Discovery of 4-Amino and 4-Hydroxy-1-aroylindoles as Potent Tubulin Polymerization Inhibitors

Jing-Ping Liou,[†] Zi-Yi Wu,[†] Ching-Chuan Kuo,[‡] Chi-Yen Chang,[‡] Pei-Yi Lu,[†] Chi-Ming Chen,[†] Hsing-Pang Hsieh,*[,¶] and Jang-Yang Chang*[,‡,§]

College of Pharmacy, Taipei Medical University, Taipei 110, Taiwan, Republic of China, National Institute of Cancer Research, National Health Research Institutes, Tainan 704, Taiwan, Republic of China, Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli 350, Taiwan, Republic of China, and Division of Hematology/Oncology, Department of Internal Medicine, National Cheng Kung University Hospital, Tainan 704, Taiwan, Republic of China

Received February 13, 2008

1-Aroylindoline, 1-aroyl-1,2,3,4-tetrahydroquinoline, and 1-aroylindole derivatives were synthesized and evaluated for anticancer activity. The 4-amino and 4-hydroxy-1-aroylindoles **26** and **27** with IC_{50} of 0.9 and 0.6 μM, respectively, exhibited antitubulin activity superior or comparable to that of colchicine and combretastatin A-4. They also showed antiproliferative activity with IC_{50} of 0.3−5.4 nM in a set of human cancer cell lines.

Introduction

One of the currently useful chemotherapy drugs in oncology is represented by antimitotic agents, for example, taxanes and vinca alkaloids.[1] However, the issues of high systemic toxicity, complex syntheses, drug resistance, and isolation procedures have encouraged scientists to develop new antimitotic agents. Recent literature reported that the antitubulin agents targeting the colchicine-binding domain rapidly depolymerize microtubules of vasculatures changing morphology in the endothelial cells of tumor's vessels to block the blood supply to tumors and can act as vascular-disrupting agents,[2] for example, **2**, **3**, and **5** (Figure 1).

The encouraging antivascular and antitumor prolife of **2** has stimulated interest in design and synthesis of a variety of derivatives or analogues.[3] One of the modified sites in combretastatin structure is the olefin functionality of *Z*-stilbene; for instance, it was replaced with a 1,3-diaryl five-member ring (oxazoline and oxadiazoline),[4] 1,2-diaryl five-member ring (triazole,[5] pyrazole, tetrazole, thiazole,[6] imidazole,[7] furan,[8] furazan[9]), carbonyl bridge,[10] and 2- or 3-carbonylthiophene.[11] Attempts to replace the double bond bridge in **1** with an amide bond resulted in dramatic loss of activity[12] (**6** vs **1**). Despite this, the amide moiety was used for isosteric replacement with an olefin group.[13] In this paper, we use **6** as a base to design a series of heterocyclic analogues of **1** to improve activity by a restricted approach connecting the amide moiety and the B-ring of **6** via a five- or six-member ring to introduce a series of 1-aroylindolines, 1-aroyl-1,2,3,4-tetrahydroquinoline, and 1-aroylindoles (Figures 2 and 3). Here, we describe the discovery of

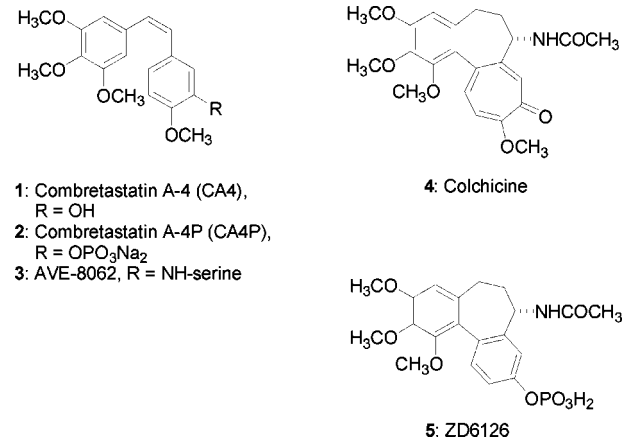


Figure 1

4-amino- and 4-hydroxy-1-aroylindoles as novel, highly potent antimitotic agents.

