行政院國家科學委員會專題研究計畫 成果報告

設計與合成具1,4-phenanthrenedione和stilbenes骨架為抗癌及抗發炎化合物
研究成果報告(精簡版)

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計 畫 主 持 人：劉景平

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處 理 方 式 ：本計畫涉及專利或其他智慧財產權，1年後可公開查詢

中 華 民 國 96年02月06日

行政院國家科學委員會補助專題研究計畫 ■成 果 報 告
□期中進度報告

（計畫名稱）

設計與合成 1,4-phenanthrene 和 stilbenes 骨架為抗癌化合物

計畫類別：■ 個別型計畫　□ 整合型計畫
計畫編號：NSC 94－2323－B－038－005－
執行期間：94 年 12 月 01 日至　95 年 11 月 30 日

計畫主持人：劉景平
共同主持人：
計畫參與人員：楊明芳、張琦豔、郭靜娟、陳繼明、張俊彥、劉景平。

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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執行單位：台北醫學大學

中　華　民　國　96 年　02 月　05 日

中文摘要

本計劃合成出一系列 2-胺基及 2'-胺基**combretastatin** (2'-aminocombretastatin) 等衍生物。該等衍生物具有抗腫瘤之活性，其中數種化合物，作用如同微管蛋白 (tubulin) 聚合作用之抑制劑，具有極佳抗微小管增生活性。化合物 11、20 及 21 抑制微管蛋白聚合作用之 IC50 值，分別為 1.6、1.7 及 1.8 μM，比秋水仙素 (colchicine) 更具有潛力，且其有效程度幾乎combretastatin A-4 相同。對於不同的人類器官之腫瘤細胞株，此三種化合物之IC_{50}值範圍為 11~44 nM，且三種化合物皆具有抗微小管增生的活性。由結構活性關係中得知，在combretastatin分子骨架上，環A或環B上的第二個位置，接上胺基的取代基($-NH_2$)，可能為這一系列化合物具有相關生物活性的重要因素之一。

英文摘要

A novel series of 2-amino and 2'-aminocombretastatin derivatives were synthesized and evaluated for antitumor activity. Several compounds had excellent antiproliferative activity as inhibitors of tubulin polymerization. Compounds **11**, **20**, and **21** with IC_{50} values of 1.6, 1.7, and 1.8 μM, respectively, exhibited more potent inhibition of tubulin polymerization than colchicine and approximately as active as combretastatin A-4. They also displayed antiproliferative activity with an IC_{50} values ranging from 11 to 44 nM in a variety of human cell lines from different organs. Structure activity relationship (SAR) information suggests that the NH_2 substituent at the 2-position of either ring A or ring B in combretastatin molecular skeleton may play an important role in the bioactivity of this series of compounds.

關鍵詞(keywords)：Inhibition of tubulin polymerization, Combretastatins, Antimitotic

報告內容

本計畫已有論文發表 *Journal of Medicinal Chemistry* **2006**, *49*, 6412-6415.

2-Amino and 2′-Aminocombretastatin Derivatives as Potent Antimitotic Agents

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A novel series of 2-amino and 2′-aminocombretastatin derivatives were synthesized and evaluated for antitumor activity. Several compounds had excellent antiproliferative activity as inhibitors of tubulin polymerization. Compounds **11**, **20**, and **21** with IC_{50} values of 1.6, 1.7, and 1.8 μM, respectively, exhibited more potent inhibition of tubulin polymerization than colchicine and approximately as active as combretastatin A-4. They also displayed antiproliferative activity with an IC_{50} values ranging from 11 to 44 nM in a variety of human cell lines from different organs. Structure activity relationship information suggests that the NH_2 substituent at the 2-position of either ring A or ring B in combretastatin molecular skeleton may play an important role in the bioactivity of this series of compounds.

Introduction

Microtubules are dynamic structures that play a crucial role in cellular division and are recognized as an important target for anticancer therapy.[1] A number of naturally occurring compounds, such as paclitaxel, epothilone A, vinblastine, combretastatin A-4, dolastatin 10, and colchicine, all exhibit their anticancer properties by interfering with the dynamics of tubulin polymerization and depolymerization, resulting in mitotic arrest.[2] Reports that drugs with binding to the colchicine domain are undergoing intensive investigation as vascular disrupting agents for cancer therapy.[3−5] For example, antitubulin agents, **3**, **5**, and **10** act as vascular-disrupting agents, rapidly depolymerizing microtubules of newly formed vasculature to shut down the blood supply to tumors. They are now undergoing human clinical trials for either single use or combination use with chemotherapy drugs[6−8] (Figure 1).

