

1-Aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides and related adducts: A quest for selective cytotoxicity for malignant cells

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Abstract

The primary objective of this study was to discover one or more clusters of compounds which are not equitoxic but display cytoselectivity toward different malignant cells. Furthermore a most important consideration is that such molecules should also display greater cytotoxic potencies to tumors than normal tissues. Two series of compounds are described which meet these criteria, namely the 1-aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides **1a–e** and 1-aryl-3-dimethylamino-2-hydroxymethyl-1-propanone hydrochlorides **2a–e**. A number of these compounds possess marked cytotoxic potencies (IC₅₀ and CC₅₀ values within the 10⁻⁶ and 10⁻⁷ molar range) which are greater than these of the reference drug melphalan. Statistical analyses demonstrated that cytotoxic potencies are influenced by the size of the aryl substituents in series **1** and to some extent by the electronic properties of the aryl groups in series **2**. The mode of action of a representative compound **1e** in HL-60 cells included inducing apoptosis and activation of caspases -3, -8, and -9.

Keywords

1-Aryl-3-dimethylamino-1-propanones; Cytotoxicity; Apoptosis; Caspases

1. Introduction

One of the major problems involved in treating different cancers with drugs is the lack of their demonstrating appreciably greater toxicity to malignancies than normal cells. Hence

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novel groups of molecules are required which are not general biocidal agents displaying equi-toxicity but vary considerably in their potencies toward different cells. In particular, these compounds should provide unequivocal evidence of being more cytotoxic to neoplastic cells rather than normal tissues. The objective of this report is to disclose that the 1-aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides **1** and the related adducts **2** are novel clusters of compounds with selective toxicity to tumors.

The reasons for preparing series **1** and the related adducts **2** and **3** included the following general considerations. First, conjugated styryl ketones are designed as thiol alkylators having little or no capacity to interact with the amino or hydroxy groups of cellular constituents.¹⁻³ Consequently these molecules may be devoid of the genotoxic properties of a number of currently available anticancer drugs⁴ since thiol groups are absent in nucleic acids. This concept of thiol-specificity represents a markedly different approach in the design of putative anticancer drugs than is often followed. Second, this novel design may lead to cytotoxics which are not cross-resistant to contemporary anticancer medication. Support for this contention comes from the observation that several drug resistant cell lines were free from cross-resistance to a series of Mannich bases of conjugated styryl ketones.⁵ Third, since enones may undergo indiscriminate thiol alkylation prior to reaching a target site, a pro-drug approach was incorporated into the project.

The specific reasons for the design of the compounds in series **1** were to create a series of molecules having the following structural features, namely (1) a conjugated enone group attached to an aryl ring, (2) a sterically unhindered β carbon atom where interactions with thiols take place, and (3) an amino group located close to the α,β -unsaturated keto pharmacophore. The reasons for the last molecular component are as follows. First, protonation of the basic center leading to a quadrivalent nitrogen atom will increase the electrophilicity to thiols as illustrated in Figure 1. Second, on occasions the extracellular pH around tumor cells may be low^{6,7} and the pH of neoplasms may be lower than in normal tissues.⁸ Since the ratio of ions to free base is dependent on the pH of the medium,⁹ the percentage of ions can be greater, close to or in the malignant cells which may lead to preferential toxicity to neoplasms. Third, the amine hydrochloride portion of **1-3** confers drug-likeness and increases water solubility.