Results and Discussion

Chemistry. Diphenylamide **6**[12] was prepared as shown in Scheme 1. To synthesize **7** and **9**, 5-methoxyindole (**28**) and 2-methyl-5-methoxyindole (**29**) were treated with sodium cyanoborohydride in acetic acid to give indoline **30** and **31**, respectively (Scheme 2). The electrophilic substitution of **30** and **31** with the 3,4,5-trimethoxybenzoyl chloride in pyridine gave the desired 1-aroylindoline **7** and **9** in 87% and 88% yield, respectively. The 1-aroyl-1,2,3,4-tetrahydroquinoline **8** was prepared in 89% yield by treatment of commercially available 1,2,3,4-tetrahydroquinoline (**32**) with 3,4,5-trimethoxybenzoyl chloride in pyridine (Scheme 3). The general method for the synthesis of 1-aroylindoles **10**[14]−**14**, **17**,[14] **20**, and **24** is depicted in Scheme 4. The direct electrophilic substitution of 3,4,5-trimethoxybenzoyl chloride at the N1-position of various commercially available indoles in the presence of KO^{t}Bu[a] gave the desired 1-aroylindoles in 25−95% yield. Compounds **15**

* To whom correspondence should be addressed. For H.-P.H.: (phone) 886-37-586-456, ext 35708; (fax) 886-37-586-456; (e-mail) hphsieh@nhri.org.tw. For J.-Y.C.: (address) 2F, No. 367, Sheng Li Road, Tainan 704, Taiwan, Republic of China; (phone) 886-6-700-0123, ext 65100; (fax) 886-6-208-3427; (e-mail) jychang@nhri.org.tw.

† Taipei Medical University.

‡ National Institute of Cancer Research, National Health Research Institutes.

¶ Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes.

§ National Cheng Kung University Hospital.

[a] Abbreviations: KO^{t}Bu, potassium *tert*-butoxide; TBDMSCl, *tert*-butyldimethylsilyl chloride; $NaBH_3CN$, sodium cyanoborohydride; K_2CO_3, potassium carbonate; DMF, *N,N*-dimethylformamide; THF, tetrahydrofuran; MDR, multidrug-resistant; TBAF, tetra-*n*-butylammonium floride.

10.1021/jm800150d CCC: $40.75
Published on Web 06/28/2008

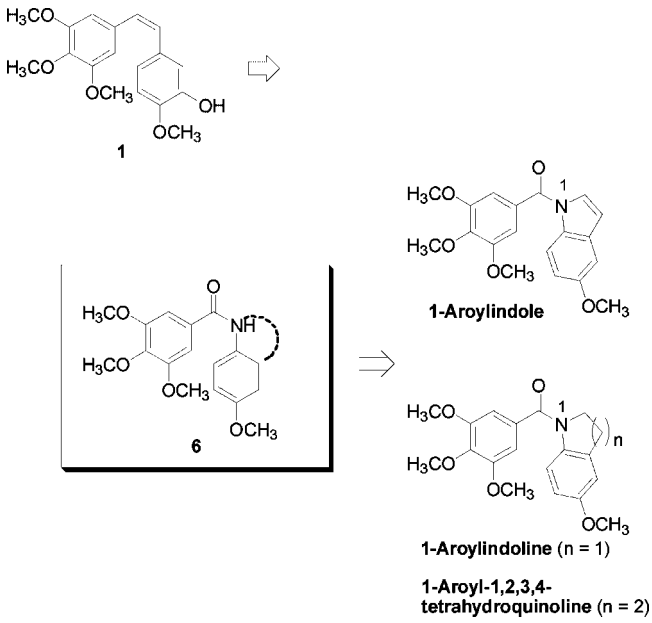
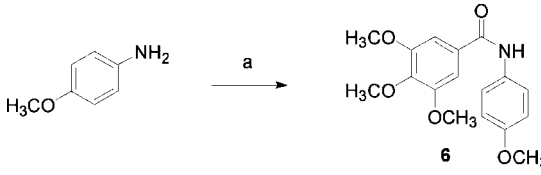


