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3,5-Bis(Phenylmethylene)-1-(N-arylmaleamoyl)-4-piperidones: A Novel Group of Cytotoxic Agents

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A series of novel 3,5-bis(phenylmethylene)-1-(N-arylmaleamoyl)-4-piperidones 3 have been synthesized which displayed potent cytotoxicity towards human Molt 4/C8 and CEM T-lymphocytes as well as murine P388 and L1210 leukemic cells. In contrast, the related N-arylmaleamic acids 4 possessed little or no cytotoxicity in these four screens. Molecular modeling revealed certain interplanar and bond angles and interatomic distances which were perceived to contribute to the observed bioactivity as well as providing suggestions for future structural modifications of the piperidones 3. Evaluation of representative compounds in series 3 and 4 on the activity of human N-myristoyltransferase revealed that, at the maximum concentration utilized, namely 250 μM, only weak inhibiting properties were displayed by some of the compounds in series 4. Various members of series 3 and 4 were well tolerated in mice.

Keywords: 4-Piperidones; Maleamic acids; Cytotoxicity; Molecular modeling; Human N-myristoyltransferase

INTRODUCTION

A number of years ago, the design, syntheses and cytotoxicity of 3,5-bis(phenylmethylene)-4-piperidone hydrochloride **1** and related compounds were described.[1] These molecules were prepared as thiol alkylators based on the preferential electrophilicity of α,β-unsaturated ketones for thiols in contrast to amino groups, which are present in nucleic acids.[2,3] Thiol groups are absent in nucleic acids and thus antineoplastic conjugated enones may be devoid of the genotoxic effects displayed by certain anticancer drugs.[4] Administration of **1** to mice led to a lowering of hepatic thiol concentrations,[1] confirming that interaction with cellular thiols occur. Recently the potencies of **1** (as the free base) towards human Molt 4/C8 and CEM T-lymphocytes as well as murine P388 and L1210 cells have been described.[5] These data are presented in Table I. In addition, conversion of **1** into the corresponding N-acryloyl amide **2** was undertaken in which an additional site for electrophilic attack with cellular thiols was created.[5] The data in Table I reveal that **2** displayed greater potency than **1** in the P388 screen while the compounds were equipotent towards the other three cell lines.

The objectives of the present investigation were as follows. First, replacement of one of the hydrogen atoms of the terminal methylene groups of **2** by an arylcarbamoyl function, leading to the formation of the compounds in **3**, was proposed for the following reasons. The Taft σ* value of the phenylcarbamoyl group is 1.56.[6] Hence the electrophilicity of the N-acyl group of **3a** will be greater than is found in **2** since the σ* figure of the β proton of the acryloyl group in **2** is 0.49.[7] Thus, in general, a greater avidity for cellular nucleophiles is predicted with series **3** compared to **2**. Furthermore, the placement of different substituents in the aryl ring adjacent to the carbamoyl group would be predicted to alter the polarity of the olefinic double bond in the N-acyl group. Hence a correlation between the cytotoxicity

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TABLE I Evaluation of **1**–**4** against human Molt 4/C8 and CEM T-lymphocytes and murine P388 and L1210 leukemic cells