The analysis of the structures of combretastatin A-4 and its derivatives shows that the polar functional group(s) is often located on the B ring,[9] for example, **4**[10] (3′-hydroxyl group), **6**[11] (2′, 3′-dihydroxyl group), and **9**[12] (3′-amino group). So, we synthesized the 2′-aminocombretastatin derivatives by introducing an amino group at the C-2 position on the B-ring of *Z*-stilbenes and then evaluated their bioactivity. Literature reports of SAR studies of combretastatin's ring A indicate that these derivatives mimic combretastatin A-3 by replacement of 3-hydroxyl group on ring A with halides[13] or simulate the resveratrol skeleton with a 3,5-dimethoxyphenyl group on the ring A[14,15] instead of 3,4,5-trimethoxyphenyl group. Because combretastatin compounds have a problem with aqueous solubility, the prodrug approach has been applied for drug candidates, such as **5**, **7**, and **10** utilizing phosphate and amino acids served as promoiety to achieve in vivo antitumor activity.[16−19] Therefore, we also modified the A-ring of *Z*-stilbene by the introduction of 2-amino group, synthesized 2-aminocombretastatins, in an attempt to increase the corresponding structure's polarity without compromising the activity. We herein describe the synthesis and structure−activity relationships of 2-amino and 2′-aminocombretastatins as potent antimitotic agents in continuation of our search for promising anticancer agents (Figure 2).

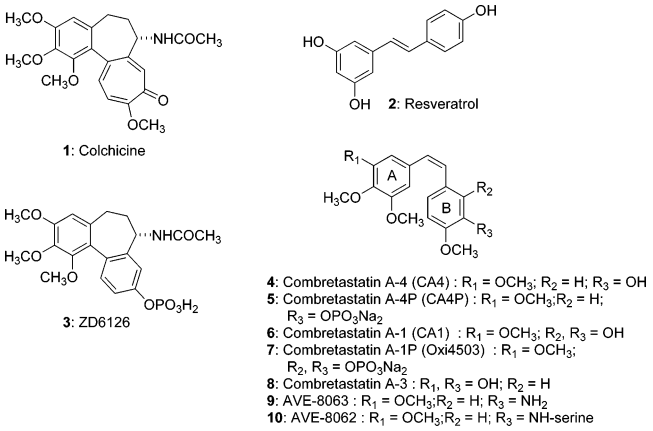

Figure 1.

Results and Discussion

Chemistry. The general methods for the synthesis of 2′-aminocombretastatins and 2-aminocombretastatins are shown in Scheme 1 and Schemes 2 and 3, respectively. The preparation involved a reaction sequence (overall 30−46% yield in two or three steps): (1) Wittig reaction of (3,4,5-trimethoxybenzyl)-phosphonium chloride (Scheme 1), (4-methoxybenzyl)phosphonium bromide (Scheme 2), and (2-nitro-3,4,5-trimethoxybenzyl)phosphonium bromide (Scheme 3) with various substituted benzaldehydes including 2-nitro or 3-nitrobenzaldehydes yielded the corresponding *Z*- and *E*-stilbenes as a ratio of about 3/1. (2) Reduction of the nitro group of *Z*-stilbenes by Zn/AcOH to afford the desired substituted 2-amino and 2′-aminocombretastatins derivatives. Ylide **31** was synthesized from the 2-nitro-3,4,5-trimethoxybenzyl bromide (**30**). The methoxy-substituted benzaldehydes **26**−**29** and **32** are commercially available. The 2-nitrobenzaldehydes **22**, **23**, **24**, **25**, and 3-(*tert*-butyldimethylsilyl)-protected isovanillin **33** were prepared in two-four steps.

Biological Evaluation. (A) In Vitro Cell Growth Inhibitory Activity. The synthesized *Z*-stilbenes **11**−**21** were evaluated for their antiproliferative activities against five types of human cancer cell lines, oral epidermoid carcinoma KB cells, colorectal

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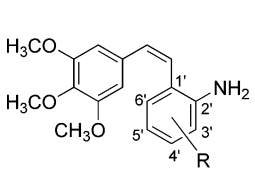
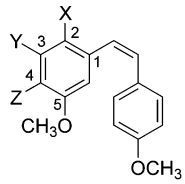
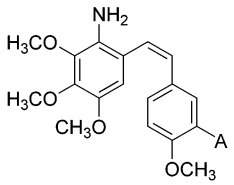


11: R = 4'-OCH_3
12: R = 5'-OCH_3



13: X = NH_2; Y, Z = OCH_3
14: X = NO_2; Y, Z = OCH_3
15: X = NH_2; Y = OCH_3; Z = H
16: X = H; Y, Z = OCH_3
17: X = H; Y = OCH_3; Z = H
18: X = NH_2; Y = H; Z = OCH_3
19: X = H; Y = Br; Z = OCH_3



