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ANTITUMOR AGENTS 201.[1] CYTOTOXICITY OF HARMINE AND β-CARBOLINE ANALOGS

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Abstract: Twenty-six β-carbolines were evaluated for in vitro cytotoxicity in a human tumor cell line panel. Harmine (**3**) showed significant activity against several cell lines including three drug-resistant KB sublines with various resistance mechanisms. α-(4-Nitrobenzylidine) harmine (**16**) had a broad cytotoxicity spectrum (ED_{50} values from 0.3–1.2 μg/mL against 1A9, KB, SaOS-2, A549, SK-MEL-2, U-87-MG, and MCF-7 cells).

In our continuing search for new plant-derived antitumor agents, the $CHCl_3$ extract of *Symplocos serchuensis*, a plant indigenous to southern China, showed potent cytotoxicity. In our initial fractionation and isolation studies, we identified harman (**2**) from this extract. This β-carboline was reported previously from the related species *S. racomosa* Roxb.[2] Norharman (**1**) and harman (**2**) have been reported to exhibit mutagenic and co-mutagenic properties,[3] and to inhibit topoisomerase I.[4] In addition, several γ-carbolines intercalate into DNA base pairs and exhibit significant in vitro cytotoxic and in vivo antitumor activities.[5] However, because few studies have been reported on the antitumor activity of this compound class against human tumor cell lines, we initiated such an investigation of 26 β-carbolines, including harman and harmine (**3**). Herein we report the results of this study.

Materials. Compounds **2**, **3**, and **16–27** were obtained from Aldrich, Inc (Milwaukee, WI). Compound **4** was obtained by boron tribromide demethylation of **3** (yield 24%). Treating an aqueous solution of **19** with NH_4OH afforded the free base **15** as crystals. 7-Alkylation (10 equiv of C_2H_5Br, $(CH_3)_2CHBr$, $CH_3(CH_2)_5Br$, $CH_3(CH_2)_9Br$, $CH_3(CH_2)_{15}Br$, ethanolic NaOH) of **4** afforded **5–9** (yield 50–60%). Compound **10** was generated by acetic anhydride-pyridine acetylation of **4** at room temperature (yield 70%). N-Alkylation of **3** (1.2 equiv of NaH, 10 equiv of CH_3I, C_2H_5I, $CH_3(CH_2)_3I$, THF-DMF, room temp, 3h) afforded **12–14** in high yield (ca. 90%). Esterification of **4** with (1*S*)-(-)-camphanic chloride in the presence

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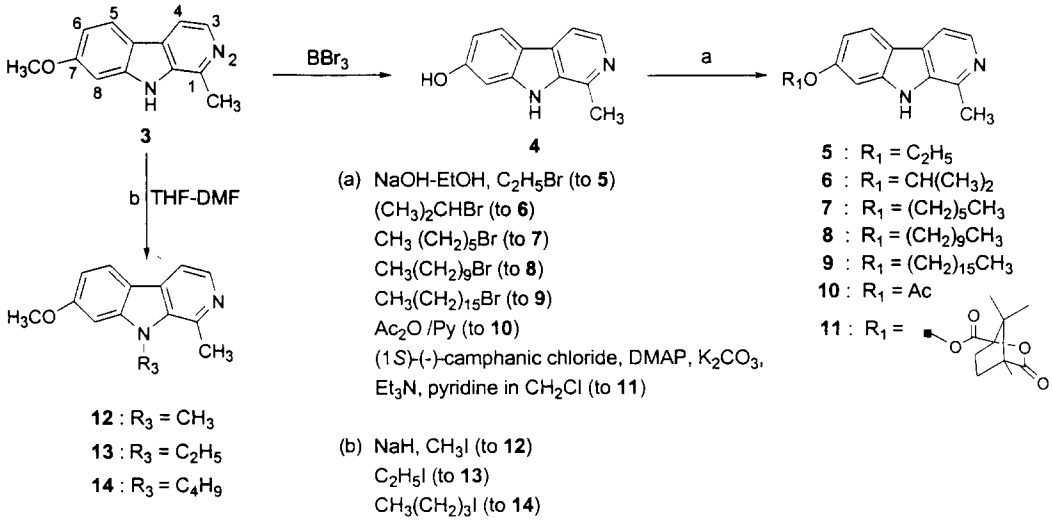
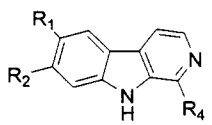
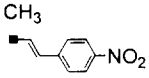
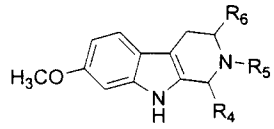
Scheme 1.

