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Antitumor Agents 268. Design, Synthesis, and Mechanistic Studies of New 9-Substituted Phenanthrene-Based Tylophorine Analogues as Potent Cytotoxic Agents

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Nineteen new phenanthrene-based tylophorine analogues with various functional groups on the piperidine moiety were designed, synthesized, and evaluated for in vitro anticancer activity against four human tumor cell lines. Analogues **15** and **21** showed approximately 2-fold enhanced inhibitory activity as compared with our prior lead compound (PBT-1). Analogues **23** and **24** with *S*- and *R*-configured substituents, respectively, at the piperidine 3′-position exhibited comparable cytotoxicity to that of PBT-1. Furthermore, mechanistic studies to investigate the effects of the new compounds on Akt protein in lung cancer cells and the NF-kB signaling pathway suggested that the compounds may exert their inhibitory activity on tumor cells through inhibition of activation of both Akt and NF-kB signaling pathway.

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Introduction

Phenanthroindolizidine and phenanthroquinolizidine alkaloids comprise a class of pentacyclic natural products isolated primarily from *Cynanchum*, *Pergularia*, and *Tylophora* species in the Asclepiadaceae family.[1,2] Many of these alkaloids show strong bioactive effects against human diseases such as cancers. For example, antofine and tylophorine (Figure 1) showed potent cytotoxic effects in antitumor screening launched by the National Cancer Institute, which has aroused great interest in exploring the synthesis and biological activities of these compounds and their derivatives. The main goal of such efforts was to obtain drug candidates with higher inhibitory potency and lower side effects, especially reduced or no central nervous system (CNS[a]) toxicity, which is associated with the natural tylophorines.[3] Although the biochemical targets of tylophorine and related bioactive principles are still unknown, recent research has indicated that the NF-κB signaling pathway and the synthesis of a number of cell cycle proteins, such as cyclin D_1, are suppressed during treatment with these alkaloids.[4,5,10]

In our previous research, we reported the synthesis and cytotoxic activity of a series of structurally simplified phenanthrene-based tylophorine (PBT) analogues, as well as structure−activity relationship conclusions. We investigated various structural building blocks, including amino acid derivatives, pyrrolidine derivatives (substituted at C-2′), piperidines (substituted at C-2′ and C-4′), and piperazine derivatives, which are connected to the core tricyclic phenanthrene structure through a methylene bridge. PBT-1, which has a 4′-hydroxymethyl piperidine moiety (Figure 1), was the most active among these compounds against four types of human cancer cell lines, including multidrug resistant (MDR) KB-VIN cells, with low IC_{50} values around 80 nM.[6,7]

The promising results obtained with a diverse but limited series of target compounds have prompted us to further explore the pharmacophores of natural tylophora alkaloids such as antofine. Our goals are to develop new potential drug candidates as well as to study the mechanism of action and identify the molecular target(s) of the new compounds. On the basis of the SAR information obtained in our previous work, we have synthesized new PBT-1 analogues with different substituents at the C-3′ and C-4′ positions of the piperidine ring. Substituents containing amino or hydroxy groups were introduced to increase the water solubility and polarity of the compounds while retaining their ability to form hydrogen bonds with the putative binding target. Because a longer side chain in the piperidine ring led to a significant reduction in efficacy,[7] we chose functional groups similar in size to the hydroxymethyl group of PBT-1. Functional groups such as methyl ester, cyano, trifluoromethyl, and methylsulfonylamino groups were also introduced at the C-3′ or C-4′ position to obtain information useful in assisting further drug structure design and optimization.

We have recently reported that PBT-1 can induce cell cycle G2/M arrest and apoptosis by suppressing Akt and NF-kB signaling pathways.[10] Therefore, we were also interested in determining whether the new cytotoxic compounds synthesized in our current study share the same mechanism as PBT-1. To answer this question, a series of active new compounds were selected for further mechanistic studies.

Tylophora natural products with a pentacyclic structural scaffold have long been known as potent antitumor agents,

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[a] Abbreviations: CNS, central nervous system; PBT, phenanthrene-based tylophorine.

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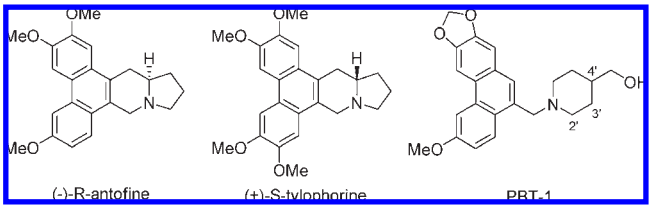


Figure 1. Structures of (−)-*R*-antofine, (+)-*S*-tylophorine, and PBT-1.

with activity against a variety of tumor cell lines. PBT derivatives are considered to be analogues of natural or synthetic *Tylophora* products. They share the tricyclic phenanthrene core structure but have only one rather than two methylene linkages to a fourth, rather than fifth, pyrrolidine or piperidine D ring. In our previous study, certain PBT analogues, along with the *Tylophora* natural products, were investigated in mechanism of action studies. The results showed that although the two compound series are structural analogues, they are not biologically functional analogues because they do not share the same spectrum of molecular targets.[11] This finding encouraged us to further study the optimal conformations of the natural products and PBT analogues and to learn whether minor structural variations affect their binding conformation and interaction with the target(s).