20: A = OH
21: A = NH_2

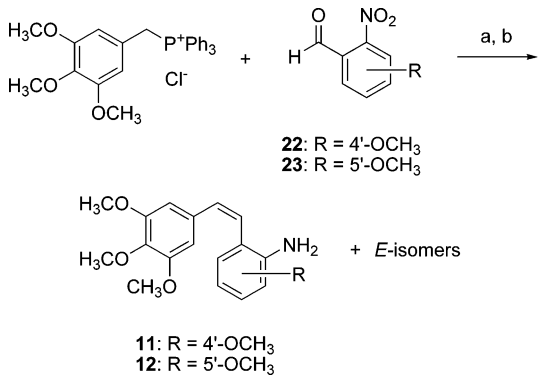
Figure 2.

carcinoma HT29 cells, nonsmall cell lung carcinoma H460 cells, and two stomach carcinoma TSGH, MKN45 cells, as well as one type of MDR-positive cell line: KB-VIN10 cells, overexpressed P-gp 170/MDR (Table 1).

First, we evaluated the cytotoxic effect of an amino group at the C-2′ position of *Z*-stilbenes. Synthesized combretastatin A-4 derivative **11**[20,21] with an amino group at C-2′ position on the B-ring exhibited potent cell growth inhibitory activity with a mean IC_{50} of 17 nM against six human cell lines. Changing the position of the methoxy group on the B ring from C-4 to C-5, as in compound **12**, resulted in a drastic loss in activity. This revealed that the position effect of methoxy substitutions at the pivotal C-4′ position of the B-ring in combretastatins significantly influences their antiproliferative activities. This is consistent with observations in the literature reports[14] on other CA-4 derivatives, which suggest that the methoxy group of the B-ring in the CA-4 family is located at the C-4′ site for maximal activity.

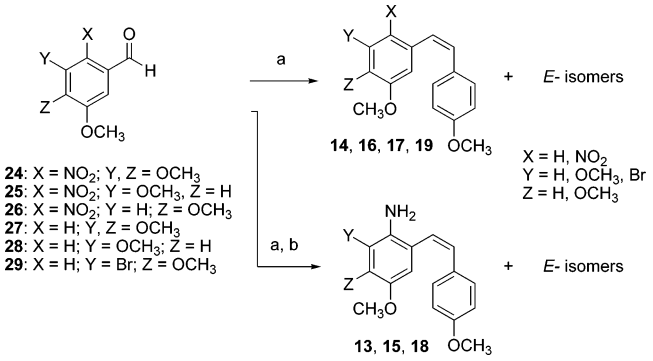
Demonstration of strong cytotoxic activity by analogues with the amino substitution at C-2′ position of *Z*-stilbenes (2′-aminocombretastatins) sparked us to construct the 2-aminocombretastatins series by introducing an amino group at the C-2 position on the A-ring. The compound **13**, namely, 2-amino-3,4,4′,5-tetramethoxy-*Z*-stilbenes, exhibited cytotoxicity activity against six cancer cell lines, with an average IC_{50} of 44 nM. An investigation of the electronic effects on the A-ring of *Z*-stilbenes by introduction of an electron-withdrawing nitro group at C-2 position (e.g., compound **14**) resulted in a significant loss of activities to micromolar range in the cell growth inhibitory assay for six human cancer cell lines. The replacement of 2-amino-3,4,5-trimethoxyphenyl moiety on the A-ring by a 2-amino-3,5-dimethoxyphenyl group (**15**) resulted in a slight decrease in cytotoxicity against several lines in comparison to compound **13** by a mean IC_{50} of 133 nM. A comparison of the substituent effect of the 2-amino group in the A-ring of *Z*-stilbenes (**13** vs **16**,[22] **15** vs **17**[14]) indicated that the 2-amino group was more tolerated in 3,5-dimethoxy-4′-methoxy-*Z*-stilbenes than in 3,4,5-trimethoxy-4′-methoxy-*Z*-stilbenes. The C3-methoxy group on A-ring in combretastatins skeleton is thought to play an important role in potency.[14] The removal of C3-methoxy from compound **13**, to synthesize compound **18**, resulted in complete loss in activity. Replacement

Scheme 1[a]



