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Antitumor agents 251: Synthesis, cytotoxic evaluation, and structure–activity relationship studies of phenanthrene-based tylophorine derivatives (PBTs) as a new class of antitumor agents

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Abstract—Polar phenanthrene-based tylophorine derivatives (PBTs) were designed, synthesized and evaluated as potential antitumor agents. These compounds contain a core phenanthrene structure and can be synthesized efficiently in excellent yield. The newly synthesized PBTs were evaluated for cytotoxic activity against the A549 human cancer cell line. Among them, *N*-(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-L-2-piperidinemethanol (**34**) and *N*-(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-5-aminopentanol (**28**) showed the highest potency with IC_{50} values of 0.16 and 0.27 μM, respectively, which are comparable to those of currently used antitumor drugs. A structure–activity relationship (SAR) study was also explored to facilitate the further development of this new compound class.

1. Introduction

Natural products have been the major source of currently available anticancer drugs. According to a review of New Chemical Entities (NCE) from 1981 to 2002,[1] approximately 74% of anticancer drugs were either natural products, natural product-based, or mimicked them in one form or another. The phenanthroindolizidine alkaloid tylophorine (**1**, Chart 1) and its analogs have been isolated primarily from the genera *Cynanchum*, *Pergularia*, and *Tylophora* in the Asclepiadaceae family,[2–5] but have also been reported from *Hypoestes verticillaris* (Acanthaceae),[6] *Cryptocarya phyllostemmon* (Lauraceae),[7] as well as *Ficus hispida* and *Ficus septica* (Moraceae).[8,9] These compounds, commonly called tylophora alkaloids, have been targets of synthesis and modification for their significant cytotoxic activities.[6,10–13] Evaluation of **1** and its analogs in the National Cancer Institute's antitumor screen showed a uniform and potent growth inhibitory effect ($GI_{50} \cong 10^{-8}$ M) against all 60 cell lines, with notable selectivity toward several refractory cell lines, including melanoma and lung tumor cell lines.[14]

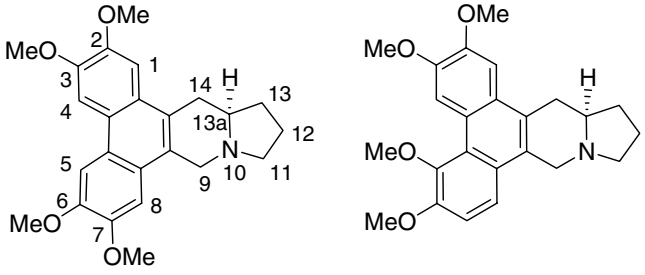

Chart 1.

Comprehensive evaluation of **1**'s antitumor activity has not been reported, and the inhibitory mechanisms on cell growth are largely unknown. Early mechanistic studies in 1960s demonstrated that tylophorine alkaloids irreversibly inhibit protein synthesis at the elongation stage of the translation cycle.[15–19] With the significant advances in the field of molecular biology in the 1990s, tylophorine alkaloids were reported to target key metabolic enzymes, including thymidylate synthase (TS)[20,21]

Keywords: Phenanthrene-based tylophorine derivatives; Antitumor agents; Cytotoxicity; *Tylophora*.
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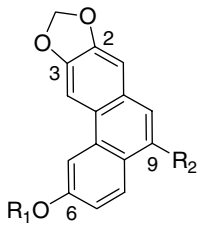
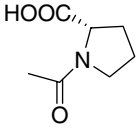
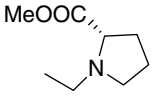
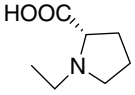
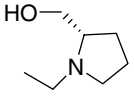
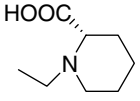
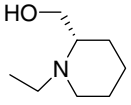
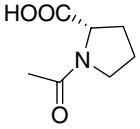
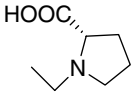
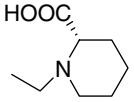
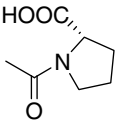
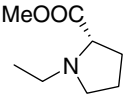
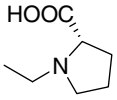
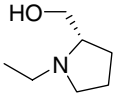
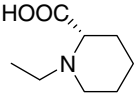
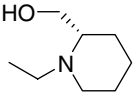
Table 1. SAR of 2,3-methylenedioxy-6-alkyloxy-9-substituted PBT analogs