In this article, we report our recent research results in the synthesis, biological evaluation, preliminary mechanistic study results, and structural conformational analysis of the most potent new compound **21** in comparison with the natural product antofine.

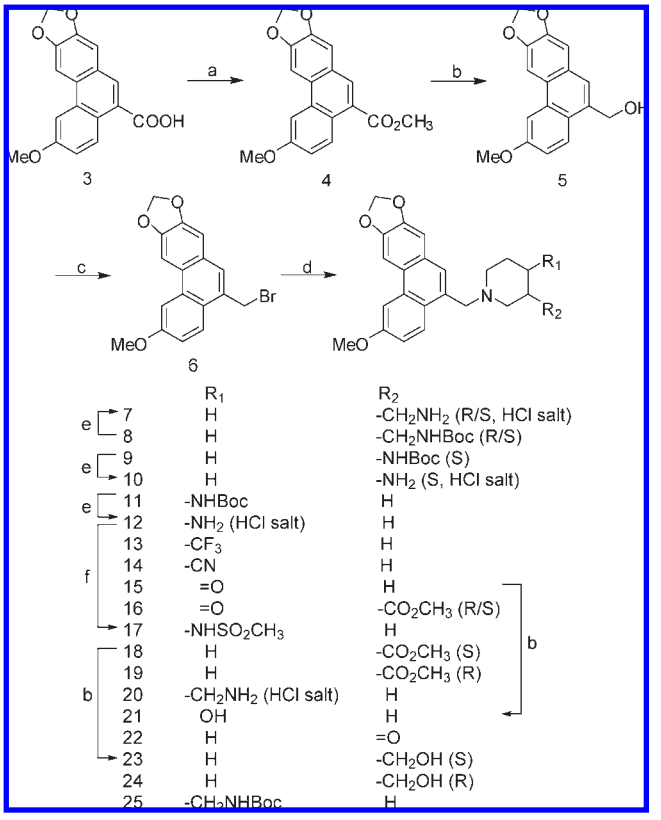
Results and Discussions

The general synthetic methods used to prepare target derivatives are shown in Scheme 1. The phenanthrene-9-carboxylic acid **3**, obtained via three steps as reported in the literature,[6] was reacted with methyl iodide using sodium bicarbonate to afford the methyl ester **4**, which was then reduced with $LiAlH_4$ at room temperature to give the alcohol **5**, followed by bromination using tribromophosphine in dichloromethane.[8] For the final step, the bromine atom of **6** was displaced by various substituted piperidines at room temperature or 60 °C to afford target products. The Boc group was removed with HCl in MeOH and sulphonylamination was carried out in CH_2Cl_2.[9] The ketone moiety was reduced with $LiAlH_4$ to form the corresponding alcohol in excellent yields.

Totally, 19 compounds were synthesized (two were *R*/*S* mixtures) and screened for in vitro anticancer activity against a panel of human tumor cell lines including KB (nasopharyngeal), A549 (lung), DU-145 (prostate), and KB-VIN (an MDR KB subline). The screening results are shown in Table 1.

Most compounds exhibited significant activity, with **15** and **21** showing the highest potency with IC_{50} values in the 0.08−0.14 μM range. Changing the position of the hydroxymethyl substituent from C-4′ in PBT-1 to C-3′ in **23** and **24** did not affect the potency; all three compounds had similar IC_{50} values. The stereochemistry of the side chain in the latter two compounds also had little effect on potency. However, compound **21** was ca. 2-fold more active compared with PBT-1, as indicated by lower IC_{50} values (0.08−0.11 compared with 0.18−0.24 μM). Because **21** has a 4′-hydroxyl rather than 4′-hydroxymethyl substituent on the piperidine ring, the augmented efficacy might be explained by a better fit into

Scheme 1[a]



[a] Reagents and conditions: (a) CH_3I, $NaHCO_3$, DMF, overnight; (b) $LiAlH_4$THF, rt; (c) PBr_3, CH_2Cl_2, 0 °C; (d) 3- or 4-substituted piperidine, DMF, TEA or K_2CO_3, 60 °C, 4 h; (e) 2 N HCl, MeOH, rt; (f) MsCl, pyridine, rt, 16 h.

the binding pocket and better interaction with the target groups, possibly through hydrogen bonding with the oxygen atom at the C-4′ position (directly or indirectly).

Interestingly, compound **15**, the oxidized form of **21**, possessed similar high potency, indicating that the oxygen atom could be used primarily as a hydrogen bond acceptor, as long as the spatial distance is favorable, while a possible covalent adduct (to the ketone) is less likely to be formed. However, when the oxo group was at the C-3′ rather than C-4′ position (**22** vs **15**), the inhibitory potency decreased by about 8-fold, possibly indicating less optimal hydrogen bonding with the target in the binding pocket. Potency decreased even more when a methyl ester was present at C-3′ in addition to an oxo group at C-4′ (compare **16** and **15**). In fact, compound **16** showed the same potency as **18** and **19**, which have only a methyl ester (cis and trans, respectively) at C-3′. Thus, the oxygen atom at C-4′ in **16** might be displaced from its optimal hydrogen bonding angle as in **15**, and thus, the oxo group might not be involved in binding.