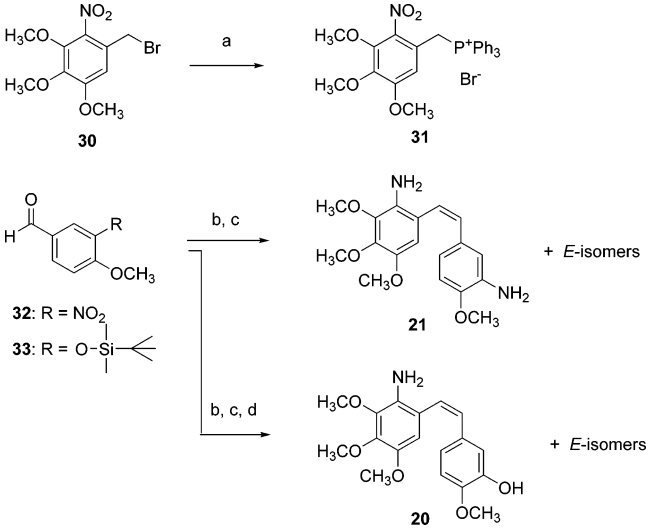
[a] Reagents and conditions: (a) *n*-BuLi, THF, −78 °C; (b) Zn, AcOH, rt.

Scheme 2[a]



[a] Reagents and conditions: (a) 4-methoxybenzyl-triphenylphosphonium bromide, *n*-BuLi, THF, −78 °C; (b) Zn, AcOH, rt.

Scheme 3[a]



[a] Reagents and conditions: (a) PPh_3, toluene, reflux; (b) **31**, *n*-BuLi, THF, −78 °C; (c) Zn, AcOH, rt.; (d) tetra-*n*-butylammonium fluoride, THF, rt.

of the C3-methoxy group of compound **16** to give 3-bromo-4,4′,5-triimethoxy-*Z*-stilbenes (**19**), however, resulted in a substantial activity in the cell growth inhibitory assay with a mean IC_{50} of 76 nM against six cancer cell lines. This observation is in agreement with other reports of 3-halo-*Z*-stilbenes combretastatin derivatives, indicating that the C3-methoxy group of combretastatins could be replaced by the halogen substituents.[13] As the addition of a C2-amino group to the A ring could apparently retain the cellular growth inhibitory

Table 1. IC_{50} Values (nM ± SD[a]) of Compounds **4**, **9**, and 2-Amino and 2′-Aminocombretastatins (**11**−**21**)

	cell type (IC_{50} nM ± SD)[a]					
compd	KB	KB-vin10	H460	HT29	TSGH	MKN45
4	1.5 ± 0.4	1.5 ± 0.4	1.9 ± 0.1	54 ± 7	38 ± 13	52 ± 8
9	7 ± 1.6	4.8 ± 0.4	9 ± 2.5	5 ± 0	7 ± 4.5	5.1 ± 0.1
11	14 ± 2.1	14 ± 1.4	19 ± 0.7	20 ± 0.7	19.5 ± 2.1	11 ± 0.1
12	>10 000	8000 ± 850	>10 000	>10 000	>10 000	6300 ± 245
13	50 ± 2	35 ± 4	54 ± 1	33 ± 6	55 ± 1	34 ± 3
14	4300 ± 128	4100 ± 78	4900 ± 210	4200 ± 680	5300 ± 396	3800 ± 265
15	154 ± 35	96 ± 15	156 ± 29	125 ± 19	186 ± 41	82 ± 11
16[22]	16 ± 4.2	11.9 ± 1.2	18.8 ± 0.3	13 ± 1.6	19.9 ± 1.2	16.6 ± 6.4
17[14]	280 ± 66	223 ± 58	330 ± 74	253 ± 51	324 ± 119	218 ± 4
18	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000
19	74 ± 25	68 ± 8	94 ± 6	70 ± 29	83 ± 32	72 ± 33
20	27 ± 3	20 ± 1	37 ±1	36 ± 5	39 ± 13	19 ± 4
21	44 ± 1	30 ± 4	41 ± 6	40 ± 2	43 ± 3	30 ± 3

[a] SD: standard deviation. All experiments were independently performed at least three times.

Table 2. Inhibition of Tubulin Polymerization and Colchicine Binding by Compounds **11**−**21**, Colchicine, and Combretastatin A-4

compd	tubulin[a] IC_{50} ± SD (μM)	colchicine binding[b] (% ± SD)
11	1.6 ± 0.2	87 ± 3
12	>10	19 ± 7
13	2.1 ± 0.3	75 ± 6
14	>10	23 ± 5
15	2.4 ± 0.3	84 ± 2
16	1.5 ± 0.2	89 ± 3
17	3.5 ± 0.4	82 ± 3
18	>10	17 ± 5
19	2.1 ± 0.2	86 ± 2
20	1.7 ± 0.2	83 ± 4
21	1.8 ± 0.1	80 ± 4
colchicine	3.2 ± 0.3	
combretastatin A-4	1.4 ± 0.2	92 ± 2