	IC_{50} (μM)			IC_{50} (μM)		
Compound	Molt 4/C8	CEM	SR[a]	P388	L1210	SR[a]
1[b]	1.67 ± 0.15	1.70 ± 0.02	1.02	0.77 ± 0.02	7.96 ± 0.11	10.3
2[b]	1.42 ± 0.27	1.48 ± 0.34	1.04	0.50 ± 0.06	8.69 ± 0.73	17.4
3a	0.95 ± 0.90	0.74 ± 0.15	1.28	0.314 ± 0.03	2.07 ± 0.27	6.59
3b	0.56 ± 0.0077	1.32 ± 0.25	2.36	0.265 ± 0.01	2.34 ± 0.01	8.83
3c	1.06 ± 0.74	1.39 ± 0.07	1.31	0.226 ± 0.01	1.89 ± 0.06	8.36
3d	0.990 ± 0.438	0.868 ± 0.229	1.14	0.294 ± 0.01	2.46 ± 0.56	8.37
3e	1.35 ± 0.24	1.46 ± 0.09	1.08	0.267 ± 0.02	2.58 ± 0.02	9.66
3f	1.72 ± 0.32	1.58 ± 0.10	1.09	0.277 ± 0.01	3.03 ± 0.24	10.9
3g	0.402 ± 0.201	0.678 ± 0.144	1.69	0.383 ± 0.03	4.96 ± 1.17	13.0
3h	0.830 ± 0.424	0.792 ± 0.039	1.05	0.473 ± 0.02	2.60 ± 0.65	5.50
4a	>500	>500	–	>50 ± 2.7	>500	–
4b	240 ± 24	290 ± 16	1.21	>50 ± 1.4	>500	–
4c	42.9 ± 1.3	56.2 ± 3.5	1.31	>50 ± 4.3	140 ± 19	–
4d	224 ± 31	204 ± 12	1.09	360 ± 10	>500	–
4e	>500	>500	–	>50 ± 2.7	>500	–
4f	245 ± 44	289 ± 4	1.18	163 ± 9.3	290 ± 40	1.78
4g	>500	>500	–	>50 ± 1.2	>500	–
4h	>500	>500	–	>50 ± 1.2	>500	–
4i	>500	>500	–	>50 ± 1.2	>500	–
Melphalan	3.24 ± 0.79	2.47 ± 0.03	1.31	0.22 ± 0.01	2.13 ± 0.03	9.68

[a] SR indicates the selectivity ratio, i.e., the ratio between the highest and lowest IC_{50} values of either the T-lymphocytes or murine leukemic cells.
[b] Evaluated as the free base. Reprinted in part from *J. Med. Chem.*, (2001) **44**, 586. Copyright 2001 American Chemical Society.

of the compounds in series **3** and the Hammett σ and/or Taft σ* values may emerge. The establishment of such a relationship would enable the design of further analogues on a rational basis.

Second, the contribution of the N-arylmaleamoyl group (AR-NHCOCH=CHCO) to the cytotoxicity of the compounds in series **3** was considered by the proposal to synthesize the maleamic acids **4** for bioevaluation. The decision was made to evaluate the compounds in series **3** and **4** in the Molt 4/C8, CEM, P388 and L1210 screens. The data could lead not only to the development of structure–activity relationships but may provide an insight into the nature of the binding sites of those compounds displaying marked cytotoxicity.

Third, a molecular target of interest in these laboratories is human N-myristoyltransferase (hNMT) which plays an important role in oncogenesis.[8] Some of the reasons for selecting this enzyme as a molecular target were summarized recently,[8] including the fact that a number of tumours have increased quantities of hNMT compared to the corresponding normal cells. In these cases, the cancer cells have a greater requirement for hNMT and thus inhibition of this enzyme may have a preferentially cytotoxic effect against tumours relative to normal tissues. Two observations are of relevance in regard to the present investigation. First, the active site of hNMT is believed to include the mercapto group of the 192-cysteine amino acid[9] and hence thiol alkylators should inhibit this enzyme. Second, the compounds in series **1**–**3** may be regarded as cyclic Mannich bases and recently several Mannich bases of α,β-unsaturated ketones, which inhibited hNMT in the presence of cAMP-dependent protein kinase, have been described.[10] Thus, the evaluation of representative compounds as inhibitors of hNMT was planned.

Finally, an estimate of the toxicity, and in particular neurotoxicity, in mice was considered in order to guide amplification of the project.

MATERIALS AND METHODS

Synthesis of Compounds

The syntheses of **1**,[1] the free base of **1**[5] and **2**[11] have been reported previously. The preparation of the compounds in series **3** and **4** has been described previously.[12]

Statistical Analyses

The σ, π and molecular refractivity (MR) values of the aryl substituents in series **3** and **4** were obtained from the literature.[13] In the case of the monosubstituted compounds, the MR figure for the hydrogen atom, namely 1.03, was added to the MR value of the aryl substituent. The σ* figure was taken from an appropriate reference.[14] The linear and semilogarithmic plots were obtained using a commercial software package.[15] The following correlations and trends towards significance were noted with the selectivity ratios (SR, see Table I) [linear (l) or semilogarithmic (sl) plot, physicochemical constant, assay, Pearson's r value, p value] namely **3**: [l, π, SR (murine), 0.644, 0.085]; **3** [sl, π, SR (murine), 0.653, 0.079],

4: [l, π, SR (human), 0.888, 0.112] and **4** [sl, π, SR (human), 0.897, 0.103].

