i.e., the tumours in question were 50% more sensitive to the compounds as compared to all cell lines. Both **2a** and **2c** displayed selective toxicity to colon tumours having approximately half of the selectivity possessed by 5-FU. Three compounds **1a**, **2a**, **c** demonstrated selective toxicity to leukemic cells having approximately one-third of the selectivity of melphalan on average. These data confirm the necessity of developing these novel compounds as candidate cytotoxic and anticancer drugs.

Finally in order to assess the anticancer potential of representative compounds, **2c**, **d** were evaluated in the hollow fibre assay [13]. In this procedure, twelve different cell lines were placed in hollow fibres and implanted intraperitoneally (IP) and subcutaneously (SC) into mice. Two doses of each compound were injected ip into the animals and after a period of time, the fibres were removed and the cytotoxicity towards the cell lines determined. Activity is denoted if a 50% reduction in the number of viable cells was achieved. The maximum score that each compound could obtain is 48 (12 cell lines × 2 doses × 2 sites of implantation). In the case of **2c**, the IP + SC scores were 0 and 2, respectively, and for **2d**, the relevant figures were 8 and 4, respectively. The criteria for activity include an IP + SC score of >10, and hence **2d** was considered to be active in this screen.

Virtually all of the new compounds prepared in this study displayed significant cytotoxicity. This study has therefore confirmed the impressive cytotoxic and anticancer potential of 4-aryl-3-arylcarbonyl-1-ethyl-4-piperidonols **1** and the related vinylogs **2**. In addition, improved potencies over the lead molecules **1a** and **2a** were achieved in certain cases. Thus the AP value of **2b** is 1.4 times that of **2a** and the selectivity of **2c** for colon cancer cells is nearly double that of **1a**. The *in vivo* activity of a representative compound **2d** afforded further evidence of the need to develop these series of compounds in the future.

3. Experimental

3.1. Synthesis of the compounds

Melting points and boiling points are uncorrected. Elemental analyses were undertaken on **1b–e**, **2b–d**, **3a–c** by Mr. K. Thoms, Department of Chemistry, University of Saskatchewan and were within 0.4% of the calculated values. ^{1}H NMR spectra of **1b–e**, **2b–d**, **3a–c** were determined using a Bruker AM 500 FT NMR machine (500 MHz) and were consistent with the proposed structures. Since detailed ^{1}H NMR spectral analyses of these groups of compounds have been presented at length previously [2], these data will be omitted in the present report. TLC undertaken using silica gel plastic-backed sheets revealed that all compounds were homogeneous using solvent systems of hexane/CH_3OH (7 : 3) for the intermediate α,β-unsaturated ketones and $CHCl_3$/CH_3OH (7 : 3) for the Mannich bases.

*3.1.1. Synthesis of **1b–e***

A mixture of the 1-aryl-1-ethanone (0.04 mol), paraformaldehyde (0.04 mol), ethylamine hydrochloride (0.01 mol), HCl (37% w/v, 0.04 ml) and CH_3CN (100 ml) was heated under reflux for 48 (**1b**, **d**), 55 (**1c**) or 19 (**1e**) h. In the case of **1d**, the precipitate was collected, washed with $(CH_3)_2CHOH$ and recrystallized from CH_3OH. For **1b**, **c**, **e**, the solvents were removed in vacuo to give oils which were treated with anhydrous $(C_2H_5)_2O$ to remove unreacted ketone. The oil was dissolved in absolute C_2H_5OH and sufficient $(C_2H_5)_2O$ was added to cause the solution to become turbid. After 2–3 days refrigeration at −4 °C, the material was collected and repeatedly recrystallized from CH_3COCH_3/CH_3OH (**1b**), $(C_2H_5)_2O$/CH_3OH (**1c**) and CH_3OH (**1e**). The m.p. (°C) and yields (%) of **1b–e** are as follows: **1b**: 214–215°, 50; **1c**: 198–199°, 30; **1d**: 212–213°, 20; **1e**: 200–202°, 24.

*3.1.2. Synthesis of **2b–d***

The intermediate 4-aryl-3-buten-2-ones required in the synthesis of **2b–d** were prepared by a literature method [14] to give 4-(3-methylphenyl)-3-buten-2-one b.p. 123–125 °C/0.5 mm, 4-(4-ethylphenyl)-3-buten-2-one b.p. 100–102 °C/0.5 mm and 4-(4-isopropylphenyl)-3-buten-2-one b.p. 90–92 °C/0.6 mm in percentage yields of 89, 75 and 75, respectively.

Compounds **2b–d** were prepared as follows. A mixture of the appropriate 4-aryl-3-buten-2-one (0.06 mol), paraformaldehyde (0.04 mol), ethylamine hydrochloride (0.01 mol), HCl (37% w/v, 0.04 ml) and C_2H_5OH (95% v/v, 100 ml) was heated under reflux for 45 (**2b**) or 72 (**2c**, **d**) h. Removal of the solvents led to an oil which was triturated with $(C_2H_5)_2O$ to remove unreacted ketone. The oil was dissolved in absolute C_2H_5OH (10 ml) and sufficient $CH_3COOC_2H_5$ was added to cause the solution to become turbid. After refrigeration at −30 °C for 3–4 days, the precipitate was collected and repeatedly recrystallized from $(C_2H_5)_2O$/CH_3OH (**2b**, **c**) or CH_3CN (**2d**). The m.p. (°C) and yields (%) of **2b–d** were as follows: **2b**: 167–169°, 18; **2c**: 165–166°, 23; **2d**: 200–202°, 27.

3.1.3. Synthesis of series **3**

Compounds **3a**, **c** were prepared as follows. A mixture of the appropriate 1-aryl-1-ethanone (0.01 mol), paraformaldehyde (0.01 mol), diethylamine hydrochloride (0.01 mol), HCl (37% w/v, 0.04 ml) and CH_3CN (100 ml) was heated under reflux for 5 h. Removal of the solvents in vacuo gave an oil, which was triturated with anhydrous $(C_2H_5)_2O$ and then CH_3COCH_3. Fractional crystallization of the resultant solids from CH_3COCH_3/C_2H_5OH led to recovery of unreacted diethylamine hydrochloride and **3a**, m.p. 143–145 °C in 60% yield and **3c**, m.p. 158–160 °C in 53% yield. Compound **3b**, m.p. 130–132 °C, was prepared in a yield of 73% in a similar manner as **3a**, **c**, except that the molar ratios of ketone, paraformaldehyde and amine hydrochloride were 0.03, 0.02 and 0.01, respectively; the time of heating under reflux was 24 h and the recrystallization solvent was CH_3CN.

3.2. Screening of the compounds

3.2.1. Evaluation using P388, L1210, Molt 4/C8, CEM and human tumour cells

Previously reported procedures were used to evaluate the compounds against P388 cells [15], L1210, Molt 4/C8 and CEM cell lines [16] as well as human tumour cell lines employed in generating the data portrayed in Table 2 [17].

3.2.2. Hollow fibre assay

A description of the hollow fibre assay has been published [13]. The tumours used were the SW-620, COLO 205 (colon), MDA-MB-231, MDA-MD-425 (breast), NCI-H23, NCI-H522 (non-small cell lung), LOX, UACC-62 (melanoma), OVCAR 3, OVCAR 5 (ovarian) and SF-295, U251 (central nervous system) cell lines. Doses of 100 and 150 mg/kg of **2c**, **d** were administered intraperitoneally once per day for 4 days. Net cell kill was noted when **2d** was evaluated against the SW-620 and H522 cell lines.

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