Antitumor Agents 253. Design, Synthesis, and Antitumor Evaluation of Novel 9-Substituted Phenanthrene-Based Tylophorine Derivatives as Potential Anticancer Agents

Linyi Wei,[†] Qian Shi,[‡] Kenneth F. Bastow,[§] Arnold Brossi,[†] Susan L. Morris-Natschke,[†] Kyoko Nakagawa-Goto,[†] Tian-Shung Wu,[||] Shiow-Lin Pan,[⊥] Che-Ming Teng,[⊥] and Kuo-Hsiung Lee*[,†]

Natural Products Research Laboratories and Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, AndroScience Corporation, 11175 Flintkote Ave., Suite F, San Diego, California 92121, National Research Institute of Chinese Medicine, Taipei, Taiwan, and Pharmacological Institute, College of Medicine, National Taiwan University, No. 1, Jen-Ai Road, Sec. 1, Taipei, Taiwan

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C9-Substituted phenanthrene-based tylophorine derivatives (PBTs) (**13**−**36**) were synthesized and evaluated as in vitro anticancer agents against the human A549 lung cancer cell line. Twelve active compounds were further examined against DU-145 (prostate), ZR-751 (breast), KB (nasopharyngeal), and KB-Vin (multidrug resistant KB subline) human cancer cell lines. They showed potent cytotoxic activity against both wild type and matched multidrug resistant KB cell lines, and displayed notable selectivity toward DU-145 (prostate) and ZR-751 (breast) cancer cell lines. The mode of action of this class may be distinctly different from that of other cancer chemotherapeutic compounds. Three PBT analogs were also evaluated in a murine model. Compound **24b** showed modest in vivo antitumor activity against human A549 xenograft in nude mice as well as potent in vitro cytotoxic activity, and thus, is a promising anticancer lead compound.

Introduction

Several plants of the *Tylophora* genus have been used medicinally as anti-inflammatory, antiarthritis, and antiamoebic agents in East Asian countries.[1−3] The biologically active constituent, (-)-*R*-tylophorine (**1**) (Chart 1), and its phenanthroindolizidine alkaloid analogs (also known as tylophora alkaloids) were isolated primarily from plants of the family Asclepiadaceae,[4−7] including members of the genera *Tylophora*, *Vincetoxicum*, *Pergularia*, and *Cynanchum*. In addition to various traditional therapeutic uses, tylophora alkaloids have been the target of synthetic modification for many years because of their profound cytotoxicity.[8−11]

Tylocrebrine (**2**) (Chart 1), a positional isomer of **1**, failed in clinic trials in 1966 due to a central nervous system (CNS) toxicity, manifested by ataxia and disorientation. This disappointing clinical result discouraged further consideration of these alkaloids for drug development. However, in the 1990s, tylophorine analogs that previously were deemed not to warrant further research were rescreened for antitumor potential by the National Cancer Institute (NCI) using a 60-tumor cell line panel. These compounds showed potent and uniform activity against 54 human tumor cell lines with mean $GI_{50} < 10^{-8}$ M. Moreover, tylophorines F (**3**) and G (**4**) were quite active toward refractory cancer cell lines including melanoma and lung cancer.[12]

Recent studies[13,14] have shown that tylophorine analogs exhibit potent cytotoxic activity against a broad range of human cancer cells and sublines resistant or cross-resistant to various conventional anticancer drugs, such as etoposide (VP-16), paclitaxel, topotecan, adriamycin, cytosine arabinoside, gemcitabine, hydroxyurea, or camptothecin. Gao et al.[13] also found that (+)-*S*-tylophorine (**5**, NSC-717335), a stereoisomer of **1**, significantly inhibits activator protein-1, cAMP response element, and nuclear factor κB (NF-κB) mediated transcription. NF-kB has been suggested to be a mechanism of drug resistance because of its antiapoptotic role.[15] Other research implicates NF-kB in the regulation of p-glycoprotein, a well-known mechanism of multidrug resistance to chemotherapy.[16] These recent discoveries imply that active tylophorine compounds may possess a novel mechanism of anticancer action.

Although the phenanthroindolizidine alkaloid tylocrebrine (**2**) previously failed in clinical trials due to CNS toxicity, the very profound cytotoxicity of these alkaloids, particularly against multidrug resistant cancer cells, sparked our interest. As we previously reported, a series of novel polar water-soluble phenanthrene-based tylophorine derivatives (PBTs[a]) showed $IC_{50} \cong 10^{-7}$ M against the A549 human lung cancer cell line.[22] These compounds could possibly have lower or no CNS toxicity because their increased polarity should prevent them from penetrating the blood−brain barrier. Preliminary structure−activity relationship (SAR) studies identified 2,3-methylenedioxy-6-methoxyphenanthrene as an optimized core structural unit that incorporated all of the favorable modifications identified at the C-2, C-3, and C-6 positions. An *N*-containing substituent at the C-9 position is also required for cytotoxic activity, and this site presents an ideal position for introducing more polar, water-solubility-enhancing moieties, such as a terminal hydroxyl or carboxylic acid. These critical SAR clues prompted us to explore additional C-9 modification to better understand the SAR of the PBT compounds.

In this report, we describe new developments toward the design and synthesis of novel C-9 substituted PBTs. Several *N*-containing cyclic and acyclic terminal-hydroxyl moieties were introduced at the C9 position in order to explore and optimize the activity profiles of novel C-9 substituted PBTs. In addition, we extended our in vitro anticancer evaluation of the most active compounds to additional significant tumor types, including a

* To whom correspondence should be addressed. Phone: 919-962-0066. Fax: 919-966-3893. E-mail: khlee@unc.edu.
[†] Natural Products Research Laboratories, University of North Carolina.
[§] Division of Medicinal Chemistry and Natural Products, University of North Carolina.
[‡] AndroScience Corp.
[||] National Research Institute of Chinese Medicine.
[⊥] National Taiwan University.

[a] Abbreviations: PBTs, phenanthrene-based tylophorine derivatives.