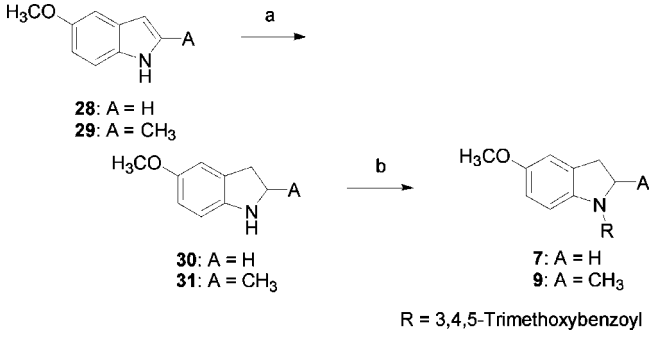
Figure 2

Scheme 1[a]



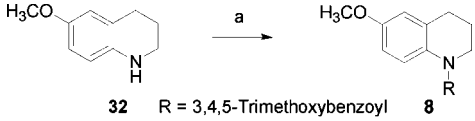
[a] Reagents and conditions: (a) 3,4,5-trimethoxybenzoyl chloride, pyridine, room temp.

Scheme 2[a]



[a] Reagents and conditions: (a) AcOH, $NaBH_3CN$; (b) 3,4,5-trimethoxybenzoyl chloride, pyridine.

Scheme 3[a]



[a] Reagents and conditions: (a) 3,4,5-trimethoxybenzoyl chloride, pyridine.

and **16**, with a C5-hydroxymethyl and C5-dimethylaminomethyl groups, respectively, were prepared by starting from the indole-5-carboxyaldehyde (**33**) as shown in Scheme 5. Dimethylamine/sodium cyanoborohydride mediated reductive amination of **33** followed by electrophilic substitution with 3,4,5-trimethoxybenzoic anhydride gave **16** in 37% yield (two steps). Compound **33** was converted into the desired the C5-hydroxymethyl **15** in 23% yield in four steps: $LiAlH_4$-mediated reduction, protection

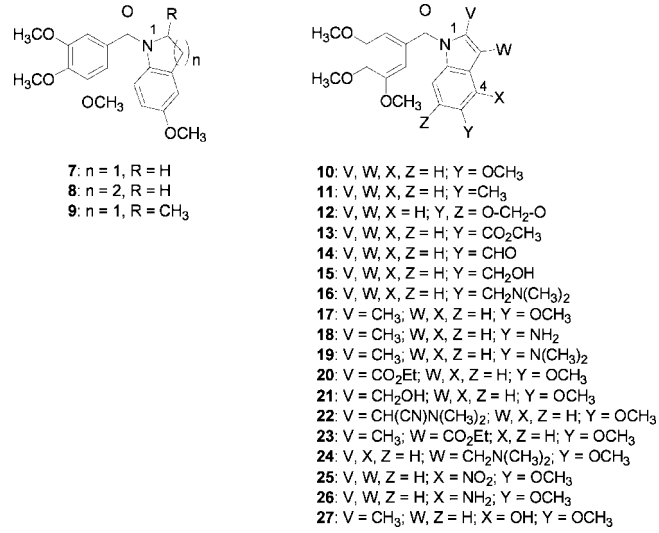
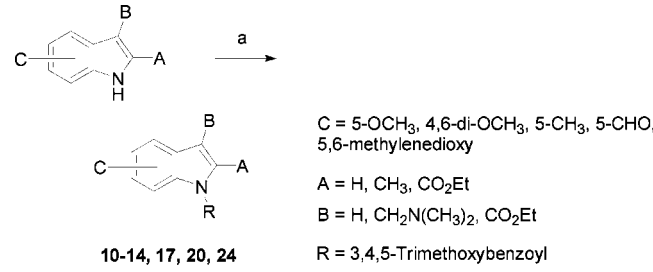


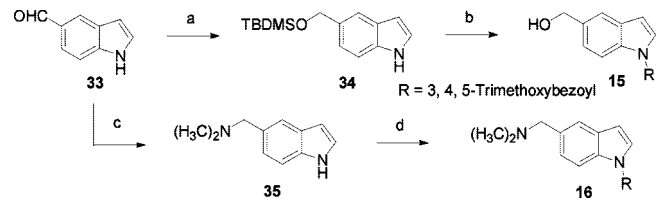
Figure 3

Scheme 4[a]



[a] Reagents and conditions: (a) 3,4,5-trimethoxybenzoic anhydride, K^{t}O-Bu, THF.