In the light of these considerations, a twofold strategy was adopted, namely Phase I (an initial examination to ascertain whether some preliminary data warranted proceeding to Phase II) and Phase II (a detailed investigation of whether the compounds display cytoselectivity and a probing of the mode of action of these novel cytotoxins). The Phase I study has been completed.¹⁰ The compounds were prepared as follows. Condensation of the appropriate aryloethanone with dimethylamine hydrochloride and excess of formaldehyde led to the formation of **1a-e**. In the presence of water, the Mannich bases **1a-e** were converted into the corresponding aminoalcohols **2a-e** while reaction of **1c** with 2-mercaptoethanol gave **3**. The IC_{50} values of the compounds in the series **1** and **2** toward human WiDr colon cancer cells were in the low micromolar range. On the other hand, **3** has very low potency ($IC_{50} = 311 \mu M$) and further analogs in series **3** were not prepared. A stability study revealed that **2c** but not **3** reverted to **1c** in solution. The purpose of the current report is to reveal the results of the Phase II investigation in which the selective toxicity of various compounds to

neoplastic cells is clearly demonstrated as well as an indication of the means whereby bioactivity is mediated (Fig. 2).

2. Bioevaluations

All of the compounds in series **1–3** were evaluated against transformed human CEM and Molt 4/C8 T-lymphocytes. In addition, these compounds were assessed against murine L1210 leukemic cells. These data are presented in Table 1. Six representative compounds **1a**, **b**, **e**, **2b**, **e**, and **3** were evaluated against approximately 54 human tumor cell lines representing different types of cancers. The cytotoxicity against these neoplasms, and in particular toward leukemic cell lines, is summarized in Table 2. The cytotoxic potencies of **1a–e**, **2a–e**, and **3** were also assessed using the following human neoplastic cells lines, namely HL-60 promyelocytic leukemic cells and HSC-2 and HSC-4 squamous cell carcinomas. In addition, evaluation toward non-malignant human HGF gingival fibroblasts, HPLF periodontal ligament fibroblasts and HPC pulp cells was undertaken. These results are portrayed in Table 3. A representative compound **1e** caused apoptosis in HL-60 cells as indicated in Figure 3. In addition, **1e** activated caspases-3, -8 and -9 in HL-60 cells and caspase-3 in HSC-2 carcinomas which are presented in Figure 3.

3. Discussion

All of the compounds **1–3** were evaluated toward CEM and Molt 4/C8 T-lymphocytes in order to discern the ability of these compounds to inhibit the growth of transformed cells of human origin. In addition, many anticancer drugs are effective toward murine L1210 cells¹¹ and therefore this bioassay was employed with a view to detecting promising lead molecules. The data are presented in Table 1. In particular, the potencies of **2a** and **2e** having IC₅₀ values of <2 μM in two-thirds of the screens as well as **1c** and **2d** with IC₅₀ figures below 10 μM in most of the assays are noteworthy.

The different cytotoxic potencies of **1a–e** may have been caused by the variation in the electronic, hydrophobic and steric properties of the aryl substituents. Accordingly linear and semilogarithmic plots were made between the IC₅₀ values of **1a–e** in each screen and the Hammett sigma, Hansch pi, and molar refractivity (MR) constants of the aryl group. Negative correlations were noted between the MR values of the aryl substituents and the IC₅₀ figures in the CEM, Molt4/C8 and L1210 bioassays ($p < 0.05$). Thus in developing series **1**, groups with greater size than the aryl substituents employed in **1a–e** should be used. A similar analysis was undertaken with **2a–e** which revealed a positive correlation between the σ constants and the IC₅₀ values in the L1210 screen. This observation reveals that in the future the placement of electron releasing groups in the aryl ring in analogs of **2a–e** should lead to compounds with greater cytotoxic properties. No other correlations were noted ($p > 0.05$).

In view of the important contribution of steric factors to cytotoxic potencies in series **1**, consideration was given to the possibility that the topography of the aryl ring may also affect the magnitude of the IC₅₀ values. Accordingly the torsion angles (θ) between the aryl rings and the adjacent carbonyl group were calculated. In the case of **1a–e**, the θ figures are -73.1 ,

–69.5, –59.5, –64.4, and –67.4, respectively. Linear and semilogarithmic plots between these figures and the IC_{50} values revealed a negative correlation in the CEM test ($p < 0.05$). Hence, the insertion of bulky substituents in the ortho and meta positions of the aryl rings when creating further analogs of series **1** may lead to compounds with increased potencies. The θ values of **2a–e** are 17.5, 24.3, 26.8, 30.7, and 15.7, respectively, but no correlations between these figures and cytotoxic potencies were noted ($p > 0.05$) confirming that no evidence was generated that steric factors contribute significantly to bioactivity in series **2**.