A lipophilic trifluoromethyl group at C-4′ (**13**) substantially decreased the inhibitory activity. Compound **14** with a cyano moiety at the same position showed significant activity but was five- to 10-fold less potent compared with compounds containing an oxo (**15**), hydroxy (**21**), or hydroxymethyl (PBT-1) group, which might be associated with the oxidation state of nitrogen and particular orientation of its lone pair of electrons.

With IC_{50} values ranging from 0.26 to 0.49 μM, **20** (4′-CH_2NH_2.HCl) showed the greatest potency among the amino compounds. Compounds with the amino group present

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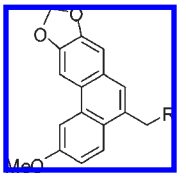
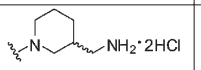
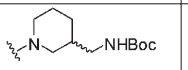
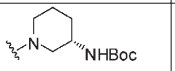
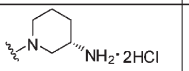
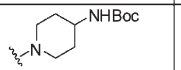
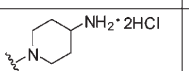
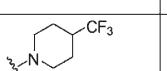
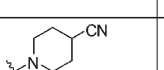
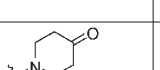
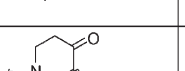
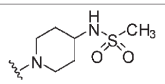
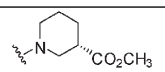
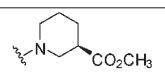
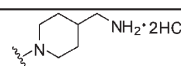
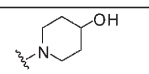
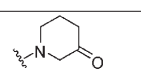
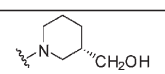
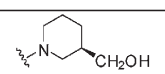
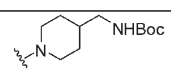
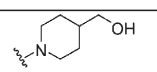
Table 1. In Vitro Anticancer Activity of PBT-1 and **7**−**25**



Compounds		IC_{50} (μM)			
		KB	KBvin	A549	DU145
7		0.73	0.84	0.62	0.82
8		1.11	1.30	1.19	1.09
9		13.85	18.09	14.58	17.23
10		8.69	8.87	8.80	9.60
11		0.86	1.06	0.88	0.93
12		0.55	0.66	0.36	0.78
13		9.30	7.48	6.57	12.56
14		1.12	1.15	0.88	1.10
15		0.11	0.14	0.11	0.11
16		1.26	1.97	1.38	1.85

Compounds		IC_{50} (μM)			
		KB	KBvin	A549	DU145
17		0.66	1.08	1.02	1.02
18		1.94	3.04	1.86	2.68
19		1.30	1.42	1.30	1.50
20		0.26	-	0.49	0.35
21		0.08	-	0.08	0.11
22		0.83	-	0.80	1.07
23		0.18	-	0.13	0.16
24		0.18	-	0.18	0.26
25		0.90	-	0.56	0.84
PBT-1		0.18	0.21	0.18	0.24
Antofine		0.036	0.025	0.022	0.025

as the HCl salt showed uniformly better activity compared with their Boc-protected precursors, perhaps resulting from elevated water solubility and side chain shortening after removal of Boc. However, the amine salts generally were less active than the corresponding compounds with a hydroxy group (PBT-1 vs **20**, **21** vs **12**, **24**/**25** vs **7**), suggesting that formation of a hydrogen bond alone cannot explain all interactions between these compounds and their targets. Compounds **7** (3′-CH_2NH_2) and **8** (3′-CH_2NHBoc) showed significantly higher inhibition than **9** (3′-NH_2) and **10** (3′-NHBoc), respectively. The extra methylene could put the hydrogen bond acceptor closer to hydrogen bond donor in the binding pocket. Comparison of **11** (4′-NH-Boc or 4′-$NHCO_2t$-Bu) and **17** (4′-$NHSO_2CH_3$) with **12** (4′-NH_2·HCl) showed only moderate decreases in potency with the former two compounds, indicating that substitutions at the C4′-NH_2 group do not significantly affect activity.

The 19 newly synthesized compounds were also examined for their effects on Akt (Figure 2) and the NF-κB signaling pathway (Figure 3) in A549 and CL1−0 cells.[10] Four compounds, **15** (YXM35), **21** (YXM41), **23** (YXM43), and **24** (YXM44), which were more active than or comparable to PBT-1 against the tested tumor cell lines, consistently showed more potent suppression of Akt activation than PBT-1 in both lung cancer cells, especially in decreasing Akt protein phosphorylation. Further assays are still needed to distinguish whether the inhibitory activity on Akt is due to transcriptional inhibition or protein stability.

In addition, when evaluated for effects on the NF-κB signaling pathway, the four compounds also showed more potent suppression of NF-κB activity than PBT-1, parallel to the results in our cell-based cytotoxicity testing, suggesting that these compounds may inhibit tumor cell growth through inhibition of both Akt activation and NF-κB related signaling pathway.