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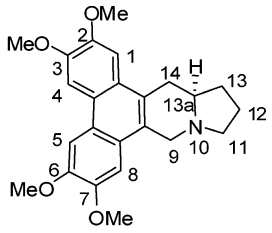
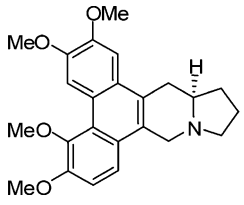
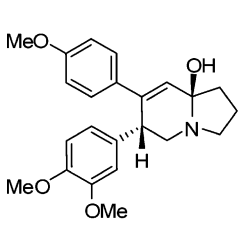
Chart 1



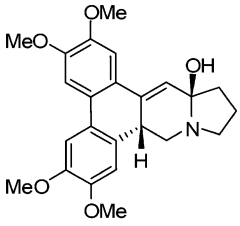
(-)-*R*-Tylophorine (**1**)



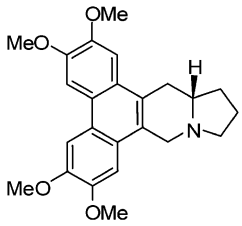
Tylocrebrine (**2**)



Tyloindicine F (**3**)

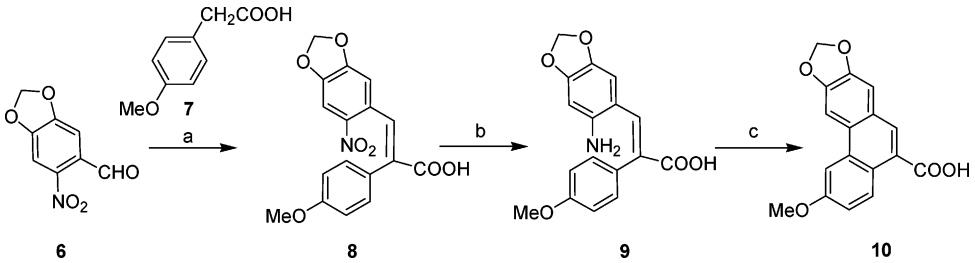


Tyloindicine G (**4**)



(+)-*S*-Tylophorine (**5**)

Scheme 1[a]



[a] Reagents and conditions: (a) Ac_2O/Et_3N; (b) $FeSO_4/NH_4OH$; (c) $NaNO_2$/fluoroboric acid, ferrocene/acetone.

multidrug resistant subline. The results are discussed relative to their mechanism of action. In addition, the in vivo anticancer effects of selected analogues were investigated in a xenograft model of human lung tumor cells (A549) in male SCID mice.

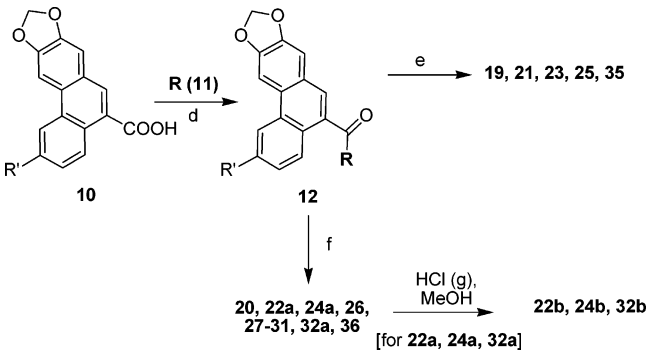
Chemistry

Compounds **13**−**32b** were synthesized from commercially available 3,4-methylenedioxy-6-nitrobenzaldehyde (**6**) and 4-methoxyphenylacetic acid (**7**) according to our previously published method. Briefly, 2,3-methylenedioxy-6-methoxyphenanthrene-9-carboxylic acid (**10**) was obtained through a Perkin reaction and an improved free-radical Pschorr cyclization (Scheme 1)[17,18] in a satisfactory overall yield of 78%. Compound **10** was then condensed with the appropriate amines (R) (**11**) in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 4-(dimethylamino)pyridine (DMAP), and 1-hydroxybenzotriazole (HOBT) (Scheme 2) to form **12**. The target carboxylic acid analogs (**19**, **21**, **23**, **25**, **35**) were generated by selective reduction of the amide carbonyl group with borane−methyl sulfide complex (BMS) followed by basic hydrolysis. The target hydroxyalkyl analogs (**20**, **22a**, **24a**, **26**−**31**, **32a**, **36**) were produced by global reduction of the amide carbonyl and terminal methyl ester with lithium aluminum hydride ($LiAlH_4$) (Scheme 2). The hydrochloride salts (**18b**, **22b**, **24b**, **32b**) were made from the respective parent compounds (**18a**, **22a**, **24a**, **32a**) by treatment with methanolic HCl (Scheme 2).

Results and Discussion

In vitro anticancer activity of the target compounds (**13**−**36**) was evaluated first in-house against the human A549 lung cancer cell line using the cell-based sulforhodamine B (SRB) microtiter

Scheme 2[a]

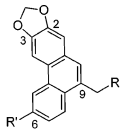


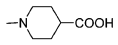
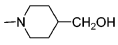
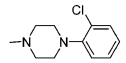
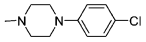
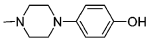
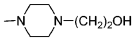
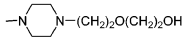
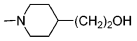
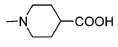
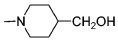
[a] Reagents and conditions: (d) EDC, DMAP, HOBt/DMF, NMM; (e) (1) BMS/THF, (2) NaOH/MeOH; (f) $LiAlH_4$/THF. See Table 1 for structures of final compounds.

plate assay.[19] Compound structures and cytotoxic activity data are shown in Table 1. Twelve active compounds were further evaluated against three additional cancer cell lines (KB, DU145, and ZR-751) and one resistant subline (KB-Vin) (Table 2). Antofine, emetine, doxorubicin, and etoposide were used as reference compounds. The data are shown in Table 2. Finally, three PBT analogs (**22b**, **24b**, and **34b**) were evaluated in vivo with a xenograft A549 model in male SCID mice

Structure−Activity Relationship (SAR) Studies. Twenty-one out of 24 PBT compounds were active against the A549 cell line, with IC_{50} values ranging from 0.08 (**24a**) to 5.2 (**19**) μM. Twelve compounds had IC_{50} values less than 1.0 μM. Thus, a wide variety of amines (alkylamine, pyrrole, piperidine, piperazine) bearing O-containing substituents (terminating in carboxyl or hydroxyl) resulted in active analogs. The stereo-

Table 1. Data for PBT Analogs **13**−**36** against the A549 Cell Line



Compound	R	R′	IC_{50} (μM) A549
13	-NH(CH$_2$)$_4$COOH	OMe	1.3
14	-NH(CH$_2$)$_5$OH	OMe	0.27
15		OMe	2.1
16		OMe	0.7
17		OMe	0.5
18a		OMe	0.16
19		OMe	5.2
20		OMe	1.1
21	-NH(CH$_2$)$_5$COOH	OMe	0.8
22a	-NH(CH$_2$)$_6$OH	OMe	0.2
23		OMe	0.23
24a		OMe	0.08
25	-NH(CH$_2$)$_{10}$COOH	OMe	3.2
26	-NH(CH$_2$)$_{11}$OH	OMe	2.6
27		OMe	NA
28		OMe	65.2
29		OMe	0.22
30		OMe	0.63
31		OMe	57.1
32a		OMe	0.15
33		$OCH_2C_6H_5$	3.2
34		$OCH_2C_6H_5$	1.3
35		$OCH_2C_6H_5$	2.1
36		$OCH_2C_6H_5$	0.7
Emetine			0.04
Doxorubicin			0.18
Etoposide			1.4
Antofine			0.008
Vincristine			0.04

chemistry (2′-pyrrole, e.g., **15** versus **19**) and geometry (2′- or 4′-piperidine, **17** versus **23**) of the O-substituent affected the specific IC_{50} values, but the differences in potency were often not large. Compounds with both carboxy and hydroxy termini were active, and reduction of a carboxylic acid to a hydroxymethyl resulted in a lower IC_{50} value (e.g., **14** versus **13**).