The question of whether masking the enone structure of **1a–e** by hydration yielding **2a–e** led to greater cytotoxic potencies was addressed by comparing the IC_{50} values of compounds bearing the same aryl substituents in each screen. Thus **1a** was compared with **2a** in the CEM assay, then in the Molt 4/C8 test and finally in the L1210 screen and so forth. Standard derivations were taken into account. The results indicated that **2a** > **1a** and **1c** > **2c** in all three bioassays while **2e** > **1e** when considering CEM and Molt 4/C8 T-lymphocytes. In the remaining cases equal potencies were observed. Hence both the nature of the aryl ring and the general structure of the series of the compounds need to be considered when developing these compounds.

A comparison was made between the IC_{50} values of the most potent compounds with the alkylating agent melphalan which is used in cancer chemotherapy. In the CEM, Molt 4/C8 and L1210 assays, **2a** possesses 1.7, 0.6, and 1.3 times the potencies of melphalan, respectively, while the relevant figures for **2e** are 2.2, 0.7, and 1.4, respectively. Clearly both of these aminoalcohols are lead molecules which should be developed further. Other compounds with 25% or more of the potencies of melphalan are **1c** (in all three screens), **1e** (L1210 assay), and **2d** (CEM and L1210 tests).

The biodata in Table 1, and also Tables 2 and 3 *vide in-fra*, confirm the lack of cytotoxic potency of **3**. However, the concept of activated soft compounds has been proposed which refers to the combination of a bioactive molecule to an inert carrier moiety.¹² Thus the attachment of a pharmacophore to **3** such as esterification of a cytotoxic acid via the hydroxy group of **3** may be a worthwhile venture in the future.

An important feature of antineoplastic agents is their ability to display preferential cytotoxicity toward malignant cells rather than normal tissues. Such a property would be displayed by compounds which are not general biocidal agents and therefore exert different potencies in bioassays. Thus a comparison between the cytotoxicity of the compounds toward transformed human CEM and Molt 4/C8 cells was undertaken. The selectivity index (SI) values of each compound were calculated using the ratio of the higher and lower IC_{50} figures and these data are presented in Table 1. Compounds in which the difference in IC_{50} values were more than doubled ($SI > 2$) were noted, namely **1a**, **d**, and all members of series **2** except **2b**. This result compares favourably with melphalan with a SI figure of 1.3.

Compounds **1a**, **b**, **e**, **2b**, **e**, and **3** were evaluated against approximately 54 human tumor cell lines which were derived from the following groups of neoplasms namely leukemia, melanoma and non-small cell lung, colon, central nervous system, ovarian, renal and prostate cancers; with the exception of **1a**, all compounds were also evaluated using various

malignant breast cells.¹³ In this determination the quantity of compound required to inhibit the growth of the cells by 50% is computed. However in those cases where 50% inhibition of growth was not achieved at the highest concentration, namely 100 μM , the figure of 100 μM was used in determining the average inhibitory concentration. Hence, the term GI_{50} rather than IC_{50} is utilized.

The biodata are presented in Table 2. The average IC_{50} figures reveal that the compounds in series **1** and **2** are potent cytotoxins in general. Of particular interest are the two compounds in series **2** with GI_{50} figures of less than 5 μM . Examination of the mean graphs¹⁴ revealed that most of the compounds displayed a greater toxicity to leukemic cells than other cell lines. With the exception of **3**, the potencies toward various leukemic cells are noteworthy; specifically **2b** has submicromolar IC_{50} values and **1a, b** possess IC_{50} figures of less than 5 μM . All of the compounds in series 1 and 2 demonstrated greater antileukemic properties than melphalan. These evaluations confirm the cytotoxic properties of series 1 and 2. In regard to selective toxicity for leukemic cells, impressive SI values were obtained for 1a, 1b and in particular **2b**.

