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Synthesis and Anti-Cancer Activity of 2-Alkylaminomethyl-5-(*E*)-Alkylidene Cyclopentanone Hydrochlorides

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Abstract—A series of 2-alkylaminomethyl-5-(*E*)-alkylidene cyclopentanone hydrochlorides (**2**), have been synthesized and evaluated as anti-cancer agents. These compounds were designed as masked α-methylenecyclopentanones, which appear in many cytotoxic or anti-cancer natural products. Most of the synthesized compounds were found to be active towards various human cancer cell lines and many showed significant subpanel selectivity. For compounds containing the same alkylidene moiety (from C_3 to C_9), the dimethylamino-methyl analogs were more active than structures possessing morpholino-, pyrrolidino-, or piperidino-methyl groups. Alteration of the alkylidene moiety had little effect on anti-cancer potency. The mass spectrum of a glutathione adduct of **2h** indicated that the mechanism of action for these anti-cancer agents may be related to the attack at the aminomethyl carbon atom by biological nucleophilic thiols.

Introduction

The presence of a methylene function alpha to the carbonyl group of γ-lactones or of cyclopentanones has given rise to promising anti-cancer and cytotoxic activities. In the former case, α-methylene-γ-lactones appear in a large number of sesquiterpenes with demonstrated anticancer properties[1-3]. The cyclopentanone framework containing an α-methylene group, which is isosteric with an α-methylene-γ-lactone, also has elicited substances with anti-cancer effects. The α-methylene cyclopentanone nucleus appears in diterpenes,[4,5] simpler natural products such as sarkomycin,[6] methylenemycin A[7] and Xanthocidin[8] as well as in many synthetic analogs.[9-13] Lee *et al.*, have discussed the importance of the O=C-C=CH_2 system in cytotoxicity.[14]

The therapeutic value of both the natural products and synthetic analogs has been limited due to their indiscriminating toxicity, and to some extent, epidermic allergic reactivity. As the methylene function may play a significant role for both anti-cancer action and related effects, many attempts have been made to mask the activity of the double bond in order to increase selectivity and reduce toxicity.[15-17] Mannich bases of cyclopentanones can produce the methylene substances upon 1,2 elimination of the amine. This masking function may have significant ramifications as the mechanism of the cytotoxic and anti-cancer activities of α-methylene-γ-lactone-containing compounds may be related to the attack by a cellular sulfhydryl enzyme on the methylene function resulting in a covalent sulfur–carbon bond.[18]

Structurally related to our synthesized compounds are Mannich bases of acrylophenones or of conjugated styryl ketones which have been reported to contain significant anti-cancer and cytotoxicity activities.[19-21] Mannich bases of ketones are prototypes of α,β-unsaturated ketones. Dimmock *et al.* attributed the cytotoxic activity of the acyclic compounds to the styryl ketone moiety.[21] A series of structure–activity relationships among α-methylene-γ-lactone-containing sesquiterpenes have shown that the presence of certain additional α,β-unsaturated carbonyl functions could significantly increase anti-cancer and cytotoxicity activity even though the α,β-unsaturated carbonyl functions themselves possessed no activity.[3,22] These studies have led us to study Mannich bases of cyclopentanone where the aminomethyl moiety and an alkylidene or arylidene group flank the ketone. For reasons described above, we prepared a series of compounds of types **1** and **2**. In a previous paper[22] we described the anti-inflammatory and anti-cancer activity of series **1** compounds. Substances **1** and **2** share a common fundamental skeleton.

In this paper we report the synthesis and anti-cancer activity of series **2** compounds. Because the mechanism of action at the molecular level could involve attack by a nucleophilic thiol on the methylene carbon, we also made a preliminary attempt to trap such an addition product.

Results and Discussion

Most of the compounds were synthesized by aldol condensation of cyclopentanone with alkyl aldehydes or alkyl ketones followed by a Mannich reaction as shown in Scheme I. The final products are summarized in Table 1.