However, changing the hydroxy terminus of the potent **29** to chlorine in **28** abolished activity and the ortho-chlorinated analog **27** was also inactive. Thus, the presence of a hydrogen-bond-acceptor/-donor group at the C-9 chain terminus appears essential for cytotoxic activity. A hydrogen-bonding interaction between the terminal group and a biological target may play an important role in binding the hypothetical target, and the spacing between this group and the remainder of the molecule is also important, on the basis of the findings below.

We investigated the optimal distance between the nitrogen and terminal polar substituent by incorporating various polar acyclic and cyclic amines. With acyclic side chains, a shorter (five- or six-carbon) spacing resulted in lower IC_{50} values than a longer (10- or 11-carbon) distance (e.g., **13** and **21** versus **25**, and **14** and **22a** versus **26**). More notably, analogs with shorter hydroxyethyl (**30**) and hydroxyphenyl (**29**) substituents on a piperazine cyclic side chain were quite active, but the longer hydroxyethylethoxy piperazine analog (**31**) lost all activity.

Anticancer Spectra and Drug-Resistance Study. In addition, we extended our in vitro anticancer evaluation of the most active compounds to additional significant tumor types and one multidrug resistant subline. Twelve active compounds [including four hydrochloride salts (**18b**, **22b**, **24b**, **32b**) of corresponding

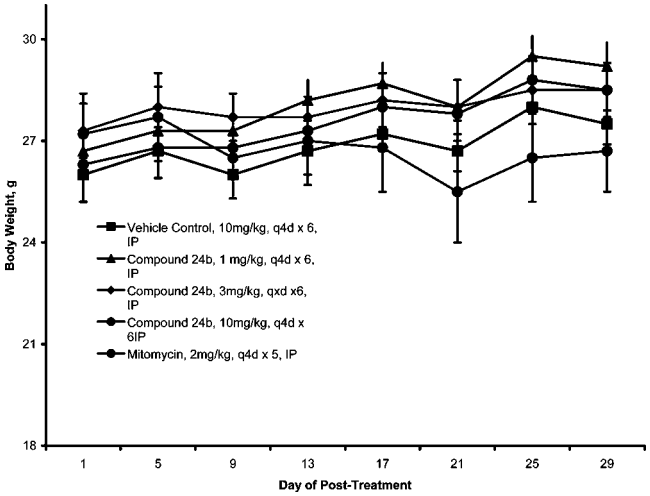


Figure 1. Body weight curves in SCID mice treated with **24b**.

Table 2. Data for Selected PBT Analogs against Five Cancer Cell Lines

	IC_{50} (μM)				
compd	A549	KB	DU-145	ZR-751	KB-VIN
18a	0.2	0.12	0.21	0.1	0.16
18b	0.16	0.12	0.09	0.08	0.09
21	0.5	0.33	0.67	0.54	0.83
22a	0.16	0.25	0.04	0.13	0.35
22b	0.13	0.23	0.07	0.08	0.16
23	0.23	0.15	0.31	0.25	0.38
24a	0.08	0.24	0.03	0.03	0.09
24b	0.07	0.07	0.05	0.04	0.04
29	0.22	0.13	0.15	0.18	0.34
30	0.63	0.50	0.25	0.23	0.51
32a	0.15	0.42	0.15	0.03	0.50
32b	0.09	0.20	0.1	0.02	0.20
antofine	0.008	0.013	0.009	0.008	0.009
emetine	0.04	0.04	0.06	0.1	>2
doxorubicin	0.18	0.18	0.13	0.04	>4
etoposide	1.4	4.8	2.4	3.1	>10
vincristine	0.04	0.006	0.085	0.04	5.3

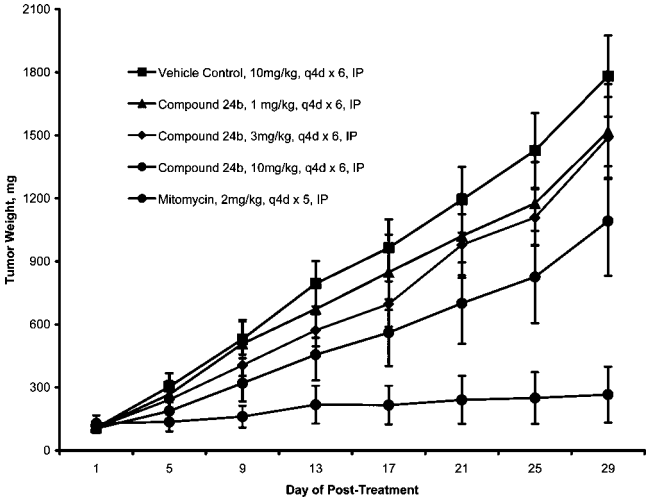


Figure 2. Tumor growth curves in SCID mice treated with **24b**.

active free bases (**18a**, **22a**, **24a**, **32a**)] were further screened against an extended panel of human tumor cell lines including DU-145 (prostate), ZR-751 (breast), KB (nasopharyngeal), and KB-Vin (multidug-resistant KB subline) in order to explore their anticancer spectra and drug-resistance profiles. Four compounds were used as reference compounds. Antofine is a positional isomer of **1** and was isolated from *Asclepiadaceae* by Dr. T. S. Wu[21] in Taiwan. Emetine is a protein synthesis inhibitor that exhibits cross-resistance with tylocrebine in mutant CHO cells.[19] Doxorubicin, vincristine, and etoposide are widely used anticancer agents for treating a range of solid tumors. The results are illustrated in Table 2. In general, all new compounds exhibited potent activity against KB (IC_{50} 0.07−0.50 μM), DU-145 (IC_{50} 0.03−0.67 μM), and ZR-751 (IC_{50} 0.02−0.54 μM) cell lines, as well as multidrug resistant subline KB-Vin (IC_{50} 0.04−0.83 μM) cells. The corresponding salts showed similar selectivity patterns and slightly better potency than the free bases, possibly due to improved water solubility. Compared to the reference compounds emetine, doxorubicin, etoposide, and vincristine, neither the C-9 substituted PBTs nor antofine showed any cross-resistance with the KB-Vin resistant cell line, suggesting that they are not substrates for p-glycoprotein and their mode of action may be distinctly different from that of other currently used cancer chemotherapeutic compounds. Compound **24a** and its salt **24b** exhibited the highest potency, with IC_{50} values less than 100 nM against the tumor cell line panel. In addition, both compounds also showed noticeable selectivity against ZR-751 (breast) cancer cells, and compound **22a** and its salt **22b** selectively inhibited DU-145 (prostate) cancer cell line replication, suggesting that further exploration of these compounds as selective anticancer drug leads will be worthwhile.