This preferential toxicity for certain neoplastic cells which have been observed may translate into a display for selective toxicity to malignant rather than normal cells. This possibility was addressed in the third bioassay involving both normal and malignant cells. All of the compounds in series **1–3** were evaluated against HGF, HPC, and HPLF normal cell lines as well as toward HL-60, HSC-2, and HSC-4 neoplasms. These data are presented in Table 3. The results confirm that the compounds in series **1** and **2** are potent cytotoxins toward malignant cells whereby 77% of the CC_{50} values are less than 10 μM . The HL-60 cells are the most sensitive to **1a–e** and **2a–e** and in the case of **1d, 2a, and 2e**, the CC_{50} figures are submicromolar toward this cell line. The CC_{50} values of most of the compounds against HL-60, HSC-2, and HSC-4 cells are lower than melphalan. Linear and semilogarithmic plots were undertaken between the CC_{50} figures generated in the HL-60, HSC-2 and HSC-4 assays with the σ , π , and MR constants of the aryl substituents and the θ values of the compounds in series **1** and **2**. Cytotoxic potencies were negatively correlated with the MR constants in the HSC-2 screen while in the case of series **2**, a positive correlation was noted between the σ values in all three cell lines. These results are similar to the observations made in reviewing the biodata in Table 1.

The SI values are clearly dependent on the cell line under consideration. Thus the percentage of compounds which possess SI figures greater than 2 is 100, 30, and 10, respectively, when considering HL-60, HSC-2, and HSC-4 cells, respectively. Of particular note are the SI values of 10 or greater displayed by **1c, d, and 2a, e** when considering the greater toxicity toward HL-60 neoplasms than normal cells. The CC_{50} values against the malignant cells vary considerably and in general the relative sensitivities to each chemical are $\text{HL-60} > \text{HSC-2} > \text{HSC-4}$. This observation demonstrates further the selective toxicity of these compounds which may reveal a disparity in the lethal effects to normal and neoplastic cells.

The final segment of this study was directed to obtaining some understanding of the way in which cytotoxicity was caused and why potencies to various malignant cells differed. Many cytotoxic agents cause apoptosis,¹⁵ which can be due to activation of caspases. Treatment of

HL-60 cells by a representative compound **1e** revealed that internucleosomal DNA was induced using 0.5, 1, and 2 μM of this Mannich base (Fig. 3A). At higher concentrations of 4, 8, and 16 μM , this phenomenon did not occur and only a smear pattern of DNA fragmentation was observed. On the other hand, there was no unequivocal evidence of DNA fragmentation using the same concentrations of **1e** with HSC-2 cells. Figure 3B indicates that activation of caspases -3, -8, and -9 occurred in HL-60 cells by **1e** but this process was virtually absent in HSC-2 cells. Thus **1e** caused apoptotic cell death in HL-60 cells but the death of HSC-2 cells is by alternative mechanisms. These results suggest that some of the compounds in series **1** and **2** cause apoptosis. In addition, a compound may cause toxicity by different mechanisms depending on the cell line. This phenomenon may be a contributing factor to the different SI values observed in this project which further enhances the potential of these compounds for further development.