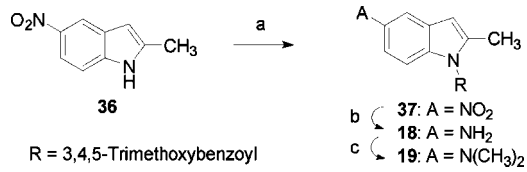
Scheme 5[a]



[a] Reagents and conditions: (a) (i) $LiAlH_4$, THF; (ii) imidazole, TBDMSCl, DMF, 0 °C; (b) (i) KO^{t}Bu, 3,4,5-trimethoxybenzoic anhydride, THF; (ii) TBAF, THF, 0 °C; (c) $(CH_3)_2NH\cdot HCl$, Et_3N, $NaBH_3CN$, EtOH; (d) KO^{t}Bu, 3,4,5-trimethoxybenzoic anhydride, THF.

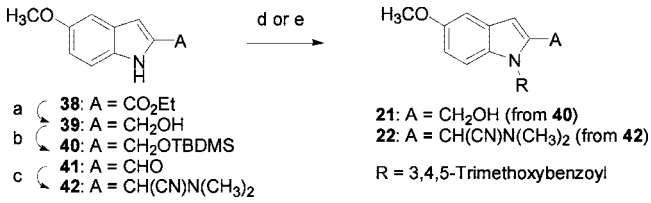
by TBDMSCl, indole N1-aroylation, and fluoride-mediated desilylation. Compound **19**, with a C5-dimethylamino substituent, was synthesized starting from the 2-methyl-5-nitroindole (**36**), which on N1-aroylation with 3,4,5-trimethoxybenzoyl chloride in the presence of NaH followed by Fe/NH_4Cl-mediated reduction afforded **18**. The amine **18** was converted into the desired C5-dimethylamino **19** in 30% yield by reaction with iodomethane in the presence of K_2CO_3 and DMF (Scheme 6). The modification at the C2-position of **10** by the hydroxy and amino moieties, giving **21** and **22**, were prepared as shown in the Schemes 7 and 8, respectively. Starting from the ethyl 5-methoxyindole-2-carboxylate (**38**), the synthesis of **21** was carried out in four steps through $LiAlH_4$-mediated reduction, protection with the *tert*-butyldimethylsilyl group, N1-aroylation with 3,4,5-trimethoxybenzoic anhydride, and deprotection with tetrabutylammonium fluoride in 21% yield (four steps, Scheme 7). Compound **22** was synthesized starting from the 5-methoxyindole-2-carboxyaldehyde (**41**), which was subjected to the

Scheme 6[a]



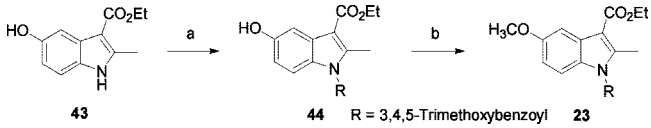
[a] Reagents and conditions: (a) NaH, 3,4,5-trimethoxybenzoyl chloride, toluene, DMF; (b) Fe, NH_4Cl, isopropanol, H_2O, reflux; (c) CH_3I, K_2CO_3, DMF.

Scheme 7[a]



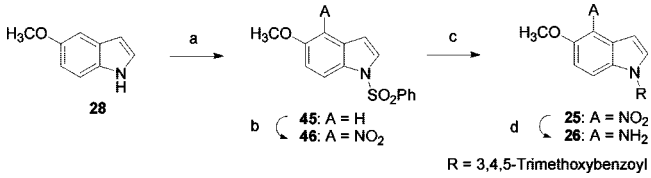
[a] Reagents and conditions: (a) $LiAlH_4$, THF, room temp; (b) imidazole, TBDMSCl, DMF, 0 °C; (c) $(CH_3)_2NH\cdot HCl$, Et_3N, $NaBH_3CN$, CH_2Cl_2; (d) (i) KO^{t}Bu, 3,4,5-trimethoxybenzoic anhydride, THF, room temp; (ii) TBAF, THF, 0 °C to room temp; (e) KO^{t}Bu, 3,4,5-trimethoxybenzoic anhydride, THF.

