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SELECTIVE CYTOTOXICITY OF CERTAIN 9-SUBSTITUTED ELLIPTICINES FOR LEUKEMIA CELLS IN A VARIETY OF LEUKEMIA CELL CULTURES

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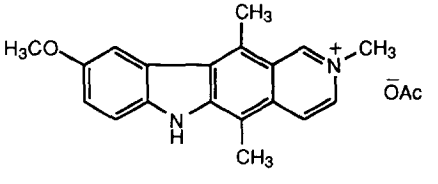
Abstract: Certain 9-substituted ellipticines, including 9-hydroxymethylellipticine, displayed selective in vitro cytotoxicity for leukemia cells in a variety of leukemia cell cultures. The selective cytotoxicity was apparent in all of the leukemia cell lines examined, which included CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, and SR cells.

The lack of selective cytotoxicity of many of anticancer agents continues to be a deterrent to their therapeutic use. In an effort to address this problem, the National Cancer Institute (NCI) has instituted an in vitro cytotoxicity screen with the goal of identifying new anticancer agents that display selective cytotoxicities against particular types of human tumor cell lines.[1-3] In prior work, the selectivity of 9-methoxy-2-methylellipticinium acetate (**1**), as well as the 9-methoxy-2-methyl-1,2-dihydroellipticine derivative (**2**), for CNS cancer cells was documented.[4,5] In view of these results, further exploration of the 9-substituted ellipticine series seemed warranted. As outlined in the present communication, additional 9-substituted ellipticines have been synthesized and their cytotoxicities examined. Although these new compounds were indeed found to be selective cytotoxic agents, the selectivity unexpectedly proved to be toward the leukemia panel instead of the CNS panel of cell cultures. It is therefore now possible to direct the selectivity of certain ellipticines through appropriate substituent choice.



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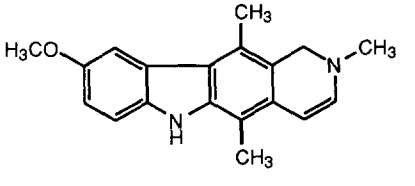

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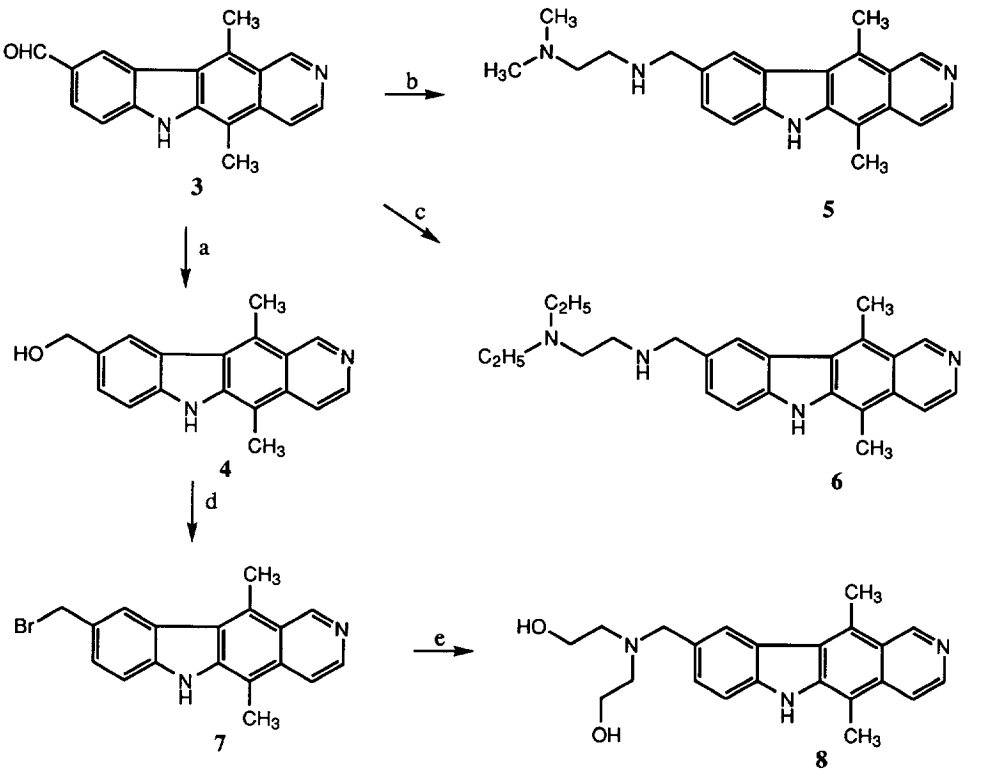
The syntheses of 9-hydroxymethylellipticine (**4**), 9-{[2-(*N,N*-dimethylamino)ethylamino]methyl}ellipticine (**5**), 9-{2-[*N,N*-(diethylamino)ethylamino]methyl}ellipticine (**6**), and 9-[*N,N*-bis(2-hydroxyethyl)amino-

