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Original article

Cytotoxic and anticonvulsant aryloxyaryl Mannich bases and related compounds

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Abstract

A series of 1-(4-aryloxyphenyl)-3-diethylamino-1-propanone hydrochlorides **3a–3e** and related compounds **3f, 3g** and **4a–4d** were synthesised. In addition, a group of 4-(4-aryloxyphenyl)-3-(4-aryloxyphenylcarbonyl)-1-ethyl-4-piperidinol hydrochlorides **6a–6e** were prepared which incorporated most of the structural features of **3a–3e**. All of these compounds displayed cytotoxic properties towards murine L1210 cells as well as human Molt 4/C8 and CEM T-lymphocytes. A number of these compounds possessed noteworthy potencies towards seven human colon cancer cell lines. Some correlations were noted between the IC_{50} values generated in the different screens and the σ, π and molar refractivity constants of the aryl substituents as well as with the volumes and solvent accessible surface areas of various basic groups. Molecular modelling of representative compounds revealed structural features, which may have contributed to the varying potencies noted. In general, the compounds in series **6** were well tolerated when administered to mice. Anticonvulsant properties were demonstrated by a number of compounds in the maximal electroshock (MES) screen when administered intraperitoneally to mice while **4c** and **6e** afforded protection in the MES test when given orally to rats.

Keywords: Mannich bases; Cytotoxicity; Molecular modelling; Structure–activity relationships; Murine toxicity; Anticonvulsant activity

1. Introduction

A major emphasis in our laboratories is on the design and syntheses of various Mannich bases as candidate cytotoxic and anticancer agents [1]. In particular, several 1-aryl-3-dialkylamino-1-propanone hydrohalides **1** displayed cytotoxic properties towards various murine and human tumour cell lines [2–4]. Investigations into the modes of action of representative compounds in series **1** disclosed the following results. Incubation of 1-(4-methoxyphenyl)-3-dimethylamino-1-propanone hydrobromide (**1**, $R^1 = OCH_3$, $R^2 = H$, $R^3 = CH_3$, X = Br) with 2-mercaptoethanol (simulating a cellular constituent containing mercapto and hydroxy groups) led to the formation of the corresponding thioether **2** [5]. Since Mannich bases often readily undergo deamination [6], extrusion of dimethylamine hydrobromide followed by thiolation likely accounts for the formation of **2** [7]. In addition, various members of series **1** inhibited respiration in isolated rat mitochondria [5]. These modes of action, namely thiol alkylation and inhibition of mitochondrial respiration, are different from the principal biochemical mechanisms displayed by currently available anticancer drugs whether the biological responses are beneficial effects or unwanted toxicity. Thus, Mannich bases such as **1** and related compounds have the potential to possess two advantages over contemporary anticancer medications. First, if the bioactivities of Mannich bases of aryl alkyl ketones are due to their being prodrugs of conjugated enones, interactions with nucleic acids should be absent. This prediction is based on the affinity of α,β-unsaturated ketones for thiols in contrast to hydroxy and amino groups, which are present in DNA and RNA [8,9]. Hence, the genotoxic side effects of traditional antineoplastic agents, such as the alkylating agents, should be absent. Sec-

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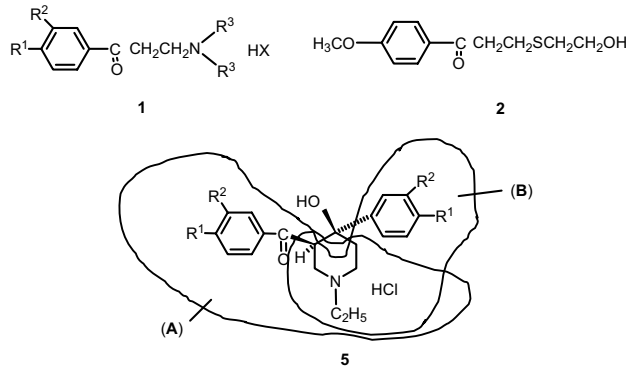

Fig. 1. Structures of the compounds in series **1, 2** and **5**.

ond, the differences in the mode of action of **1** and analogues from the anticancer drugs used today should enable cross-resistance to be absent. The observation that some melphalan-resistant tumour cells were not cross-resistant to various Mannich bases of conjugated styryl ketones [10] reinforces this conclusion.

The objective of the present investigation was to expand series **1** in the following ways. The aryl ring in series **1** may be designated the proximal ring. It is likely that this group either in **1**, or in the α,β-unsaturated ketones released from the Mannich bases, interacts at a binding site by van der Waals bonding. Such a putative area on the receptor may be referred to as the proximal aryl binding site. It is possible that an additional aryl binding site close to the proximal one exists. In order to evaluate this hypothesis, the decision was made to attach a second aryl moiety (subsequently referred to as the distal aryl ring) via a spacer group to the proximal aryl ring. Should the biodata of these compounds prove to be noteworthy, the relative positions of the proximal and distal aryl rings could be altered by changing the nature of the spacer group. These considerations led to the decision to launch an investigation involving the preparation of a number of prototypic molecules. Thus, the preparation and bioevaluation of series **3** was planned.

