Supporting Information

4- And 5-Aroylindoles as Novel Classes of Potent Antitubulin Agents

Contents:

General. (A) **Chemistry.** Melting points were determined on a Büchi (B-545) melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were obtained with the Varian Mercury-400 spectrometer (operating at 400 MHz and at 100 MHz, respectively), and the Bruker DRX-500 spectrometer (operating at 500 MHz and at 125 MHz, respectively), with chemical shift in parts per million (ppm, δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were measured with a Finnigan (MAT-95XL) electron impact (EI) mass spectrometer. Elemental analyses were performed on an Elementar vario EL Ⅲ. Flash column chromatography was done using silica gel (Merck Kieselgel 60, No. 9385, 230-400 mesh ASTM). All reactions were carried out under an atmosphere of dry nitrogen.

(B) **Biology. (a) Materials.** Regents for cell culture were obtained from Gibco-BRL Life Technologies (Gaitherburg, MD). Microtubule-associated protein (MAP)-rich tubulin was purchased from Cytoskeleton, Inc. (Denver, CO). [^{3}H]Colchicine (specific activity, 60-87 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA).

(b) Cell Growth Inhibitory Assay. Human oral epidermoid carcinoma KB cells, colorectal carcinoma HT29 cells, non small cell lung carcinoma H460 cells, and two stomach carcinoma TSGH, MKN45 cells were maintained in RPMI-1640 medium supplied with 5% fetal bovine serum. KB-VIN10 cells were maintained in growth medium supplemented with 10 nM vincristine, generated from vincristine-driven selection, and displayed overexpression of P-gp170/MDR. Cell in logarithmic phase were cultured at a density of 5000 cells/mL/well in a 24-well plate. KB-VIN10 cells were cultured in drug-free medium for 3 days prior to use. The cells were exposed to various concentrations of the test drugs for 72 h. The methylene blue dye assay was used to evaluate the effect of the test compounds on cell growth as described

previously.[1] The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically as a comparison with the control. Compounds were examined in at least three independent experiments, and the values shown for these compounds are the mean and standard deviation of these data.

(c) Tubulin Polymerization in Vitro Assay.[2,3] Turbidimetric assays of microtubules were performed as described by Bollag et al.[4] MAP-rich tubulin (2 mg/mL) in 100 μL buffer containing 100 mM PIPES (pH 6.9), 2 mM $MgCl_2$, 1 mM GTP, and 2% (v/v) dimethyl sulfoxide were placed in 96-well microtiter plate in the presence of test compounds. The increase in absorbance was measured at 350 nm in a PowerWave X Microplate Reader (BIO-TEK Instruments, Winooski, VT) at 37 °C and recorded every 30 s for 30 min. The area under the curve (AUC) used to determine the concentration that inhibited tubulin polymerization to 50% (IC_{50}). The AUC of the untreated control and 10μM of colchicine was set to 100% and 0% polymerization, respectively, and the IC_{50} was calculated by nonlinear regression in at least three experiments.

(d) [^{3}H] Colchicine Binding Assay.[2,3] The assay was basically performed according to the method of Lambeir and Engelborghs.[5] The 1 μM tubulin was incubated with 5.0 μM [^{3}H]-colchicine at either 1.0 or 5.0 μM concentrations of test compounds in a buffer containing 0.05 M PIPES (pH 6.9), 1mM $MgCl_2$, and 1 mM GTP. After incubating at room temperature for 1 h, the samples were centrifuged through Sephadex G-50 columns (Amersham Biosciences, Piscataway, NJ). The eluates in the flow-through were analyzed for radioactivity by scintillation counting.

Table 3. Elemental analyses of compounds **9**, **10**, **13**-**15**, and **18**

Compd	formula	calculated			found		
		%C	%H	%N	%C	%H	%N
9	$C_{18}H_{17}NO_4 \cdot 0.25H_2O$	68.45	5.58	4.43	68.12	5.29	4.54
10	$C_{18}H_{17}NO_4$	69.44	5.50	4.50	69.21	5.64	4.36
13	$C_{19}H_{19}NO_4$	70.14	5.89	4.31	69.85	5.94	4.48
14	$C_{19}H_{19}NO_4$	70.14	5.89	4.31	70.36	5.72	4.51
15	$C_{20}H_{21}NO_4$	70.78	6.24	4.13	70.89	6.03	4.29
18	$C_{19}H_{19}NO_5 \cdot 0.5H_2O$	65.13	5.75	4.00	64.76	5.78	4.38

Spectral Data and Procedure of compounds **6-8, 11, 12, 16, 17,** and **19-21**.