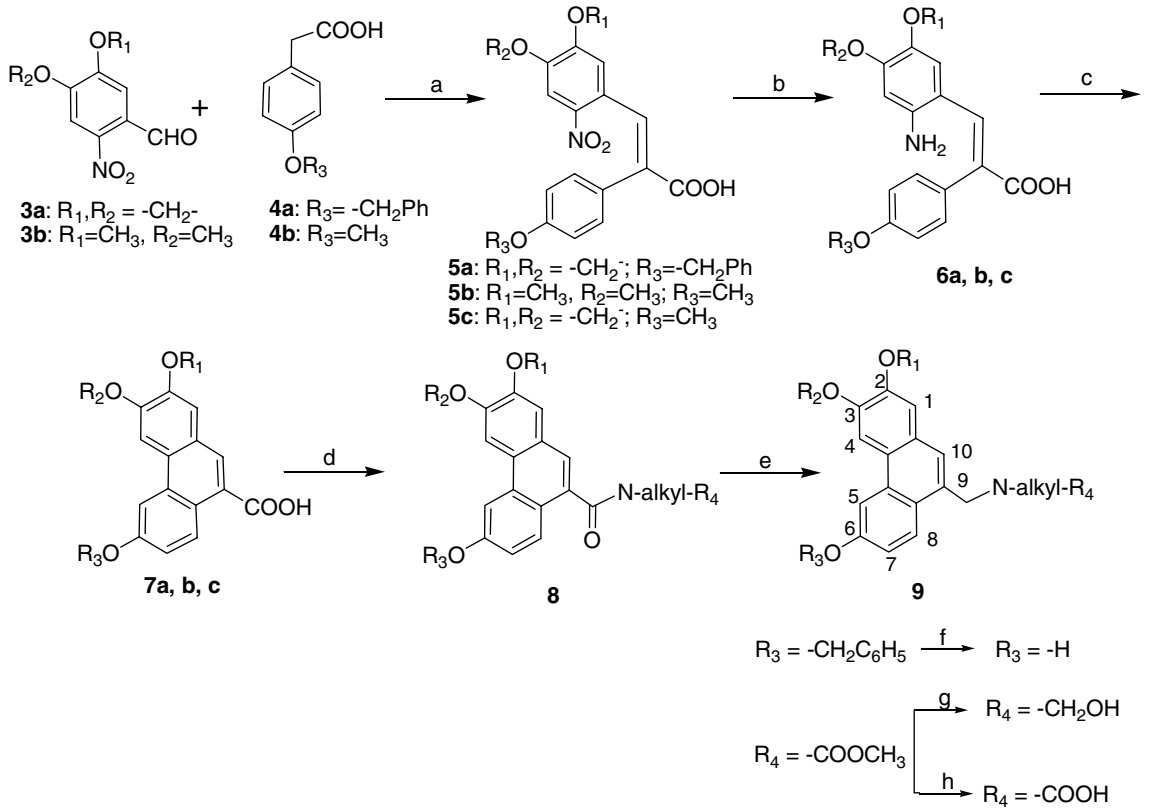


Compound	R_1	R_2	IC_{50}[a,b] (μM)
7a	$-CH_2C_6H_5$	–COOH	NA
7b	$-CH_3$	–COOH	NA
10	$-CH_2C_6H_5$	$-CONH(CH_2)_5COOMe$	41.2
11	$-CH_2C_6H_5$	$-CONH(CH_2)_5COOH$	41.2
12	$-CH_2C_6H_5$	$-CH_2NH(CH_2)_5COOH$	1.6
13	$-CH_2C_6H_5$	$-CH_2NH(CH_2)_5CH_2OH$	1.1
14	$-CH_2C_6H_5$	$-CH_2NH(CH_2)_4COOMe$	17.0
15	$-CH_2C_6H_5$	$-CH_2NH(CH_2)_4COOH$	2.2
16	$-CH_2C_6H_5$		42.6
17	$-CH_2C_6H_5$		32.1
18	$-CH_2C_6H_5$		4.4
19	$-CH_2C_6H_5$		1.8
20	$-CH_2C_6H_5$		3.2
21	$-CH_2C_6H_5$		1.3
22	–H		39.7
23	–H		41.2
24	–H		39.7
25	CH_3	$-CONH(CH_2)_4COOH$	73.3
26	CH_3	$-CH_2NH(CH_2)_4COOMe$	25.3
27	CH_3	$-CH_2NH(CH_2)_4COOH$	1.3
28	CH_3	$-CH_2NH(CH_2)_4CH_2OH$	0.27
29	CH_3		5.3
30	CH_3		73.8
31	CH_3		2.1
32	CH_3		0.7
33	CH_3		0.5
34	CH_3		0.16

[a] Etoposide (VP-16) used as positive control, IC_{50} = 1.4 μM.
[b] NA, not active.

and dihydrofolate reductase.[22] In addition, they were also found to induce cell apoptosis.[23] In 2004, Gao et al.[24] found that tylophorine derivatives significantly inhibit activator protein-1, CRE, and NF-κB mediated transcription. These recent discoveries emphasize that tylophorine derivatives merit development as a new antitumor drug class distinctly different from current cancer chemotherapeutic agents.

In the early 1960s, tylocrebrine (**2**), a positional isomer of tylophorine, was advanced to clinical trials but failed due to its significant CNS toxicity, manifested as disorientation and ataxia.[13] Stærk et al.[25] proposed that the CNS side effects could possibly be minimized by use of more polar analogs that would be unable to pass the blood–brain barrier. Our preliminary screening results showed that polar intermediate compound **15**, an amino acid precursor of tylophorine, exhibited significant cytotoxic activity against a human lung cancer cell line with an IC_{50} value of 2.2 μM. This discovery led us to explore a new series of novel phenanthrene-based tylophorine derivatives (PBTs). Successful application of the Pschorr cyclization[26] and coupling reaction in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) enabled efficient and concise access to more polar PBTs with overall yields greater than 50%. In comparison to the reported total synthesis of tylophorine,[27] our PBT synthesis avoids the cyclization step (limiting synthetic factor) and makes the parallel and large-scale synthesis of PBTs feasible, which could potentially enhance the development of these



Scheme 1. Reagents and conditions: (a) Ac_2O/Et_3N; (b) $FeSO_4/NH_4OH$; (c) $NaNO_2$/fluoroboric acid; ferrocene/acetone; (d) EDC, DMAP, HOBt/DMF; (e) BMS/THF; (f) H_2, Pd/C/MeOH; (g) $LiAlH_4$/THF; (h) NaOH/MeOH (1:1).
Structures of target compounds **10**–**42**, including R_1, R_2, R_3 and *N*-alkyl, are found in Tables 1–3.

For general structure **8** with $R_4 = COOMe$: **7a** $\xrightarrow{d}$ **10**
For general structure **8** with $R_4 = COOH$: **7a/b** $\xrightarrow{d+h}$ **11, 16, 25, 29**
For general structure **10** with $R_4 = COOMe$: **7a/b** $\xrightarrow{d+e}$ **14, 17, 26, 30**
For general structure **10** with $R_4 = COOH$: **7a/b/c** $\xrightarrow{d+e+h}$ **12, 15, 18, 20, 27, 31, 33, 35, 37**
For general structure **10** with $R_4 = CH_2OH$: **7a/b/c** $\xrightarrow[d+e+h+g]{}$ **13, 19, 21, 28, 32, 34, 36, 38**
For $R_3 = OH$, $R_4 = COOH$: **16, 18, 20** $\xrightarrow{f}$ **22, 23, 24**
For seco PBT analogs: **5c** $\xrightarrow{d+h}$ **39** $\xrightarrow{e}$ **40** $\xrightarrow{g}$ **41**

compounds into drugs. In addition, by opening the indolizidine ring, polar chemical moieties can easily be introduced into the phenanthrene skeleton in conjunction with Stærk's proposal.

Therefore, as part of our studies in plant-derived antitumor agents, we have initiated the design and synthesis of new tylophorine analogs. This paper describes our initial design and synthesis of novel polar PBTs, cytotoxicity evaluation against the human A549 lung cancer cell line (Table 1), and structure–activity relationships (SAR) of this new compound class. The goal of this study is to generate and optimize phenanthrene derivatives as promising clinical trial candidates for treating cancer.

2. Chemistry

Compounds **10**–**42** were synthesized following the efficient reported procedure[26] outlined in Scheme 1. A commercially available substituted *O*-nitrobenzaldehyde (**3a**,**b**) was treated with 4-methoxy or -benzyloxyphenyl acetic acid (**4a**,**b**) in the presence of Ac_2O (Perkin condensation) to yield an intermediate nitro-substituted cinnamic acid (**5a**–**c**). The nitro group of **5** was converted to an amine using ammoniacal ferrous sulfate ($FeSO_4$) to provide the amino-substituted cinnamic acid (**6a**–**c**). Phenanthrenes **7a**–**c**, which are the key intermediates in our method, were formed by an improved free-radical Pschorr cyclization[26] in high yield. Compound **6** was treated with sodium nitrite in 48% fluoroboric acid, and the resulting diazonium tetrafluoroborate was then efficiently cyclized to the phenanthrene-9-carboxylic acid (**7**) using catalytic ferrocene. Intermediates **7a**–**c** were then condensed with the appropriate protected cyclic or acyclic amino acid in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 4-(dimethylamino)pyridine (DMAP), and 1-hydroxybenzotriazole (HOBT) to give the amido-substituted phenanthrene (**8**). These mild conditions avoid the production of degradation products resulting from the acidic instability of the methylenedioxy group. Selective reduction of the amide carbonyl group to a methylene was achieved by using borane-methyl sulfide

complex (BMS, 2.0 M solution in THF) to provide **9**. The C-9 side-chain methyl ester was converted to carboxylic acid and hydroxymethyl groups by basic hydrolysis and lithium aluminum hydride reduction, respectively. The 6-phenolic analogs were prepared by hydrogenolysis (H_2, Pd/C) of the benzyloxy-protecting group.