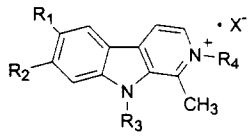




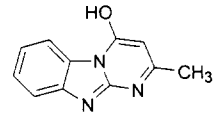
1 : $R_1 = R_2 = R_4 = H$
2 : $R_1 = R_2 = H$, $R_4 = CH_3$
15 : $R_1 = Br$, $R_2 = OCH_3$, $R_4 = CH_3$
16 : $R_1 = H$, $R_2 = OCH_3$, $R_4 =$ 



23 : $R_4 = CH_3$, $R_5 = NO$, $R_6 = H_2$
24 : $R_4 = (CH_2)_7CH_3$, $R_5 = H$, $R_6 = COOH$
25 : $R_4 = CH(C_2H_5)C_2H_5$, $R_5 = H$, $R_6 = COOH$



17 : $R_1 = R_2 = R_3 = R_4 = H$, $X = CH_3SO_4$
18 : $R_1 = R_3 = H$, $R_2 = OCH_3$, $R_4 = CH_3$, $X = I$
19 : $R_1 = Br$, $R_2 = OCH_3$, $R_3 = R_4 = H$, $X = Br$
20 : $R_1 = H$, $R_2 = OCH_3$, $R_3 = C_3H_7$, $R_4 = CH_3$, $X = I$
21 : $R_1 = R_3 = H$, $R_2 = OCH_3$, $R_4 = C_3H_7$, $X = I$
22 : $R_1 = R_3 = R_4 = H$, $R_2 = OCH_3$, $X = Br$; 3,4-dihydro



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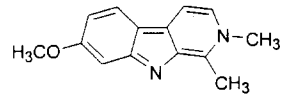

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Table 1. In vitro *cytotoxicity* of harmine and β-carboline analogs

Compounds	ED_{50} (μg/mL)[a]										
	KB[b]	A549[b]	CAKI-1[b]	MCF-7[b]	1A9[b]	SaOS-2[b]	HOS[b]	HCT-8[b]	SK-MEL-2[b]	U-87-MG[b]	HEL[b]
2	8.9	9.3	8.0	19.0	6.1	ND[c]	ND	ND	>20	19.0	9.8
3	2.2	2.4	1.9	10.5	1.6	ND	ND	ND	18.5	14.5	1.9
4	4.4	6.5	9.2	9.2	3.8	ND	ND	ND	ND	4.2	ND
5	10.2	5.0	9.0	5.0	8.0	20.0	ND	ND	9.5	ND	ND
6	12.8	17.0	12.3	11.9	7.9	ND	10.2	NA[d]	NA	16.4	ND
7	2.4	4.4	2.2	3.1	1.9	ND	2.5	3.8	>5	4.2	ND
8	2.0	1.2	5.0	3.8	1.4	ND	6.7	4.1	ND	5.4	ND
9	NA	5	NA	NA	8	ND	5	NA	ND	NA	ND
10	19.5	4.3	10.2	7.5	4.5	>20	ND	ND	5.0	ND	ND
11	>10	17.0	>20	20.0	19.5	>20	ND	ND	>20	ND	ND
12	2.2	4.2	5.0	4.3	1.8	ND	ND	>20	ND	7.8	ND
13	1.8	4.2	7.4	5.3	1.8	ND	ND	>20	ND	7.4	ND
14	7.8	6.0	11.0	9.5	2.2	ND	ND	>20	ND	12.3	ND
15	7.9	13.6	6.7	14.6	2.3	ND	6.5	16.0	>20	14.0	ND
16	0.5	0.9	7.0	1.2	0.3	0.9	ND	ND	1.0	1.2	ND
17	10.5	>20	>20	ND	>20	ND	ND	ND	ND	ND	>20
18	7.2	0.9	8.9	6.2	7.0	8.2	ND	ND	8.4	6.2	ND
19	3.5	3.9	7.3	5.6	1.9	6.8	ND	ND	5.0	6.7	ND
20	1.8	4.0	9.2	>20	<1.3	ND	ND	ND	18.0	>20	15.0
21	5.0	15.0	15.0	ND	2.0	ND	ND	ND	ND	ND	9.5
22	9.7	9.8	>10	10.0	>10	6.8	ND	ND	>10	9.8	ND
23	4.7	>10	>10	>10	>10	>10	ND	ND	>10	>10	ND
24	>20	>20	>20	ND	>20	ND	ND	ND	ND	ND	NA
25	20	>20	>20	ND	>20	ND	ND	ND	ND	ND	>20
26	>10	>10	>10	>10	>10	>10	ND	ND	>10	NA	ND
27	6.4	2.7	7.8	5.6	7.0	9.6	ND	ND	10.0	9.0	ND