The conformations of **21** and antofine were energy-minimized and superimposed, and the results are shown in Figure 4. It was obvious that the side chain orientation of **21**, the PBT-analogue, was distinct from the remainder of the natural product antofine. Thus, the conformational variation

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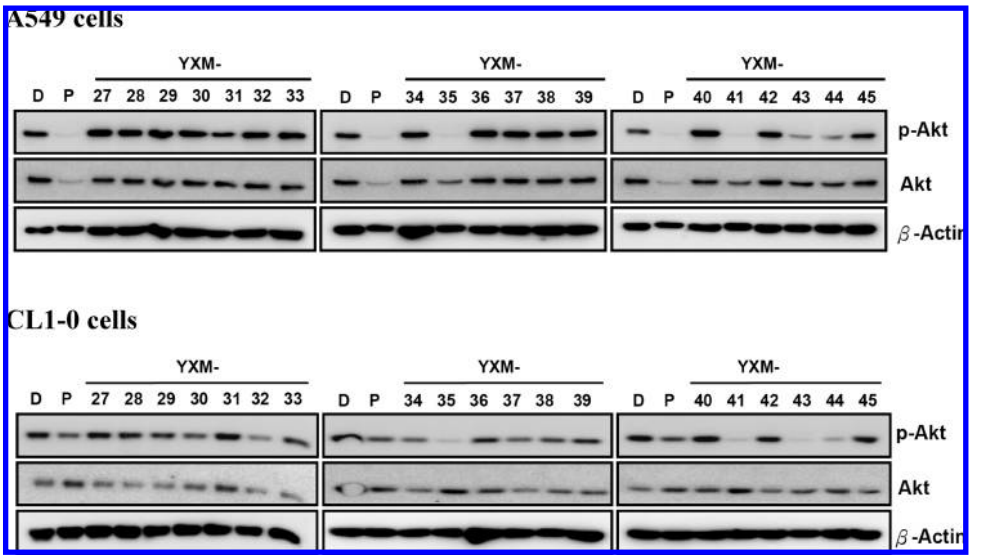

Figure 2. Inhibitory effects of PBT analogues on Akt and its phosphorylation in A549 and CL1−0 cells.

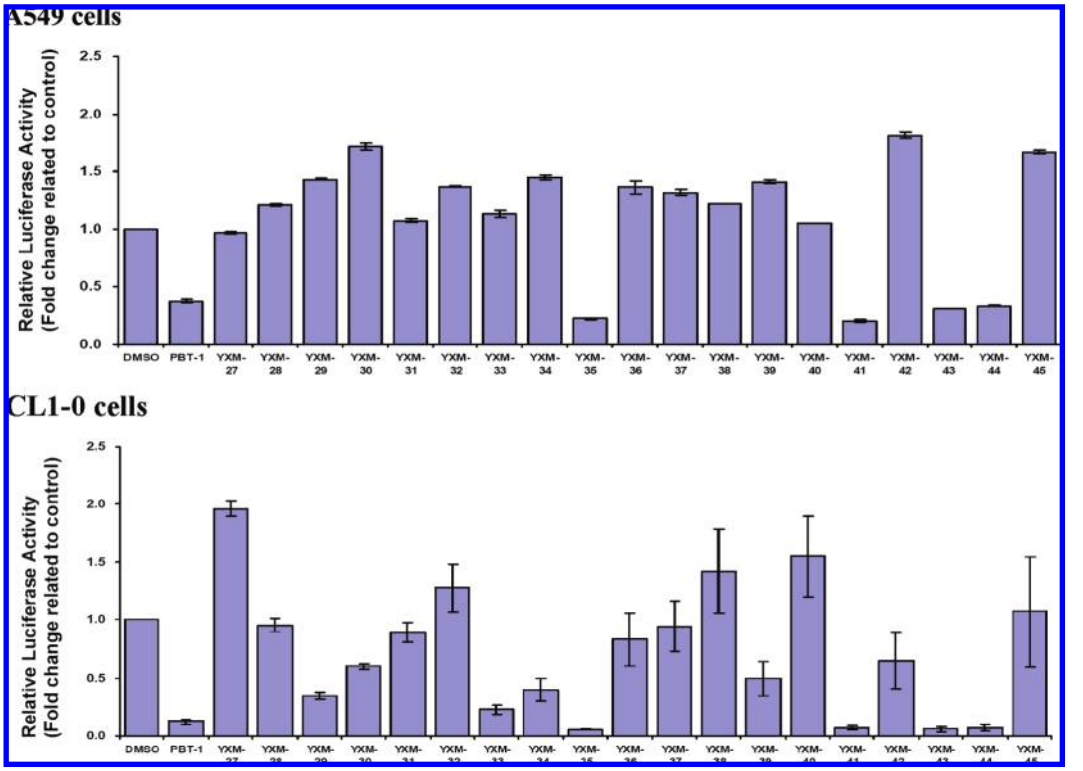

Figure 3. Inhibitory effects of PBT analogues on NF-κB signaling pathway in A549 and CL1−0 cells.

between antofine and our PBT analogues may affect the binding affinity in the binding site(s) and further affect the interaction between drugs and target molecule(s).