Scheme 8[a]



[a] Reagents and conditions: (a) (i) imidazole, TBDMSCl, DMF, 0 °C; (ii) KO^{t}Bu, 3,4,5-trimethoxybenzoic anhydride, THF; (iii) TBAF, THF, 0 °C; (b) CH_3I, K_2CO_3, DMF.

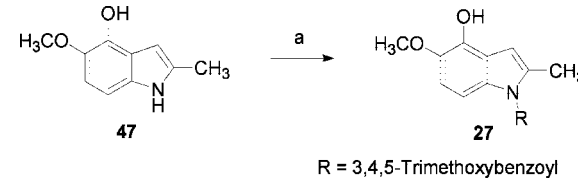
Scheme 9[a]



[a] Reagents and conditions: (a) KOH, tetrabutylammonium bisulfate, $PhSO_2Cl$, CH_2Cl_2; (b) HNO_3; (c) (i) NaOH, EtOH, reflux; (ii) KO^{t}Bu, 3,4,5-trimethoxybenzoic anhydride, THF; (d) Fe, NH_4Cl, isopropanol, H_2O, reflux.

reductive amination of C2-aldehyde in the presence of $(CH_3)_2NH$ and $NaBH_3CN$ to give unexpected **42** that followed by indole N1-aroylation with 3,4,5-trimethoxybenzoic anhydride, affording **22** (Scheme 7). Compound **23**, the C3-ethyl carboxylate analogue of **17**, was prepared starting from commercially available **43** by treating with the TBDMSCl/imidazole, 3,4,5-trimethoxybenzoic anhydride/KO^{t}Bu, and then tetrabutylammonium fluoride to give **44**. The O-methyation of **44** in the presence of CH_3I and K_2CO_3 afforded the desired **23** in 52% yield (Scheme 8). Compound **26**, with an additional amino group at the 4-position of indole in **10**, was prepared by starting from the 5-methoxyindole (**28**) in five steps, as shown in the Scheme 9. N1-Benzenesulfonyl protection of **28** followed by nitration[15] gave the 5-methoxy-4-nitro-substituted indole **46**. The N1-benzenesulfonyl of **46** was deprotected with NaOH and then treated with the 3,4,5-trimethoxybenzoic anhydride in the presence of KO^{t}Bu and THF and then subjected to Fe-mediated reduction to give the desired **26** in 16% overall yield (five steps). Compound **27**, with an additional hydroxyl group at the 4-position of 1-aroylindole **17**, was synthesized from the key material **47**, which was prepared by the literature methodology.[16] The 4-hydroxy-2-methyl-5-methoxyindole (**47**) was converted into the 4-hydroxy-1-aroylindole **27** in three steps via the protection with *tert*-butyldimethylsilyl group, N1-aroylation with 3,4,5-trimethoxybenzoic anhydride, and fluoride ion-mediated deprotection to afford the desired **27** in 19% yield (Scheme 10).

Scheme 10[a]



[a] Reagents and conditions: (a) (i) TBDMSCl, Et_3N, CH_2Cl_2; (ii) KO^{t}Bu, 3,4,5-trimethoxybenzoic anhydride, THF, room temp; (iii) TBAF, THF, 0 °C.

Table 1. IC_{50} Values of Compounds **6**−**27**, **4**, and **1**

	cell type (IC_{50} ± SD,[a] nM)			
compd	KB	H460	MKN45	KB-vin10
6	>5000	>5000	>5000	>5000
7	89 ± 2.9	98 ± 1	53 ± 1.4	84 ± 11
8	596 ± 123	652 ± 32	395 ± 51	586 ± 82
9	370 ± 33	413 ± 6	257 ± 30	372 ± 18
10	208 ± 58	232 ± 21	198 ± 13	197 ± 20
11	371 ± 111	353 ± 75	314 ± 35	400 ± 80
12	575 ± 55	559 ± 81	521 ± 51	587 ± 42
13	>5000	>5000	>5000	>5000
14	>5000	>5000	>5000	>5000
15	>5000	>5000	>5000	>5000
16	>5000	>5000	>5000	>5000
17	18 ± 6	23 ± 7	17 ± 3	11 ± 4
18	345 ± 17	334 ± 84	156 ± 54	312 ± 20
19	42 ± 21	52 ± 7	41 ± 13	38 ± 14
20	230 ± 71	198 ± 1.4	101 ± 19	185 ± 21
21	>5000	>5000	>5000	>5000
22	>5000	>5000	>5000	>5000
23	>5000	>5000	>5000	>5000
24	>5000	>5000	>5000	>5000
25	>5000	>5000	>5000	>5000
26	1.2 ± 0.4	5.4 ± 1.2	3.1 ± 2.3	2.4 ± 1.3
27	0.3 ± 0.3	0.5 ± 0.4	0.6 ± 0.8	0.3 ± 0.2
4	11.4 ± 1	20 ± 2	11 ± 1	125 ± 10
1	2.2 ± 0.3	2.8 ± 0.8	5.6 ± 0.2	1.8 ± 0.4