3. Results and discussion

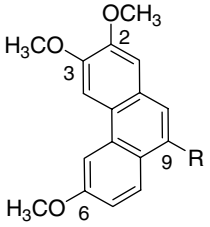
Tables 1–3 summarize the structures of newly synthesized PBTs (**7a**–**c**, **10**–**42**) and their cytotoxic activity (IC_{50}) against the A549 lung cancer cell line. Etoposide (VP-16) was used as the reference compound.

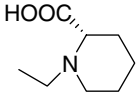
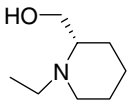
All three intermediate phenanthrene-9-carboxylic acids **7a**–**c** were inactive. Thus, the rigid, planar phenanthrene system is not sufficient for cytotoxic activity, and an appropriate C-9 side chain is crucial. However, the phenanthrene system is required for cytotoxic activity, as the seco analogs **39**–**42**, which contain an active proline side chain (see discussion below) but only a stilbene skeleton, were also inactive.

Active analogs contained both cyclic (pyrrole/proline, piperidine/pipecolinic acid) and acylic (aminopentanoic, aminohexanoic acids) nitrogen-containing side chains at the C-9 position of phenanthrene. However, the linkage between the nitrogen and the phenanthrene was very critical. Reduction of the amide carbonyl to methylene could dramatically increase the cytotoxic activity as shown in the comparison of **16** (L-proline amide, carbonyl linkage, IC_{50} 42.6 μM) and **18** (methylene linkage, IC_{50} 4.4 μM). This observation (carbonyl, unfavorable or less favorable; methylene, favorable) also could be seen in comparison of **11/12** and **25/27**. Differences in conformational constraint could possibly explain this observation. The carbonyl is conjugated with the phenanthrene ring and, thus, extends the coplanarity, while the methylene substituent can rotate freely around the C–C bond. The former geometric restriction appears to disfavor cytotoxic activity and may prevent the molecule from attaining the optimal conformation for binding to an assumed biological target. In addition, the basicity of the nitrogen would differ between the amide (carbonyl linkage) and amine (methylene linkage) analogs.

On the phenanthrene skeleton, changing the benzyloxy moiety at the C-6 position to a hydroxy group was extremely detrimental to activity (cf. **18/23** and **20/24**). In addition, active compounds with a 6-benzyloxy moiety (**15** and **18**–**21**, IC_{50} 1.3–4.4 μM) were generally slightly less potent than corresponding analogs with a 6-methoxy group (**27** and **31**–**34**, IC_{50} 0.16–2.1 μM). Thus, the rank order of potency at the phenanthrene C-6 position was methoxy > benzyloxy ≫ hydroxyl, suggesting that this position cannot tolerate the intro-

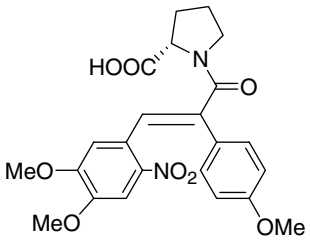
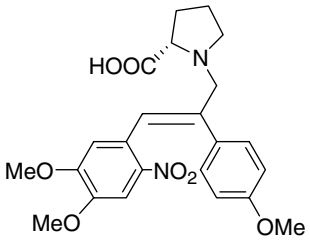
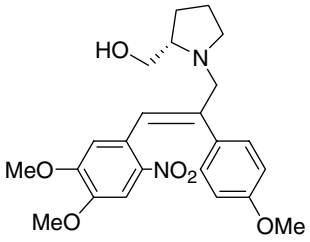
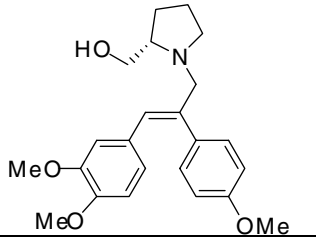
Table 2. SAR of 2,3,6-trimethoxy-9-substituted PBT analogs



Compound	R	IC_{50}[a,b] (μM)
7c	–COOH	NA
35	$-CH_2NH(CH_2)_5COOH$	9.7
36	$-CH_2NH(CH_2)_5CH_2OH$	2.7
37		9.7
38		6.3

[a] Etoposide (VP-16) used as positive control, IC_{50} = 1.4 μM.
[b] NA, not active.

Table 3. SAR of seco-PBT analogs

Compound	Structure	IC_{50}[a] (μM)
39		80
40		45.2
41		11.7
42		52.2

[a] Etoposide (VP-16) used as positive control, IC_{50} = 1.4 μM.

duction of a polar moiety. A hydrophilic moiety may be disfavored because a hydrophobic interaction occurs at this position between the compound and biological target that is essential for enhanced cytotoxic activity.

Interestingly, active compounds with a 2,3-methylenedioxy group (**33** and **34**, IC_{50} 0.5 and 0.16 μM) were up to 40 times more potent than the corresponding compounds with a 2,3-dimethoxy moiety (**37** and **38**, IC_{50} 9.7 and 6.3 μM). Thus, the five-membered methylenedioxy ring extension at the phenanthrene C2-C3 is quite favorable for cytotoxic activity.

In the amino side chain, when the terminal carboxylic acid group was masked as the methyl ester, the cytotoxic activity was diminished drastically or abolished (e.g., **14/15**, **17/18**, **26/27**, and **30/31**). However, reduction of the carboxylic acid to the hydroxymethyl generally increased activity, both in cyclic (**18/19**, **20/21**, **31/32**, **33/34**, and **37/38**) and acyclic (**12/13**, **27/28**, and **35/36**) analogs. Thus, the rank order of potency of the terminal polar substituent was hydroxy > carboxylic acid ≫ methyl ester. Hydrogen bond donor/acceptor capability or polarity may affect the activity.

In summary, the favorable modifications for these novel PBTs are as follows. (1) A planar phenanthrene system is required, but not sufficient for cytotoxic activity. (2) A *N*-hydrophilic substituent at the C-9 position is essential for the enhanced cytotoxicity and should be linked through a methylene rather than a carbonyl group. (3) This C-9 *N*-hydrophilic substituent is ideal for the introduction of a polar moiety. Analogs containing terminal carboxylic acid or hydroxymethyl groups are more favorable than those with methyl esters. (4) On the phenanthrene skeleton, a methoxy substituent best fits both the steric and electronic requirements at the C-6 position and is preferred over benzyloxy and hydroxy groups. (5) Adding a methylenedioxy ring at the 2,3 position of the planar phenanthrene system can dramatically enhance the cytotoxic activity and led to the most potent derivatives.

4. Conclusion

In this study, a total of 33 novel phenanthrene-based tylophorine derivatives (PBTs) were synthesized and evaluated for cytotoxic activity against human A549 lung cancer cells. Among these compounds, *N*-(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-L-2-piperidinemethanol (**34**) and *N*-(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-5-aminopentanol (**28**) were the most potent compounds designed and synthesized to date with IC_{50} values of 0.16 and 0.27 μM, respectively. These two compounds incorporated all of the favorable modifications identified to date. They possess a novel structure and showed remarkable IC_{50} values in the sub-micromolar range, comparable with front-line antineoplastic drugs such as etoposide, suggesting that this new compound class is worthy of further development as potential antitumor clinical trial candidates. We are extending these preliminary structural and biological studies to include both additional compounds and cancer cell lines.