Molecular Modeling

The charge densities on **3a**–**h** were calculated using CS Chem 3D Pro®.[16] The charges on certain atoms indicated in Figure 2 were as follows: 14: 0.037 ± 0.003; 5: −0.013 ± 0.002; 3: 0.006 ± 0.001; 7: 0.008 ± 0.002; 22: −0.047 ± 0.006 and 23: −0.057 ± 0.007. The torsion and bond angles, as well as the interatomic distances, were determined using the Chem 3D Ultra programme.[17] The atoms used in determining the torsion angles in series **1**–**3** are indicated in parentheses, namely θ_1 (C5–C14–C15–C20), θ_2 (C3–C7–C8–C13), θ_3 (O2–C21–C22–C23), θ_4 (C22–C23–C24–O3) and θ_5 (C24–N25–C26–C31). In the case of series **4**, the atoms used for determining the torsion angles were θ_3 (O2–C1–C2–C3), θ_4 (C2–C3–C4–O3) and θ_5 (C4–N5–C6–C11).

Cytotoxicity Evaluations

The compounds were evaluated in the Molt 4/C8, CEM and L1210 screens using a reported procedure.[18] In brief, various concentrations of compounds in suitable solvents were incubated at 37°C with Molt 4/C8, CEM and L1210 cells and the percentage survival was noted after 48 h. Control experiments in which the compounds were omitted were also undertaken. All tests and controls were carried out in triplicate at each concentration of the compound. The P388 assay was undertaken using a method which has been described previously[19] and was performed in a similar manner as the Molt 4/C8, CEM and L1210 assays.

N-myristoyltransferase Assays

Evaluation of **1**, **3a**, **c**–**e**, **g** and **4a**, **c**–**e**, **g** for their effect on hNMT was undertaken by a literature procedure.[10] In brief, *Escherichia coli* DH5α with recombinant pT-7.hNMT was grown in LB medium at 37°C to stationary phase to yield hNMT, which was purified by a previously described methodology[20]and the assays were carried out in the presence of cAMP-dependent protein kinase derived peptide.[21,22] This Gly–Asn–Ala–Ala–Ala–Ala–Lys–Lys–Arg–Arg peptide is based on the amino-terminal sequence of the type II catalytic subunit of cAMP-dependent protein kinase which was obtained from Research Genetics, Huntsville, U.S.A. The IC_{50} figures (in μM) obtained were as follows: **4a**: 202.5 ± 8.10; **4d**: 245.5 ± 6.36; **4e**: 52.5 ± 7.77 and **4g**: 433.5 ± 21.67. The concentration of **4c** required to cause 50% stimulation of hNMT was 241.6 ± 27.18 μM.

Antifungal Evaluation

Compounds **1**, **3a** and **4a**,**e**,**f** were evaluated against three isolates of *Aspergillus fumigatus* (ATCC 208995–208997) and one isolate of *Candida albicans* (ATCC 90028) using the broth microdilution assay.[23] The MIC value of voriconazole was 0.25 μg/ml against all four isolates.

Toxicity and Anticonvulsant Screens

The enones **1**, **3b**,**d**–**h** and **4a**–**i** were examined for murine toxicity using a reported procedure.[24] In brief, the compounds were administered to mice by the intraperitoneal route using doses of 30, 100 and 300 mg/kg. The animals were observed after 0.5 and 4 h. Neurotoxicity, which was determined by the rotorod method,[25] was observed after administration of the following compounds (number of animals displaying neurotoxicity/number of animals tested, dose in mg/kg, time in hours in parentheses), namely **3b** (2/8, 100, 0.5; 1/2, 30, 4; 1/4, 100, 4), **3d** (1/4, 300, 0.5), **3h** (1/8, 100, 0.5) and **4b** (1/4, 300, 0.5). The Anticonvulsant Screening Program of the National Institute of Neurological Disorders and Stroke, USA requires that all mice be housed, fed and handled in ways that are consistent with the recommendations of the National Research Council Publication "Guide for the Care and Use of Laboratory Animals". All mice were euthanized in accordance with the policies of the Institute of Laboratory Resources dealing with the humane care of laboratory animals.