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

Biological Evaluation. (A) In Vitro Cell Growth Inhibitory Activity. The synthesized 1-aroylindolines **7** and **9**, 1-aroyl-1,2,3,4-tetrahydroquinoline **8**, and 1-aroylindoles **10**−**27** were evaluated for antiproliferative activities against three types of human cancer cell lines, oral epidermoid carcinoma KB cells, non-small-cell lung carcinoma H460 cells, and stomach carcinoma MKN45 cells, as well as one type of MDR-positive cell line, KB-vin10 cells, overexpressed P-gp 170/MDR (Table 1).

We first evaluated the effect of adding a five- or six-member ring upon the B-ring of **6**, i.e., to cyclize the amide bond and B-ring in **6**, for cytotoxicity activity. Indoline **7**, tetrahydroquinoline **8**, and indole **10** were evaluated for antiproliferative activity. Structure−activity relationships indicated that the five-member ring based heterocycles, 1-aroylindoline (**7**) and 1-aroylindole (**10**), and the six-member ring based heterocycles, 1-aroyltetrahydroquinoline (**8**), all show stronger activity than the parent (**6**), with mean IC_{50} values of 81, 208, 557 nM against four lines, respectively. On the basis of these results of five-member-ring-containing heterocyclic analogues showing good cellular growth inhibitory activity, the substituted 1-aroylindoline

derivative (**9**) and 1-aroylindole derivatives (**11**−**27**) were further prepared and evaluated for activity.

In the SAR study of **1**, the *p*-methoxy substitution of the B-ring of *Z*-stilbene plays a pivotal role for activity.[10a,12] So we prepared **11**−**16** with a methyl, methylenedioxy, methylcarboxylate, carboxyaldehyde, hydroxymethyl, and dimethylaminomethyl groups at the 5- or 5,6-postion of 1-aroylindoles. Compounds **11** and **12**, with electron-donating groups C5-methyl and C5,6-methylenedioxy groups, respectively, slightly reduced the activity compared to **10**. But changing the C5-methoxy group of **10** to electron-withdrawing CO_2CH_3 and CHO groups in **13** and **14**, respectively, resulted in drastic loss of activity. Compounds **15** and **16**, with a CH_2OH and $CH_2N(CH_3)_2$ group at C5-postion, respectively, displayed no activity, thus revealing that the steric effect of substituents at C5-position may influence cellular growth inhibitory activity. In order to investigate the effect of substitution at C2-position of 1-aroylindoles, **17**, **20**, **21**, and **22** with an alkyl, ester, alcohol, and amine functionalities, respectively, on the 2-indole were tested for their antiproliferative activity. Compound **17**, with a C2-methyl substitution on the 1-aroylindole **10**, showed the most potent activity, changing to the C2-ethyl carboxylate substitution (**20**), which resulted in moderate activity with mean IC_{50} of 178 nM against four lines comparable to the that of **10**, while changing to the alcohol or amino functionalities (**21** and **22**) decreased the activity drastically. On the basis of these results (C2-modification), the introduction of an alkyl or ester functional group at the C2-position of 1-aroylindoles seems to be preferable in activity. The introduction of a methyl group at the C2-position of 1-aroylindoles exerted a potency increase that intrigued us to explore whether 1-aroylindolines also exhibit this effect. Contrary to expectation, **9** with an additional methyl group in 1-aroylindoline **7** showed a decreased growth inhibition by a >3-fold magnitude in four cell lines (**9** vs **7**). As the C2-ethyl carboxylate substitution in 1-aroylindoles (**20**) exhibited moderate cytotoxicity, **23** and **24**, with the CO_2Et and $CH_2N(CH_3)_2$ groups at the C3-position, respectively, were also prepared. But no activity was observed in **23** and **24**. A literature report[12] showing that the 4-methoxy group in the B-ring of **1** skeleton can be replaced with a 4-*N*,*N*-dimethylamino group inspired us to synthesize **19**, with the C2-methyl and C5-dimethylamino groups on the indole ring. Indeed, **19** demonstrated strong antiproliferative activity in four cell lines, with mean IC_{50} values of 43 nM.