[a]Cytotoxicity as ED_{50} for each cell line, is the concentration of compound that caused a 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay. [b]Cell lines include epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A549), renal cancer (CAKI-1), breast cancer (MCF-7), ovarian cancer (1A9), osteosarcoma (SaOS-2), osteosarcoma (HOS), ileocecal carcinoma (HCT-8), melanoma cancer (SK-EL-2), glioblastoma (U-87-MG), and embryonic lung fibroblast (HEL). [c]ND= not tested. [d]NA = not active at the highest concentration tested.

of DMAP, K_2CO_3, Et_3N and dry pyridine and CH_2Cl_2 afforded **11** in 20% yield.

Cytotoxicity Assays. In vitro cytotoxicity assays were carried out using microtitre plate cultures and sulforhodamine B (SRB) staining according to procedures described in Rubinstein et al.[6] Cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μg/mL Kanamycin. Cultures were propagated at 37 °C in a humified atmosphere containing 5% CO_2. Cell lines were obtained from the Lineberger Comprehensive Cancer Center (UNC-CH) except the KB-resistant sub-lines. Their isolation and characterization are described in detail elsewhere.[7,8] Drug stock solutions were prepared in DMSO. The final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentrations without effect on cell replication. The human tumor cell line panel consisted of epiderimoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), renal cancer (CAKI-1), breast cancer (MCF-7), melanoma cancer (SK-MEL-2), ovarian cancer (1A9), glioblastoma (U-87-MG), osteosarcoma (SaOS-2), osteosarcoma (HOS), and embryonic lung fibroblast (HEL). The human KB drug-resistant sub-line panel included KB-7d (pleotrophic resistance including MRP multidrug resistant protein), KB-VIN (expressing P-glycoprotein), and KB-CPT (pleotrophic mechanism including reduced level of topoisomerase I).

Results and Discussion. Twenty-six β-carboline derivatives (**2–27**) were tested for cytotoxicity against a panel of human tumor cell lines. The results are summarized in Table 1. Harmine (**3**) showed potent cytotoxic activity against KB, A549, CAKI-1, 1A9 and HEL cells with ED_{50} values of 2.2, 2.4, 1.9, 1.6, and 1.9 μg/mL. Compounds **2**, **11**, **17**, and **22–26** were inactive. Compound **16**, which has an α-4-nitrobenzylidene at the 1-position, was the most active compound in this study.

In addition, we examined **2**, **3**, and **20** in three drug-resistant KB cell lines (Table 2), including KB-7d, KB-VIN, KB-CPT. Compound **3** (harmine) exhibited cytotoxic effects in three drug-resistant cell lines, but again, **2** was not active. Compound **20** was cytotoxic against the KB-CPT cell line, but KB-VIN and KB-7d cells were cross-resistant, suggesting that this analog may be a substrate for the two types of drug-efflux pump expressed.