4. Conclusions

The biodata presented in Table 1 reveal that the amino-alcohols **2a** and **2e** are clearly lead molecules in terms of both cytotoxic potencies and SI values. When evaluations of representative compounds against a number of human tumor cell lines took place, **2b** emerged as a noteworthy candidate for future development in terms of potency and selective toxicity especially to leukemic cells (Table 2). An important feature of most of these compounds in series **1** and **2** is their lethal effects toward promyelocytic leukemic HL-60 cells as the figures in Table 3 reveal. In particular **1d**, **2a**, and **2e** have submicromolar CC_{50} values. Of considerable importance is the observation of the selective toxicity for HL-60 cells displayed by various molecules, especially **1c**, **d** and **2a**, **e** with SI figures of 10 or more. A representative compound **1e** caused apoptosis in HL-60 but not HSC-2 cells. In addition, while **1e** activated caspases -3, -8, and -9 in HL-60 cells, this effect was virtually absent in HSC-2 cells. This observation of different mechanisms of action is mandatory when compounds are to be developed which are tumor-specific and spare normal tissues. This study has demonstrated the need to develop these prototypic molecules in series **1** and **2**. In particular the very favourable properties of **2a** and **2e** both in terms of cytotoxic potencies and selective toxicity to different cells should be noted.

5. Experimental protocols

5.1. Chemistry

5.1.1. Synthesis of compounds—The compounds in series **1–3** were prepared by the methodologies described previously.¹⁰

5.1.2. Molecular modeling—Molecular modeling used a BioMedCache programme.¹⁶

5.1.3. Statistical analyses—The Hammett sigma, Hansch pi, and molar refractivity constants were obtained from the literature.¹⁷ The linear and semilogarithmic plots were made using a commercial statistics package.¹⁸ The significant *p* values (<0.05) that are generated from the data presented in Table 1 when the IC_{50} values of the compounds in series **1** were plotted against various physicochemical parameters are as follows [bioassay, physical constant, linear(l) or semilogarithmic(sl) plot in parentheses]: 0.023 (CEM, MR, l),

0.005 (Molt4/C8, MR, l), 0.025 (Molt4/C8, MR, sl), 0.015 (L1210, MR, l), 0.046 (L1210, MR, sl), 0.040 (CEM, θ , sl). In the case of series **2**, the relevant figure is as follows: 0.009 (L1210, σ , l).

The related p value (<0.05) obtained from the CC_{50} figures of the compounds in series **1** evaluated against HL-60, HSC-2, and HSC-4 cells is as follows: 0.045 (HSC-2, MR, l). In the case of series **2**, the relevant figures are as follows: 0.035 (HL-60, σ , l), 0.035 (HSC-2, σ , l), 0.019 (HSC-2, σ , sl), 0.036 (HSC-4, σ , l), 0.017 (HSC-4, σ , sl).

5.2. Bioassays

5.2.1. Evaluation of 1a–e, 2a–e, and 3 against transformed CEM and Molt 4/C8 T-lymphocytes and murine L1210 leukemic cells—The methodology for these bioassays using CEM, Molt 4/C8 and L1210 cells has been described previously.¹⁹ In brief, different concentrations of each compound were incubated at 37 °C for 72 h (CEM and Molt 4/C8 T-lymphocytes) or 48 h (L1210 cells).

5.2.2. Examination of 1a, b, e, 2b, e, and 3 against approximately 54 human tumor cell lines—A literature procedure was utilized when assaying various compounds against a number of human tumor cell lines.¹³ The concentrations of the compounds were 10^{-8} – 10^{-4} M except in the case of melphalan quantities of $10^{-7.6}$ – $10^{-3.6}$ M were employed. The number of cell lines whose growth was not inhibited by 50% at the maximum concentration of 10^{-4} M was not achieved/ total number of cell lines examined were 5/53 (**1b**), 4/49 (**1e**), and 54/55 (**3**).