RESULTS

The preparation of the compounds in series **3** and **4** has been described recently[12] using the methods outlined in Figure 1. Molecular modeling was employed in order to compute the charge densities on the atoms of the three olefinic groups of **3a**–**h** as well as for determining various torsion and bond angles and interatomic distances in the compounds in series **1**–**4**. The evaluation of **1**–**4** against Molt 4/C8, CEM, P388 and L1210 cells is presented in Table I. The piperidone **1** and **3a**,**c**–**e**,**g** had no effect on hNMT, using concentrations up to and including 250 μM. On the other hand, the IC_{50} figures for the inhibition of hNMT by **4a**,**d**,**e**,**g** were 203, 246, 53 and 434 μM, respectively, while **4c** caused stimulation of the activity of this enzyme. Compounds **1**, **3a** and **4a**,**e**,**f** possessed minimum inhibitory concentration (MIC) figures in excess of 25 μg/mL when evaluated against three strains of *Aspergillus fumigatus* and one strain of *Candida albicans*. Doses of 30, 100 and 300 mg/kg of **3b**,**d**–**h** and **4a**–**i** were administered by the intraperitoneal route to mice.

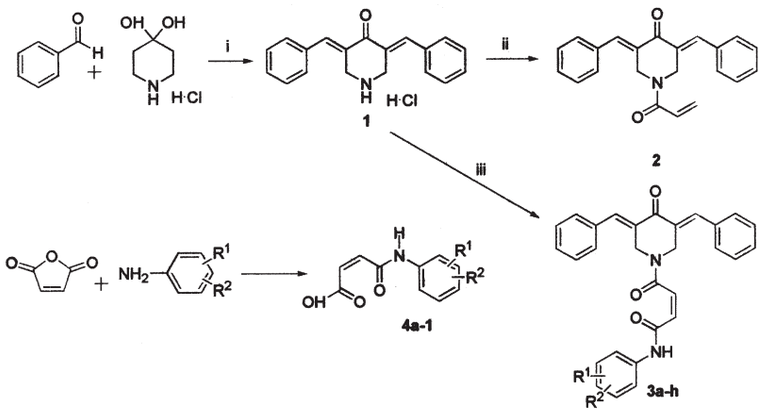

FIGURE 1 Synthetic routes for the preparation of series **1**–**4**; i = HCl, ii = K_2CO_3/ClCOCH=CH_2, iii = $N(C_2H_5)_3$/$ClCOOC_2H_5$/**4a**–**h**. The aryl substituents R^1 and R^2 were as follows: **a**: $R^1 = R^2 = H$; **b**: R^1 = 4-Cl, R^2 = H; **c**: R^1 = 3-Cl, R^2 = 4-Cl; **d**: R^1=4-NO_2, R^2 = H; **e**: R^1 = 4-CH_3, R^2 = H; **f**: R^1 = 3-CH_3, R^2 = 4-CH_3; **g**: R^1 = 2-CH_3, R^2 = 6-CH_3; **h**: R^1 = 4-OCH_3, R^2 = H; **i**: R^1 = 4-$COCH_3$, R^2 = H.

After 0.5 and 4 hours, no lethal effects for any of the compounds were observed, while neurotoxicity was noted in **3b**,**d**,**h** and **4b**.

DISCUSSION

In order to evaluate the cytotoxicity of the maleamoyl derivaties **3** and **4**, the compounds were examined using two human cell lines, namely Molt 4/C8 and CEM T-lymphocytes as well as murine P388 and L1210 cells. The data are presented in Table I. Previous studies revealed that P388 cells are often more sensitive to conjugated enones than are L1210, Molt 4/C8 and CEM cells.[5,26] Hence the maximum concentration of compounds used in the P388 screen was 50 μM in general, whereas a figure of 500 μM was employed in the three other tests.