5. Experimental

5.1. General information

Melting points were measured using a Fisher Johns melting apparatus without correction. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a 400 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. Mass spectra were recorded on a PE-Sciex API-3000 LC/MS/MS instrument equipped with a Turbo IonsSpray ion source. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. All active target compounds were analyzed for C, H, N and gave values within ±0.4% of the theoretical values (Table 4). 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 as medium-pressure column chromatography. Silica gel (200–400 mesh) from Aldrich, Inc. was used for column chromatography. 4-Benzyloxyphenylacetic acid and 3,4-

Table 4. Elemental analysis data for active PBT analogs

Compound	Chemical formula	Analysis	Anal. Calcd (%)			Found (%)		
			C	H	N	C	H	N
12	$C_{29}H_{29}O_5N$	C, H, N	73.87	6.20	2.97	73.89	6.16	2.97
13	$C_{29}H_{31}O_4N$	C, H, N	76.12	6.83	3.06	76.15	6.78	3.06
15	$C_{28}H_{27}O_5N$	C, H, N	73.51	5.95	3.06	73.52	5.91	3.06
18	$C_{28}H_{25}O_5N{\cdot}1.0\ H_20$	C, H, N	71.02	5.75	2.96	70.91	5.66	2.99
19	$C_{28}H_{27}O_4N$	C, H, N	76.17	6.16	3.17	76.54	6.21	3.20
20	$C_{29}H_{27}O_5N$	C, H, N	74.18	5.80	2.98	74.20	5.76	2.96
21	$C_{29}H_{29}O_4N$	C, H, N	76.46	6.42	3.07	76.82	5.96	3.09
27	$C_{22}H_{23}O_5N$	C, H, N	69.28	6.08	3.67	69.38	6.17	3.61
28	$C_{22}H_{25}O_4N$	C, H, N	71.9	6.86	3.81	69.97	6.62	3.75
31	$C_{22}H_{21}O_5N{\cdot}0.5\ H_20$	C, H, N	68.03	5.71	3.61	68.01	5.65	3.52
32	$C_{22}H_{23}O_4N$	C, H, N	72.31	6.34	3.83	72.56	6.22	3.72
33	$C_{23}H_{23}O_5N$	C, H, N	74.18	5.80	2.98	73.99	5.79	2.87
34	$C_{23}H_{25}O_4N{\cdot}1.5\ H_2O$	C, H, N	67.96	6.94	3.45	68.01	6.92	3.44

methylenedioxy-6-nitrobenzaldehyde were purchased from TCI. L-Pipecolinic acid and isonipecotic acid were commercially available from Lancaster. All other chemicals were obtained from Aldrich, Inc. and Fisher, Inc.

5.2. General synthetic procedures

5.2.1. Coupling reaction (a). A solution of 3,4-methylenedioxy-6-nitrobenzaldehyde (12 mmol), triethyl amine (12 mmol), and 4-benzyloxy- or 4-methoxy-phenylacetic acid (17 mmol) in anhydrous acetic anhydride (17 mL) was refluxed with stirring under Ar for 40 min. Water (30 mL) was added to the reaction mixture and the temperature was maintained at 90–100 °C during the addition. The reaction mixture was cooled to rt and the solid was collected by filtration and recrystallized from EtOH.

5.2.2. Reduction of the nitro group (b). To a solution of the nitrocinnamic acid (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 recrystallized from EtOH to yield the aminocinnamic acid.

5.2.3. Ring closure (c). A solution composed of the aminocinnamic acid (3 mmol), NaOH (33 mmol), and $NaNO_2$ in water (10 mL) was added dropwise over 30 min with stirring to 48% fluoroboric acid (43 mmol) at 0–5 °C. The mixture was stirred for 1 h, after which sat. aqueous sulfamic acid was added until the mixture tested negative to starch-iodide paper. The crude solid was collected by filtration, dissolved in anhydrous acetone (10 mL), and then added dropwise to ferrocene (0.06 g, 0.3 mmol) in anhydrous acetone with stirring over a 15 min period at rt. 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 phenanthrene-9-carboxylic acid.

5.2.4. Peptide bond condensation reaction (d). To a solution of phenanthrene-9-carboxylic acid (4 mmol), 4-(dimethylamino) pyridine (DMAP) (2 mmol), and 1-hydroxybenzotriazole (HOBT) (4 mmol) in DMF (20 mL) was added 4-methylmorpholine (NMM) (1.03 mL). After the mixture was stirred at 0 °C for 15 min, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (4.4 mmol) was added in portions. After 30 min stirring, the appropriate amino acid methyl ester[†] (4.4 mmol) was added. The reaction mixture was stirred overnight at rt 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, filtered, and concentrated in vacuo. The crude product was chromatographed using Biotage Flash and Isco Companion systems using a 40 g silica cartridge and EtOAc/hexane as eluant.

5.2.5. Carbonyl reduction reaction (e). To a stirred solution of 9-amido-substituted phenanthrene (2 mmol) in THF (20 mL) was added dropwise borane-methyl sulfide (BMS) (4 mL, 2.0 M solution in THF). The reaction mixture was stirred at rt overnight and quenched with 1 N HCl. THF was removed in vacuo, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was dried, filtered, and evaporated to afford the target product. The crude product was chromatographed using Biotage Flash and Isco Companion systems using MeOH/CH_2Cl_2 as eluant.

5.2.6. Catalytic hydrogenolysis of benzyl group (g). A solution of benzyloxy derivative (1 mmol) and Pd/C (10%) was hydrogenated in a Parr apparatus (30 psi) for 2 h. The reaction mixture was filtered through Celite, and filtrate was concentrated in vacuo and was chromatographed using Biotage Flash and Isco Companion systems using MeOH/CH_2Cl_2 as eluant.

5.2.7. $LiAlH_4$ reduction (g). To a suspension of methyl ester (1 mmol) in anhydrous THF (15 mL) was added $LiAlH_4$ (1 g) in portions at 0 °C. The reaction mixture was warmed to rt and heated to reflux for 4 h. After cooling to 0 °C, the reaction mixture was quenched with MeOH. 10% Rochelle salt was added to the mixture, which was then 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 using MeOH/CH_2Cl_2 as eluant.

5.2.8. Methyl ester hydrolysis reaction (h). A solution of ester in a 1:1 mixture of 4 N NaOH and MeOH 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 using MeOH/CH_2Cl_2 as eluant.

5.2.9. 2,3-Methylenedioxy-6-benzyloxy-phenanthrene-9-carboxylic acid (7a). 75% yield over two steps; mp 263–265 °C; ^{1}H NMR (400.13 MHz) δ 8.40 (d, J = 4 Hz, 1H), 7.92 (d, J = 2 Hz, 1H), 7.88 (s, 1H), 7.52 (m, 2H), 7.38 (m, 5H), 7.26 (dd, J = 4 Hz, 2 Hz, 1H), 6.14 (d, J = 4 Hz, 2H), 5.35 (d, J = 7 Hz, 2H); ESI MS m/z: 373 $(M+H)^+$.