Compound **2u** was synthesized as shown in Scheme II. This substance was prepared to test our hypothesis on the possible mechanism of action involving the aminomethyl moiety.

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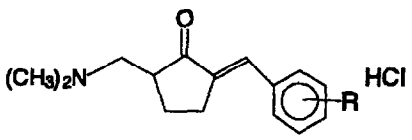

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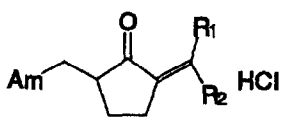
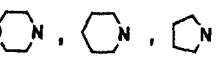


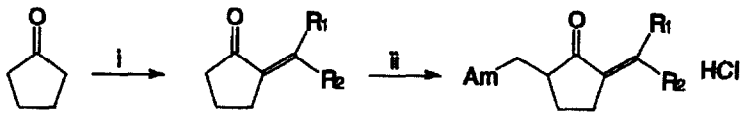
2

R = Alkyl, Alkoxyl, Hydroxyl

Am = $(CH_3)_2N$, 

R_1 = H, CH_3, CH_2CH_3

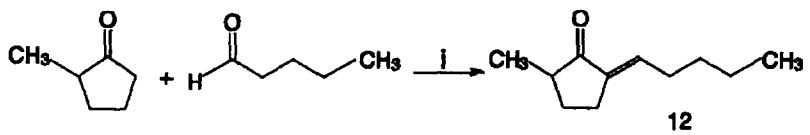
R_2 = CH_3, $CH(CH_3)_2$, $(CH_2)_{3-6}CH_3$



3-11 2a-t

i. R_1R_2CO/OH, r.t.; ii. AmH·HCl/$(CH_2O)_n$/H^+, reflux.

Scheme I.



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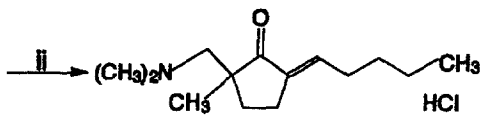


2u

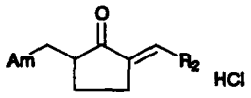
i. OH^-, r.t.; ii. $(CH_3)_2NH$·HCl/$(CH_2O)_n$/H^+, reflux.

Scheme II.

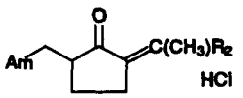
Compounds **2a–2p** were assigned stereochemically as the *E*-form based on the following reasoning. ^1H NMR results showed that there was only one group of signals related to the proton adjacent to the vinyl carbon, which showed a triplet in compounds **2a**, **2f**, **2g**, **2i**, **2k** and **2m** (Table 1).[23] The long distance coupling effect (J = 2 Hz) of the $-CH_2-$ in the cyclopentanone ring could be observed with compounds **2h**, **2j**, **2l**, **2n**, and **2p**, which showed a triplet of triplets and that of compounds **2b–2d** and **2o**, which showed a doublet of triplets.[24] These observations indicate that there is only one isomer in these compounds. To strengthen this argument, the ^1H NMR results of some alkylidene-cyclopentanone derivatives of prostaglandins demonstrated a significant difference for the chemical shift of the proton adjacent to the exo-double bond in the *E*-form relative to the *Z*-form; for the *E*-isomer, $\delta > 6.0$ ppm, but for the *Z*-isomer, $\delta < 6.0$ ppm.[25] The chemical shift of these protons for compounds **2a–2p** ranges from 6.64 to 6.80 ppm. The ^1H NMR data strongly suggest that these substances are in the *E*-form. Compound **2t** was found to be a 1:1 mixture of *E*- and *Z*-isomers according to its ^1H NMR spectrum.