Initially comments will be made regarding the cytotoxicity of the compounds in series **3**. First, the very high potencies of **3a**–**h** indicate that the 3,5-bis(phenylmethylene)-1-(N-arylmaleamoyl)-4-piperidones are a novel class of potent cytotoxics. Of the 32 IC_{50} figures generated for **3a**–**h** in the four screens, the values are less than 1 μM, and between 1 and 2 μM, in 17 and 8 cases, respectively, while the remaining evaluations resulted in IC_{50} figures of less than 5 μM. Second, the potencies of **3a**–**h** were compared to the figures generated for the established anticancer agent melphalan. All of these compounds were significantly more potent than melphalan towards both human T-lymphocyte cell lines. Of particular note were **3b** and **3g**, which have approximately 6 and 8 times, respectively, the potency of melphalan in the Molt 4/C8 screen. In addition, **3a** and **3g** were a little more than three times more potent than the reference drug in the CEM test. In the case of the murine cell lines, **3c** possessed greater antileukemic activity than melphalan towards L1210 cells, while **3c** (P388 test) and **3a**,**d**,**h** (L1210 screen) were equipotent with this drug. The remaining compounds displayed marginally inferior potencies to melphalan. Thus, in consideration of the IC_{50} values of the compounds in series **3** towards both T-lymphocytes and murine cell lines, **3a**–**d**,**g**,**h**, in particular, are clearly useful lead molecules. In addition, comparisons between the potencies of **3a**–**h** with both **1** and **2** were made (the specific screen under consideration is given in parentheses). The data in Table I reveal that **3b**,**d**,**g**,**h** (Molt 4/C8), **3a**–**e**,**g**,**h** (CEM), **3a**–**h** (P388) and **3a**–**h** (L1210) possess greater potencies than **1**, i.e., in 84% of the comparisons made. When the IC_{50} figures of **3a**–**h** and **2** were compared in all four screens, **3b**,**g** (Molt 4/C8), **3a**,**d**,**g**,**h** (CEM), **3a**–**g** (P388) and **3a**–**h** (L1210) displayed the greater potencies, i.e., in 66% of the comparisons made. In the remaining cases, the compounds in **3** were equipotent with either **1** or **2**. Thus, in 75% of the cases the attachment of arylmaleamoyl and arylcarbamoyl groups to **1** and **2**, respectively, to give rise to series **3** led to potency increases. In addition, **3a**–**h** are structurally divergent from currently employed anticancer agents. Hence they may be of value in inhibiting the growth of tumours which are resistant to alkylating agents, a viewpoint which is supported by the observation that certain melphalan-resistant cells were free from cross-resistance to various Mannich bases of α,β-unsaturated ketones.[27]

Several investigations were undertaken with a view to discern the reasons for the significant potencies of **3a**–**h**. These procedures involved: first, a comparison of the potencies of the compounds in series **3** and **4**; second, molecular modeling of various congeners; and finally, a quantitative structure–activity relationship (QSAR) study.

Bioactivity is unlikely to be due to hydrolysis of the acyl group attached to the piperidyl nitrogen atom in series **3**, which would lead to **1** and the corresponding

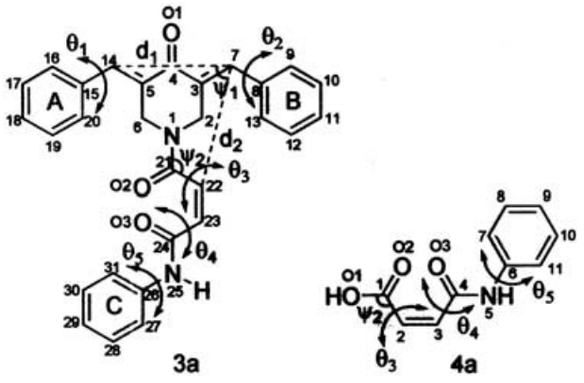

FIGURE 2 Designation of the torsion angles ($\theta_1-\theta_5$) and bond angles (ψ_1 and ψ_2) in **3a** and **4a**.

N-arylmaleamic acids for the following reasons. In the first place, compounds **3** are, in general, more potent than **1** and secondly, the data in Table I reveal the extremely low potencies of the N-arylmaleamic acids **4**. Thus the bioactivity of **3a**–**h** is likely due to the intact molecules.