An additional line of investigation involved the syntheses of the compounds in series **4** for the following reasons. If antineoplastic activity was displayed by the compounds in series **3**, the result could be due principally to the intact molecules or to the α,β-unsaturated ketones released on deamination. In the first case, i.e. if cytotoxicity is due to the Mannich bases per se, then interaction of the basic group (or the related protonated species) with a binding site is likely. This interaction will be controlled to a significant extent by the sizes of the basic or protonated basic groups, which in turn would be predicted to affect potencies. The sizes of the diethylamino, dimethylamino, pyrrolidino, piperidino and morpholino groups (the corresponding protonated species are given in parentheses) which are found in **3b** and **4a–4d**, respectively, were determined by molecular modelling and found to be 87.5 (89.6), 55.5 (57.8), 78.0 (80.1), 86.2 (88.4) and 94.9 (96.5) $Å^3$, respectively. In addition, the solvent accessible surface area figures for these five substituents (protonated forms are in parentheses) also determined by modelling are 234.4 (247.9), 163.6 (184.9), 196.7 (218.5), 208.8 (229.9) and 220.0 (241.1), respectively. Thus investigations pertaining to possible correlations between the sizes of the terminal groups in **3b** and **4a–4d** with potencies was planned. On the other hand, if the antineoplastic activity would be principally due to the α,β-unsaturated ketone de-

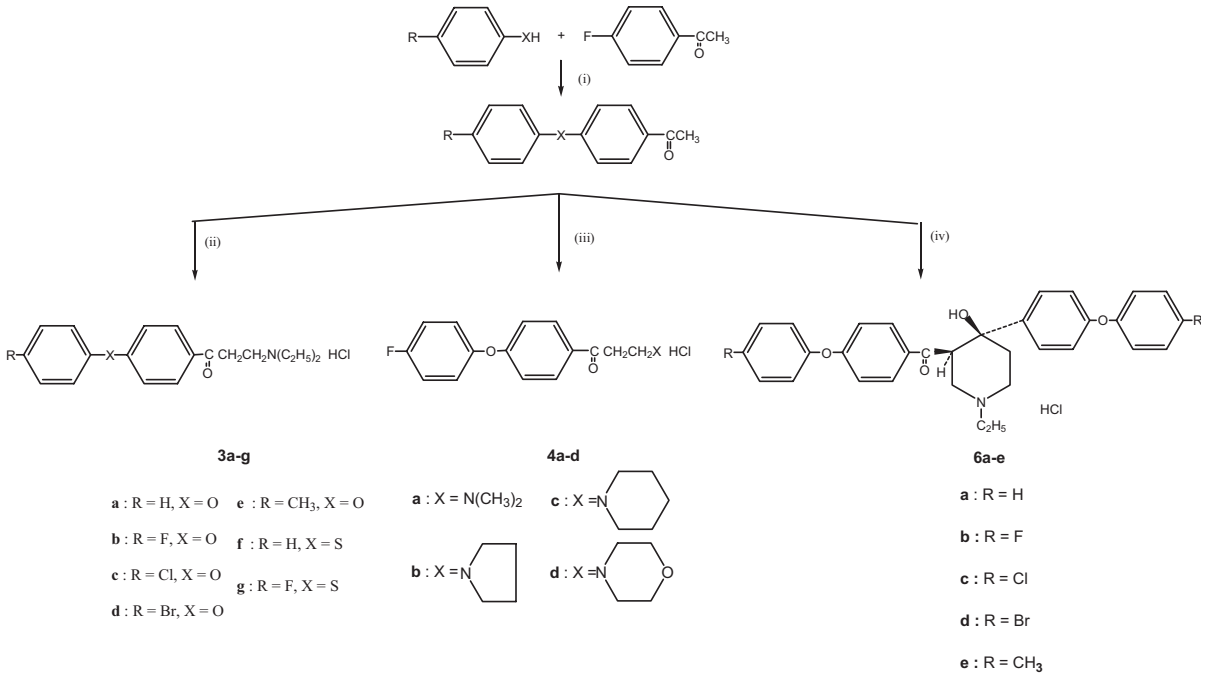

Fig. 2. The synthetic routes used in preparing the compounds in series **3, 4** and **6**. The reactants (i)–(iv) were as follows: (i) K_2CO_3; (ii) $HCHO/HN(C_2H_5)_2HCl$; (iii) HCHO/various amine hydrochlorides (**4a–4c**), 4-methylenemorpholinium chloride (**4d**) and (iv) $HCHO/C_2H_5NH_2HCl$.

rived from **3b** and **4a–4d**, then the rates of release of this enone may govern the potencies of these compounds. The pK_a values of diethylamine, dimethylamine, pyrrolidine, piperidine and morpholine are 10.84, 10.73, 11.31, 11.12 and 8.50 [11], respectively. Since the rates of deamination are inversely proportional to the pK_a figures of the basic group, the possibility of a correlation between the potencies of these compounds and the rates of release of an identical enone was planned to be investigated.

Several years ago, some 3-aroyl-4-aryl-1-ethyl-4-piperidinol hydrochlorides **5** were prepared as rigid analogues of **1** ($R^3 = C_2H_5$) [4]. These substituted piperidines **5** may be considered as possessing two portions of the molecules designated as (**A**) and (**B**) both of which incorporate most of the structural features of the acyclic analogues. In general, when the same substituents were present in the aryl ring, the compounds in both series exhibited similar IC_{50} values when evaluated towards murine L1210 cells and a panel of human tumour cell lines [4]. Thus in the present investigation, the preparation of **6a–6e** was proposed with a view to comparing the potencies of these compounds with **3a–3e**, respectively. Furthermore the structures of the piperidines **5** and **6** do not permit deamination to occur and hence any bioactivity observed is likely due to the Mannich bases per se.

Previous studies with certain acyclic Mannich bases revealed side effects of neurotoxicity (NT) and lethality [12,13]. In addition, anticonvulsant properties were demonstrated implying that penetration of the CNS occurred. Thus, an evaluation of the in vivo tolerability of the compounds proposed in this study was planned.

In summary, the principal objectives of the current investigation were to explore the potential of the series of compounds **3, 4** and **6** as candidate cytotoxic agents and also to determine the extent of murine toxicity displayed by these compounds. The data generated may provide guidelines as to which clusters of compounds should be developed further as candidate anticancer agents.