In Vivo Study. In the xenograft model of human lung tumor cells (A549, ATCC CCL-185) in male SCID mice, hydrochloride salts **22b**, **24b**, and **32b** at 10, 3, and 1 mg/kg were administered by intraperitoneal (ip) injection at a 4-day interval (q4d) for a total of six doses. The tumor size, body weight, and signs of overt toxicity following test compound dosing were monitored and recorded for the duration of the experiment (29 days). Tumor growth inhibition was calculated as (treatment/control) × 100%.

In comparison with the vehicle-treated control group, none of the three compounds were associated with any changes in body weight or overt toxicity (Figure 1, **24b** shown). Administration of **22b** and **32b** under the above conditions also did not cause any significant inhibition of tumor weight relative to the vehicle control during the experiment period of 29 days. However, analog **24b** at 10 mg/kg caused significant tumor inhibition on day 5 and moderate growth inhibition from day 9 to day 29 (the tumor growth data in terms of percent tumor weight relative to the vehicle control group are shown Figure 2).

Conclusions

Novel 9-substituted 2,3-methylenedioxy-6-methoxy-PBTs were synthesized and exhibited potent cytotoxic activity against a limited but diverse panel of human tumor cell lines, including a multidrug-resistant variant. The IC_{50} values were in the submicromolar range, comparable with the activity of the two frontline antineoplastic drugs used as positive controls, and the new compounds had a superior drug-resistance profile. Combination of a 2,3-methylenedioxy-6-methoxyphenanthrene skeleton with a C-9 cyclic piperidine ring and para terminal hydroxyl side chain generated the most potent PBTs. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-L-4-piperidinemethanol (**24a**) had IC_{50} values less than 100 nM against the cell line panel, and the activity was maintained by formation of the corresponding hydrochloride salt (**24b**). These PBT salts are the first reported water-soluble tylophorine derivatives with significant cytotoxic activity, particularly against multidrug-resistant cells. In addition, analog **24b** showed activity in a murine model without overt toxicity to the animal and, thus, has promise as a lead anticancer compound. Studies will continue to further establish the suitability of PBT analogs as clinical trial candidates for cancer (especially refractory cancer) treatment.

Experimental Section

Melting points were measured using a Fisher Johns melting apparatus without correction. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker 400 MHz spectrometer using TMS as internal standard. $CDCl_3$ was used as the solvent unless indicated. Mass spectra were recorded on a PE-Sciex API-3000 LC/MS/MS instrument equipped with a Turbo IonSpray ion source. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. All active target compounds were analyzed for C, H, N. Thin-layer chromatography (TLC) was performed on PLC silica gel 60 F_{254} plates (0.5 mm, Merck). Biotage Flash+ and Isco Companion systems were used for medium-pressure column chromatography. Silica gel (200−400 mesh) from Aldrich, Inc. was used for column chromatography. 4-Benzyloxyphenylacetic acid and 3,4-methylenedioxy-6-nitrobenzaldehyde were purchased from TCI. Isonipecotic acid and L-pipecolinic acid were commercially available from Lancaster. All other chemicals were obtained from Aldrich, Inc. and Fisher, Inc.

4,5-Methylenedioxy-(4-methoxyphenyl)-2-nitrocinnamic Acid (8). A solution of **6** (12 mmol), triethylamine (12 mmol), and **7** (17 mmol) in acetic anhydride (20 mL) was refluxed with stirring under Ar for 40 min.[20] Water (30 mL) was added to the reaction mixture, and during the addition the temperature was maintained between 90 and 100 °C. The reaction mixture was cooled to room temperature and the resulting solid was collected by filtration and recrystallized from EtOH: 91% yield; yellow powder; mp 184−185 °C; ^{1}H NMR (400.13 MHz) δ 7.92 (s, 1H), 7.53 (s, 1H), 7.01 (d, J = 2 Hz, 2H), 6.73 (d, J = 2 Hz, 2H), 6.22 (s, 1H), 5.96 (s, 2H), 3.74 (s, 3H); ESI MS m/z 344 $(M + H)^+$.

4,5-Methylenedioxy-(4-methoxyphenyl)-2-aminocinnamic Acid (9). To a solution of **8** (7 mmol) in 10% aqueous NH_4OH (100 mL) was added ferrous sulfate heptahydrate (15 g) dissolved in distilled water (100 mL) and concentrated aqueous NH_4OH (100 mL). The reaction mixture was refluxed for 1.5 h, cooled to 40 °C, filtered through Celite, and acidified with HOAc (100 mL). The resulting solid was collected by filtration, and recrystallization from

EtOH yielded the aminostilbenic acid: 95% yield; yellow powder; mp 165−166 °C; ^{1}H NMR (400.13 MHz) δ 7.74(s, 1H), 7.08 (d, J = 2 Hz, 2H), 6.80 (d, J = 2 Hz, 2H), 6.17 (s, 1H), 6.06 (s, 1H), 5.70 (s, 2H), 3.80 (s, 2H), 3.73 (s, 3H); ESI MS m/z 314 $(M + H)^+$.

2,3-Methylenedioxy-6-methoxyphenanthrene-9-carboxylic Acid (10). A solution composed of **9** (3 mmol), NaOH (33 mmol), and $NaNO_2$ in water (10 mL) was added to 48% fluoroboric acid (43 mmol) dropwise over 30 min with stirring at 0−5 °C. The mixture was further stirred for 1 h, after which sulfamic acid was added until the mixture showed a negative result on starch−iodide paper. The crude solid was collected by filtration and then dissolved in anhydrous acetone (10 mL). This solution was added dropwise with stirring to a solution of ferrocene (0.056 g, 0.3 mmol) in acetone over a 15 min period at room temperature. After an additional 15 min of stirring, the green reaction mixture was added to water (100 mL). A light-yellow precipitate was collected and the trace amount of ferrocene was removed in vacuo to afford the phenanthroic acid: 92% yield; white powder; mp 293−295 °C; ^{1}H NMR (400.13 MHz) δ 7.67 (s, 1H), 7.60 (d, J = 4 Hz, 1H), 7.22 (dd, J = 4 Hz, 2 Hz, 1H), 6.92 (s, 1H), 6.89 (d, J = 2 Hz, 1H), 6.70 (s, 1H), 5.98 (s, 2H), 3.79 (s, 3H); ESI MS m/z 297 $(M + H)^+$.

General Procedure for the Preparation of Methyl Esters of Amino Acids (R) 11. A. Cycloalkylamino Acids. To a solution of cycloalkylamino acid (4 mmol) in dry MeOH (4 mL) was added dropwise $SOCl_2$ (0.4 mL) at −30 °C. The reaction mixture was warmed to room temperature and heated to reflux for 1 h. Then the solvent was removed in vacuo and the product was used in the next reaction without further purification.