Conclusions

Nineteen novel PBT-based analogues with different substituents on the C-3′ and C-4′ positions of the piperidine ring were designed and synthesized. Among them, four compounds **15**, **21**, **23**, and **24**, showed comparable activity and were more potent than the remaining compounds against four tumor cells. Preliminary mechanism studies indicated that, although these new compounds are analogues of PBT-1, they might not share the same mechanism to fully interact with the target molecule(s). The inhibitory activity of the new compounds against tumor cell growth may be induced by inhibition of the activation of Akt and NF-κB signaling pathway in tumor cells. Conformation analysis indicated that the PBT analogues possess a different side chain orientation from that of the natural product antofine. Further studies are warranted to confirm the biological effects of the compounds. In summary, **15** and **21** were identified as new more active PBT derivatives and are the basis for our ongoing ligand-based design efforts.

Experimental Section

Melting points were measured using a Fisher Johns melting apparatus without correction. Proton nuclear magnetic resonance

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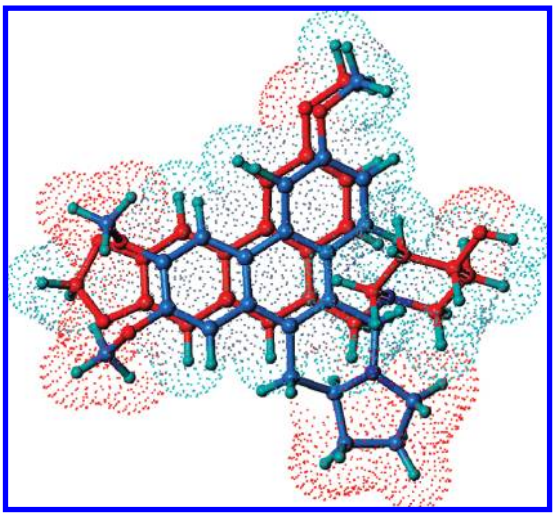

Figure 4. Conformational comparison of compound **21** (red) and antofine (blue). Energy minimization and superimposition of the two molecules were done by SYBYL8.0. VdW dotted surface were generated for each molecule. Atom coloring: gray, carbon; green, hydrogen; blue, nitrogen; red, oxygen. Method for energy minimization: Powell. Max iterations were 1000. All other parameters were defaulted.

(^{1}H NMR) spectra were measured on a 300 MHz Gemini spectrometer using TMS as internal standard. The solvent used was CDC_{l3} unless indicated. Mass spectra were recorded on a Shimazu-2010 LC/MS/MS instrument equipped with a Turbo IonsSpray ion source.

2,3-Methylenedioxy-6-methoxyphenanthrene-9-carboxylic Acid Methyl Ester (4). The carboxylic acid (1.16 g, 3.92 mmol) was suspended in 30 mL of DMF, to which $NaHCO_3$ (527 mg, 6.27 mmol) and CH_3I (0.49 mL, 7.80 mmol) were added. The mixture was stirred overnight at room temperature before 20 mL water was added to quench the reaction. EtOAc was used for extraction. The organic layers were collected and washed with water and brine and dried over $MgSO_4$. The methyl ester was found to be clean enough for the next step without further purification (quantitative). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.27 (d, J = 9.30 Hz, 1H), 7.90 (s, 1H), 7.81 (d, J = 2.40 Hz, 1H), 7.40 (s, 1H), 7.22 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.16 (s, 1H), 6.10 (s, 2H), 4.00 (s, 3H), 3.90 (s, 3H). ESI MS m/z 310.95 $(M+H)^+$.

2,3-Methylenedioxy-6-methoxy-9-hydroxylmethyl-phenanthrene (5). The ester (500 mg, 1.61 mmol) in 30 mL THF was added to $LiAlH_4$ in 20 mL THF in an ice bath, which was then stirred at room temperature for 2 h. Water (5 mL) and 5 mL of 2 N NaOH were added, and the mixture was filtered. The solvent was removed under vacuum and redissolved in CH_2Cl_2, which was washed with brine and dried over $MgSO_4$. The product was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (d, J = 9.30 Hz, 1H), 7.88 (s, 1H), 7.81 (d, J = 2.40 Hz, 1H), 7.40 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.15 (s, 1H), 6.09 (s, 2H), 4.96 (s, 2H), 4.01 (s, 3H). ESI MS m/z 283.00 $(M+H)^+$.

2,3-Methylenedioxy-6-methoxy-9-bromomethyl-phenanthrene (6). At 0 °C, PBr_3 (1.18 mL, 12.56 mmol) was added dropwise to **5** (885 mg, 3.14 mmol) in CH_2Cl_2. The ice bath was then removed and the mixture was stirred at room temperature for 3 h before 20 mL sat. $NaHCO_3$ was added. The mixture was extracted using CH_2Cl_2 and washed with brine and dried over $MgSO_4$. Chromatography gave 700 mg product (65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, J = 9.30 Hz, 1H), 7.87 (s, 1H), 7.79 (d, J = 2.40 Hz, 1H), 7.39 (s, 1H), 7.22 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.15 (s, 1H), 6.08 (s, 2H), 4.68 (s, 2H), 4.00 (s, 3H), ESI MS m/z 346.05 $(M+H)^+$.