[a] Inhibition of tubulin polymerization. [b] Inhibition of [^{3}H]colchicine binding. Tubulin was at 1 μM; both [^{3}H]colchicine and inhibitor were at 5 μM.

activity, we further studied the C2-amino-substituted derivatives of combretastatin A-4 and **9**, compounds **20** and **21**, respectively. 2-Aminocombretastatins, namely, 2-amino-3′-hydroxy-3,4,4′,5-tetramethoxy-*Z*-stilbenes (**20**) and 2,3′-diamino-3,4,4′,5-tetramethoxy-*Z*-stilbenes (**21**), showed potent antiproliferative activity by a mean IC_{50} of 29 and 38 nM, respectively, in all six human cancer cell lines.

A comparison between cellular growth inhibitory activity of **9**, **11**, **13**, **15**, **20**, **21**, and combretastatin A-4 revealed that the introduction of an amino group at the C-2 or C-3 position on the B-ring and at the C-2 position on the A-ring is beneficial for potency. Hence, it may be assumed that an amino group located at the C-2 position, either on the A ring or B ring, and the C-3 position on the B ring, seems to perform in a requisite role of the inhibition of cellular growth. Furthermore, newly synthesized 2-aminocombretastatins **13**, **15**, **20**−**21**, and 2′-aminocombretastatins **11** overcome MDR-positive resistant cell line (KB-VIN10), indicating 2-amino and 2′-aminocombretastatin derivatives are not substrate for efflux pump.

(B) Inhibition of Tubulin Polymerization and Colchicine Binding Activity. To investigate whether the activities of these 2-amino and 2′-amino-*Z*-stilbenes compounds were related to interactions with microtubulin system, all compounds **11**−**21** and reference compounds (colchicine and combretastatin A-4) were evaluated for in vitro tubulin polymerization inhibitory activities and colchicine binding activities (Table 2). The results showed that compound cytotoxicities correlated with the inhibition of tubulin polymerization and colchicine binding affinity. As shown in Table 2, **11**, **20**, and **21** were effective in inhibiting tubulin assembly, with IC_{50} of 1.6, 1.7, and 1.8 μM, respectively. These values were slightly inferior or comparable to combretastatin A-4 (IC_{50} = 1.4 μM) and superior to the IC_{50} values of 3.2 μM for colchicine. In the colchicine binding assay, 2-amino and 2′-amino-*Z*-stilbenes derivatives were bound to the colchicine binding site, which suggested that 2-amino and 2′-aminocombretastatins displayed antiproliferative activity resulting from effective inhibition of tubulin polymerization at the colchicine binding site.

Conclusion

We have synthesized a series of 2-amino and 2′-aminocombretastatins, which compounds **12**, **13**−**15**, and **18**−**21** are new combretastatin derivatives. The synthesized 2-amino and 2′-aminocombretastatins, compounds **11**, **13**, **20**, and **21**, are potent cytotoxic agents and inhibitors of tubulin polymerization through the colchicine binding site on microtubules. The compounds **11**, **13**, **20**, and **21** display antiproliferative activity, with IC_{50} values ranging from 11 to 55 nM in a variety of human cell lines from different organs. They also showed comparable or similar antitubulin activities (IC_{50} = 1.6, 2.1, 1.7, and 1.8 μM, respectively) to combretastatin A-4 and colchicine (IC_{50} = 1.4 and 3.2 μM, respectively). SAR data indicated that the NH_2 substituent at position 2 of ring A or ring B in combretastatin moieties apparently plays an important role in the activity of this series of compounds. The introduction of a polar amino group in the C2-position of combretastatin A-4 and **9** gave compound **20** and **21**, respectively, which resulted in a substantial activity. This information can be applied to other related combretastatin and colchicine analogue modifications for increasing their polarity without compromising activity.

Experimental Section

General Procedure for the Preparation of Substituted 2-Amino and 2′-Aminocombretastatins Derivatives (11−13, 15, 18, 20−21). 2′-Amino-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (11). *n*-BuLi (1.6 M in hexane, 3.5 mL) was added dropwise to a well-stirred suspension of 3,4,5-trimethoxybenzyltriphenylphosphonium chloride (1.32 g, 2.76 mmol) in THF (30 mL) at −78 °C. The stirring was continued at −78 °C for 30 min and at room temperature for 1 h. The reaction mixture was recooled to −78 °C and then was added to a solution of 4-methoxy-2-nitrobenzaldehyde (**22**) in THF (16 mL) by an addition funnel. The stirring was continued at −78 °C for 1 h and at room temperature for 18 h. Ice water (30 mL) and ethyl acetate (10 mL) were added to the mixture. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (15 mL × 2) and methylene chloride (15 mL × 2). The combined organic layers were dried and evaporated to give a residue, which was further treated with Zn (9 g, 0.138 mol) in AcOH (50 mL) at room temperature for 1 h. The reaction mixture was filtrated and then evaporated to give a residue that was purified by