In an effort to increase the corresponding 1-aroylindoles structure's polarity, the 4-amino and 4-hydroxy-substituted 1-aroylindoles **26** and **27** were prepared to mimic the structure of **3** and **2** with a amino and hydroxy group at the C3′-position of B-ring of *Z*-stilbene, respectively. Compound **26**, namely, 4-amino-5-methoxy-1-(3′,4′,5′-trimethoxybenzoyl)indole, displayed a mean IC_{50} of 3.0 nM against four cell lines comparable to the reference compound **1** (IC_{50} = 3.1 nM). Compound **27**, with a hydroxyl group at C-4 position of indole ring, namely, 4-hydroxyl-2-methyl-5-methoxy-1-(3′,4′,5′-trimethoxybenzoyl)indole, showed mean a IC_{50} of 0.42 nM in all four lines, thus exhibiting stronger cytotoxicity than **1**. Interestingly, **26** and **27** with additional amino and hydroxyl groups showed approximately >10-fold improvement in IC_{50} values over analogues **10** and **17**, respectively (**26** vs **10**; **27** vs **17**). Hence, it may be concluded that the introduction of an amino or hydroxyl group at the C-4 position of 1-aroylindoles, in addition to a methoxy group at C-5 position, is important for maximal cytotoxicity.

Table 2. Inhibition of Tubulin Polymerization and Colchicine Binding by Compounds **7**, **8**, **10**, **19**, **26**, **27**, **4**, and **1**

compd	tubulin[a] $IC_{50} \pm$ SD (μM)	colchicine binding[b] ± SD (%) 1 μM inhibitor	5 μM inhibitor
7	2.6 ± 0.3	27 ± 2	55 ± 3
8	>5		
10	2.5 ± 0.6	45 ± 3	72 ± 2
19	1.5 ± 0.5	72 ± 1	89 ± 1
26	0.9 ± 0.2	71 ± 2	93 ± 1
27	0.6 ± 0.1	94 ± 1	97 ± 0.5
4	3.0 ± 0.4		
1	1.1 ± 0.3	85 ± 0.2	93 ± 2

[a] Inhibition of tubulin polymerization.[17] Tubulin was at 1 μM. [b] Inhibition of [^{3}H]colchicine binding. [^{3}H]Colchicine was at 5 μM.

(B) Inhibition of Tubulin Polymerization and Colchicine Binding Activity. To examine whether 1-aroylindoline, 1-aroyltetrahydroquinoline, and 1-aroylindoles were tubulin inhibitors through the colchicine-binding site, the selected compounds **7**, **8**, **10**, **19**, **26**, **27** and reference compounds (**1** and **4**) were evaluated for antitubulin activity and the ability to compete for the colchicine-binding site (Table 2). Results indicated that the compounds' antiproliferative activity positively correlated with the inhibition of tubulin polymerization. Compounds **19**, **26**, and **27** were efficacious in inhibiting microtubulin assembly, with IC_{50} values of 1.5, 0.9, 0.6 μM, respectively. In the [^{3}H]colchicine binding assay, data indicated the 4-amino-1-aroylindoles (**26**) and 4-hydroxy-1-aroylindoles (**27**) were strongly bound to the colchicine-binding domain on microtubulin. Compound **27** showed substantial activity. It inhibited colchicine binding by 94% (**27** was 1 μM with colchicine at 5 μM) and 97% (**27** was 5 μM with colchicine at 5 μM).