The *in vitro* anti-cancer activity of the compounds was evaluated by the National Cancer Institute (NCI) against a panel of eight human cancer cell lines: leukemia, non-small cell lung, small cell lung, colon, CNS, melanoma, ovarian and renal.[26] Results are presented in terms of GI_{50}, TGI and LC_{50}, which represent the concentrations required to inhibit cell growth by 50 %, 0 and −50 %, respectively and they characterize the growth-inhibiting, cytostatic and cytotoxic properties of the compounds. This new *in vitro* screening method is different from the traditional *in vitro* screening method in which only an IC_{50} parameter was used for cytotoxicity. To simplify the data, and for SAR comparisons, we summarized the average values of log GI_{50}, log TGI and log LC_{50} of subpanels as shown in Table 2.

Table 1. Some physical and NMR data for 2-alkylaminomethyl-5-(E) alkylidene cyclopentanone hydrochlorides



2a-p



2q-t

Compound[a]	R_2	Am	mp,°C	Yield %[b]	Vinyl H, ppm
2a	$-(CH_2)_2CH_3$	$(CH_3)_2N-$	135-137 EtOH	35	6.64 (t)
2b	$-CH(CH_3)_2$	$(CH_3)_2N-$	147-149 EtOH	36	6.46 (dt)
2c	$-CH(CH_3)_2$	morpholino	139-141 EtOH	46	6.47 (dt)
2d	$-CH(CH_3)_2$	pyrrolidino	121-123 EtOH	35	6.48 (dt)
2e	$-(CH_2)_3CH_3$	$(CH_3)_2N-$	155-157 MeOH-Acetone	52	6.65 (tt)
2f	$-(CH_2)_3CH_3$	morpholino	136-138 EtOH	48	6.65 (t)
2g	$-(CH_2)_3CH_3$	piperidino	130-132 EtOH	35	6.64 (t)
2h	$-(CH_2)_4CH_3$	$(CH_3)_2N-$	144-146 EtOH	31	6.66 (tt)
2i	$-(CH_2)_4CH_3$	morpholino	131-133 EtOH	45	6.65 (t)
2j	$-(CH_2)_5CH_3$	$(CH_3)_2N-$	141-143 EtOH-Acetone	42	6.65 (tt)
2k	$-(CH_2)_5CH_3$	pyrrolidino	131-133 EtOH	32	6.65 (t)
2l	$-(CH_2)_6CH_3$	$(CH_3)_2N-$	138-140 EtOH	34	6.65 (tt)
2m	$-(CH_2)_7CH_3$	$(CH_3)_2N-$	130-132 EtOH	42	6.66 (t)
2n	$-(CH_2)_7CH_3$	morpholino	112-114 EtOH	34	6.65 (tt)
2o	$-(CH_2)_8CH_3$	$(CH_3)_2N-$	125-126 EtOH	31	6.66 (dt)
2p	$-(CH_2)_8CH_3$	morpholino	123-124 EtOH	31	6.65 (tt)
2q	$-CH_3$	$(CH_3)_2N-$	173-175 EtOH	55	
2r	$-CH_3$	morpholino	166-167 EtOH	56	
2s	$-CH_3$	pyrrolidino	158-160 EtOH	73	
2t	$-CH_2CH_3$	morpholino	152-153 EtOH	57	

[a]Spectroscopic data including ^1H NMR, IR and MS were consistent with the assigned structures; elemental analyses were within ± 0.4 % of the theoretical values.
[b]Yields are based on the Mannich reaction.

Most of the compounds show selectivity towards leukemia, colon cancer, melanoma and renal cancer at the levels of GI_{50} and TGI. In other words, at the levels of GI_{50} and TGI among the eight panels of human cancers, leukemia, colon cancer, melanoma and renal cancer are the more sensitive panels to which series **2** compounds show growth-inhibiting and cytostatic activity. However, at the LC_{50} level, or in terms of cytotoxicity, leukemia is the less sensitive panel, whereas ovarian cancer, CNS cancer and non-small cell lung cancer are the more sensitive subpanels. MG-MID values indicate compound **2o** is the most potent substance upon comparison of GI_{50}, TGI and LC_{50} with those other compounds. Compounds **2a**, **2b**, **2e**, **2f**, **2j**, **2m**, **2o** and **2p**, have appreciable activity based on MG-MID > 5.2 at the GI_{50} level, > 4.8 for TCI and > 4.4 for LC_{50}.