B. Acyclic Alkylamino Acids. To a solution of dry MeOH (3 mL) was added dropwise acetyl chloride (0.45 mL) at 0 °C. After 10 min of stirring, the amino acid was added to the solution in portions. The mixture was warmed to room temperature and then heated to reflux for 2 h. The solvent was removed in vacuo and the product was used in the next reaction without further purification.

General Procedure for the Preparation of 12. To a solution of **10** (4 mmol), DMAP (2 mmol), HOBT (4 mmol), and the appropriate amino acid methyl ester (4.4 mmol) in 20 mL of DMF was added *N*-methylmorpholine (NMM) (1.03 mL). After the mixture was stirred at 0 °C for 15 min, EDC (4.4 mmol) was added in portions. The reaction mixture was stirred overnight at room temperature and partitioned between EtOAc and water. The organic layer was washed with brine, saturated $NaHCO_3$, and 1 N HCl; dried over Na_2SO_4; and concentrated in vacuo. The crude product was chromatographed using Biotage Flash+ and Isco Companion systems on a 40 g silica cartridge with EtOAc/hexane as eluant.

General Procedure for the Preparation of 19, 21, 23, 25. To a stirred solution of **12** (2 mmol) in THF (20 mL) was added dropwise BMS (4 mL, 2.0 M solution in THF). The reaction mixture was stirred at room temperature overnight and quenched with 1 N HCl. After removing THF in vacuo, the residue was partitioned between CH_2Cl_2 and water. The organic layer was dried, filtered, and evaporated to afford the 9-ylmethyl ester. The crude ester was chromatographed using Biotage Flash+ and Isco Companion systems with MeOH/CH_2Cl_2 as eluant. A solution of pure ester, 4 N NaOH, and MeOH (1:1) was refluxed for 4 h. The reaction mixture was acidified and partitioned between 10% MeOH/CH_2Cl_2 and 1 N HCl. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude product was chromatographed using Biotage Flash+ and Isco Companion systems with MeOH/CH_2Cl_2 as eluant to give the final target compound.

General Procedure for the Preparation of 20, 22a, 24a, 26, 27−31, 32a. To a suspension of **12** (1 mmol) in 15 mL of dry THF was added $LiAlH_4$ (1 g) in portions at 0 °C. After addition, the reaction mixture was refluxed for 4 h and then cooled to 0 °C. The reaction mixture was quenched with MeOH, and then 10% Rochelle salt was added. The reaction mixture was extracted with water and 10% MeOH/CH_2Cl_2. The organic layer was dried over Na_2SO_4, and the crude product was chromatographed using Biotage Flash+ and Isco Companion systems with MeOH/CH_2Cl_2 as eluant to give the final target compound. Compounds **33**−**36** were prepared in a similar manner from the 6-benzyloxyphenanthrene precursors.

General Procedure for Preparation of Hydrochloride Salts. To a suspension of the free base (3 mmol) in EtOH (15 mL) was added dropwise 1 equiv of 6 N HCl. After the free base was well-dissolved, the solvent was removed in vacuo and the resulting hydrochloride salt was dried over phosphorus pentoxide.

The physical and spectral data for **13**−**18a**, **33**, and **34** have been reported previously.[22]

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-L-2-piperidinem ethanol hydrochloride (18b):** mp 193−194 °C; ^{1}H NMR (400.13 MHz) δ 8.14 (d, J = 4 Hz, 1H), 8.00 (s, 1H), 7.78 (d, J = 2 Hz, 1H), 7.49 (s, 1H), 7.20 (dd, J = 4, 2 Hz, 1H), 7.15 (s, 1H), 6.04 (s, 2H), 4.20 (s, 2H), 3.90 (s, 3H), 3.72 (d, J = 17 Hz, 2H), 3.04 (m, 1H), 2.68 (m, 2H), 1.83 (m, 6H); ESI MS m/z 380 $(M + H)^+$. Anal. ($C_{23}H_{25}O_4N$·HCl·2.0H_2O) C, H, N,

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-D-proline (19):** General procedures d and e from **10** (81%); white powder; mp 152−153 °C; ^{1}H NMR (400.13 MHz) δ 8.21 (d, J = 4 Hz, 1H), 7.77 (s, 1H), 7.72 (d, J = 2 Hz, 1H), 7.56 (s, 1H), 7.23 (dd, J = 4, 2 Hz, 1H), 7.10 (s, 1H), 6.04 (s, 2H), 4.05 (s, 2H), 3.96 (s, 3H), 3.16 (m, 1H), 2.36 (m, 2H), 2.14 (m, 2H), 1.81 (m, 2H); ESI MS m/z 380 $(M + H)^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-D-prolinol (20):** General procedures d and f from **10** (95%); yellow syrup, recrystallization from EtOH gave white powder; mp 130−132 °C; ^{1}H NMR (400.13 MHz) δ 8.16 (d, J = 4 Hz, 1H), 7.88 (s, 1H), 7.80 (d, J = 2 Hz, 1H), 7.46 (s, 1H), 7.23 (dd, J = 4, 2 Hz, 1H), 7.14 (s, 1H), 6.07 (s, 2H), 4.03 (s, 2H), 3.94 (s, 3H), 3.70 (d, J = 17 Hz, 2H), 3.16 (m, 1H), 2.38 (m, 2H), 2.23 (m, 2H), 1.95 (m, 2H); ESI MS m/z 366 $(M + H)^+$. Anal. ($C_{22}H_{23}O_4N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-6-aminohexanoic acid (21):** General procedures d and e from **10** (78%); white powder; mp 167−169 °C; ^{1}H NMR (400.13 MHz) δ 7.88 (d, J = 4 Hz, 1H), 7.76 (s, 1H), 7.71 (d, J = 2 Hz, 1H), 7.44 (s, 1H), 7.13 (dd, J = 4, 2 Hz, 1H), 7.05 (s, 1H), 5.99 (s, 2H), 3.87 (s, 2H), 3.82 (s, 3H), 2.75 (m, 2H), 2.23 (t, J = 6 Hz, 2H), 1.53 (m, 2H), 1.44 (m, 2H), 1.28 (m, 2H); ESI MS m/z 396 $(M + H)^+$. Anal. ($C_{23}H_{25}O_5N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-6-aminohexanol (22a):** General procedures d and f from **10** (90%); light green syrup, recrystallization from EtOH gave pale green powder; mp 145−147 °C; ^{1}H NMR (400.13 MHz) δ 7.90 (d, J = 4 Hz, 1H), 7.84 (s, 1H), 7.78 (d, J = 2 Hz, 1H), 7.51 (s, 1H), 7.21 (dd, J = 4, 2 Hz, 1H), 7.14 (s, 1H), 6.03 (s, 2H), 4.34 (s, 2H), 3.94 (s, 3H), 3.48 (t, J = 6 Hz, 2H), 2.76 (m, 2H), 1.58 (m, 2H), 1.45 (m, 2H, 1.26 (m, 2H), 1.20 (m, 2H); MS (ESI) ESI MS m/z 382 $(M + H)^+$. Anal. ($C_{23}H_{27}O_4N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-6-aminohexanol hydrochloride (22b):** White powder; mp 187−188 °C; ^{1}H NMR (400.13 MHz) δ 8.14 (d, J = 4 Hz, 1H), 7.82 (s, 1H), 7.76 (d, J = 2 Hz, 1H), 7.50 (s, 1H), 7.18 (dd, J = 4, 2 Hz, 1H), 7.13 (s, 1H), 6.08 (s, 2H), 4.67 (s, 2H), 3.98 (s, 3H), 3.60 (t, J = 6 Hz, 2H), 3.44 (m, 2H), 1.66 (m, 2H), 1.59 (m, 2H), 1.40 (m, 2H), 1.33 (m, 2H); ESI MS m/z 382 $(M + H)^+$. Anal. ($C_{23}H_{27}O_4N$·HCl·1.5H_2O) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-L-4-piperidinecarboxylic acid (23):** General procedures **d** and **e** from **10** (85%); light yellow powder; mp 238−240 °C; ^{1}H NMR (400.13 MHz) δ 8.30 (d, J = 4 Hz, 1H), 7.92 (s, 1H), 7.83 (d, J = 1 Hz, 1H), 7.42 (s, 1H), 7.22 (dd, J = 4, 2,4 Hz, 1H), 7.17 (s, 1H), 6.09 (s, 2H), 4.04 (s, 2H), 3.66 (s, 3H), 2.98 (m, 1H), 2.58 (m, 4H), 1.75 (m, 4H); ESI MS m/z 394 $(M + H)^+$. Anal. ($C_{23}H_{23}O_5N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-L-4-piperidinem ethanol (24a):** General procedures **d** and **f** from **10** (92%); light green syrup, recrystallization from EtOH gave white powder; mp 130−132 °C; ^{1}H NMR (400.13 MHz) δ 8.26 (d, J = 4 Hz, 1H), 7.87 (s, 1H), 7.78 (d, J = 2 Hz, 1H), 7.38 (s, 1H), 7.19 (dd, J = 4, 2 Hz, 1H), 7.14 (s, 1H), 6.05 (s, 2H), 3.97 (s, 3H),