5.2.10. 2,3-Methylenedioxy-6-methoxy-phenanthrene-9-carboxylic acid (7b). 78% 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 $(M+H)^+$.

[†] For a cycloalkylamino acid, $SOCl_2$ (0.4 mL) was added dropwise to the amino acid (4 mmol) in anhydrous MeOH (4 mL) at −30 °C. The reaction mixture was warmed to rt and refluxed for 1 h. For an acyclic alkylamino acid, acetyl chloride (0.45 mL) was added dropwise to anhydrous MeOH (3 mL) at 0 °C. After 10 min stirring, the amino acid was added to the solution in portions. The mixture was warmed to rt and refluxed for 2 h. Then the solvent was removed in vacuo and the resulting methyl ester was used without further purification.

5.2.11. 2,3,6-Trimethoxyphenanthrene-9-carboxylic acid (7c). 79% yield; white powder; mp 241–243 °C; ^{1}H NMR (400.13 MHz) δ 8.93 (d, J = 4 Hz, 1H), 8.33 (s, 1H), 7.84 (d, J = 2Hz, 1H), 7.82 (s, 1H), 7.23 (s, 1H), 7.22 (dd, J = 4 Hz, 2 Hz, 1H), 4.07 (s, 3H), 3.98 (s,3H), 3.97 (s, 3H); ESI MS m/z: 314 $(M+H)^+$.

5.2.12. Methyl *N*-(2,3-methylenedioxy-6-benzyloxy-phenanthr-9-ylcarbonyl)-6-aminohexanoate (10). General procedure **d** from **7a** and methyl 6-aminohexanoate (92%); colorless syrup; ^{1}H NMR (400.13 MHz) δ 8.09 (d, J = 4 Hz, 1H), 7.81 (d, J = 2 Hz, 1H), 7.82 (s, 1H), 7.74 (d, J = 4 Hz, 2H), 7.45 (s, 1H), 7.33 (t, J = 4 Hz, 2H), 7.26 (m, 1H), 7.20 (dd, J = 4 Hz, 2 Hz, 1H), 7.09 (s, 1H), 6.03 (s, 2H), 5.18 (s, 2H), 3.41(s, 3H), 3.28 (m, 2H), 2.28 (t, J = 6 Hz, 2H), 1.61 (m, 2H), 1.56 (m, 2H), 1.38 (m, 2H); ESI MS m/z: 500 $(M+H)^+$.

5.2.13. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylcarbonyl)-6-aminohexanoic acid (11). General procedure **h** from **10** (100%); white needles; mp 198–200 °C; ^{1}H NMR (400.13 MHz) δ 8.06 (d, J = 4 Hz, 1H), 7.79 (d, J = 2 Hz, 1H), 7.75 (s, 1H), 7.48 (s, 1H), 7.40 (d, J = 4 Hz, 2H), 7.30 (t, J = 4 Hz, 2H), 7.23 (m, 1H), 7.16 (dd, J = 4 Hz, 2 Hz, 1H), 7.09 (s, 1H), 6.00 (s, 2H), 5.15 (s, 2H), 3.23 (m, 2H), 2.23 (t, J = 6 Hz, 2H), 1.58 (m, 4H), 1.36 (m, 2H); ESI MS m/z: 486 $(M+H)^+$.

5.2.14. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-6-aminohexanoic acid (12). General procedures **e** and **h** from **10** (90%); white powder, recrystallized from EtOH to give white needles; mp 184–186 °C; ^{1}H NMR (400.13 MHz) δ 7.90 (d, J = 4 Hz, 1H), 7.87 (d, J = 2 Hz, 1H), 7.77 (s, 1H), 7.60 (s, 1H), 7.44 (d, J = 4 Hz, 2H), 7.34 (t, J = 4 Hz, 2H), 7.30 (dd, J = 4 Hz, 2 Hz, 1H), 7.28 (m, 1H), 7.16 (s, 1H), 6.03 (s, 2H), 5.19 (s, 2H), 4.46 (s, 2H), 2.83 (m, 2H), 2.26 (t, J = 6 Hz, 2H), 1.90 (m,2H), 1.68 (m, 2H), 1.28 (m, 2H); ESI MS m/z: 472 $(M+H)^+$. Anal. ($C_{29}H_{29}O_5N$) C, H, N.

5.2.15. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-6-aminohexanol (13). General procedure **g** from **12** (95%); white powder; mp 175–177 °C; ^{1}H NMR (400.13 MHz) δ 7.90 (d, J = 4 Hz, 1H), 7.86 (d, J = 2 Hz, 1H), 7.76 (s, 1H), 7.45 (s, 1H), 7.44 (d, J = 4 Hz, 2H), 7.32 (t, J = 4 Hz, 2H), 7.26 (m, 1H), 7.24 (dd, J = 4 Hz, 2 Hz, 1H), 7.14 (s, 1H), 6.03 (s, 2H), 5.22 (s, 2H), 4.24 (s, 2H), 3.53 (t, J = 6 Hz, 2H), 2.72 (t, J = 6 Hz, 2H), 1.58 (m, 2H), 1.47 (m, 2H), 1.29 (m, 4H); ESI MS m/z: 458 $(M+H)^+$. Anal. ($C_{29}H_{31}O_4N$) C, H, N.

5.2.16. Methyl *N*-(2,3-methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-5-aminopentanoate (14). General procedures **d** and **e** from **7a** and methyl 5-aminopentanoate (93%); colorless syrup; ^{1}H NMR (400.13 MHz) δ 7.97 (d, J = 4 Hz, 1H), 7.83 (d, J = 2 Hz, 1H), 7.77 (s, 1H), 7.46 (s, 1H), 7.45 (d, J = 4 Hz, 2H), 7.35 (t, J = 4 Hz, 2H), 7.27 (m, 1H), 7.20 (dd, J = 4 Hz, 2 Hz, 1H), 7.07 (s, 1H), 6.01 (s, 2H), 5.17 (s, 2H), 4.95 (s, 2H), 3.50 (s, 3H), 3.01 (m, 2H), 2.42 (t, J = 6 Hz, 2H), 1.67 (m, 2H), 1.59 (m, 2H); ESI MS m/z: 472 $(M+H)^+$.

5.2.17. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-5-aminopentanoic acid (15). General procedure **h** from **14** (93%); white powder, recrystallized from EtOH to give white needles; mp 197–199 °C; ^{1}H NMR (400.13 MHz) δ 7.89 (d, J = 4 Hz, 1H), 7.88 (d, J = 2 Hz, 1H), 7.77 (s, 1H), 7.46 (s, 1H), 7.45 (d, J = 4 Hz, 2H), 7.34 (t, J = 4 Hz, 2H), 7.29 (dd, J = 4 Hz, 2 Hz, 1H), 7.27 (m, 1H), 7.16 (s, 1H), 6.05 (s, 2H), 5.21 (s, 2H), 4.42 (s, 2H), 2.89 (m, 2H), 2.27 (t, J = 6 Hz, 2H), 1.89 (m, 2H), 1.68 (m, 2H); ESI MS m/z: 458 $(M+H)^+$. Anal. ($C_{28}H_{27}O_5N$) C, H, N.