Conclusion

Synthesis and structure−activity relationship of synthetic anticancer agents 1-aroylindoline, 1-aroyltetrahydroquinoline, and 1-aroylindoles skeleton were described. The synthesized 1-aroylindoline (**7**) and 1-aroylindoles (**19**, **26**, and **27**) are potent cytotoxic agents and antitubulin agents acting through the colchicine-binding site on tubulin. Compounds **19**, **26**, and **27** displayed antiproliferative activity with IC_{50} values of 38−52, 1.2−5.4, and 0.3−0.6 nM, respectively, in a variety of human cancer cell lines from different organs. They also showed substantial antitubulin activity with IC_{50} values of 1.5, 0.9, and 0.6 μM, respectively. SAR data revealed that the introduction of amino or hydroxyl group at the C4-position of 1-aroylindole series significantly increased activity than the parent (**26** vs **10**; **27** vs **17**). Thus, the amino or hydroxyl substituents located at position 4 of 1-aroylindole moieties apparently plays an important role in the activity of this series of compounds. Utilizing a restricted approach of drug design, we effectively converted inactive diphenylamide **6** into active compounds, for example, 1-aroylindoline (**7** and **9**), 1-aroyltetrahydroquinoline (**8**), and 1-aroylindoles (**10**−**12**, **17**−**20**, **26**, and **27**), with substantial activity. This information maybe can be applied to other corresponding tubulin inhibitors or combretastatin analogues modification.

Experimental Section

4-Hydroxy-5-methoxy-2-methyl-1-(3′,4′,5′-trimethoxybenzoyl)indole (27). To a solution of **47** (1.46 g, 8.24 mmol) and triethylamine (2.29 mL, 16.48 mmol) in CH_2Cl_2 (20 mL) was added *tert*-butyldimethylsilyl chloride (1.86 g, 12.36 mmol) at room temperature. After the mixture was stirred for 18 h, the reaction was quenched with water and the layer was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer was dried over anhydrous

$MgSO_4$ and concentrated under reduced pressure to give a yellow oil residue, which was treated with potassium *tert*-butoxide (1.39 g, 12.36 mmol) in the presence of THF (50 mL) and stirred for 15 min at room temperature. The 3,4,5-trimethoxybenzoyl chloride (2.14 g, 9.27 mmol) was added to the reaction mixture. After 1 h, the solvent was evaporated and the residue was neutralized with $NaHCO_{3(sat.)}$ and then extracted with EtOAc (20 mL × 2) and CH_2Cl_2 (20 mL × 2). The combined organic layers were dried over $MgSO_4$ and evaporated to give a residue, which was dissolved in THF (20 mL) and then was subjected to 1.0 M tetra-*n*-butylammonium floride/THF (12.4 mL) with stirring at 0 °C for 1 h. The reaction mixture was evaporated and purified by silica gel chromatography (EtOAc/*n*-hexane = 1: 3; recrystallized by EtOAc/*n*-hexane) to afford **27** as a yellow crystalline solid: yield 19%, mp 153−155 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.82 (s, 6H), 3.88 (s, 3H), 3.95 (s, 3H), 5.76 (br, 1H), 6.53 (s, 1H), 6.63 (d, J = 8.9 Hz, 1H), 6.69 (d, J = 8.9 Hz, 1H), 6.95 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.8, 56.3, 57.1, 61.1, 105.1, 105.7, 107.0, 107.8, 118.5, 130.3, 133.5, 137.0, 137.6, 141.1, 141.8, 153.1, 169.3. MS (EI) *m/z*: 371 (M^+, 44%), 195 (100%). HRMS (EI) for $C_{20}H_{21}NO_6$ (M^+): calcd, 371.1371; found, 371.1370. Anal. ($C_{20}H_{21}NO_6 \cdot 0.5H_2O$) C, H, N.

Acknowledgment. This research was supported by the National Science Council of the Republic of China (Grants NSC 96-2320-B-038-003, NSC 96-2752-B-400-001-PAE, and NSC-95-2113-M-400-001-MY3) and by National Health Research Institutes, Taiwan (Grant CA-097-PP-02).

Supporting Information Available: Spectral data of **6**−**26**, **37**, **42**, **44**, **46** and details for synthesis and biological evaluations. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM800150D