5.2.3. Evaluation of the compounds in series 1–3 against normal and malignant human tumor cell lines—The methodology used in assessing the cytotoxicity of various compounds to the normal HGF, HPC, and normal HPLF cell lines as well as neoplastic HL-60, HSC-2, and HSC-4 cells has been described previously²⁰ and recently summarized.²¹

5.2.4. Evaluation of the ability of 1e to cause apoptosis and activate caspases in HSC-2 and HL-60 cells—The methodology of evaluating whether apoptosis and activation of caspases –3, –8, and –9 occurs have been previously described²² and summarized.²¹

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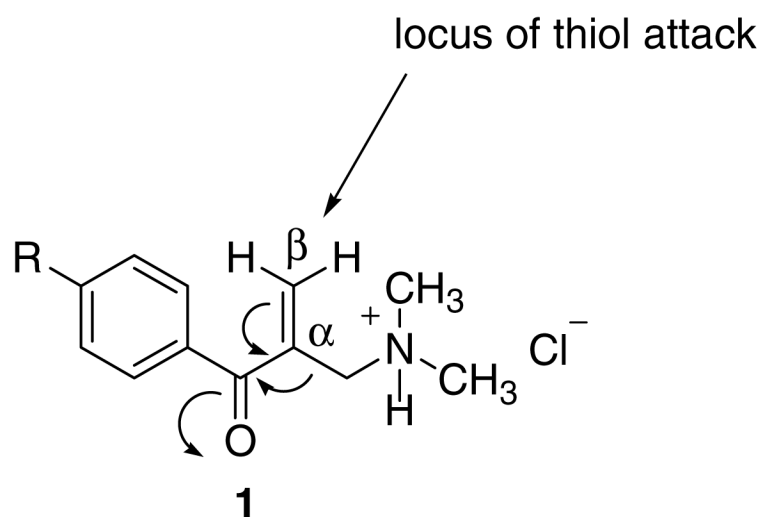


Figure 1.
Design of the thiol alkylator **1**.

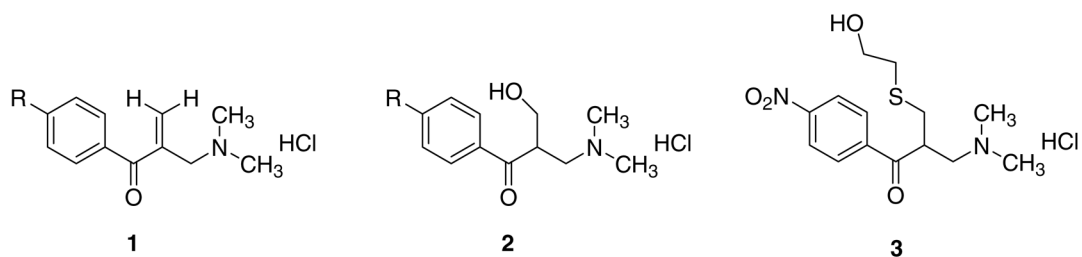


Figure 2.

Structures of series 1–3. The aryl substituents in series 1 and 2 are as follows, namely (a) R = H; (b) R = Cl; (c) R = NO₂; (d) R = CH₃; (e) R = OCH₃.

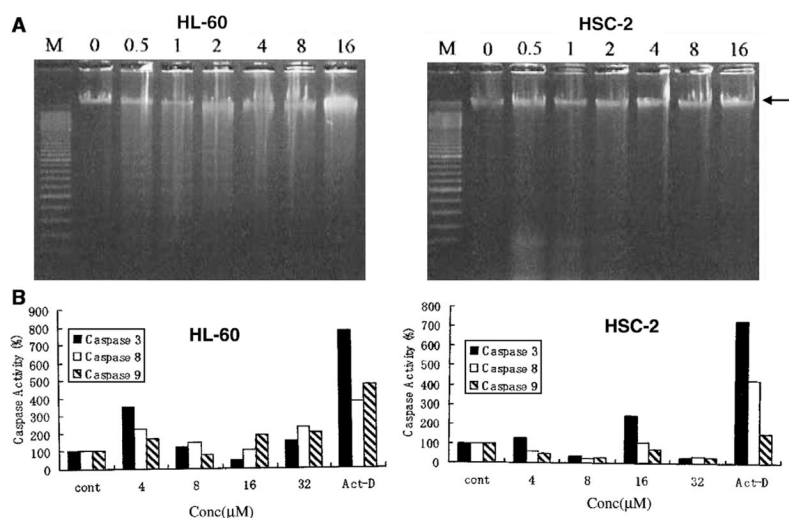


Figure 3.