5.2.18. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylcarbonyl)-L-proline (16). General procedures **d** and **h** from **7a** and L-proline methyl ester (95%); white powder; mp 221–223 °C; ^{1}H NMR (400.13 MHz) δ 8.00 (d, J = 4Hz, 1H), 7.85 (d, J = 2 Hz, 1H), 7.81 (s, 1H), 7.46 (s, 1H), 7.45 (d, J = 4 Hz, 2H), 7.36 (t, J = 4 Hz, 2H), 7.30 (m, 1H), 7.23 (dd, J = 4 Hz, 2 Hz, 1H), 7.13 (s, 1H), 6.04 (s, 2H), 5.19 (s, 2H), 4.71 (t, J = 7 Hz, 1H), 3.62 (m, 2H), 2.21 (m, 2H), 1.83 (m, 2H); ESI MS m/z: 470 $(M+H)^+$.

5.2.19. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-L-proline methyl ester (17). General procedures **d** and **e** from **7a** and L-proline methyl ester (85%); brown syrup; ^{1}H NMR (400.13 MHz) δ 7.97 (d, J = 4 Hz, 1H), 7.83 (d, J = 2 Hz, 1H), 7.80 (s, 1H), 7.71 (s, 1H), 7.59 (d, J = 4 Hz, 2H), 7.47 (t, J = 4 Hz, 2H), 7.36 (m, 1H), 7.22 (dd, J = 4 Hz, 2 Hz, 1H), 7.11 (s, 1H), 6.06 (s, 2H), 5.20 (s, 2H), 4.75 (s, 2H), 3.67 (s, 3H), 3.26 (d, J = 17 Hz, 1H), 2.45 (m, 2H), 1.96 (m, 2H), 1.68 (m, 2H); ESI MS m/z: 470 $(M+H)^+$.

5.2.20. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-L-proline (18). General procedure **h** from **17** (100%); white powder; mp 147–149 °C; ^{1}H NMR (400.13 MHz) δ 8.11 (d, J = 4 Hz, 1H), 7.84 (d, J = 2 Hz, 1H), 7.75 (s, 1H), 7.42 (s, 1H), 7.40 (d, J = 4 Hz, 2H), 7.30 (t, J = 4 Hz, 2H), 7.27 (m, 1H), 7.23 (dd, J = 4 Hz, 2 Hz, 1H), 7.14 (s, 1H), 6.01 (s, 2H), 5.16 (s, 2H), 4.30 (s, 2H), 3.24 (m, 1H), 2.48 (m, 2H), 2.0 (m, 2H), 1.68 (m, 2H); ESI MS m/z: 456 $(M+H)^+$. Anal. ($C_{28}H_{25}O_5N{\cdot}1.0\ H_2O$) C, H, N.

5.2.21. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-L-prolinol (19). General procedure **g** from **18** (95%); colorless oil, recrystallization from EtOH gave white powder; mp 122–124 °C; ^{1}H NMR (400.13 MHz) δ 8.13 (d, J = 4 Hz, 1H), 7.90 (d, J = 2 Hz, 1H), 7.84 (s, 1H), 7.51 (d, J = 4 Hz, 2H), 7.42 (s, 1H), 7.41 (t, J = 4 Hz, 2H), 7.34 (m, 1H), 7.29 (dd, J = 4 Hz, 2 Hz, 1H), 7.14 (s, 1H), 6.06 (s, 2H), 5.24 (s, 2H), 4.36 (d, J = 7 Hz, 2H), 3.78 (d, J = 17 Hz, 2H), 3.41 (m, 1H), 2.42 (m, 2H), 1.83 (m, 2H), 1.66 (m, 6H); ESI MS m/z: 442 (M+H)+. Anal. ($C_{28}H_{27}O_4N$) C, H, N.

5.2.22. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-L-2-piperidinecarboxylic acid (20). General procedures **d**, **e**, and **h** from **7a** and methyl L-2-piperidinecarboxylate (83%); white powder; mp 168–170 °C; ^{1}H NMR (400.13 MHz) δ 8.26 (d, J = 4 Hz, 1H), 7.85 (d, J = 2 Hz, 1H), 7.77 (s, 1H), 7.56 (s, 1H), 7.45 (d,

J = 4 Hz, 2H), 7.35 (t, J = 4 Hz, 2H), 7.30 (m, 1H), 7.26 (dd, J = 4 Hz, 2 Hz, 1H), 7.12 (s, 1H), 6.05 (s, 2H), 5.20 (s, 2H), 4.24 (s, 2H), 3.16 (m, 1H), 2.26 (m, 2H), 1.80 (m, 2H), 1.64 (m, 4H); ESI MS m/z: 470 $(M+H)^+$. Anal. ($C_{29}H_{27}O_5N$) C, H, N.

5.2.23. *N*-(2,3-Methylenedioxy-6-benzyloxy-phenanthr-9-ylmethyl)-L-piperidinemethanol (21). General procedure **g** from **20** (95%); light brown oil, recrystallization from EtOH gave white powder; mp 151–152 °C ; ^{1}H NMR (400.13 MHz) δ 8.17 (d, J = 4 Hz, 1H), 7.89 (d, J = 2 Hz, 1H), 7.83 (s, 1H), 7.51 (d, J = 4 Hz, 2H), 7.43 (s, 1H), 7.40 (t, J = 4 Hz, 2H), 7.34 (m, 1H), 7.27 (dd, J = 4 Hz, 2 Hz, 1H), 7.14 (s, 1H), 6.06 (s, 2H), 5.23 (s, 2H), 4.45 (d, J = 7 Hz, 2H), 3.46 (d, J = 17 Hz, 2H), 2.85 (m, 1H), 2.37 (m, 2H), 1.69 (m, 6H); ESI MS m/z: 456 $(M+H)^+$. Anal. ($C_{29}H_{29}O_4N$) C, H, N.

5.2.24. *N*-(2,3-Methylenedioxy-6-hydroxy-phenanthr-9-ylcarbonyl)-L-proline (22). General procedure **f** from **16** (92%); white powder; mp 230–231 °C; ^{1}H NMR (400.13 MHz) δ 7.73 (d, J = 4 Hz, 1H), 7.67 (s, 1H), 7.50 (d, J = 2 Hz, 1H), 7.35 (s, 1H), 7.21 (dd, J = 4 Hz, 2 Hz, 1H), 7.06 (m, 1H), 6.01 (s, 2H), 4.67 (t, J = 7 Hz, 1H), 3.22 (m, 2H), 2.38 (m, 2H), 2.18 (m, 2H), 1.86 (m, 2H); ESI MS m/z: 380 $(M+H)^+$.

5.2.25. *N*-(2,3-Methylenedioxy-6-hydroxy-phenanthr-9-ylmethyl)-L-proline (23). General procedure **f** from **18** (95%); white powder; mp 205–206 °C; ^{1}H NMR (400.13 MHz) δ 8.07 (d, J = 4 Hz, 1H), 7.82 (s, 1H), 7.80 (d, J = 2 Hz, 1H), 7.42 (s, 1H), 7.21 (dd, J = 4 Hz, 2 Hz, 1H), 7.10 (s, 1H), 6.04 (s, 2H), 4.09 (s, 2H), 3.16 (m, 1H), 2.38 (m, 2H), 2.23 (m, 2H), 1.95 (m, 2H); ESI MS m/z: 366 $(M+H)^+$.