3.81(s, 2H), 3.43 (d, J = 7 Hz, 2H), 2.97 (m, 2H), 2.02 (m, 2H), 1.65 (m, 2H), 1.48 (m, 1H), 1.26 (m, 2H); ^{13}C NMR (Varian 300,) δ 157.8 (C-6), 147.6 (C-2 or C-3), 147.5 (C-2 or C-3), 131.8, 131.1, 128.3, 127.2, 125.7, 125.6, 125.3, 115.7, 105.6, 103.6, 101.2, 100.8, 68.1 (CH_2OH), 62.1 (CH_2N), 55.3 (4′-hydroxymethylpiperidinyl C-2′ or C-6′), 53.7 (OCH_3), 38.8 (4′-hydroxymethylpiperidinyl C-4′), 28.9 (4′-hydroxymethylpiperidinyl C-3′ or C-5′); ESI MS m/z 380 (M + H)$^+$. Anal. ($C_{23}H_{25}O_4N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-L-4-piperidinem ethanol hydrochloride (24b):** White powder; mp 256−258 °C; ^{1}H NMR (400.13 MHz) δ 8.00 (d, J = 4 Hz, 1H), 7.85 (s, 1H), 7.82 (s, 1H),7.77 (d, J = 2 Hz, 1H), 7.22 (dd, J = 4, 2 Hz, 1H), 7.20 (s, 1H), 6.03 (d, J = 4 Hz, 2H), 4.59 (s, 2H), 3.92 (s, 3H), 3.46 (d, J = 4 Hz, 2H), 3.31 (m, 2H), 2.77 (m, 2H), 1.76 (m, 4H), 1.68 (m, 1H); ESI MS m/z:380 (M + H)$^+$. Anal. ($C_{23}H_{25}O_4N$·HCl·H_2O) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-11-aminoundecanoic acid (25):** General procedures **d** and **e** from **10** (75%); white powder; mp 146−148 °C; ^{1}H NMR (400.13 MHz) δ 7.98 (d, J = 4 Hz, 1H), 7.80 (s, 1H), 7.72 (d, J = 2 Hz, 1H), 7.50 (s, 1H), 7.11 (dd, J = 4, 2 Hz, 1H), 7.06 (s, 1H), 6.05 (s, 2H), 4.02 (s, 3H), 3.86 (s, 2H), 2.93 (m, 2H), 2.2 (m, 2H), 1.67 (m, 2H), 1.53 (m, 2H), 1.21 (m, 12H); ESI MS m/z 466 (M + H)$^+$. Anal. ($C_{28}H_{35}O_5N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-6-aminoundecanol (26):** General procedures **d** and **f** from **10** (90%); light green syrup, recrystallization from EtOH gave a pale green powder; mp 122−124 °C; ^{1}H NMR (400.13 MHz) δ 7.86 (d, J = 4 Hz, 1H), 7.67 (s, 1H), 7.40 (d, J = 2 Hz, 1H), 7.43 (s, 1H), 7.18 (dd, J = 4, 2 Hz, 1H), 7.08 (s, 1H), 6.01 (s, 2H), 4.32 (s, 2H), 3.88 (s, 3H), 3.68 (t, J = 6 Hz, 2H), 2.86 (m, 2H), 1.68 (m, 2H), 1.58 (m, 2H), 1.26 (m, 14H); ESI MS m/z 452 (M + H)$^+$. Anal. ($C_{28}H_{37}O_4N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-*N*′-(4-chlorophenyl)piperazine (27):** General procedures d and f from **10** (87%); white powder; mp 196−198 °C; ^{1}H NMR (400.13 MHz) δ 8.32 (d, J = 4 Hz, 1H), 7.91 (s, 1H), 7.81 (d, J = 2 Hz, 1H), 7.44 (s, 1H), 7.32 (dd, J = 4, 2 Hz, 1H), 7.22 (d, J = 9 Hz, 1H), 7.18 (s, 1H), 7.16 (d, J = 9 Hz, 1H), 7.00 (m, 1H), 6.93 (m, 1H), 6.08 (s, 2H), 4.30 (s, 2H), 3.94 (s, 3H), 3.68 (t, J = 6 Hz, 4H), 3.05 (m, 4H); ESI MS m/z 461.5 (M + H)$^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-*N*′-(2-chlorophenyl)piperazine (28):** General procedures d and f from **10** (80%); white powder; mp 222−223 °C; ^{1}H NMR (400.13 MHz) δ 8.30 (d, J = 4 Hz, 1H), 7.90 (s, 1H), 7.81 (d, J = 2 Hz, 1H), 7.50 (s, 1H), 7.22 (dd, J = 4, 2 Hz, 1H), 7.19 (s, 1H), 7.17 (d, J = 9 Hz, 2H), 6.89 (d, J = 9 Hz, 2H), 6.09 (s, 2H), 4.68 (s, 2H), 3.90 (s, 3H), 3.65 (t, J = 8 Hz, 4H), 3.14 (m, 4H); ESI MS m/z 461.5 (M + H)$^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-*N*′-(4-hydroxyphenyl)piperazine (29):** General procedures **d** and **f** from **10** (82%); white powder; mp 225−227 °C; ^{1}H NMR (400.13 MHz) δ 8.10 (d, J = 4 Hz, 1H), 7.90 (s, 1H), 7.82 (d, J = 2 Hz, 1H), 7.49 (s, 1H), 7.22 (dd, J = 4, 2 Hz, 1H), 7.14 (s, 1H), 6.76 (d, J = 9 Hz, 2H), 6.61 (d, J = 9 Hz, 2H), 6.12 (s, 2H), 4.43 (s, 2H), 3.85 (s, 3H), 3.21 (t, J = 8 Hz, 4H), 2.83 (m, 4H); ESI MS m/z 443 (M + H)$^+$. Anal. ($C_{27}H_{26}O_4N_2$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-*N*′-(2-hydroxyethyl)piperazine (30):** General procedures **d** and **f** from **10** (86%); white powder; mp 142−144 °C; ^{1}H NMR (400.13 MHz) δ 8.18 (d, J = 4 Hz, 1H), 7.85 (s, 1H), 7.76 (d, J = 2 Hz, 1H), 7.32(s, 1H), 7.16 (dd, J = 4, 2 Hz, 1H), 7.10 (s, 1H), 6.05 (s, 2H), 3.96 (s, 3H), 3.82 (s,2H), 3.70 (t, J = 6 Hz, 2H), 2.70 (m, 8H), 2.61 (m, 2H); ESI MS m/z 395 (M + H)$^+$. Anal. ($C_{23}H_{26}O_4N_2$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-*N*′-(2-hydroxyethylethoxy)piperazine (31):** General procedures **d** and **f** from **10** (78%); white powder; mp 145−147 °C; ^{1}H NMR (400.13 MHz) δ 8.10 (d, J = 4 Hz, 1H), 7.80 (s, 1H), 7.75 (d, J = 2 Hz, 1H), 7.46(s, 1H), 7.14 (dd, J = 4, 2 Hz, 1H), 7.09 (s, 1H), 6.05 (s, 2H), 3.96 (s, 3H), 3.89 (s,2H), 3.79 (t, J = 6 Hz, 2H), 3.61 (t, J = 6 Hz, 2H), 3.49 (t, J = 6 Hz, 2H), 3.12 (m, 8H), 2.87 (m, 2H); ESI MS m/z 439 (M + H)$^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4-piperidineethanol (32a):** General procedures **d** and **f** from **10** (88%); white powder; mp 148−149 °C; ^{1}H NMR (400.13 MHz) δ 8.08 (d, J = 4 Hz, 1H), 7.90 (s, 1H), 7.81 (d, J = 2 Hz, 1H), 7.45 (s, 1H), 7.22 (dd, J = 4, 2 Hz, 1H), 7.17 (s, 1H), 6.09 (s, 2H), 4.14 (s, 2H), 3.98 (s, 3H), 3.52 (t, J = 6 Hz, 2H), 3.00 (m, 2H), 2.58 (m, 2H), 1.65 (m, 4H), 1.55 (m, 1H), 1.43 (m, 2H); ESI MS m/z 394 (M + H)$^+$. Anal. ($C_{24}H_{27}O_4N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4-piperidineethanol hydrochloride (32b):** White powder; mp 153−155 °C; ^{1}H NMR (400.13 MHz) δ δ 8.07 (d, J = 4 Hz, 1H), 7.94 (s, 1H), 7.83 (d, J = 2 Hz, 1H), 7.50 (s, 1H), 7.22 (dd, J = 4, 2 Hz, 1H), 7.15 (s, 1H), 6.10 (s, 2H), 4.56 (s,2H), 3.98 (s, 3H), 3.56 (t, J = 6 Hz, 2H), 3.44 (m, 2H), 2.84 (m, 2H), 1.85 (m, 4H), 1.73 (m, 1H), 1.45 (m, 2H); ESI MS m/z 394 (M + H)$^+$. Anal. ($C_{24}H_{27}O_4N$·HCl·2.0H_2O) C, H, N,