silica gel flash column chromatography (ethyl acetate/*n*-hexane = 1:2) to afford **11** as a pale yellow solid, yield 41%; mp 94.0−94.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (s, 6H), 3.74 (s, 3H), 3.79 (s, 3H,), 6.25 (d, J = 2.4 Hz, 1H), 6.30 (dd, J = 8.4, 2.4 Hz, 1H), 6.42 (d, J = 12 Hz, 1H), 6.49 (d, J = 14.8 Hz, 3H), 7.03 (d, J = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.1, 55.7, 60.7, 100.6, 104.2, 105.7, 115.985, 125.6, 130.5, 130.8, 132.2, 137.2, 144.8, 152.6, 160.0. MS (EI) *m*/*z* 315 (M^+, 90%), 300 (100%). HRMS (EI) calcd for $C_{18}H_{21}NO_4$ (M^+), 315.1471; found, 315.1471. Anal. ($C_{18}H_{21}NO_4$) C, H, N.

2-Amino-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (13). The title compound was obtained in 39% overall yield from (4-methoxybenzyl)-triphenylphosphonium bromide and 2-nitro-3,4,5-trimethoxybenzaldehyde (**24**). ^{1}H NMR (500 MHz, CD_3OD) δ 3.52 (s, 3H), 3.73 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 6.35 (d, J = 11.9 Hz, 1H), 6.45 (s, 1H), 6.55 (d, J = 12.0 Hz, 1H), 6.74 (d, J = 8.6 Hz, 2H), 7.15 (d, J = 8.6 Hz, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 55.6, 56.9, 60.8, 61.3, 110.3, 114.5, 120.0, 125.0, 130.8, 131.2, 131.4, 133.6, 142.8, 143.4, 146.6, 160.4. MS (EI) *m*/*z* 315 (M^+, 100%), 300 (58%). HRMS (EI) calcd for $C_{18}H_{21}NO_4$ (M^+), 315.1469; found, 315.1470. Anal. ($C_{18}H_{21}NO_4$) C, H, N.

2-Amino-3,4′,5-trimethoxy-(*Z*)-stilbene (15). The title compound was obtained in 40% overall yield from (4-methoxybenzyl)-triphenylphosphonium bromide and 3,5-dimethoxy-2-nitrobenzaldehyde (**25**); mp 83.2−86.3 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.63 (s, 3H), 3.75 (s, 3H), 3.83 (s, 3H), 6.31 (d, J = 2.3 Hz, 1H), 6.39 (s, 1H), 6.41 (d, J = 12.9 Hz, 1H), 6.58 (d, J = 12.0 Hz, 1H), 6.73 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.0, 55.5, 98.4, 103.9, 113.4, 123.5, 124.2, 127.2, 129.2, 130.0, 130.8, 148.4, 152.1, 158.8. MS (EI) *m*/*z* 285 (M^+, 100%), 270 (29%). HRMS (EI) calcd for $C_{17}H_{19}NO_3$ (M^+), 285.1369; found, 285.1367. Anal. ($C_{17}H_{19}NO_3$) C, H, N.

2,3′-Diamino-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (21). The title compound was obtained in 34% overall yield from 2-nitro-3,4,5-(trimethoxybenzyl)triphenylphosphonium bromide (**31**) and 4-methoxy-3-nitrobenzaldehyde (**32**); mp 97.3−98.1 °C. ^{1}H NMR (500 MHz, acetone-d_6) δ 3.54 (s, 3H), 3.77 (s, 3H), 3.79 (s, 3H), 3.80 (s, 3H), 4.01 (s, 2H), 4.25 (s, 2H), 6.26 (d, J = 12.1 Hz), 6.39 (d, J = 12.1 Hz, 1H), 6.53 (s, 1H), 6.56 (dd, J = 8.2, 1.7 Hz, 1H), 6.66 (d, J = 8.2 Hz, 1H), 6.68 (d, J = 1.8 Hz, 1H). ^{13}C NMR (125 MHz, acetone-d_6) δ 56.1, 57.1, 61.0, 61.4, 110.5, 111.2, 117.0, 120.4, 120.9, 124.9, 131.3, 132.1, 133.6, 137.3, 143.0, 143.5, 146.8, 148.8. MS (EI) *m*/*z* 330 (M^+, 100%), 315 (27%). HRMS (EI) calcd for $C_{18}H_{22}N_2O_4$ (M^+), 330.1578; found, 330.1570. Anal. ($C_{18}H_{22}N_2O_4$) C, H, N.