5.2.26. *N*-(2,3-Methylenedioxy-6-hydroxy-phenanthr-9-ylmethyl)-L-2-piperidinecarboxylic acid (24). General procedure **f** from **20** (95%); white powder; mp 211–213 °C; ^{1}H NMR (400.13 MHz) δ 8.07 (d, J = 4 Hz, 1H), 7.80 (s, 1H), 7.76 (d, J = 2 Hz, 1H), 7.50 (s, 1H), 7.22 (dd, J = 4 Hz, 2 Hz, 1H), 7.12 (s, 1H), 6.05 (s, 2H),4.06 (s, 2H), 3.12 (m, 1H), 2.38 (m, 2H), 1.88 (m, 2H), 1.64 (m, 4H); ESI MS m/z: 380 $(M+H)^+$.

5.2.27. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylcarbonyl)-5-aminopentanoic acid (25). General procedures **d** and **h** from **7b** and methyl 5-aminopentanoate (100%); white powder; mp 137–138 °C; ^{1}H NMR (400.13 MHz) δ 7.92 (d, J = 4 Hz, 1H), 7.82 (s, 1H), 7.75 (s, 1H), 7.71 (d, J = 2 Hz, 1H), 7.13 (dd, J = 4 Hz, 2 Hz, 1H), 7.09 (s, 1H), 6.00 (s, 2H), 3.89 (s, 3H), 3.23 (m, 2H), 2.23 (t, J = 6 Hz, 2H), 1.58 (m, 4H); ESI MS m/z: 412 $(M+H)^+$.

5.2.28. Methyl *N*-(2,3-methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-5-aminopentanoate (26). General procedures **d** and **e** from **7b** (90%); colorless syrup; ^{1}H NMR (400.13 MHz) δ 8.10 (d, J = 4 Hz, 1H), 7.82 (s, 1H), 7.70 (d, J = 2 Hz, 1H), 7.52 (s, 1H), 7.12 (dd, J = 4 Hz, 2 Hz, 1H), 7.08 (s, 1H), 6.04 (s, 2H), 3.89 (s, 3H), 3.80 (s, 2H), 3.56 (s, 3H), 2.80 (m, 2H), 2.33 (t, J = 6 Hz, 2H), 1.64 (m, 4H); ESI MS m/z: 396 $(M+H)^+$.

5.2.29. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-5-aminopentanoic acid (27). General procedure **h** from **26** (99%); white powder; mp 145–146 °C; ^{1}H NMR (400.13 MHz) δ 8.06 (d, J = 4 Hz, 1H), 7.79 (s, 1H), 7.75 (d, J = 2 Hz, 1H), 7.48 (s, 1H), 7.22 (dd, J = 4 Hz, 2 Hz, 1H), 7.10 (s, 1H), 6.03 (s, 2H), 4.02 (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); ESI MS m/z: 382 $(M+H)^+$. Anal. ($C_{22}H_{23}O_5N$) C, H, N.

5.2.30. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-5-aminopentanol (28). General procedure **g** from **27** (97%); colorless oil, recrystallization from EtOH gave white powder; mp 125–126 °C; ^{1}H NMR (400.13 MHz) δ 8.00 (d, J = 4 Hz, 1H), 7.76 (s, 1H), 7.72 (d, J = 2 Hz, 1H), 7.44 (s, 1H), 7.13 (dd, J = 4 Hz, 2 Hz, 1H), 7.05 (s, 1H), 6.04 (s, 2H), 3.95 (s, 2H), 3.93 (s, 3H), 3.58 (t, J = 6 Hz, 2H), 2.75 (m, 2H), 1.55 (m, 2H), 1.44 (m, 2H), 1.30 (m, 2H); ESI MS m/z: 368 $(M+H)^+$. Anal. ($C_{22}H_{25}O_4N$) C, H, N.

5.2.31. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylcarbonyl)-L-proline (29). General procedures **d** and **h** from **7b** and L-proline methyl ester (93%); white powder; mp 207–208 °C; ^{1}H NMR (400.13 MHz) δ 7.78(d, J = 4 Hz, 1H), 7.70 (s, 1H), 7.54 (d, J = 2 Hz, 1H), 7.35 (s, 1H), 7.20 (dd, J = 4 Hz, 2 Hz, 1H), 7.14 (m, 1H), 6.01 (s, 2H), 4.67 (m, 1H), 3.90 (s, 3H), 3.22 (m, 2H), 2.38 (m, 2H), 1.86 (m, 2H); ESI MS m/z: 394 $(M+H)^+$.

5.2.32. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-L-proline methyl ester (30). General procedures **d** and **e** from **7a** (85%); white syrup; ^{1}H NMR (400.13 MHz) δ 8.20 (d, J = 4 Hz, 1H), 7.80 (s, 1H), 7.70 (d, J = 2 Hz, 1H), 7.52 (s, 1H), 7.21 (dd, J = 4 Hz, 2 Hz, 1H), 7.13 (s, 1H), 6.05 (s, 2H), 4.02 (s, 2H), 3.89 (s, 3H), 3.67 (s, 3H), 3.14 (m, 1H), 2.32 (m, 2H), 2.14 (m, 2H), 1.86 (m, 2H); ESI MS m/z: 394 $(M+H)^+$.

5.2.33. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-L-proline (31). General procedure **h** from **30** (100%); white powder; mp 145–146 °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 Hz, 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 $(M+H)^+$. Anal. ($C_{22}H_{21}O_5N{\cdot}0.5\ H_2O$) C, H, N.

5.2.34. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-L-prolinol (32). General procedure **g** from **31** (95%); white powder; mp 138–139 °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 Hz, 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.

5.2.35. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-L-2-piperidinecarboxylic acid (33). General procedures **d**, **e**, and **h** from **7b** and methyl L-2-piperidinecarboxylate (80%); white powder; mp 171–172 °C; ^{1}H

NMR (400.13 MHz) δ 8.21 (d, J = 4Hz, 1H), 7.77 (s, 1H), 7.72 (d, J = 2 Hz, 1H), 7.56 (s, 1H), 7.23 (dd, J = 4 Hz, 2 Hz, 1H), 7.10 (s, 1H), 6.04 (s, 2H), 4.08 (s, 2H), 3.96 (s, 3H), 3.08 (m, 1H), 2.38 (m, 2H), 1.84 (m, 2H), 1.60 (m, 4H); ESI MS m/z: 394 $(M+H)^+$. Anal. ($C_{23}H_{23}O_5N$) C, H, N.

5.2.36. *N*-(2,3-Methylenedioxy-6-methoxy-phenanthr-9-ylmethyl)-L-2-piperidinemethanol (34). General procedure **g** from **33** (98%); white powder; mp 155–157 °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), 7.47 (s, 1H), 7.22 (dd, J = 4 Hz, 2 Hz, 1H), 7.15 (s, 1H), 6.07 (s, 2H), 4.06 (s, 2H), 3.98 (s, 3H), 3.68 (d, J = 7 Hz, 2H), 2.87 (m, 1H), 2.40 (m, 2H), 1.64 (m, 6H); ESI MS m/z: 380 $(M+H)^+$. Anal. ($C_{23}H_{25}O_4N{\cdot}1.5\ H_2O$) C, H, N.