methyl]ellipticine (**8**) are outlined in Scheme 1. Sodium borohydride reduction of 9-formylellipticine (**3**)[6] afforded 9-hydroxymethylellipticine (**4**).[7] Reaction of **3** with the appropriate amines, followed by in situ sodium cyanoborohydride reduction of the resulting Schiff bases, gave the desired products **5**[7] and **6**. Treatment of 9-hydroxymethylellipticine (**4**) with HBr yielded 9-bromomethylellipticine (**7**), which was converted to ellipticine derivative **8**.[7]

Scheme 1[a]



[a]Reagents and conditions: (a) $NaBH_4$, MeOH, 10 °C, 1 h; (b) (1) $(CH_3)_2NCH_2CH_2NH_2$, EtOH, 4 Å molecular sieves, 23 °C, 24 h, (2) $NaCNBH_3$, 23 °C, 1 h; (c) $(C_2H_5)_2NCH_2CH_2NH_2$, EtOH, 4 Å molecular sieves, 23 °C, 24 h, (2) $NaCNBH_3$, 23 °C, 24 h; (d) HBr, AcOH, 23 °C, 1 h; (e) $(HOCH_2CH_2)_2NH$, Et_3N, DMF, 23 °C, 24 h.

Although compounds **4**, **5**, **6**, and **8** all displayed selective cytotoxicity for leukemia cell cultures, the activity of the 9-hydroxymethyl compound **4** was the most impressive in terms of selectivity. The GI_{50} (concentration for 50% growth inhibition), TGI (concentration for total growth inhibition), and LC_{50} (concentration for 50% cell kill) values for **4** in representative cell lines are shown in Table 1. The selectivity for the leukemia panel is apparent at both the GI_{50} and TGI levels. In contrast, ellipticine itself did not possess any apparent selective cytotoxicity for any of the cancer cell culture panels.

Table 1. Cytotoxicity of 9-Hydroxymethylellipticine (**4**)

Panel/Cell line	ED_{50} (μM)[a]	TGI (μM)[a]	LC_{50} (μM)[a]
leukemia			
CCRF-CEM	0.06	1.01	>100
HL-60 (TB)	0.02	0.11	0.9
K-562	0.03	0.11	>100
MOLT-4	0.01	0.04	4.09
RPMI-8226	0.03	0.22	5.57
SR	0.04	0.49	--
non-small cell lung cancer			
NCI-H23	0.68	9.44	79.0
NCI-H322M	0.62	11.5	35.3
NCI-H522	0.30	2.95	>100
colon cancer			
COLO 205	0.24	2.20	10.2
HCT-116	0.05	--	>100
KM-12	0.16	1.52	7.38
CNS cancer			
SNB-19	0.21	11.5	56.2
SNB-75	0.49	2.63	14.9
U251	0.28	>100	>100
melanoma			
LOX IMVI	0.37	3.03	>100
MALME-3M	0.64	7.35	0.82
M14	0.60	3.65	34.1
ovarian cancer			
IGROV1	0.18	11.7	62.0
OVCAR-3	0.70	52.1	46.2
OVCAR-8	0.42	11.3	55.2
renal cancer			
CAKI-1	0.37	13.4	52.6
RXF 393	1.61	3.89	9.43
SN12C	0.22	3.47	1.91
prostrate cancer			
PC-3	0.45	3.60	23.3
DU-145	1.29	11.9	35.0
breast cancer			
MDA-MB-435	0.24	1.61	6.02
MDA-N	0.35	1.69	4.33
T-47D	1.42	18.4	78.2