2. Chemistry

The aryloxyaryl or arylthioaryl methyl ketones required in the preparation of **3b–3g, 4a–4d** and **6b–6e** were synthesised from the appropriate phenol or thiophenol and 4-fluoroacetophenone. Various aryloxyaryl or arylthioaryl ketones, formaldehyde and different secondary amine hydrochlorides underwent the Mannich reaction leading to the formation of **3a–3g** and **4a–4c**. The synthesis of **4d** was achieved from the reaction between 1-(4-fluorophenyloxyphenyl)-1-ethanone and 4-methylenemorpholinium chloride. Condensation of ethylamine hydrochloride with excess of both the appropriate aryloxyaryl ketone and formaldehyde led to the cyclized products **6a–6e** whose structures were confirmed by high resolution ^{1}H NMR spectroscopy. Molecular modelling was undertaken with **3a** and **6a**, and various interatomic distances as well as a bond angle and a torsion angle were obtained. These data are presented in Table 3.

3. Bioevaluations

All of the compounds in series **3, 4** and **6** were evaluated against murine L1210 leukaemic cells as well as human Molt 4/C8 and CEM T-lymphocytes. These data along with the figures for **7** and **8** (vide infra) and a reference compound melphalan are presented in Table 1. The majority of the compounds, as well as **7** and **8** and the reference drugs 5-fluorouracil and melphalan, were screened towards seven human colon cancer cell lines. The results obtained are summarised in Table 2. Evaluation of **3a–3f, 4a–4d** and **6a–6e** for NT and lethality was undertaken. These compounds were also examined for anticonvulsant activity in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens.

4. Results and discussion

The initial evaluation of the Mannich bases **3, 4** and **6** utilised murine L1210 cells, which have been employed extensively in evaluating candidate antineoplastic agents. The choice to use Molt 4/C8 and CEM T-lymphocytes was made in order to investigate whether the compounds displayed cytotoxic properties towards two human cell lines. The data are presented in Table 1.

The first question addressed was whether the attachment of aryloxy or arylthio groups to the aryl ring of **1** ($R^1 = R^2 = H$, $R^3 = C_2H_5$, X = Cl referred to hereafter as **7**) led to an increase in cytotoxicity. The results in Table 1 reveal that **7** was more potent than **3a–3g** (L1210 test), **3a–3e, 3g** (Molt 4/C8 screen) and **3g** (CEM assay) while in the remaining cases, equal potencies were observed. Thus in the case of these three cell lines, the attachment of aryloxy or arylthio groups to **7** led to reduction in potencies in two-thirds of the comparisons made.

The IC_{50} values of **3a, 3b** and **3f, 3g** were examined with a view to ascertaining whether an oxygen or sulphur spacer atom between the proximal and distal aryl rings in series **3** was preferred. In four of the six comparisons, the analogue containing the oxygen spacer had greater cytotoxicity and in one case, equal potencies were observed suggesting that an oxygen atom between the aryl rings was preferred. This result may have been due to the variation in the relative positions of the two aryl rings, which were dependent on the nature of the spacer atom, since molecular modelling revealed that the aryl ring–oxygen (sulphur)–aryl ring bond angles in diphenylether and diphenylthioether were 48.2° and –55.9°, respectively.

A number of statistical evaluations were undertaken by constructing linear and semilogarithmic plots between vari-

Table 1
Evaluation of the compounds in series **3, 4, 6–8** against murine L1210 cells and human Molt 4/C8 and CEM T-lymphocytes

Compound	IC_{50} (μM) [a]		
	L1210	Molt 4/C8	CEM
3a	50.5 ± 4.4	43.8 ± 2.8	43.3 ± 3.0
3b	46.0 ± 2.1	43.6 ± 0.66	37.4 ± 2.2
3c	51.5 ± 2.3	39.0 ± 11.7	43.5 ± 0.8
3d	165 ± 108	104 ± 32	38.7 ± 1.7
3e	46.9 ± 3.8	43.8 ± 1.5	40.8 ± 1.3
3f	38.2 ± 2.5	133 ± 121	72.8 ± 52.6
3g	212 ± 37	182 ± 9	129 ± 35
4a	88.2 ± 81.1	97.5 ± 33.1	40.2 ± 2.5
4b	52.7 ± 12.5	36.0 ± 9.3	108 ± 50
4c	41.3 ± 1.5	43.8 ± 4.7	38.2 ± 4.2
4d	48.0 ± 3.9	62.3 ± 19.9	37.1 ± 1.1
6a	15.1 ± 1.8	16.3 ± 2.7	20.7 ± 1.3
6b	8.69 ± 1.2	13.2 ± 2.7	12.3 ± 6.6
6c	9.11 ± 0.61	8.58 ± 0.47	7.33 ± 0.35
6d	8.15 ± 0.27	8.94 ± 1.18	7.66 ± 0.59
6e	8.75 ± 0.18	22.3 ± 16.4	22.0 ± 7.8
7	20.3 ± 3.1	17.4 ± 4.1	38.5 ± 34.3
8	6.94 ± 3.08	12.5 ± 6.6	15.8 ± 1.8
Melphalan	2.13 ± 0.03	3.24 ± 0.79	2.47 ± 0.79

[a] The IC_{50} figures indicate the concentrations required to inhibit cell proliferation by 50%.

ous physicochemical parameters and cytotoxic potencies. Any relationships observed were classified as highly significant ($P < 0.01$ and $P < 0.001$), significant ($P < 0.05$) or trends towards significance ($P < 0.1$ and $P < 0.15$). Details, including Pearson's correlation coefficients (i.e. "*r*" values), are given in Section 6.

The potencies of **3a–3e** are likely influenced by the magnitude of the physicochemical properties of the aryl substituents. Plots were made between the IC_{50} values in each of the three screens and the σ, π and molar refractivity (MR) constants of the aryl substituents, which reflect the electronic, hydrophobic and steric properties, respectively, of the aryl substituents. However, no correlations were noted ($P > 0.15$).