5.2.37. *N*-(2,3,6-Trimethoxyphenanthr-9-ylmethyl)-6-aminohexanoic acid (35). General procedures **d**, **e**, and **h** from **7b** and methyl 6-aminohexanoate (77%); brown powder; mp 133–134 °C; ^{1}H NMR (400.13 MHz) δ 7.90 (d, J = 4 Hz, 1H), 7.78 (s, 1H), 7.72 (d, J = 2 Hz, 1H), 7.52 (s, 1H), 7.20 (dd, J = 4 Hz, 2 Hz, 1H), 7.08 (s, 1H), 3.92 (s, 9H), 3.71 (s, 2H), 2.87 (m, 2H), 2.23 (m, 2H), 1.55 (m, 2H), 1.46 (m, 2H), 1.28 (m, 2H); ESI MS m/z: 412 $(M+H)^+$.

5.2.38. *N*-(2,3,6-Trimethoxyphenanthr-9-ylmethyl)-6-aminohexanol (36). General procedure **g** from **35** (92%); yellow oil; ^{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 Hz, 2 Hz, 1H), 7.05 (s, 1H), 5.99 (s, 2H), 3.94 (s, 9H), 3.80 (s, 2H), 3.73 (t, J = 6 Hz, 2H), 2.75 (m, 2H), 1.50 (m, 4H), 1.28 (m, 4H); ESI MS m/z: 398 $(M+H)^+$.

5.2.39. *N*-(2,3,6-Trimethoxyphenanthr-9-ylmethyl)-L-2-piperidinecarboxylic acid (37). General procedures **d**, **e**, and **h** from **7c** and methyl L-2-piperidinecarboxylate (75%); light yellow powder; mp 187–188 °C; ^{1}H NMR (400.13 MHz) δ 8.21 (d, J = 4 Hz, 1H), 7.78 (s, 1H), 7.71 (d, J = 2 Hz, 1H), 7.56 (s, 1H), 7.23 (dd, J = 4 Hz, 2 Hz, 1H), 7.10 (s, 1H), 4.10 (s, 2H), 3.98 (s, 9H), 3.12 (m, 1H), 2.42 (m, 2H), 1.80 (m, 2H), 1.60 (m, 4H); ESI MS m/z: 410 $(M+H)^+$.

5.2.40. *N*-(2,3,6-Trimethoxyphenanthr-9-ylmethyl)-L-2-piperidinemethanol (38). General procedure **g** from **37** (95%); brown oil, recrystallization from EtOH gave yellow powder; mp 135–137 °C; ^{1}H NMR (400.13 MHz) δ 8.21 (d, J = 4 Hz, 1H), 7.78 (s, 1H), 7.72 (d, J = 2 Hz, 1H), 7.50 (s, 1H), 7.20 (dd, J = 4 Hz, 2 Hz, 1H), 7.14 (s, 1H), 6.07 (s, 2H), 4.10 (s, 2H), 3.96 (s, 9H), 3.74 (d, J = 17 Hz, 2H), 2.87 (m, 1H), 2.41 (m, 2H), 1.66 (m, 6H); ESI MS m/z: 396 $(M+H)^+$.

5.2.41. 1-[3-(4,5-Dimethoxy-2-nitrophenyl)-2-(4-methoxyphenyl)-acryloyl]-pyrrolidine-2-carboxylic acid (39). General procedures **d** and **h** from **5b** and methyl pyrrolidine-2-carboxylate (90%); brown powder; mp 120–122 °C; ^{1}H NMR (400.13 MHz) δ7.67 (s, 1H), 7.26 (d, J = 4 Hz, 2H), 7.08 (s, 1H), 6.73 (d, J = 4Hz, 2H), 6.44 (s, 1H), 4.63 (t, J = 7 Hz, 1H), 3.92 (s, 6H), 3.73 (s, 3H), 3.41(t, J = 7 Hz, 2H), 2.22 (m, 2H), 1.92 (m, 2H); ESI MS m/z: 443 $(M+H)^+$.

5.2.42. 1-[3-(4,5-Dimethoxy-2-nitrophenyl)-2-(4-methoxyphenyl)-allyl]-pyrrolidine-2-carboxylic acid (40). General procedure **e** from **39** (90%); brown syrup; ^{1}H NMR (400.13 MHz) δ 7.61 (s, 1H), 7.24 (d, J = 4Hz, 2H), 7.01 (s, 1H), 6.77 (d, J = 4 Hz, 2H), 6.64 (s, 1H), 4.36 (d, J = 7 Hz, 2H), 3.89 (s, 3H), 3.72 (t, J = 7 Hz, 1H), 3.69 (s, 3H), 3.42 (s, 3H), 3.24 (m, 2H), 2.18 (m, 2H), 1.84 (m, 2H); ESI MS m/z: 429 $(M+H)^+$.

5.2.43. {1-[3-(4,5-Dimethoxy-2-nitrophenyl)-2-(4-methoxyphenyl)-allyl]-pyrrolidin-2-yl}-methanol (41). General procedure **g** from **40** (90%); dark oil; ^{1}H NMR (400.13 MHz) δ 7.59 (s, 1H), 7.23 (d, J = 4 Hz, 2H), 7.00 (s, 1H), 6.71 (d, J = 4 Hz, 2H), 6.23 (s, 1H), 4.17 (d, J = 7 Hz, 2H), 3.89 (s, 3H), 3.78 (d, J = 7 Hz, 2H), 3.69 (s, 3H), 3.58 (m, 1H), 3.41 (s, 3H), 3.16 (m, 2H), 1.91 (m, 2H), 1.80 (m, 2H); ESI MS m/z: 415 $(M+H)^+$.

5.2.44. {1-[3-(3,4-Dimethoxyphenyl)-2-(4-methoxyphenyl)allyl]-pyrrolidin-2-yl}-methanol (42). Silmilar procedure as **40** (77% for two steps); yellow syrup; ^{1}H NMR (400.13 MHz) δ 7.11 (d, J = 5 Hz, 2H), 6.89 (d, J = 5 Hz, 2H), 6.63 (s, 1H), 6.58 (d, J = 3 Hz, 2H), 6.39 (s, 1H), 4.06 (d, J = 7Hz, 2H), 3.90 (d, J = 6 Hz, 2H), 3.71 (s, 3H), 3.69 (s, 3H), 3.65 (m, 1H), 3.39 (s, 3H), 3.27 (m, 2H), 1.84 (m, 2H), 1.70 (m, 2H); ESI MS m/z: 370 $(M+H)^+$.

5.3. Cell growth inhibition assay

The human A549 lung cancer cell line was used for the cytotoxicity screening of PBT derivatives using the cell-based sulforhodamine B (SRB) microtiter plate assay.[28] Compound stock solutions were prepared in DMSO with the final solvent concentration $\leqslant$2% 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. Duration of compound exposure was 3 days. The IC_{50} value (the concentration that reduced the cell number by 50%) was interpolated from dose–response data. Each test was performed in triplicate with variation less than 5%. The IC_{50} values determined in each independent test varied less than 10%.

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