(A) Evaluation of the effects of **1e** in HL-60 and HSC-2 cells (5×10^5 cells) on DNA fragmentation after incubation for 6 h with the concentration indicated (in μM). M is the molecular weight marker of DNA. The arrow indicates a large DNA fragment. Reproducible results were obtained when repeating the experiment. (B) The effect of **1e** on the activation of caspases in neoplastic cells (4×10^4 cells) after incubation for 4 h. Each value is the mean of two or three experiments.

Table 1

Evaluation of series 1–3 against human CEM and Molt 4/C8 T-lymphocytes and murine L1210 leukemic cells

| Compound | IC ₅₀ (μM) ^a | | SI ^b | IC ₅₀ (μM) ^a |
|------------------------|------------------------------------|-------------|-----------------|------------------------------------|
| | CEM | Molt 4/C8 | | |
| 1a | 65.6 ± 33.8 | 146 ± 91 | 2.2 | 52.2 ± 15.8 |
| 1b | 32.6 ± 7.0 | 30.2 ± 19.0 | 1.1 | 26.5 ± 21.7 |
| 1c | 7.91 ± 0.53 | 8.95 ± 0.20 | 1.1 | 8.61 ± 1.85 |
| 1d | 11.4 ± 1.9 | 35.5 ± 7.4 | 3.1 | 11.1 ± 0.9 |
| 1e | 10.6 ± 8.5 | 21.3 ± 14.0 | 2.0 | 5.08 ± 4.52 |
| 2a | 1.42 ± 0.07 | 5.84 ± 1.89 | 4.1 | 1.65 ± 0.10 |
| 2b | 33.1 ± 19.5 | 30.9 ± 18.6 | 1.1 | 22.0 ± 18.5 |
| 2c | 12.2 ± 0.0 | 36.8 ± 2.6 | 3.0 | 42.6 ± 1.7 |
| 2d | 8.22 ± 2.28 | 21.0 ± 13.0 | 2.6 | 6.55 ± 4.29 |
| 2e | 1.15 ± 0.03 | 4.90 ± 2.19 | 4.3 | 1.57 ± 0.05 |
| 3 | >500 | >500 | ~1.0 | >500 |
| Melphalan ^c | 2.47 ± 0.03 | 3.24 ± 0.79 | 1.3 | 2.13 ± 0.03 |

^aThe IC₅₀ value is the concentration of compound required to inhibit the growth of the cells by 50%.

^bThe letters SI refer to the selectivity index of each compound which is the ratio of the highest to lowest IC₅₀ values of each compound toward the two T-lymphocytes.

^cThe data for melphalan is taken from Ref. 23 copyright (2006) with permission of Elsevier.

Table 2

Evaluation of **1a**, **b**, **e**, **2b**, **e**, **3**, and melphalan against a panel of human tumor cell lines^a

| Compound | All cell lines | | Leukemic cell lines, IC ₅₀ (μM) ^b | | | | SI ^d | |
|-----------|--|-----------------|---|------|-----------|------|-----------------|------|
| | Average GI ₅₀ (μM) ^b | SI ^c | CCRF-CEM | K562 | RPMI-8226 | SR | | |
| 1a | 7.94 | 67.6 | 0.40 | 2.19 | 1.91 | 0.55 | 1.26 | 6.3 |
| 1b | >20.4 | >30.2 | 3.31 | 3.47 | — | 3.98 | 3.59 | >5.7 |
| 1e | >10.2 | >269 | 0.37 | 1.05 | 21.4 | 14.5 | 9.33 | >1.1 |
| 2b | 4.68 | 251 | 0.32 | 0.33 | — | 0.63 | 0.43 | 10.9 |
| 2e | 4.57 | 2512 | — | 0.49 | 20.0 | — | 10.3 | 0.4 |
| 3 | >97.7 | >2.63 | >100 | >100 | 38.0 | >100 | >84.5 | ~1.2 |
| Melphalan | 26.9 | 118 | 6.17 | 43.7 | 66.1 | 1.86 | 29.5 | 0.9 |