The volumes and SASA figures of the protonated form of the basic groups of **3b, 4a–4d** were negatively correlated with the IC_{50} figures in the L1210 screen ($P < 0.05$). In addition, the volumes ($P < 0.05$) and SASA values ($P < 0.1$) of the protonated amines found in **3b, 4a–4d** were negatively correlated with the IC_{50} values in the L1210 assay. No correlations were noted when the two T-lymphocytes were evaluated. Thus, future molecular modifications should consider increasing the size of the basic group, such as using hexamethyleneimine in the Mannich reaction.

Table 2
The cytotoxicity of representative compounds in series **3, 4, 6–8** towards various human colon cancer cell lines

Compound	IC_{50} (μM) [a]							
	COLO 205	HCC-2998	HCT-116	HCT-15	HT29	KM12	SW-620	Average potency
3a	17.8	17.8	17.8	17.4	19.1	13.2	17.4	17.2
3b	19.1	20.0	16.6	18.2	20.0	12.3	18.2	17.8
3c	24.5	19.1	17.8	18.2	21.4	14.5	21.9	19.6
3e	23.4	19.1	17.4	18.6	19.1	11.0	18.6	18.2
3g	20.9	32.4	18.2	17.4	21.9	15.8	20.0	20.9
4b	22.9	44.7	60.3	41.7	32.4	30.2	79.4	44.5
4d	22.9	37.2	22.4	19.1	18.6	17.4	30.2	24.0
6a	13.5	6.61	9.33	12.3	6.92	4.90	12.0	9.34
6b	16.6	11.5	13.5	11.5	10.7	4.37	15.5	12.0
6c	13.2	12.6	3.80	7.41	5.13	2.57	12.9	8.23
6e	12.0	7.94	6.46	3.98	3.31	6.31	12.9	7.56
7 [b]	–	56.2	17.4	20.0	28.2	43.7	20.0	30.9
8	21.4	>100	17.0	19.1	20.4	41.7	15.8	>33.6
Melphalan	32.4	52.5	39.8	36.3	70.8	57.5	26.9	45.2
5-Fluorouracil	7.41	0.91	5.37	12.0	7.94	12.0	25.7	10.2

[a] The IC_{50} figures indicate the concentrations required to inhibit cell proliferation by 50%.
[b] Evaluated as the hydrobromide salt.

In order to determine whether the relative rates of deamination of **3b, 4a–4d** correlated with cytotoxic potencies, plots were constructed between the pK_a values of the liberated amines and the IC_{50} figures in the L1210, Molt 4/C8 and CEM assays. No correlations were observed ($P > 0.15$).

The data for **6a–6e** in Table 1 revealed that these compounds were, in general, the most potent Mannich bases towards L1210, Molt 4/C8 and CEM cells. The average IC_{50} values for **6a–6e** in these three screens were 9.96, 13.9 and 14.0 μM, respectively. These figures reflect potencies which are approximately one-fifth that of melphalan in these three assays. Comparisons were made between the IC_{50} values of each of the compounds **6a–6e** with the analogues in series **3** which had the same aryl substituent, e.g. the figures for **6a** were compared with the data for **3a, 6b** with **3b** and so forth. In each case, greater potencies were displayed by the compounds in series **6**. Furthermore, the average IC_{50} values of **3a–3e** in each of the L1210, Molt 4/C8 and CEM assays were 72.0, 54.8 and 40.7 μM, respectively. Hence the potency of **6a–6e** were seven, four and three times that of **3a–3e** in each of these three cytotoxicity screens. Thus from these initial data **6a–6e**, which contain some of the structural features of series **3a–3e**, represent an important structural modification of series **3**. In addition, plots were made between the IC_{50} values of **6a–6e** in each of these three screens and the σ, π and MR values of the aryl substituents. The results indicated that the σ constants were negatively correlated with the IC_{50} values in the Molt 4/C8 ($P < 0.001$) and CEM ($P < 0.05$) tests, while no other correlations ($P > 0.15$) were noted. Thus, the placement of strongly electron-withdrawing substituents into the distal aryl rings, such as the 4-trifluoromethyl and 4-nitro groups, which possess σ_p values of 0.54 and 0.78, respectively [14], should be considered when amplifying the project.

An examination was made of the effect on potencies by adding aryloxy substituents onto the phenyl groups of **5**, $R^1 = R^2 = H$ (referred to hereafter as **8**), which led to series **6**. The average IC_{50} values of **6a–6e** in the L1210, Molt 4/C8 and CEM screens were 9.96, 13.9 and 14.0 μM, respectively, which were virtually identical to the figures obtained for **8**. Thus, in the case of series **6**, the attachment of aryloxy groups to the proximal rings of **8** had negligible effects on potencies when these three cell lines were considered.

One of the interests of this laboratory is the discovery of compounds possessing antineoplastic activity towards colon cancers [3,4]. In the present investigation, over two-thirds of the compounds described in this report were evaluated against seven human colon cancer cell lines. These data are presented in Table 2. Comparisons were made between the IC_{50} values of different groups of compounds in order to glean some understanding of the structural features governing potencies. The results obtained using the colon cancer cells revealed some similarities and also various differences from the data summarised in Table 1.