[a]The cytotoxicity GI_{50}, TGI, and LC_{50} values are the concentrations corresponding to 50% growth inhibition, total growth inhibition, and 50% cell kill, respectively.

Representative cytotoxicity results for **4**, **5**, **6**, and **8** in a limited number of cancer cell cultures are provided in Table 2. It is apparent that each of these compounds has significant cytotoxicity, and that they all possess greater cytotoxicity in leukemia cells. The underlying reasons for the selectivities of these compounds for leukemia cells, as well as the selectivities of **1** and **2** for CNS cancer cells, are presently uncertain. Since **2** is readily oxidized to **1**, the CNS selectivities of these compounds might be related to the presence of a quaternary nitrogen.[5] On the other hand, **4**, **5**, **6**, and **8** may eliminate water or amines to form a reactive iminoquinomethane species that could possibly bind covalently to biological nucleophiles. Additional studies are indicated in order to elucidate the chemistry and pharmacological properties of these cytotoxic ellipticines.

Table 2. Cytotoxicities of **4**, **5**, **6**, and **8** (GI_{50} values in μM)[a]

Panel/Cell line	4	5	6	8
leukemia				
MOLT-4	0.01	0.10	0.01	0.02
non-small cell lung cancer				
NCI-H322M	0.62	0.25	0.06	0.22
colon cancer				
HCT-116	0.05	0.17	0.05	0.10
CNS cancer				
U251	0.28	0.28	0.18	0.26
melanoma				
LOX IMVI	0.37	0.16	0.12	0.15
ovarian cancer				
IGROV1	0.18	0.15	0.04	0.12
renal cancer				
CAKI-1	0.37	0.26	0.16	0.25
prostrate cancer				
PC-3	0.45	0.38	0.21	0.34
breast cancer				
MDA-N	0.35	0.28	0.11	0.37

[a]The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition.

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7. Analytical data: **4** mp > 260 °C (dec), ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1 H) 9.70 (s, 1 H), 8.42 (d, 1 H, J = 6 Hz), 8.35 (s, 1 H), 7.93 (d, 1 H, J = 6 Hz), 7.52 (s, 2 H), 5.23 (t, 1 H, J = 6 Hz), 3.29 (s, 3 H), 2.79 (s, 3 H); **5** mp 249-254 °C (dec), ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.37 (s, 1 H), 9.69 (s, 1 H), 8.43 (d, 1 H, J = 6 Hz), 8.38 (s, 1 H), 7.92 (d, 1 H, J = 6 Hz), 7.52 (s, 2 H), 3.98 (s, 2 H), 3.29 (s, 3 H), 2.78 (s, 3 H), 2.71 (t, 2 H, J = 6 Hz), 2.41 (t, 2 H, J = 6 Hz), 2.15 (s, 6 H); **8** mp 160-163 °C (dec), ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.38 (s, 1 H), 9.70 (s, 1 H), 8.43 (d, 1 H, J = 6 Hz), 8.38 (s, 1 H), 7.92 (d, 1 H, J = 6 Hz), 7.53 (s, 2 H), 4.55 (br s, 2 H), 3.97 (s, 2 H), 3.56 (m, 4 H), 3.28 (s, 3 H), 2.79 (s, 3 H), 2.73 (br t, 4 H).

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