General Procedure for the Synthesis of 9-Substituted Phenanthrene. Substituted piperidine (in excess) was added to a mixture of bromide and TEA in DMF, which was stirred at room temperature or 60 °C overnight. DMF was then removed under reduced pressure, and CH_2Cl_2 was used for extraction. The organic layers were combined and sequentially washed with 1 N HCl, sat. $NaHCO_3$, and brine and dried over $MgSO_4$. Chromatography afforded the final products (Yield: 50−90%). HCl salt was prepared using 2 M aqueous HCl in MeOH at room temperature.

(*R*/*S*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-3′-aminomethyl-piperidine Hydrochloride (7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.28 (d, J = 9.00 Hz, 1H), 7.90 (s, 1H), 7.80 (d, J = 2.40 Hz, 1H), 7.39 (s, 1H), 7.22 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.16 (s, 1H), 6.08 (s, 2H), 4.00 (s, 3H), 3.81 (m, 2H), 2.95−2.88 (m, 1H), 2.82−2.80 (m, 1H), 2.57 (d, J = 6.30 Hz, 2H), 2.10−2.04 (m, 2H), 1.94 (m, 2H), 1.75 (m, 2H), 1.01 (m, 1H). ESI MS m/z 379.10 $(M+H)^+$. Anal. ($C_{23}H_{26}N_2O_3 \cdot 2HCl \cdot 2H_2O$) C, N, O,

(*R*/*S*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-3′-*N*-Boc-aminomethyl-piperidine (8). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (d, J = 9.00 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.40 Hz, 1H), 7.39 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.16 (s, 1H), 6.09 (s, 2H), 4.46 (s, 1H), 4.00 (s, 3H), 3.80 (m, 2H), 3.00 (m, 2H), 2.80 (m, 2H), 2.10 (m, 2H), 1.89 (m, 2H), 1.62 (m, 2H), 1.38 (s, 9H), 1.05 (m, 1H). ESI MS m/z 479.15 $(M+H)^+$.

(*S*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-3′-*N*-Boc-aminopiperidine (9). White solid; mp 133−135 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.28 (d, J = 8.70 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.40 Hz, 1H), 7.40 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 1.80 Hz, 1H), 7.17 (s, 1H), 6.09 (s, 2H), 4.44 (s, 1H), 4.01 (s, 3H), 3.86 (s, 2H), 2.54 (m, 2H), 2.46 (m, 1H), 2.28 (m, 1H), 1.59 (m, 2H), 1.53 (m, 2H), 1.44 (s, 9H), 1.26 (m, 1H). ESI MS m/z 465.15 $(M+H)^+$.

(*S*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-3′-aminopiperidine Hydrochloride (10). White solid; mp 265−267 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, J = 9.00 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.70 Hz, 1H), 7.42 (s, 1H), 7.22 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.16 (s, 1H), 6.09 (s, 2H), 4.00 (s, 3H), 3.90 (m, 2H), 2.88 (m, 1H), 2.75 (m, 1H), 2.63 (m, 1H), 2.18 (m, 2H), 2.05 (m, 2H), 1.58−1.52 (m, 1H), 1.28 (m 1H), ESI MS m/z 365.05 $(M+H)^+$. Anal. ($C_{22}H_{24}N_2O_3 \cdot 2HCl \cdot 2H_2O$) C, N, O,

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-*N*-Boc-aminopiperidine (11).** White solid; mp 211−213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (d, J = 9.30 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.70 Hz, 1H), 7.39 (s, 1H), 7.22 (dd, J = 9.00 Hz, J = 2.70 Hz, 1H), 7.17 (s, 1H), 6.09 (s, 2H), 4.41 (s, 1H), 4.01 (s, 3H), 3.84 (s, 2H), 3.49 (m, 1H), 2.89 (m, 2H), 2.17 (m, 2H), 1.90 (m, 2H), 1.44 (s, 9H), 1.39 (m, 2H). ESI MS m/z 465.15 $(M+H)^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-aminopiperidine Hydrochloride (12).** White solid; mp 261−263 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (d, J = 9.00 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.70 Hz, 1H), 7.40 (s, 1H), 7.22 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.17 (s, 1H), 6.09 (s, 2H), 4.01 (s, 3H), 3.84 (s, 2H), 2.93 (m, 2H), 2.72−2.63 (m, 1H), 2.14−2.06 (m, 2H), 1.78 (m, 2H), 1.42−1.31 (m, 2H). ESI MS m/z 365.10 $(M+H)^+$. Anal. ($C_{22}H_{24}N_2O_3 \cdot 2HCl \cdot 3H_2O$) C, N, O,