***N*-(2,3-Methylenedioxy-6-benzyloxyphenanthr-9-ylmethyl)-L-4-piperidinecarboxylic acid (35):** Similar to procedures **d** and **e** (77%); white powder; mp 183−185 °C; ^{1}H NMR (400.13 MHz) δ 8.05 (d, J = 4 Hz, 1H), 7.89 (d, J = 2 Hz, 1H), 7.81 (s, 1H), 7.52 (d, J = 4 Hz, 2H), 7.43 (s, 1H), 7.40 (t, J = 4 Hz, 2H), 7.33 (m, 1H), 7.26 (dd, J = 4, 2 Hz, 1H), 7.15 (s, 1H), 6.08 (s, 2H), 5.26 (s, 2H), 3.92 (s, 2H), 2.89 (m, 1H), 2.36 (m, 4H), 1.83 (m, 4H); ESI MS m/z 470 (M + H)$^+$. Anal. ($C_{29}H_{27}O_5N$) C, H, N.

***N*-(2,3-Methylenedioxy-6-benzyloxyphenanthr-9-ylmethyl)-L-4-piperidinemethanol (36):** Similar to procedures **d** and **f** (93%); white powder; mp 120−122 °C; ^{1}H NMR (400.13 MHz) δ 8.25 (d, J = 4 Hz, 1H), 7.89 (d, J = 2 Hz, 1H), 7.84 (s, 1H), 7.51 (d, J = 4 Hz, 2H), 7.44 (t, J = 4 Hz, 2H), 7.33 (m, 1H), 7.26 (dd, J = 4, 2 Hz, 1H), 7.15 (s, 1H), 6.07 (s, 2H), 5.28 (s, 2H), 3.95 (s, 2H), 3.47 (d, J = 3 Hz, 2H), 2.12 (m, 4H), 1.69 (m, 1H), 1.48 (m, 4H); ESI MS m/z 456 (M + H)$^+$. Anal. ($C_{29}H_{29}O_4N$) C, H, N.

Cell Growth Inhibition Assay. The sulforhodamine B assay was used according to the procedures developed and validated at NCI.[19] The in vitro anticancer activities are expressed as IC_{50} values, which is the test compound concentration (μM) that reduced the cell number by 50% after 72-h of continuous treatment. The values were interpolated from dose−response data. Each test was performed in triplicate with a variation of less than 5%. The IC_{50} values determined in each of the independent tests varied less than 10%. Compound stock solutions were prepared in DMSO with the final solvent concentration ≤1% DMSO (v/v), a concentration without effect on cell replication. The cells were cultured at 37 °C in RPMI-1640 supplemented with 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 2% (w/v) sodium bicarbonate, 10% (v/v) fetal bovine serum, and 100 μg/mL kanamycin in a humidified atmosphere containing 5% CO_2.