The most potent compounds among the substances **2a–2t** that contain the same alkylidene moiety, specifically C_3–C_9, are those containing the dimethylaminomethyl group.

Table 2. *In vitro* anti-cancer data[a]

Compound	LEU	LNS	SCL	COL	CNS	MEL	OVA	REN	MG-MID[b]
2a	-5.67	-5.12	-4.87	-5.43	-5.09	-5.17	-5.06	-5.25	-5.23
	-5.23	-4.83	-4.53	-5.04	-4.74	-4.77	-4.72	-4.92	-4.87
	-4.29	-4.47	-4.19	-4.59	-4.42	-4.35	-4.36	-4.57	-4.44
2b	-5.92	-5.17	-5.41	-5.74	-4.86	-5.34	-5.06	-5.49	-5.38
	-5.39	-4.82	-4.79	-5.28	-4.50	-4.91	-4.76	-5.05	-4.96
	-4.40	-4.50	-4.13	-4.69	-4.13	-4.52	-4.37	-4.59	-4.48
2c	-5.53	-4.78	-4.66	-5.13	-4.49	-4.85	-4.88	-4.88	-4.91
	-4.96	-4.46	-4.34	-4.72	-4.24	-4.49	-4.60	-4.47	-4.54
	-4.00	-4.24	-4.01	-4.18	-4.04	-4.19	-4.32	-4.22	-4.18
2d	-5.53	-4.81	-4.75	-5.07	-4.52	-4.86	-4.71	-4.93	-4.91
	-4.94	-4.50	-4.42	-4.68	-4.34	-4.48	-4.39	-4.53	-4.55
	-4.04	-4.33	-4.09	-4.18	-4.21	-4.21	-4.16	-4.27	-4.21
2e	-5.92	-5.12	-5.54	-5.69	-5.00	-5.52	-5.14	-5.50	-5.40
	-4.83	-4.78	-5.08	-5.36	-4.62	-5.10	-4.82	-5.17	-4.98
	-4.00	-4.45	-4.66	-4.98	-4.28	-4.59	-4.52	-4.85	-4.55
2f	-6.09	-5.22	-5.24	-6.00	-5.24	-5.47	-4.99	-5.38	-5.42
	-5.25	-4.91	-4.40	-5.18	-4.88	-5.01	-4.63	-5.07	-5.00
	-4.00	-4.60	-4.02	-4.58	-4.61	-4.60	-4.25	-4.73	-4.51
2g	-4.83	-4.97	-4.74	-5.39	-4.86	-5.06	-4.78	-5.08	-5.11
	-5.03	-4.68	-4.31	-4.88	-4.49	-4.66	-4.56	-4.63	-4.69
	-4.00	-4.39	-4.00	-4.36	-4.18	-4.33	-4.23	-4.49	-4.28
2h	-5.98	-4.95	-4.88	-5.24	-4.85	-4.94	-4.94	-5.01	-5.09
	-4.62	-4.63	-4.59	-4.84	-4.53	-4.58	-4.66	-4.70	-4.64
	-4.00	-4.32	-4.10	-4.36	-4.23	-4.26	-4.41	-4.40	-4.27
2i	-5.60	-4.76	-4.66	-5.03	-4.78	-4.75	-4.74	-4.90	-4.91