If the compounds in series **3** exert their cytotoxicity, at least in part, by interaction with cellular thiols, then the sites of action would be predicted to be on the most electron-deficient carbon atom on each of the three olefinic groups located in **3a**–**h**. Furthermore, the theory of sequential cytotoxicity predicts that compounds which undergo a sequence of chemical reactions *in vitro* and *in vivo* may be more harmful to neoplastic cells than the corresponding normal tissues.[28,29] Hence different electronic charges on the carbon atoms which are proposed to interact with thiols would be an additional positive feature of the N-arylmaleamoyl-3,5-bis(phenylmethylene)-4-piperidones **3**. Extended Huckel charges were obtained on the following carbon atoms of **3a**–**h**, as indicated in Figure 2 (average charges are in parentheses), namely 14 (0.037), 5 (−0.013), 3 (0.006), 7 (0.008), 22 (−0.047) and 23 (−0.057). Thus, thiolation would be predicted to take place on carbon atoms 14, 7 and 22. The marked differences in charges on these three atoms suggested that thiolation will take place in the sequence of carbon atoms 14, 7 and finally 22. Thus, the compounds in series **3** have the potential of fulfilling the criteria of sequential cytotoxicity, which may have been a contributing factor to their cytotoxicity. In addition, development of these compounds may reveal a preferential toxicity for neoplastic tissues compared to the related normal cells.

The determination of the reasons for the potent cytotoxicity of **3a**–**h** was further addressed by considering the shapes of these compounds as determined by molecular modeling. The objectives were as follows. First, marked differences between the topography of **3a**–**h** were evaluated and, if detected, consideration would be given as to whether such differences were correlated with cytotoxicity. Second, a study of the shapes of these molecules could enable suggestions to be made regarding the syntheses of further analogues for bioevaluation. Third, comparison between those structural features that were common to the compounds in series **1**–**4** may provide some explanations for the variations in observed cytotoxicities. Various torsion angles $\theta_1-\theta_5$ and bond angles ψ_1 and ψ_2, as illustrated in Figure 2 for the representative compounds **3a** and **4a**, were determined. In addition, the interatomic distances between the C7 and C14 atoms (d_1) and C7 and C22 atoms (d_2) as well as the distance that the C14 atom was above or below the plane of the C7 atom (d_3) were obtained. The reasons for the choice of these specific measurements are as follows. The torsion angles $\theta_1-\theta_5$ were determined on the basis of certain reports of the importance of coplanarity, or lack thereof, of portions of molecules with adjacent linkages which are usually unsaturated groups.[30] The bond angles ψ_1 and ψ_2 gave estimates of the location of the N-acyl group in relation to the rigid 3,5-bis(phenylmethylene)-4-piperidone moiety. If cytotoxicity was due, at least to some extent, to reaction with cellular thiols, then the relative positions of carbon atoms 7, 14 and 22, as revealed by the d_1-d_3 values, may afford an insight into the interatomic distances of these molecules, which enhance potency. The data generated are presented in Table II.

The data in Table II reveal that among the compounds in series **3**, the $\theta_1-\theta_5$, d_2 and d_3 figures for **3g** differed noticeably from **3a**–**f**,**h**. This effect is probably due to the two ortho substituents in aryl ring C which created a θ_5 value of −18°C in contrast to an average θ_5 value of 2.10 for **3a**–**f**,**h**. This distortion of **3g** likely caused the other variations in the θ values as well as the d_2 and d_3 figures of this compound compared to its congeners. The average IC_{50} figures for **3a**–**h** towards Molt 4/C8 and CEM T-lymphocytes were computed. Compound **3g** demonstrated the highest potency with an average IC_{50} figure of 0.54 μM; the average value for series **3** being 1.04 μM. Thus, the high potency of **3g** may have resulted, at least in part, from the conformation of the molecule. Consequently, future synthetic chemical endeavours should place one or more ortho substituents in ring C of the N-acylpiperidones **3**.

A comparison of the topography of series **3** with the related analogues **1**, **2** and **4** was made. While the θ_1 and θ_2 values of **1** are widely disparate, the presence of a N-acyl group in **2** and **3** reduced this difference considerably. The addition of a N-arylcarbamoyl group to **2**, leading to **3**, greatly

TABLE II Certain torsion (θ) and bond (ψ) angles as well as interatomic distances (d) present in the compounds in series **1**–**4**