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-trifluoromethyl-piperidine (13).** White solid; mp 146−148 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (d, J = 9.00 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.40 Hz, 1H), 7.39 (s, 1H), 7.26 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.17 (s, 1H), 6.09 (s, 2H), 4.01 (s, 3H), 3.85 (s, 2H), 3.05 (m, 1H), 2.05−2.01 (m, 4H), 1.79 (m, 2H), 1.63 (m, 2H). ESI MS m/z 418.05 $(M+H)^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-cyanopiperidine (14).** White solid; mp 157−159 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (d, J = 9.00 Hz, 1H), 7.91 (s, 1H), 7.81 (d, J = 2.70 Hz, 1H), 7.38 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 2.70 Hz, 1H), 7.16 (s, 1H), 6.10 (s, 2H), 4.01 (s, 3H), 3.86 (s, 2H), 2.72 (m, 2H), 2.65 (m, 1H), 2.40 (m, 2H), 1.90−1.79 (m, 4H). ESI MS m/z 375.05 $(M+H)^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-piperidone (15).** White solid; mp 161−163 °C. ^{1}H NMR (300 MHz,

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$CDCl_3$): δ 8.32 (d, J = 9.30 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.40 Hz, 1H), 7.40 (s, 1H), 7.21 (dd, J = 9.00 Hz, J = 2.70 Hz, 1H), 7.16 (s, 1H), 6.09 (s, 2H), 4.01 (s, 3H), 3.97 (s, 2H), 2.83 (t, J = 6 Hz, 4H), 2.44 (t, J = 6 Hz, 4H). ESI MS m/z 364.05 $(M+H)^+$.

(*R*/*S*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-piperidone-3′-carboxylic Acid Methyl Ester (16). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29−8.25 (d, J = 9.00 Hz, 1H), 7.92 (s, 1H), 7.82 (d, J = 2.40 Hz, 1H), 7.44 (s, 1H), 7.25−7.23 (m, 1H), 7.18 (s, 1H), 6.10 (s, 2H), 4.03−3.99 (s, 6H), 3.71 (s, 2H), 3.26−2.96 (m, 3H), 2.88−2.67 (m, 2H), 2.66−2.47 (m, 1H), 2.39−2.35 (m, 1H). ESI MS m/z 422.00 $(M+H)^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-methanesulfonylamino-piperidine (17).** White solid; mp 197−199 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (d, J = 9.00 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.70 Hz, 1H), 7.40 (s, 1H), 7.19 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.17 (s, 1H), 6.18 (m, 1H), 6.10 (s, 2H), 4.01 (s, 3H), 3.89 (s, 2H), 2.92 (m, 3H), 2.28−2.21 (m, 2H), 1.93 (m, 2H), 1.75 (m, 2H), 1.55 (m, 2H), 1.25 (m, 1H). ESI MS m/z 443.05 $(M+H)^+$.

(*S*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-piperidine-3′-carboxylic Acid Methyl Ester (18). White solid; mp 71−72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (d, J = 9.00 Hz, 1H), 7.90 (s, 1H), 7.81 (d, J = 2.70 Hz, 1H), 7.38 (s, 1H), 7.21 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.16 (s, 1H), 6.08 (s, 2H), 4.00 (s, 3H), 3.84 (s, 2H), 3.57 (s, 3H), 3.00 (m, 1H), 2.74 (m, 1H), 2.56 (m, 1H), 2.35 (m, 1H), 2.16 (m, 1H), 1.88 (m, 1H), 1.69 (m, 1H), 1.54 (m, 2H). ESI MS m/z 408.10 $(M+H)^+$.

(*R*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-piperidine-3′-carboxylic Acid Methyl Ester (19). White solid; mp 69−71 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (d, J = 9.00 Hz, 1H), 7.90 (s, 1H), 7.81 (d, J = 2.40 Hz, 1H), 7.38 (s, 1H), 7.22 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.16 (s, 1H), 6.08 (s, 2H), 4.00 (s, 3H), 3.84 (s, 2H), 3.57 (s, 3H), 3.00 (m, 1H), 2.74 (m, 1H), 2.56 (m, 1H), 2.35 (m, 1H), 2.17 (m, 1H), 1.88 (m, 1H), 1.69 (m, 1H), 1.54 (m, 2H). ESI MS m/z 408.05 $(M+H)^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-aminomethyl-piperidine Hydrochloride (20).** White solid; mp 223−225 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (d, J = 9.30 Hz, 1H), 7.92 (s, 1H), 7.82 (d, J = 2.70 Hz, 1H), 7.42 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 2.70 Hz, 1H), 7.18 (s, 1H), 6.09 (s, 2H), 4.01 (s, 3H), 3.85 (s, 2H), 3.01 (m, 2H), 2.58 (d, J = 6.0 Hz, 2H), 2.04 (m, 2H), 1.70 (m, 2H), 1.30−1.16 (m, 3H). ESI MS m/z 379.10 $(M+H)^+$. Anal. ($C_{23}H_{26}N_2O_3 \cdot 2HCl \cdot 3H_2O$) C, N, O,