Table 2. Activity of selected compounds against drug resistant tumor cell lines.

	ED_{50} (μg/ml)[a]			
Compound	**KB**[b]	**Kb-7d**[b]	**KB-VIN**[b]	**KB-CPT**[b]
2	8.9	16.5	17.5	15.0
3	2.2	3.7	2.5	4.3
20	1.8	35.5	>40(11)	3.0

[a]Cytotoxicity as ED_{50} for each cell line, is the concentration of compound that caused a 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay. [b]The human KB drug-resistant SVB-lines, including KB-7d (pleotrophic resistance including MRP multidrug resistant protein), KB-VIN (expressing P-glycoprotein) and KB-CPT (pleotrophic mechanism including reduced level of topoisomerase I) are described elsewhere.[6,7]

From the above data, the following conclusions were drawn. (a) Introducing an oxygenated substituent

at C-7 led to enhanced cytotoxic activity (compare **3** with unsubstituted **2**). (b) The length of the C-7 alkoxy chain affected both cytotoxicity and cell line specificity. Compounds **7** (hexyloxy) and **8** (decyloxy) were more active than **3** (methoxy) against the MCF-7 (ED_{50} 3.1 and 3.8 μg/mL, respectively) cell line and **8** was more active against the A549 (ED_{50} 1.2 μg/mL) cell line. In other cell lines, the three compounds were comparable. Conversely, **5** (ethoxy), **6** (isopropoxy), and **9** (hexadecyloxy) were not significantly cytotoxic in any cell line. Therefore, cytotoxicity was maximal with a six to ten carbon C-7 alkoxy group. (c) N-Alkylated **3**-derivatives (**12–14**, **20**) showed strong cytotoxic effects against KB and 1A9 cell lines. (d) Bromination of **3** at C-6 gave **15** (6-bromoharmine), which showed selective activity against the 1A9 cell line. (e) The 3,4-dihydro-β-carbolines (**17**, **22–25**) were inactive. However, **27**, an N-methyl derivative, was selectively active against the A549 cell line with an ED_{50} value of 2.7 μg/mL. Thus aromaticity of the pyridine ring may be important for antitumor activity. (f) The N-alkyl salts (**18–21**) showed cell line specific cytotoxicity. For example, harmine methiodide (**18**) showed selective activity against the A549 cell line. (g) Substitution at the 1-position by α-4-nitrobenzylidene led to (**16**), which exhibited increased activity against most of the cell lines tested.

In summary, the position and nature of substituents on the harmine nucleus seem to modulate antitumor activity. Within the series of β-carboline derivatives (**2–26**), selective activities against KB, A549 and 1A9 cell lines were observed. Synthesis of additional analogs and mechanism of action studies are ongoing to develop more potent compounds.

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References and Notes:

1. For the previous paper in the series, see Ohyama, M.; Tanaka, T.; Ito, T.; Iinuma, M.; Bastow, K.F.; Lee, K. H. "Antitumor Agents 200. Cytotoxicity of Naturally Occurring Resveratrol Oligomers and Their Acetate Derivatives," *Bioorg. Med. Chem.* **1999**, *9*, 3057.
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9. (**9**): colorless needles, mp: 110–113 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7 Hz), 1.27 (12H, m), 1.48 (2H, m), 1.83 (2H, m), 4.03 (2H, t, *J* = 7 Hz), 6.89 (1H, dd, *J* = 2,8 Hz), 6.93 (1H, d, *J* = 2 Hz), 7.72 (2H, d, *J* = 5 Hz), 8.70 (1H, br s) *J* = 2 Hz), 6.79 (1H, br s), 7.05 (2H, d, *J* = 8 Hz), 7.08 (2H, d, *J* = 8 Hz), 7.22 (2H, d, *J* = 8 Hz); FABMS: *m/z* 338.2354, $C_{22}H_{30}N_2O$, requires 338.2358.