Compound	θ_1°	θ_2°	θ_3°	θ_4°	θ_5°	ψ_1°	ψ_2°	d_1(°A)	d_2(°A)	d_3(°A)
1	37.1	−54.0	–	–	–	–	–	–	–	–
2	43.5	−43.7	−29.9	–	–	119.7	76.7	4.84	4.39	5.67
3a	45.0	−48.9	−50.0	91.6	2.85	118.2	74.6	4.87	4.38	5.52
3b	45.2	−48.7	−50.2	90.4	2.99	118.1	74.6	4.87	4.38	5.52
3c	45.5	−48.6	−51.0	89.5	2.03	117.9	74.7	4.87	4.37	5.50
3d	46.1	−48.7	−50.8	89.0	2.10	117.8	75.0	4.87	4.38	5.51
3e	46.1	−48.5	−51.3	88.6	1.93	117.9	75.0	4.87	4.39	5.52
3f	46.1	−48.5	−52.0	88.7	1.42	118.0	75.1	4.87	4.38	5.51
3g	43.1	−46.5	−46.9	105.1	−18.0	117.2	74.5	4.87	4.12	5.22
3h	46.2	−48.4	−51.2	89.5	1.38	118.2	75.1	4.87	4.39	5.53
4a	–	–	−32.1	−59.6	−5.63	120.0	–	–	–	–
4b	–	–	−31.4	−60.9	−4.32	120.0	–	–	–	–
4c	–	–	−31.4	−60.2	−5.71	120.0	–	–	–	–
4d	–	–	−30.9	−60.6	−5.09	120.1	–	–	–	–
4e	–	–	−30.7	−60.7	−4.92	119.9	–	–	–	–
4f	–	–	−29.9	−61.5	−5.19	119.9	–	–	–	–
4g	–	–	−20.4	−68.1	−11.8	119.9	–	–	–	–
4h	–	–	−30.6	−62.1	−4.84	119.9	–	–	–	–
4i	–	–	−29.9	−63.4	−5.32	119.8	–	–	–	–

increased the θ_3 figure. It is possible that the magnitude of the θ_1–θ_3 figures influenced potency. In any event, in the future other groups should replace the terminal proton of the acryloyl group of **2** and measurement of the θ_1–θ_3 values may lead to a better understanding of the importance of these torsion angles to potency.

The fact that, in general, **3a**–**h** were more potent that **1** revealed that the arylmaleamoyl group contributed to the bioactivity. In other words, the compounds in series **4** should be able to align at the same location as the N-maleamoyl group present in **3**. However, the data in Table II indicated that the θ_3–θ_5 figures in series **3** and **4** are markedly divergent, which may explain the huge differences in potencies between these two series of compounds. In harmony with the observations made with **3g**, the presence of two ortho substituents in **4g** is likely the cause of θ_3–θ_5 figures of this compound differing considerably from **4a**–**f**,**h**,**i**.

Future modifications of series **3** should include the preparation of compounds that are likely to lead to considerable changes in the θ_1–θ_5, ψ_1, ψ_2 and d_1–d_3 values. Such a study could include placing substituents in the ortho positions of aryl rings A, B and C, altering the stereochemistry of the C22–C23 olefinic linkage and replacing the hydrogen atoms on the C22, C23 and N25 atoms by bulkier groups. A comparison of these determinations obtained by molecular modeling with the IC_{50} data may lead to conclusions regarding the shapes of these molecules which influence cytotoxicity.

A further probing for structure–activity relationships (SAR) in the novel cytotoxics **3** was performed as follows. Linear and semilogarithmic plots were made between the IC_{50} values in each of the four screens and the Hammett σ and/or Taft σ* values, the Hansch π figures and the MR constants of the substituents in the aryl ring attached to the carbamoyl group. The objective was to determine whether correlations between cytotoxicity and the electronic (σ, σ*), hydrophobic (π) and/or steric (MR) properties of the aryl substituents were established; such relationships would be of value in guiding amplification of the series of compounds. In the case of series **3**, neither correlations ($p > 0.1$) nor trends towards significance ($p > 0.15$) were noted. The absence of specific IC_{50} figures in two-thirds of the bioevaluations in series **4** meant that there was an insufficient number of data points for an analysis to be undertaken in most cases. However, in those instances when plots were generated, no correlations ($p > 0.1$) nor trends towards significance ($p > 0.15$) were observed.