No datum was available for the evaluation of **7** towards COLO 205 cells, while the average IC_{50} value of this compound towards the remaining six neoplasms was 30.9 μM. The average IC_{50} value of **3a–3c, 3e, 3g** toward the same cell lines was 18.4 μM. This observation revealed that the attachments of aryloxy or arylthio groups to the aromatic ring of **7** led to compounds possessing nearly double the potency of **7** towards colon cancer cell lines. The 4-fluorophenoxy Mannich base **3b** possessed marginally greater potencies than the corresponding sulphur analogue **3g**, which is in tandem with the results obtained in the L1210, Molt 4/C8 and CEM screens. The relative potencies of the acyclic Mannich bases containing diethylamino (**3b**), pyrrolidino (**4b**) and morpholino (**4d**) groups were **3b >4d >4b**. Thus the two most potent compounds, i.e. **3b** and **4d**, had larger volumes and solvent accessible surface areas as well as lower pK_a values than the pyrrolidino analogue **4b**. Plots were constructed between the IC_{50} values of **3a–3c, 3e** towards each of the colon cell lines as well as the average potencies and the σ, π and MR values of the aryl substituents. Positive correlations were noted between the IC_{50} figures generated towards COLO 205 cell lines and the π ($P < 0.01$) and MR ($P < 0.05$) values. In addition, positive trends towards significance were observed between the σ constants and the IC_{50} figures generated towards HT29 ($P < 0.1$) and KM12 ($P < 0.1$) cells as well as between the π values and SW20 cells ($P < 0.15$) and average potencies ($P < 0.1$). Thus, future modifications of series **3** should consider the insertion of small, hydrophilic groups into the aryl ring with a view to increasing cytotoxic potencies.

The average IC_{50} values of **3a–3c, 3e** and **6a–6c, 6e** were 18.2 and 9.28 μM, respectively, indicating that the piperidines **6** possessed double the potencies of the acyclic derivatives **3**. In addition, the average potency of **6a–6c, 6e** was more than three times the figure of **8**, revealing that the insertion of aryloxyaryl groups into the phenyl rings of **8** led to substantial increases in cytotoxic potencies. A negative correlation was noted between the σ values of the aryl substituents of **6a–6c, 6e** and the IC_{50} values towards KM12 cells when linear ($P < 0.01$) and semilogarithmic ($P < 0.05$) plots were made. Negative trends towards significance were observed with the π and MR values and HCT-116 cells ($P < 0.15$) and between the MR figures and HCT-15 cell lines ($P < 0.10$). Thus, development of series **6** should include the use of strongly electron-withdrawing aryl substituents, which are preferably hydrophobic and large in size.

The data in Table 2 revealed that the potencies of the compounds in series **3, 4** and **6** were greater than that of the established anticancer alkylating agent melphalan. The maximum activity was displayed by **6a–6c, 6e** which, on average, were nearly five times more potent than melphalan. Furthermore, comparisons were made with 5-fluorouracil which finds extensive use in treating colon cancers [15]. Each of the piperidines **6a, 6c, 6e** had lower average IC_{50} figures than 5-fluorouracil indicating that development of these compounds with specificity for colonic neoplasms is clearly warranted.

An investigation was undertaken with a view to determining the reasons for the greater potencies of the piperidines **6**

than of the acyclic analogues **3**. The approach taken was to compare the shapes of two representative compounds **3a** and **6a**. The assumptions were made that the observed bioactivities of the compounds in series **3** and **6** were due to interactions at the same binding site and also that the relative locations of two aryl rings and the nitrogen atom principally accounted for the disparity in potencies between the two series of compounds. In other words, interactions could occur at three areas of a putative binding site designated B1, B2 and B3 as indicated in Fig. 3(a).

The compounds in series **3** and **6** likely exist as a mixture of the free bases and protonated species. The Mannich bases **3a** and **6a** were modelled with quaternary nitrogen atoms although conceivably interaction at the binding site could be achieved by the free bases. In the case of **6a**, both aryloxyaryl groups may be orientated in such a way as to interact at the three areas of the proposed binding site; these arrangements are illustrated in Fig. 3(b,c) and are referred to as **6a-A** and **6a-B**, respectively.

The possibility exists that **6a** in one or both of its orientations has a better fit than **3a** at the different areas on the binding site. This hypothesis implies that certain crucial interatomic distances differed between **3a** and **6a**. In order to evaluate this possibility, carbon atoms were placed in the centres of the aryl rings A and B (**3a, 6a-A, 6a-B**) and C and D (**6a-A, 6a-B**) and designated C^A, C^B, C^C and C^D, respectively. In addition, axis 1 was constructed by extending the plane of the carbon atoms marked with asterisks in ring A (**3a, 6a-A**) and ring C (**6a-B**) as indicated in Fig. 3(b,c). The following distances were measured, namely d_1 [C^A–C^B (**3a, 6a-A**) or C^C–C^D (**6a-B**)], d_2 [C^A–N (**3a, 6a-A**) or C^C–N (**6a-B**), d_3 [C^B–N (**3a, 6a-A**) or C^D–N (**6a-B**)] and d_4 [the perpendicular distances from N to axis 1 (**3a, 6a-A, 6a-B**)]. Furthermore, as indicated in Fig. 3(c), the bond angle ψ_1, comprising the two aryl rings separated by an oxygen atom, as well as the torsion angle θ_1, created by the proximal rings and the adjacent oxygen atom, were measured. The results are presented in Table 3.

The interatomic distances d_1 were virtually identical in **3a, 6a-A** and **6a-B**. The increased flexibility of **3a** probably accounts for its d_2 distance being larger than is found in either orientations of **6a**. The d_3 distances of **3a** and **6a-B** are very similar, while the figure for **6a-A** is 6% larger than the span for **3a**. The major difference in the alignment of **3a** and **6a** at a binding site is the distance of the nitrogen atom from axis 1. Thus the d_4 figures for the orientations **6a-A** and **6a-B** are 20% and 12%, respectively, greater than the case of **3a** and may contribute significantly to the differences in potencies between the compounds in series **3** and **6**. The molecular modelling studies suggest that use of ketones containing one or more methylene groups between the carbonyl function and the proximal aryl ring in the Mannich reaction will lead to even greater d_4 distances. Such compounds may have increased potencies towards a variety of tumour cell lines.