^aThe compounds were evaluated against several groups of human tumors including leukemia.

^bThe GI₅₀ and IC₅₀ values refer to the quantities of compounds required to inhibit 50% of the growth of the cells as explained in the text.

^cThe selectivity index (SI) figures are the differences between the highest and lowest GI₅₀ values.

^dThe selectivity index (SI) figures refer to the quotients of the average GI₅₀ value of all the cell lines and the average IC₅₀ figure for leukemic cells.

Table 3

Evaluation of **1a–e**, **2a–e**, **3**, and melphalan against normal and malignant cells

| Compound | Normal cells, CC ₅₀ (μM) ^a | | | | | Tumor cells, CC ₅₀ (μM) ^a | | | | |
|------------------------|--|------|------|------|-------|---|-------|-----------------|-------|-----------------|
| | HGF | HPC | HPLF | ave | HL-60 | SJ ^b | HSC-2 | SJ ^b | HSC-4 | SP ^b |
| 1a | 26 | 31 | 52 | 36 | 12 | 3.0 | 29 | 1.2 | 33 | 1.1 |
| 1b | 6.1 | 6.0 | 9.2 | 7.1 | <3.1 | >2.3 | 5.1 | 1.4 | 6.4 | 1.1 |
| 1c | 19.3 | 30 | 26 | 25 | 1.6 | 16 | 8.2 | 3.1 | 19 | 1.3 |
| 1d | 6.1 | 6.9 | 8.1 | 7.0 | 0.45 | 16 | 3.4 | 2.1 | 7.6 | 0.9 |
| 1e | 6.8 | 5.1 | 9.2 | 7.0 | <3.4 | >2.1 | 5.9 | 1.2 | 6.1 | 1.2 |
| 2a | 4.4 | 5.5 | 5.2 | 5.0 | 0.41 | 12 | 5.2 | 1.0 | 6.5 | 0.8 |
| 2b | 5.8 | 5.4 | 12 | 7.7 | <3.1 | >2.5 | 6.0 | 1.3 | 8.0 | 1.0 |
| 2c | 271 | 227 | 176 | 225 | 35 | 6.4 | 73 | 3.1 | 99 | 2.3 |
| 2d | 4.9 | 5.7 | 9.8 | 6.8 | <3.1 | >2.2 | 6.3 | 1.1 | 5.8 | 1.2 |
| 2e | 4.0 | 5.3 | 4.9 | 4.7 | 0.46 | 10 | 2.5 | 1.9 | 5.2 | 0.9 |
| 3 | 390 | 400 | 400 | 397 | 353 | 1.1 | 165 | 2.4 | 264 | 1.5 |
| Melphalan ^c | >200 | >200 | >200 | >200 | 6.0 | >33 | 35 | >5.7 | 81 | >2.5 |

^aThe CC₅₀ value is the concentration of the compound required to kill 50% of the cells. The figures quoted are the average of two independent determinations which differed by less than 5%. The highest concentrations employed were 400 μM except solubility considerations precluded the use of more than 200 μM of melphalan.

^bThe letters SI refer to the selectivity index which was obtained by dividing the average CC₅₀ value for the three normal cells by the CC₅₀ figure of each malignant cell line.

^cThe data for melphalan was taken from Ref. 21 copyright (2007) with permission of Elsevier.