	-4.91	-4.58	-4.28	-4.57	-4.43	-4.39	-4.45	-4.58	-4.51
	-4.00	-4.21	-4.00	-4.14	-4.14	-4.15	-4.15	-4.32	-4.17
2j	-5.74	-4.98	-5.39	-5.56	-5.14	-5.30	-4.92	-5.48	-5.29
	-5.25	-4.64	-5.07	-5.17	-4.70	-4.96	-4.60	-5.13	-4.91
	-4.06	-4.33	-4.75	-4.77	-4.39	-4.55	-4.34	-4.77	-4.48
2k	-5.48	-4.79	-4.88	-4.99	-4.60	-4.80	-4.74	-4.80	-4.83
	-4.00	-4.50	-4.57	-4.52	-4.36	-4.51	-4.45	-4.54	-4.45
	-4.00	-4.21	-4.26	-4.18	-4.14	-4.22	-4.17	-4.26	-4.18
2l	-5.65	-4.80	-4.73	-4.92	-4.78	-4.78	-4.73	-5.00	-4.92
	-5.17	-4.48	-4.33	-4.52	-4.50	-4.50	-4.46	-4.58	-4.57
	-4.14	-4.20	-4.00	-4.18	-4.21	-4.22	-4.19	-4.31	-4.21
2m	-5.69	-5.12	-5.44	-5.49	-5.38	-5.42	-5.15	-5.31	-5.36
	-5.15	-4.76	-5.02	-5.08	-4.96	-4.96	-4.78	-4.91	-4.94
	-4.24	-4.38	-4.58	-4.59	-4.56	-4.52	-4.47	-4.51	-4.49
2n	-5.70	-4.97	-4.88	-5.26	-5.07	-5.01	-4.94	-5.17	-5.13
	-5.23	-4.69	-4.40	-4.75	-4.57	-4.65	-4.62	-4.72	-4.72
	-4.04	-4.31	-4.00	-4.26	-4.25	-4.29	-4.29	-4.42	-4.29
2o	-6.00	-5.54	-5.71	-5.69	-5.61	-5.69	-5.24	-5.77	-5.67
	-5.19	-5.13	-5.35	-5.35	-5.25	-5.33	-4.94	-5.41	-5.26
	-4.07	-4.73	-5.07	-4.98	-4.77	-4.94	-4.59	-5.01	-4.78
2p	-5.67	-5.10	-5.52	-5.47	-5.33	-5.34	-5.09	-5.30	-5.33
	-5.19	-4.76	-4.09	-5.08	-4.92	-4.91	-4.74	-4.93	-4.93
	-4.00	-4.39	-4.63	-4.65	-4.53	-4.54	-4.44	-4.59	-4.49
2q	-5.11	-4.68	-4.78	-4.83	-4.59	-4.70	-4.44	-4.68	-4.72
	-4.56	-4.48	-4.42	-4.42	-4.23	-4.39	-4.27	-4.37	-4.39
	-4.07	-4.33	-4.05	-4.14	-4.14	-4.16	-4.10	-4.17	-4.17
2r	-5.81	-5.13	-4.80	-5.21	-4.78	-4.99	-4.92	-5.09	-5.11
	-5.33	-4.77	-4.39	-4.75	-4.41	-4.63	-4.61	-4.73	-4.72
	-4.13	-4.19	-4.00	-4.25	-4.12	-4.34	-4.18	-4.31	-4.26