The next phase of the study was directed at determining whether a site of action of representative compounds was hNMT. The piperidones **3a**,**c**–**e**,**g** had no effect on this enzyme. In contrast, the analogues in series **4** caused either inhibition (**4a**,**d**,**e**,**g**) or stimulation (**4c**) of hNMT. The observation that **1** was devoid of hNMT-inhibiting properties suggested that replacement of the hydroxy group in series **4** by the bulky 3,5-bis (phenylmethylene)-4-oxopiperidinyl group, leading to **3**, prevented alignment of the N-arylmaleamoyl group of **3** on the enzyme. A very recent disclosure from this laboratory revealed that stimulation of hNMT was accomplished by certain compounds which were believed to form hydrogen bonds with substrates.[31] It is conceivable that **4c** exerted its effect in the same way, although the reasons for its behaviour being different from **4a**,**d**,**e**,**g** are unclear. Further investigations are required in order to understand the mechanism(s) of these compounds on hNMT at the cellular level and how the signal pathway

is affected in normal and tumour cells. These experiments reveal that there are no simple correlations between cytotoxic potencies and effects on hNMT.

The final stage of the investigation was the determination of whether the compounds in series **1**–**4** demonstrated differential toxicities. In other words, were marked differences in the biological effects on different cells noted? A demonstration of selectivity may reveal candidate drugs with preferential toxicity for malignant rather than normal cells.[32] In order to address this issue, three different approaches were undertaken: first, a comparison of the IC_{50} values towards both T-lymphocytes and murine leukemic cells; second, evaluation of representative compounds for antifungal properties and third, whether toxicity to mice could be demonstrated.

Comparisons between the IC_{50} figures of the compounds in series **1**–**4** towards the Molt 4/C8 and CEM T-lymphocytes are presented in Table I as selectivity ratio (SR) values. All of the compounds in series **3** displayed higher SR values than the analogues **1** and **2**. In particular, **3b** and **3g** possessed greater SR figures than melphalan while the SR data of this established anticancer drug and both **3c** and **4c** were the same. The ratios of the IC_{50} figures for the murine cell lines are also given in Table I. Both **3f** and **3g** showed greater selectivity than **1**, while none of the compounds in series **1**, **3** and **4** surpassed the figure of 17.4 for **2**. The enones **1**, **2**, **3f** and **3g** had higher SR values than melphalan. The evidence from the SR values indicated that selectivity for different cells exists, especially when murine neoplasms were considered. In particular, **3b**,**c**,**f**, and **3g** were identified as useful lead molecules in this regard. In addition, linear and semilogarithmic plots were constructed between the SR values of the compounds in series **3** and **4** with the σ/σ^*, π and MR constants of the aryl substituents. The π values correlated positively with the murine SR figures in series **3** ($p < 0.1$) and a positive trend towards significance was noted with the human SR values in series **4** ($p < 0.15$). No other correlations were noted ($p > 0.15$). Thus incorporation of markedly lipophilic groups into ring C should be undertaken when extending this series of compounds.

Second, five compounds having differing cytotoxic properties were evaluated for antifungal properties. The enones **1**, **3a** and **4a**,**e**,**f** possessed MIC values in excess of 100-fold of the MIC value of the antifungal agent voriconazole that was required to inhibit the growth of strains of *A. fumigatus* and *C. albicans*. This observation suggests that the compounds prepared in this study are not general biocidal agents.

Third, important for a candidate anticancer agent is the nature and extent of any mammalian toxicity displayed. In the present study, doses up to and including 300 mg/kg of **3b**,**d**–**h** and **4a**–**i** were administered to mice and the animals were examined after 0.5 and 4 hours. No mortalities were noted. The only observed side effect was neurotoxicity (**3b**,**d**,**h**,**4f**) in less than half of the animals after 0.5 h, which had disappeared at the end of the 4 h except in the case of **3b**.

Thus, in general, the novel compounds described in this study demonstrated selective toxicities based on their differential toxicities towards human T-lymphocytes and murine leukemic cells, a lack of antifungal activity and the absence of significant toxicity in mice. These observations reinforce the potential of the N-acylpiperidones **3** as candidate anticancer agents.

Some of the conclusions that may be drawn from this study are as follows. First, the compounds in series **3** are novel cytotoxic agents, some of which were more potent than melphalan or were equiactive with this established drug. Second, evidence was obtained that these compounds demonstrated selective toxicity for neoplastic cells and are not general biocidal agents. Third, molecular modeling of the compounds prepared in this study led to suggestions for the syntheses and bioevaluation of additional analogues. Fourth, the site(s) of action of these compounds are unlikely to include hNMT and further investigations as to the manner in which bioactivity is mediated needs to be undertaken.

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