The bond angles ψ_1 were virtually identical. However, the data in Table 3 indicate that the torsion angles θ_1 for **6a** were, on average, 20% greater than the corresponding measurement for **3a**. Thus in the future, the placement of substituents

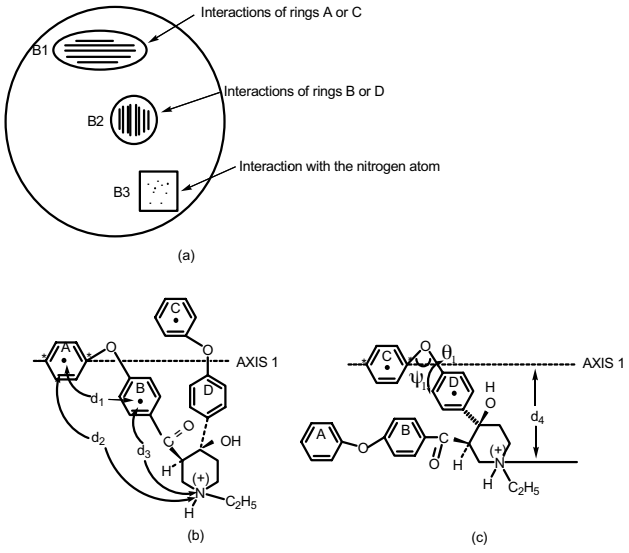

Fig. 3. (a) The putative areas of the binding site of **6a**. (b) The orientation of **6a** is so that rings A and B and the nitrogen atom align at B1, B2 and B3. (c) The orientation of **6a** is so that rings C and D align at B1, B2 and B3.

Table 3
Some interatomic distances d_1–d_4 (Å), bond angles ψ_1 (°) and torsion angles θ_1 (°) of **3a** and **6a** determined by molecular modelling

Structure	d_1	d_2	d_3	d_4	ψ_1	θ_1
3a	4.81	10.31	5.87	5.61	117.2	44.7
6a-A	4.80	9.89	6.20	6.72	117.1	–49.5
6a-B	4.78	10.03	5.75	6.26	116.3	57.4

of varying sizes in the *ortho* position of the aryl ring attached to the oxygen atom would be predicted to increase the θ_1 figures. It is conceivable that such molecular modifications of **6a** would lead to compounds displaying greater potencies than **6a**; in addition, correlations between the magnitude of the θ_1 values and potencies may emerge.

A final portion of this study involved the evaluation of whether NT and/or lethal properties were present in the compounds of series **3, 4** and **6** in order to guide future aspects of the project. In addition, their inclusion in the MES and scPTZ screens was planned since, if protection was observed, an indication of the ability of the compounds to penetrate the CNS would be demonstrated. Should this be the case, the potential of the compounds to treat brain tumours in vivo exists. On the other hand, CNS penetration may lead to unwanted toxicity. Accordingly, doses of 30, 100 and 300 mg/kg of **3a–3f, 4a–4d** and **6a–6e** were administered intraperitoneally to mice and the animals were observed at 0.5 and 4 h. The doses indicated in the following discussion reflect the quantity of compound required to elicit anticonvulsant activity in 50% or more of the animals.

After 0.5 h, NT was detected for **3e** and **4c** (30 mg/kg) and for the remaining compounds of series **3** and **4** when a dose of 100 mg/kg was used. After 0.5 h, deaths in some or all of the animals were caused by all of the compounds in series **3** and **4** at 100 mg/kg (**3a, 3b, 4b, 4c**) or 300 mg/kg (**3c–3f, 4a, 4d**). Neither NT nor mortalities were observed when doses up to and including 300 mg/kg of **6a–6e** were administered to mice. Anticonvulsant activity in the MES screen was observed by various compounds. After 0.5 h, protection was afforded by **3a–3f** and **4b, 4c** (30 mg/kg) and **4a, 4d** (100 mg/kg). When the mice were examined 4 h after administration of the compounds, **4b, 4c** (30 mg/kg), **3b–3d, 4d** (100 mg/kg) and **6a–6c, 6e** (300 mg/kg) demonstrated anticonvulsant activity. None of the compounds afforded protection in the scPTZ screen.

Three representative compounds, namely **3f, 4b** and **4c**, which gave complete protection in the MES screen at the minimum dose of 30 mg/kg 0.5 h after administration, were examined using doses of 3 and 10 mg/kg with a view to determine whether bioactivity would be demonstrated at lower doses. After 0.5 h, **3f** displayed neither anticonvulsant activity nor toxicity while **4b** and **4c** afforded protection in the MES screen unaccompanied by murine toxicity when 10 mg/kg of each compound was administered. A quantitative evaluation of **4c** revealed that the ED_{50} and TD_{50} figures (95% confidence intervals in parentheses) were 7.37 (6.64–8.13) and 26.5 (25.2–28.1) mg/kg, respectively, indicating a protection index (TD_{50}/ED_{50}) of 3.60. The ED_{50} and TD_{50} values of a reference drug phenytoin were 6.32 and 41.2 mg/kg, respectively [16], which generated a protection index of 6.52 or approximately twice that of **4c**. Compounds **4b, 4c** and **6e** were administered orally to rats using doses of 12.5 mg/kg (**4b**) and 30 mg/kg (**4c, 6e**). The animals were observed after 0.25, 0.5, 1, 2 and 4 h in the MES screen and for NT. Protection was displayed by **4c** (0.25, 1 and 2 h) and **6e** (4 h). No toxicity was noted by visual observation.

Some of the conclusions that may be drawn from the toxicity and anticonvulsant screens were as follows. First, the data afford noteworthy evidence that mice tolerated single doses up to and including 300 mg/kg of the compounds of series **6**, in marked contrast to members of series **3** and **4** which demonstrated NT and lethality. Second, anticonvulsant activity in the MES screen of the latter compounds was observed, and bearing in mind the oral activity of **4c**, some future development may be worthwhile with **3** and **4** as candidate anticonvulsants.