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-hydroxylpiperidine (21).** White solid; mp 93−95 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (d, J = 9.00 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.40 Hz, 1H), 7.40 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 2.70 Hz, 1H), 7.17 (s, 1H), 6.09 (s, 2H), 4.01 (s, 3H), 3.85 (s, 2H), 3.72 (m, 1H), 2.84 (m, 2H), 2.23 (m, 2H), 1.89 (m, 2H), 1.67−1.56 (m, 2H). ESI MS m/z 366.05 $(M+H)^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-3′-piperidone (22).** White solid; 140 °C (decomposed). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (d, J = 9.00 Hz, 1H), 7.92 (s, 1H), 7.82 (d, J = 2.40 Hz, 1H), 7.39 (s, 1H), 7.22 (dd, J = 9.30 Hz, J = 2.70 Hz, 1H), 7.17 (s, 1H), 6.10 (s, 2H), 4.01 (s, 3H), 3.94 (s, 2H), 3.12 (s, 2H), 2.71 (t, J = 5.40 Hz, 2H), 2.34 (t, J = 7.20 Hz, 2H), 1.20 (t, J = 7.20 Hz, 2H). ESI MS m/z 364.10 $(M+H)^+$.

(*S*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-3′-hydroxylmethyl-piperidine (23). White solid; mp 100−102 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (d, J = 9.30 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.70 Hz, 1H), 7.38 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.16 (s, 1H), 6.08 (s, 2H), 4.00 (s, 3H), 3.82 (s, 2H), 3.62−3.48 (m, 2H), 2.81 (m, 1H), 2.61 (m, 1H), 2.31 (m, 1H), 2.21 (m, 1H), 1.84−1.71 (m, 2H), 1.66 (m, 1H), 1.60−1.53 (m, 1H), 1.25 (m, 1H). ESI MS m/z 380.05 $(M+H)^+$.

(*R*)-*N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-3′-hydroxylmethyl-piperidine (24). White solid; mp 65−67 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (d, J = 9.00 Hz, 1H), 7.89 (s, 1H), 7.81 (d, J = 2.70 Hz, 1H), 7.40 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 2.40 Hz, 1H), 7.16 (s, 1H), 6.08 (s, 2H), 3.99 (s, 3H), 3.88 (s, 2H), 3.61−3.47 (m, 2H), 2.89 (m, 1H), 2.67 (m, 1H), 2.33 (m, 1H), 2.24 (m, 1H), 1.82−1.56 (m, 4H), 1.23 (m, 1H). ESI MS m/z 380.05 $(M+H)^+$.

***N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-4′-*N*-Boc-aminomethyl-piperidine (25).** White solid; mp 211−213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, J = 9.30 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 2.40 Hz, 1H), 7.40 (s, 1H), 7.23 (dd, J = 9.00 Hz, J = 2.70 Hz, 1H), 7.17 (s, 1H), 6.09 (s, 2H), 4.57 (s, 1H), 4.01 (s, 3H), 3.84 (s, 2H), 3.02−2.94 (m, 4H), 2.02 (m, 2H), 1.65 (m, 2H), 1.43 (s, 9H), 1.29−1.22 (m, 3H). ESI MS m/z 479.15 $(M+H)^+$.

Cell Growth Inhibition Assay. The sulforhodamine B assay was used according to the procedures developed and validated at NCI. The in vitro anticancer activities are expressed as IC_{50} values, which is the test compound concentration (μg/mL) that reduced the cell number by 50% after 72 h of 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% CO2.

NF-κB Luciferase Assay. Cells were cultured in 12-well plates and transiently cotransfected with 0.2 μg of a pNF-κB-Luc vector (Stratagene, La Jolla, CA) and 0.2 μg of pSV-β-galactosidase dissolved in 3 μL lipofectamine or lipofectamine 2000 (Invitrogen, Carlsbad, CA) as the internal control. The plasmids were transfected according to the manufacturer's instructions. After 6 h, the medium was changed to complete medium and cultured for 6 h, and then the transfected cells were treated with different compounds in complete medium for 24 h. Cell extracts were harvested using 150 μL of lysis buffer (Tropix, Inc., Bedford, MA) per well. To measure the luciferase and β-galactosidase activities, cell extracts (20 μL each) were assayed separately using the Luciferase Assay Kit and Galacto-Light Plus system (Tropix, Inc.), respectively. Luciferase activity was measured and analyzed using an FB12 luminometer (Zylux Corporation, Oak Ridge, TN).

Western Blot Analysis. Cells were treated with different compounds for 48 h. Equal amounts (50 μg) of cell lysate were separated by 10% SDS-PAGE and transferred to a polyvinylidene membrane (Millipore, Billerica, MA). The membrane was probed with antibodies directed against p-Akt, Akt, and β-actin (Sigma, St Louis, MO). Antibodies were diluted in TBS (pH 7.5) containing 0.05% (v/v) Tween 20, and 5% (w/v) dried milk. Blots were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences, Uppsala, Sweden). Bound antibodies were visualized by electrochemical luminescence staining with autoradiographic detection using Kodak X-Omat Blue film (PerkinElmer Life Science, Boston, MA) or Typhoon9410 variable mode imager (Amersham BioScience, Piscataway, NJ).

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