Table 2. *Continued*.

Compound	LEU	LNS	SCL	COL	CNS	MEL	OVA	REN	MG-MID[b]
2s	-4.75	-4.23	-4.55	-4.60	-4.25	-4.42	-4.33	-4.56	-4.45
	-4.32	-4.12	-4.22	-4.31	-4.09	-4.21	-4.20	-4.32	-4.22
	-4.03	-4.06	-4.08	-4.10	-4.03	-4.07	-4.11	-4.14	-4.08
2t	-5.05	-4.64	-5.01	-4.78	-4.56	-4.74	-4.74	-4.99	-4.79
	-4.33	-4.38	-4.61	-4.43	-4.37	-4.46	-4.45	-4.64	-4.44
	-4.00	-4.15	-4.28	-4.15	-4.12	-4.21	-4.18	-4.30	-4.17
2u	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00[c]

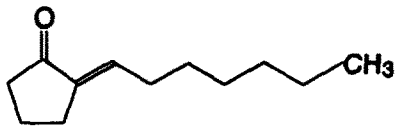
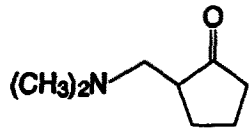
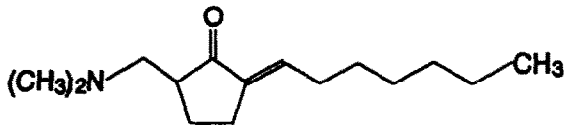
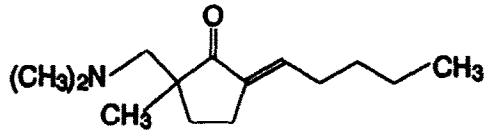
[a]NCl data. LEU = leukemia, LNS = non-small cell lung, SCL = small cell lung, COL = colon, CNS = central nervous system, MEL = melanoma, OVA = ovarian, REN = renal. The first, second and third rows of data for each compound represent log GI_{50}, log TCl and log LC_{50} respectively. These values represent the molar concentrations causing a 50 % reduction in cell growth (GI_{50}), a cytostatic effect or 0 % growth (TGI) and a cytocidal effect or -50 % cell growth (LC_{50}).
[b]Meangraph-midpoints values.
[c]LC_{50} data only.

These observations are noted for each of the subsequent series, **2b–2d**; **2e–2g**; **2h**, **2i**; **2j**, **2k**; **2m**, **2n**; **2o**, **2p**. In each of these series, greater activity appears when Am = $-N(CH_3)_2$, compared to the morpholino, pyrrolidino, or piperidino groups. However, in the disubstituted alkylidene series **2q–2t**, the dimethylaminomethyl compounds are no longer the most potent in the series. Overall, the alteration of the alkylidene moiety, from a short chain to a long chain, from a straight chain to a branched chain, from a low lipophilicity chain to a high lipophilicity chain, has little effect on anti-cancer potency.

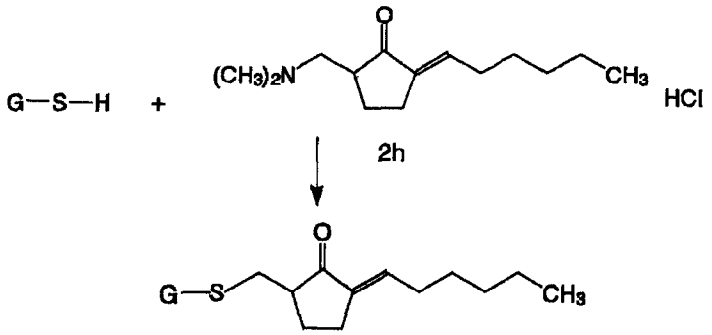
A further attempt was made to strengthen our hypotheses of structural requirements for high potency using cultured cells. The cytotoxicity results in L1210 cells in Table 3 demonstrated that the Mannich base of cyclopentanone, i.e. 2-dimethylaminomethyl cyclopentanone hydrochloride (**13**), was quite low in cytotoxic action and 2-heptylidene cyclopentanone (**7**), possessed no activity. Thus, the alkylidene moiety can increase the cytotoxicity of the cyclopentanone Mannich base, as shown in compound **2j**.

With regard to the potential mechanism of action, we treated compound **2h** with glutathione at 37 °C in an aqueous solution. A mass spectrum, $[M+H]^+ = 486$, of an unstable product was consistent with the structure illustrated. We propose the mechanism of this reaction to proceed via an elimination–Michael addition process—

Table 3. Cytotoxic activity toward the L1210 cell line

Compound	Structure[a]	IC_{50}(uM)[b]
7		>50.0
13		19.7
2j		5.3
2u		>50.0

[a]Compounds **13**, **2j** and **2u** were tested as their hydrochloride salts.
[b]The concentration required to inhibit cell growth by 50 %.



Scheme III.

that is, deamination first takes place to form an α-methylene group, followed by a 1,4-Michael addition. This two step process is favored over a mechanism involving a direct displacement of the amine, based on the observations described below.