5. Conclusions

This study reports the synthesis and some bioevaluations of three series of novel compounds **3, 4** and **6**. Cytotoxic evaluation using murine L1210 lymphocytic leukaemia cells, human Molt 4/C8 and CEM T-lymphocytes and human colon cancer cell lines revealed that all three series of compounds possessed cytotoxic properties. However, the piperidines **6** were more potent than **3** and **4** and are clearly useful lead molecules. This conclusion was enhanced further by the excellent tolerance of the compounds in series **6** when administered to mice. Thus, while **3** and **4** displayed moderate cytotoxic properties and possess interesting anticonvulsant activity, it is series **6**, which should be considered as a template for future development of candidate antineoplastic agents. Correlations were established between some of the tumour cell lines and one or more physicochemical constants of the aryl ring as well as the volumes and SASA figures of the basic groups. These observations, along with the data provided by molecular modelling, provided guidelines for future molecular modifications of these novel compounds.

6. Experimental protocols

6.1. Chemistry

Melting points (m.p.) are uncorrected. Temperatures were recorded in Celsius degrees. Elemental analyses (carbon, hydrogen and nitrogen) were undertaken by Mr. K. Thoms, Department of Chemistry, University of Saskatchewan, on **3a–3g, 4a–4d** and **6a–6e** and were within 0.4% of the calculated values. Compounds **3a, 3b** and **6b** were obtained as the monohydrates and **3e** as the hemihydrate. The yields were based on the lowest molar fractions of the reactants. 1-(4-Phenyloxyphenyl)-1-ethanone was obtained from the Aldrich Chemical Company, Milwaukee, USA. TLC using silica gel plastic-backed sheets with a fluorescent indicator revealed that the compounds were homogenous. ^{1}H NMR spectra were determined using Varian T-60 and Bruker AM 300 FT instruments. Spectra obtained on some of the intermediates and on all of the compounds submitted for bioevaluation confirmed the identity of the products. The protons of the proximal and distal aryl rings are designated as

unprimed and primed, respectively. The recrystallisation solvents were designated by the letters A–I and were as follows, namely A, acetone–ethanol; B, diethyl ether–ethanol; C, *n*-hexane–acetone; D, acetonitrile; E, diethyl ether–methanol; F, methanol; G, ethanol; H, acetone–methanol and I, diethyl ether–acetone.

*6.1.1. Synthesis of series **3** and **4***

The 1-(4-aryloxyaryl)-1-ethanones and 1-(4-arylthioaryl)-1-ethanones required for the syntheses of **3b–3g, 4a–4d** and **6b–6e** were prepared as follows. A mixture of the appropriate phenol or thiophenol (0.11 mol), 4-fluoroacetophenone (0.10 mmol), anhydrous potassium carbonate (0.15 mol) and dimethylacetamide (75 ml) was heated under reflux for approximately 12 h at 160 °C under nitrogen. On cooling, water (100 ml) was added and the mixture was extracted with chloroform (2 × 100 ml) and the combined organic extracts were washed with aqueous sodium hydroxide solution (4% w/v) and water. The solution was dried using anhydrous magnesium sulphate and evaporation of the solvent led to the isolation of an oil which was used without further purification. The ^{1}H NMR spectrum (60 MHz) of a representative intermediate, namely 1-(4-fluorophenyloxyphenyl)-1-ethanone was as follows: δ ($CDCl_3$) 7.8–8.0 (m, 2H, *ortho* H), 6.8–7.2 (m, 2H, *meta* H and 4′H, *ortho* and *meta* H), 2.5 (s, 3H, CH_3).

The preparation of a representative Mannich base **3a** was as follows. A mixture of 1-(4-phenyloxyphenyl)-1-ethanone (0.015 mol), paraformaldehyde (0.035 mol), diethylamine hydrochloride (0.01 mol), hydrochloric acid (36.5% w/v, 0.04 ml) and acetonitrile (50 ml) was heated under reflux for 24 h. Removal of the solvent gave an oil which was triturated several times with diethyl ether. The residue obtained was recrystallised from acetone to give **3a**, m.p. 98–99 °C in 75% yield. A similar route was utilised to give the following compounds (molar ratio of ketone, paraformaldehyde and amine, time of heating under reflux, recrystallisation solvent, m.p. and % yield in parentheses), namely **3b** (0.03, 0.02, 0.01, 46 h, A, 131–133 °C, 64), **3c** (0.015, 0.03, 0.01, 14 h, B, 125–127 °C, 37), **3d** (0.015, 0.03, 0.01, 14 h, B, 126–128 °C, 50), **3e** (0.03, 0.03, 0.01, 24 h, C, 134–136 °C, 78), **3f** (0.03, 0.05, 0.01, 48 h, C, 94–96 °C (Ref. [17] m.p. 90–92 °C), 72), **3g** (0.03, 0.05, 0.01, 48 h, C, 136–138 °C, 76), **4a** (0.02, 0.02, 0.01, 24 h, D, 162–164 °C, 34), **4b** (0.025, 0.025, 0.01, 24 h, E, 165–167 °C, 34) and **4c** (0.01, 0.02, 0.01, 6 h, D, 174–175 °C, 49).

The preparation of **4d** was undertaken as follows. A mixture of 4-methylenemorpholinium chloride (0.01 mol), which was prepared by a literature procedure [18], 1-(4-flurorophenyloxyphenyl)-1-ethanone (0.01 mol) and acetonitrile (~50 ml) was heated under reflux for 2 h. On cooling, the precipitate was collected, washed with cold acetonitrile and dry diethyl ether. Recrystallisation from acetone gave **4d**, m.p. 184–185 °C in 70% yield.

The ^{1}H NMR spectrum (60 MHz) of a representative compound **4c** was as follows: δ ($CDCl_3$): 7.9–8.1 (m, 2H, *ortho* H), 6.8–7.2 (m, 2H, *meta* H and 4′H *ortho* and *meta* H), 2.7–3.9 (m, 8H, $COCH_2CH_2$; 2-CH_2, 6-CH_2 of piperidine ring), 1.5–2.5 (br m, 4H, 3-CH_2, 5-CH_2 of piperidine ring), 1.2–1.4 (m, 2H, 4-CH_2 of piperidine ring).