Antitumor Activity in Vivo. Severe combined immune deficiency (SCID) male mice were used. Viable human lung tumor cells (A549) at a dose of 1.5×10^7 cells in 0.2 mL were injected subcutaneously into the dorsal side of the test animals. When the tumor grew and reached ≥5 mm in diameter (designated as day 1), the tumor-bearing animals were divided into 14 groups (six animals in each group) for antitumor studies. For the inhibition of tumor growth, when the A549 tumor grew to reach ≥5 mm, PBT analogs at 1, 3, and 10 mg/kg were administered at 4-day intervals by ip injection for a total of six doses using the dosing volume of 10 mL/kg. Concurrently, mitomycin at 2 mg/kg was given at 4-day intervals for a total of five doses by ip injection. The animals were observed for signs of overt toxicity after dosing. Body weight and tumor size were measured and recorded every 4 days during the experiment period of 29 days. Tumor weight (mg) was estimated according to the formula length × (width)^2 × 0.5 in mm^3, assuming its specific gravity to be 1. Tumor growth inhibition was calculated as *T*/*C* (treatment/control) by the following formula: $T/C = (T_n - T_1)/(C_n - C_1) \times 100\%$; if $(T_n - T_1) < 0$, then $T/C = (T_n - T_1)/T_1 \times 100\%$, where C_1 (C_n) is the tumor weight at day 1 (day n) in the

control group and T_1 (T_n) is the tumor weight at day 1 (day n) in the treated group. A T/C value <42% indicates significant antitumor activity.

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Supporting Information Available: Elemental analysis data for compounds **18b**, **20**−**26**, **29**, **30**, **32**, **35**, and **36**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jiangsu New Medical College. *Dictionary of Chinese Traditional Medicine*; Shanghai: Shanghai Science and Technology Publishing House, 1977; p 1747.
(2) Baumgartner, B.; Erdelmeier, C. A. J.; Wright, A. D.; Rali, T.; Sticher, O. An antimicrobial alkaloid from *Ficus septica*. *Phytochemistry* **1990**, *29*, 33327−33330.
(3) Wu, P. L. V. R. K.; Su, C.-H.; Kuoh, C.-S.; Wu, T.-S. Phenanthroindolizidine alkaloids and their cytotoxicity from the leaves of *Ficus septica*. *Heterocycles* **2002**, *57*, 2401.
(4) Gellert, E. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Academic Press: New York, 1987; pp 55−132.
(5) Gellert, E. The indolizidine alkaloids. *J. Nat. Prod.* **1982**, *45*, 50−73.
(6) Govindachari, T. R. In *The Alkaloids, Chemistry and Pharmacology*; Manske, R. H. F., Ed.; Academic Press: New York, 1976; Vol. 9, pp 517−528.
(7) Bick, I. R. C.; Sinchai, W. In *The Alkaloids, Chemistry and Pharmacology*; Manske, R. H. F., Rodrigo, R. G. A, Eds.; Academic Press: New York, 1981; Vol. 19, pp 193−220.
(8) Gellert, E.; Rudzats, R. Antileukemia activity of tylocrebrine. *J. Med. Chem.* **1964**, *7*, 361−362.
(9) Rao, K. V.; Wilson, R. A.; Cummings, B. Alkaloids of tylophora. 3. New alkaloids of *Tylophora indica* (Burm) Merrill and *Tylophora dalzellii* Hook f. *J. Pharm. Sci.* **1971**, *60*, 1725−1726.
(10) Suffness, M.; Cordell, G. A. In *The Alkaloids, Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: New York, 1985; Vol. 25, pp 3−355.
(11) Tanner, U.; Wiegrebe, W. Alkaloids of *Cynanchum vincetoxicum*: efficacy against MDA-MB-231 mammary carcinoma cells. *Arch. Pharm. (Weinheim)* **1993**, *326*, 67−72.
(12) The 60-cell line NCI test data, along with in vivo data, can be accessed from the NSC numbers: http://dtp.nci.nih.gov/dtpstandard/dwindex/index.jsp.
(13) Gao, W.; Lam, W.; Zhong, S.; Kaczmarek, C.; Baker, D. C.; Cheng, Y.-C. Novel mode of action of tylophorine analogs as antitumor compounds. *Cancer Res.* **2004**, *64*, 678−688.
(14) Stærk, D.; Lykkeberg, A. K.; Christensen, J.; Budnik, B. A.; Abe, F.; Jaroszewski, J. W.; In vitro cytotoxic activity of phenanthroindolizidine alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanakae* against drug-sensitive and multidrug-resistant cancer cells. *J. Nat. Prod.* **2002**, *65*, 1299−1302.
(15) Wang, C. Y.; Cusack, J. C., Jr.; Liu, R.; Baldwin, A. S., Jr. Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kB. *Nat. Med.* **1999**, *5*, 412−417.
(16) Cusack, J. C., Jr.; Liu, R.; Baldwin, A. S., Jr. Inducible chemoresistance to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin (CPT-11) in colorectal cancer cells and a xenograft model is overcome by inhibition of nuclear factor-kB activation. *Cancer Res.* **2000**, *60*, 2323−2330.
(17) Wassmundt, F. W.; Kiesman, W. F. Soluble catalysts for improved Pschorr cyclizations. *J Org. Chem.* **1995**, *60*, 196−201.
(18) Lebrun, S.; Couture, A.; Deniau, E.; Grandclaudon, P. Total syntheses of (±)-cryptopleurine, (±)-antofine and (±)-deoxypergularinine. *Tetrahedron* **1999**, *55*, 2659−2670.
(19) Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; kehan, P.; Scudiero, D. A.; Monks, M. R. Comparison of in vitro anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. *J. Natl. Cancer Inst.* **1990**, *82*, 1113−1118.
(20) Abe, F.; Hirokawa, M.; Yamauchi, T.; Honda, K.; Hayashi, N.; Ishii, M.; Imagawa, S.; Iwahana, M. Further investigation of phenanthroindolizidine alkaloids from *Tylophora tanakae*. *Chem. Pharm. Bull.* **1998**, *46*, 767−769.
(21) Wu, P. L.; Rao K. V.; Su, C.-H.; Kuoh, C.-S.; Wu, T.-S. Phenanthroindolizidine alkaloids and their cytotoxicity from the leaves of *Ficus septica*. *Heterocycles* **2002**, *57*, 2401−2408.
(22) Wei, L.; Brossi, A.; Kendall, R.; Bastow, K. F.; Morris-Natschke, S. L.; Shi, Q.; Lee, K. H. Antitumor agents 251. Synthesis, cytotoxic evaluation, and structure−activity relationship studies of phenanthrene-based tylophorine derivatives (PBTs) as a new class of anticancer agents. *Bioorg. Med. Chem.* **2006**, *14*, 6560−6569.

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