To test our hypothesis, we synthesized **2u**. The presence of the α-methyl group obviates the formation of the α-methylene function from the Mannich base. As expected, the α-methylated analog eliminated the anti-cancer activity and cytotoxicity as shown in Table 2 and Table 3 for compound **2u**. These results provide some evidence for the hypothesis that the series **2** compounds act as masked α-methylenecyclopentanones which are capable of interacting with biological nucleophiles, quite possibly thiol-containing enzymes or other proteins. These results suggest that series **2** compounds produce anti-cancer effects due to an alkylating property.

In summary, the presence of an unsaturated group in conjugation with the carbonyl moiety increases anti-cancer potency. Significant changes in activity are not apparent in altering the attached alkylidene structure. If mechanism of action is related to alkylation of biological nucleophiles, attack on the incipient methylene function seems to be more plausible than 1,4-addition at the alkylidene site.

Experimental Section

Melting points were not corrected. IR Spectra were recorded on an IR-27G spectrometer. The ^1H NMR spectra were measured on a BRUKER AC(E)-250 spectrometer with an internal standard of tetramethylsilane. Mass spectra were recorded on a GMS-D300 mass spectrometer. Elemental analyses were performed on EA-MOD 1106 elemental analyzer.

General procedure for synthesis of 2-alkylidenecyclopentanones (3–9)

To a mixture of 0.5 mol of cyclopentanone in 80 mL of 1 % NaOH, 0.25 mol of alkylaldehyde was added dropwise at about 30 °C with stirring. The resulting mixture was stirred for 2 h at room temperature and then neutralized with 36 % aqueous acetic acid and extracted with benzene. The combined extractions were mixed with 1.0 mL of phosphoric acid and refluxed under a water separator for 2 h and then the solvent was removed on a rotary evaporator to give a residue. The residue was distilled to give the corresponding 2-alkylidenecyclopentanones.

3: 2-Butylidene cyclopentanone, yield 69 %, bp 96–100 °C/6 mmHg;
4: 2-Pentylidene cyclopentanone, yield 75 %, bp 98–102 °C/6 mmHg;
5: 2-Hexylidene cyclopentanone, yield 77 %, bp 125–130 °C/10 mmHg;
6: 2-Heptylidene cyclopentanone, yield 72 %, bp 112–116 °C/1 mmHg;
7: 2-Octylidene cyclopentanone, yield 73 %, bp 120–125 °C/4 mmHg;
8: 2-Nonylidene cyclopentanone, yield 75 %, bp 145–150 °C/4 mmHg;
9: 2-Decylidene cyclopentanone, yield 63 %, bp 135–140 °C/3 mmHg.

*Synthesis of 2-(1-methylethylidene)cyclopentanone (**10**)*

To a mixture of 190 mL of acetone and 36.0 g of NaOH in 600 mL of water, 60.0 g of cyclopentanone was added under vigorous stirring at room temperature. The mixture was stirred for 12 h at room temperature and then neutralized with 36 % aqueous acetic acid and extracted with ethyl ether. The combined extractions were dried over anhydrous $MgSO_4$. After the solvent was removed, the residue was distilled to give 62.6 g of colorless liquid, bp 85–88 °C/10 mmHg, yield 60 %.

2-(1-Methylpropylidene)cyclopentanone (**11**) was synthesized by a similar procedure as above, yield 21 %, bp 110–115 °C/12 mmHg.

General procedure for synthesis of Mannich bases of 2-alkylidene cyclopentanones (2a–2t)

A mixture of 0.05 mol of 2-alkylidenecyclopentanone, 0.05 mol of secondary amine hydrochloride, 3.8 g (0.13 mol) of paraformaldehyde, 50 mL of absolute ethanol and 0.5 ml of concentrated HCl was refluxed with stirring. Two hours later, an additional 1.5 g (0.05 mol) of paraformaldehyde was added and refluxing was continued for another 2 h. The resulting solution was left to stand overnight to give white crystals (at times ethyl ether was added in order to give crystals), which were collected by filtration and recrystallized from an appropriate solvent, to give sufficiently pure products. Results are summarized in Table 1.