2-Amino-3′-hydroxy-3,4,4′,5- tetramethoxy-(*Z*)-stilbene (20). The title compound was obtained in 30% overall yield from 2-nitro-(3,4,5-trimethoxybenzyl)triphenylphosphonium bromide (**31**) and 3-(*tert*-butyldimethylsilyloxy)-4-methoxybenzaldehyde (**33**) according to the above procedure and one extra procedure, which was 3 equiv of tetra-*n*-butylammonium fluoride/THF at room temperature, stirring for 1 h. ^{1}H NMR (500 MHz, CD_3OD) δ 3.55 (s, 3H), 3.79 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 6.33 (d, J = 12 Hz, 1H), 6.49 (s, 1H, H-6), 6.50 (d, J = 11.9 Hz, 1H), 6.71 (dd, J = 8.2, 1.9 Hz, 1H), 6.74 (d, J = 1.8 Hz, 1H), 6.77 (d, J = 8.2 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ 56.3, 57.0, 60.9, 61.3, 110.4, 112.3, 116.5, 120.0, 122.0, 125.1, 131.4, 131.5, 133.5, 142.9, 143.4, 146.7, 147.1, 148.5. MS (EI) *m*/*z* 331 (M^+, 100%), 284 (25%). HRMS (EI) calcd for $C_{18}H_{21}NO_5$ (M^+), 331.1422; found, 331.1421. Anal. ($C_{18}H_{21}NO_5$) C, H, N.

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Supporting Information Available: Spectral data of compounds **12**, **14**, **16**−**19**, **22**−**25**, **30**, **31**, **33** and experimental procedures for biological evaluations. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. Tubulin as a Target for Anticancer Drugs: Agents Which Interact with the Mitotic Spindle. *Med. Res. Rev.* **1998**, *18*, 259−296.
(2) Hamel, E. Antimitotic Natural Products and Their Interactions with Tubulin. *Med. Res. Rev.* **1996**, *16*, 207−231.
(3) Jordan, M. A.; Wilson, L. Microtubules as a Target for Anticancer Drugs. *Nat. Rev. Cancer* **2004**, *4*, 253−265.
(4) Siemann, D. W.; Bibby, M. C.; Dark, G. G.; Dicker, A. P.; Eskens, F. A. L. M.; Horsman, M. R.; Marme, D.; LoRusso, P. M. Differentiation and Definition of Vascular-Targeted Therapies. *Clin. Cancer Res.* **2005**, *11*, 416−420.
(5) Siemann, D. W. *Vascular-Targeted Therapies in Oncology*; John Wiley & Sons: New York, 2006.
(6) Chaplin, D. J.; Horsman, M. R.; Siemann, D. W. Current Development Status of Small-Molecule Vascular Disrupting Agents. *Curr. Opin. Invest. Drugs* **2006**, *7*, 522−528.
(7) Beerepoot, L. V.; Radema, S. A.; Witteveen, E. O.; Thomas, T.; Wheeler, C.; Kempin, S.; Voest, E. E. Phase I Clinical Evaluation of Weekly Administration of the Novel Vascular-Targeting Agent, ZD6126, in Patients With Solid Tumors. *J. Clin. Oncol.* **2006**, *24*, 1491−1498.
(8) Thorpe, P. E. Vascular Targeting Agents as Cancer Therapeutics. *Clin. Cancer Res.* **2004**, *10*, 415−427.
(9) Cirla, A.; Mann, J. Combretastatins: from Natural Products to Drug Discovery. *Nat. Prod. Rep.* **2003**, *20*, 558−564.
(10) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. Isolation and Structure of Combretastatin. *Can. J. Chem.* **1982**, *60*, 1374−1376.
(11) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. Isolation, Structure and Synthesis of Combretastatins A-1 and B-1, Potent New Inhibitors of Microtubule Assembly, Derived from *Combretum caffrum*. *J. Nat. Prod.* **1987**, *50*, 119−131.
(12) Morinaga, Y.; Nihei, Y.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. Novel Combretastatin Analogues Effective against Murine Solid Tumors: Design and Structure−Activity Relationships. *J. Med. Chem.* **1998**, *41*, 3022−3032.
(13) Pettit, G. R.; Minardi, M. D.; Rosenberg, H. J.; Hamel, E.; Bibby, M. C.; Martin, S. W.; Jung, M. K.; Pettit, R. K.; Cuthbertson, T. J.; Chapuis, J.-C. Antineoplastic Agents. 509. Synthesis of Fluorocombstatin Phosphate and Related 3-Halostilbenes. *J. Nat. Prod.* **2005**, *68*, 1450−1458.
(14) Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H. M.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Analogues of (*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane as Potential Cytotoxic and Antimitotic Agents. *J. Med. Chem.* **1992**, *35*, 2293−2306.
(15) Pettit, G. R.; Grealish, M. P.; Jung, M. K.; Hamel, E.; Pettit, R. K.; Chapuis, J. C.; Schmidt, J. M. Antineoplastic Agents. 465. Structural Modification of Resveratrol: Sodium Resverastatin Phosphate. *J. Med. Chem.* **2002**, *45*, 2534−2542.
(16) Tozer, G. M.; Kanthou, C.; Baguley, B. C. Disrupting Tumour Blood Vessels. *Nat. Rev. Cancer* **2005**, *5*, 423−435.
(17) Pettit, G. R.; Temple, C., Jr.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Rener, G. A.; Bansal, N. Antineoplastic Agents 322. Synthesis of Combretastatin A-4 prodrugs. *Anticancer Drug Des.* **1995**, *10*, 299−309.
(18) Pettit, G. R.; Lippert III, J. W. Antineoplastic Agents 429. Syntheses of Combretastatin A-1 and Combretastatin B-1 prodrugs. *Anticancer Drug Des.* **2000**, *15*, 203-216.
(19) Hori, K.; Saito, S.; Nihei, Y.; Suzuki, M.; Sato, Y. Antitumor Effects Due to Irreversible Stoppage of Tumor Tissue Blood Flow: Evaluation of A Novel Combretastatin A-4 Derivative, AC7700. *Jpn. J. Cancer Res.* **1999**, *90*, 1026−1038.
(20) Chaplin, D. J.; Garner, C. M., III; Kane, R. R.; Pinney, K. G.; Prezioso, J. A. Functionalized Stilbene Derivatives as Improved Vascular Targeting Agents. WO 03035008 A2, 2003.
(21) Monk, K. A.; Siles, R.; Hadimani, M. B.; Mugabe, B. E.; Ackely, J. F.; Studerus, S. W.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Rhodes, M. R.; Pettit, G. R.; Pinney, K. G. Design, Synthesis, and Biological Evaluation of Combretastatin Nitrogen-containing Derivatives as Inhibitors of Tubulin Assembly and Vascular Disrupting Agents. *Bioorg. Med. Chem.* **2006**, *14*, 3231−3244.
(22) Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Stilbene and Dihydrostilbene Derivatives as Potential Anticancer Agents That Inhibit Tubulin Polymerization. *J. Med. Chem.* **1991**, *34*, 2579−2588.