*6.1.2. Synthesis of series **6***

A mixture of the appropriate 1-(4-aryloxyphenyl)-1-ethanone (0.04 mol), paraformaldehyde (0.08 mol), ethylamine hydrochloride (0.01 mol), hydrochloric acid (37% w/v, 0.04 ml) and isopropanol (100 ml) was heated under reflux for varying periods of time (vide infra). Evaporation in vacuo gave a residue which was triturated with dry diethyl ether and upon recrystallisation led to the following compounds (time of heating under reflux, recrystallisation solvent, m.p. and % yield in parentheses), namely **6a** (48 h, F, 180–182 °C, 38), **6b** (48 h, G, 172–174 °C, 25), **6c** (72 h, H, 179–181 °C, 18), **6d** (72 h, F, 191–193 °C, 22) and **6e** (29 h, I, 175–177 °C, 20). The ^{1}H NMR spectrum (300 MHz) of a representative compound **6a** is given below. In this case, the hydrogen atoms of the aroyl proximal and distal aryl rings are primed and unprimed, respectively, while the proximal and distal aryl hydrogen atoms of the group at position 4 of the piperidine ring are double and triple primed, respectively. The subscripts a and e refer to hydrogen atoms in the axial and equatorial conformations, respectively. δ ($CDCl_3$): 8.02–8.10 (d, 2H, *ortho* H), 7.30–7.45 (m, 2H, *meta* H; 2′H, *meta* H), 7.15–7.30 (m, 3‴H, *ortho* and *para* H), 6.95–7.05 (m, 3′H, *ortho* and *para* H), 6.75–6.85 (d, 2″H, *ortho* H), 6.90–6.95 (m, 2‴H, *meta* H; 2″H, *meta* H), 5.55 (dd, 1H, $C3H_a$, J $3_a/2_a$ = 11.8 Hz, J $3_a/2_a$ = 3.7 Hz), 5.10 (d, 1H, OH, J = 2.54 Hz), 3.40–3.48 (m, 2H, $C2H_e$, $C6H_e$), 3.25–3.35 (m, 2H, $C2H_a$, $C6H_a$), 3.00–3.20 (m, 2H, N CH_2CH_3), 2.74–2.90 (m, 1H, $C5H_a$), 1.92 (br d, 1H, $C5H_e$, J = 14.7 Hz), 1.50 (t, 3H, NCH_2CH_3, J = 7.3 Hz).

*6.1.3. Syntheses of **7** and **8***

The synthesis of **7** and the corresponding hydrobromide salt as well as **8** has been described previously [4].

6.1.4. Statistical analyses

The SASA values and volumes of the diethylamino, dimethylamino, pyrrolidino, piperidino and morpholino groups (along with the related protonated species) were obtained from MacroModel 4.5 [19]. The σ, π and MR values were taken from Ref. [14]. Linear and semilogarithmic plots were made using a commercial software package [20].

For each group of compounds where correlations and trends towards significance were noted, the physicochemical constant, assay, linear (l) or semilogarithmic (sl) plots, Pearson's correlation coefficient and *P*-value are indicated as follows: **3b, 4a–4d**: volume of basic group (protonated), L1210, l, –0.935, 0.020; volume of basic group (protonated), L1210, sl, –0.925, 0.024; volume of basic group (free base), L1210, l, –0.931, 0.022; volume of basic group (free base), L1210, sl, –0.921, 0.026; SASA (protonated), L1210, l, –0.910, 0.032; SASA (protonated), L1210, sl, –0.900, 0.037;

SASA (free base), L1210, l, –0.875, 0.052; SASA (free base), L1210, sl, –0.867, 0.057; **6a–6e**: σ, Molt 4/C8, l, –0.995, 0.0001; σ, Molt 4/C8, sl, –0.996, 0.001; σ, CEM, l, –0.942, 0.017; σ, CEM, sl, –0.946, 0.015; **3a–3c, 3e**: σ, HT29, l, 0.905, 0.095; σ, HT29, sl, 0.906, 0.094; σ, KM12, l, 0.921, 0.079; σ, KM12, sl, 0.920, 0.080; π, COLO 205, l, 0.999, 0.001; π, COLO 205, sl, 0.998, 0.002; π, SW-620, sl, 0.842, 0.146; π, average potency of human colon cancer cell lines (AP), l, 0.910, 0.090; π, AP, sl, 0.915, 0.085; MR, COLO 205, l, 0.980, 0.020; MR, COLO 205, sl, 0.977, 0.023; **6a–6c, 6e**: σ, KM12, l, –0.997, 0.003; σ, KM12, sl, –0.977, 0.023; π, HCT-116, sl, –0.871, 0.129; MR, HCT-116, l, –0.893, 0.107; MR, HCT-116, sl, –0.898, 0.102; MR, HCT-15, l, –0.905, 0.095.

6.1.5. Molecular modelling

Models of compounds were built using MacroModel 8.0 [21] followed by a Monte Carlo search for the lowest energy conformations using an Amber force field of 1000 initial conformations. Measurements were made on the lowest energy conformations.

6.2. Bioevaluations

6.2.1. Cytotoxicity determinations

Literature methodologies were followed when evaluating various compounds against L1210, Molt 4/C8 and CEM cells [22] as well as human colon cancer cell lines [23].

6.2.2. Evaluation of various compounds for toxicity and anticonvulsant properties

The screening of **3a–3f, 4a–4d** and **6a–6e** for murine toxicity and anticonvulsant properties was undertaken by a literature procedure [24]. Initially, doses of 30, 100 and 300 mg/kg of each compound were injected into mice using the intraperitoneal route. The animals were observed after 0.5 and 4 h. NT was determined by the rotorod procedure [25] and visual observations. Anticonvulsant properties were assessed using the MES and scPTZ screens.

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