Synthesis of 2-methyl-2-dimethylaminomethyl-5-pentylidene cyclopentanone hydrochloride (2u)

A mixture of 5.0 g (0.03 mol) of 2-methyl-5-pentylidene cyclopentanone (synthesized in a similar procedure as described for 2-alkylidenecyclopentanone, bp 82–90 °C/2 mmHg), 3.6 g (0.045 mol) of dimethylamine hydrochloride, 3.6 g (0.12 mol) of paraformaldehyde, 50 mL of ethanol and 0.2 mL of concentrated HCl was refluxed under stirring for 19 h, and then 0.9 g (0.03 mol) of paraformaldehyde was added and refluxing was continued for 6 h. The resulting solution was concentrated *in vacuo* to give a residue. The residue was dissolved in water and extracted with ethyl ether. The aqueous layer was made alkaline with 5 % NaOH and extracted with ethyl ether. The ethyl ether extraction was dried over anhydrous $MgSO_4$. Dry HCl gas was bubbled through the dried ethyl ether solution to give a white precipitate. The white precipitate was crystallized from ethanol to give 1.0 g of white platelets, mp 194–196 °C (dec.). IR (KBr) cm^{-1}: 1690 (C=O), 1630 (C=C). ^1H NMR ($CDCl_3$) ppm: 0.89 (t, 3H, CH_2CH_3), 1.16 (s, 3H, CH_3), 1.20–1.38 (m, 4H, $CH_2CH_2CH_3$), 1.38–1.62 (m, 1H), 1.90 (br, 1H), 2.19 (t, 2H, $CH_2CH_2CH_2CH_3$), 2.42 (d, 3H, $NHCH_3$), 2.52–2.70 (m, 1H), 2.85 (d, 3H, $NHCH_3$), 3.16–3.46 (m, 2H), 3.79 (d, 1H), 7.43 (br, 1H, C=CH), 12.25 (br, 1H, NH).

Reaction of 2-dimethylaminomethyl-5-hexylidene cyclopentanone hydrochloride (2h) with glutathione

A solution of 130 mg (0.5 mmol) of 2-dimethylaminomethyl-5-hexylidenecyclopentanone hydrochloride (1 g) in 10 mL of H_2O was mixed with a solution of 153 mg (0.5 mmol) of glutathione in 10 mL H_2O. The resulting solution was stirred at 37 °C for 12 h. The solvent was removed by lyophilization to give 215 mg of white solid, no further purification was attempted, FDMS: 486 [M+H].

In vitro *cytotoxicity against L1210*

L1210 Leukemia cells were grown in nutrient medium RPM11640 supplemented with 2 mM L-glutamine, 200 IU/mL penicillin, 50 μg/mL streptomycin, and 20 % heat inactivated horse serum. They were incubated in a 5 % CO_2 atm at 37 °C. The compounds were dissolved in saline and added to the cells in the exponential phase of growth at an initial concentration of 0.8×10^5 cells/mL. The cells were counted in triplicate after 48 h and results were expressed as the concentration which inhibited cell growth by 50 % (IC_{50}) as compared to controls.

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23. We suggest that the vinyl proton in compounds **2a**, **2f**, **2g**, **2i**, **2k** and **2m** is a triplet of triplets but resolution at 250 Hz was insufficient to make such an observation.

24. ^1H NMR data for compound **2b** typifies the spectra for the other structures: 1.05 (d, 6H, $CH(CH_3)_2$), 1.64–1.82 (m, 1H), 2.42–2.68 (m, 2H), 2.72–3.20 (m, 10H), 3.44–3.62 (m, 1H), 6.46 (dt) 1H, C=CH-), 12.29 (br, 1H, NH) ppm.

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