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可供推廣之研發成果資料表

■ 可申請專利　■ 可技術移轉　　日期：96年02月05日

國科會補助計畫	計畫名稱:**設計與合成 1,4-phenanthrene 和 stilbenes 骨架為抗癌化合物** 計畫主持人：劉景平 計畫編號：NSC 94-2323-B-038-005　　學門領域：藥學
技術/創作名稱	***Z*-stilbenes 衍生物及其醫藥組合物**
發明人/創作人	劉景平、張俊彥
技術說明	中文： 藉由在 *Z*-stilbenes 骨架中導入極性的胺基官能基，發現可保持其活性，同時可改善此類化合物最嚴重的水溶性問題。目前 Z-stilbenes 化合物，用做抗癌用途已有許多成功例子，如天然物的 combretastatinA-4P (Phase II-Phase III)，AVE8062 (Phase I/II)，Oxi-4503 (Phase I)等，這些化合物都必須以前驅藥(prodrug)的型式存在，才能達到抗癌活性，本研究主要在 Z-stilbenes 的 A 環導入胺基形成全新結構，保有強效活性，且改善其極性更 water soluble，而可能可以不用前驅藥形式來達到抗癌活性。
	英文： A Novel Classes of 2-Amino-Z-stilbenes as Potent Anti-cancer Agents.
可利用之產業及可開發之產品	抗癌藥、眼科用藥。
技術特點	1. 新結構具強效活性、增加此類化合物之極性水溶性。 2. 新的結構化合物(New substances) 3. 小分子(分子量< 450) 4. Inhibitor of tubulin polymerization 5. Antivascular activity.
推廣及運用的價值	抗癌藥。

※ 1. 每項研發成果請填寫一式二份，一份隨成果報告送繳本會，一份送　貴單位研發成果推廣單位（如技術移轉中心）。

※ **2. 本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。**

※ 3. 本表若不敷使用，請自行影印使用。