1-(3', 4', 5'-Trimethoxybenzoyl)indole (6). To a solution of **22** (0.5g, 4.26 mmol) in THF (20 ml), potassium *tert*-butoxide (0.71g, 6.40 mmol) was added and stirred at room temperature for 20 min. The 3, 4, 5-Trimethoxybenzoyl chloride (1.47g, 6.40 mmol) was added to the reaction mixture in one portion. After 16h, the solvent was evaporated, and the residue was extracted with CH_2Cl_2 (15 ml x 3). The combined organic extracts were dried over $MgSO_4$ and evaporated to give a residue, which was chromatographed over silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford **6**, yield 85%; mp 102.1-103.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.89 (s, 6H), 3.94 (s, 3H), 6.62 (d, J = 3.7 Hz, 1H), 6.98 (s, 2H), 7.31 (t, J = 7.6 Hz, 1H), 7.36-7.39 (m, 2H), 7.60 (d, J = 7.6 Hz, 1H), 8.35 (d, J = 8.2 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.2, 60.9, 106.7, 108.4, 116.2, 120.8, 123.8, 124.8, 127.4, 129.4, 130.6, 135.9, 141.1, 153.1, 168.1. MS (EI) *m/z*: 311 (M^+, 29%), 195 (100%). HRMS (EI) calcd for $C_{18}H_{17}NO_4$ (M^+), 311.1160; found, 311.1159.

2-(3', 4', 5'-Trimethoxybenzoyl)indole (7). The title compound was obtained in 34% overall yield from 2-formylindole (**23**) and 3, 4, 5-trimethoxyphenylmagnesium bromide in a manner similar to that described for the preparation of **9**; mp 150.8-151.7°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (s, 6H), 3.96 (s, 3H), 7.16-7.20 (m, 2H), 7.27 (s, 2H), 7.38 (t, J = 7.2 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 9.43 (s, 1H). MS (EI) *m/z*: 311 (M^+, 100%), 296 (12%). HRMS (EI) calcd for $C_{18}H_{17}NO_4$ (M^+), 311.1152; found, 311.1155.

3-(3', 4', 5'-Trimethoxybenzoyl)indole (8). The title compound was obtained in 38% overall yield from 3-formylindole (**24**) and 3, 4, 5-trimethoxyphenylmagnesium bromide in a manner similar to that described for the preparation of **9**; mp 132.5-133.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 6H), 3.92 (s, 3H), 7.11 (s, 2H), 7.27-7.32 (m, 2H), 7.40-7.44 (m, 1H), 7.73 (d, J = 2.8 Hz, 1H), 8.37-8.39 (m, 1H), 9.28 (s, 1H). MS (EI) *m/z*: 311 (M^+, 100%), 144 (77%). HRMS (EI) calcd for $C_{18}H_{17}NO_4$ (M^+), 311.1159; found, 311.1158.

6-(3', 4', 5'-Trimethoxybenzoyl)indole (11). The title compound was obtained in 33% overall yield from 6-formylindole (**27**) and 3, 4, 5-trimethoxyphenylmagnesium bromide in a manner similar to that described for the preparation of **9**; mp 131.3-134.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 6H), 3.94 (s, 3H), 6.64 (m, 1H), 7.08 (s, 2H), 7.42 (t, J = 2.8 Hz, 1H), 7.62 (dd, J = 8.4, 1.6 Hz, 1H), 7.71 (d, J = 8.4, 1H), 7.95 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.2, 60.9, 103.0, 107.6, 114.2, 120.1, 122.0, 127.9, 131.3, 131.4, 133.9, 135.0, 141.3, 152.7, 196.4. MS (EI) *m/z*: 311 (M^+, 100%), 144 (52%), 116 (28%). HRMS (EI) calcd for $C_{18}H_{17}NO_4$ (M^+), 311.1152; found, 311.1155.

7-(3', 4', 5'-Trimethoxybenzoyl)indole (12). The title compound was obtained in 30% overall yield from 7-formylindole (**28**) and 3, 4, 5-trimethoxyphenylmagnesium bromide in a manner similar to that described for the preparation of **9**; mp 114.1-115.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 6H), 3.95 (s, 3H), 6.61 (m, 1H), 7.03 (s, 2H), 7.14 (t, J = 7.6 Hz, 1H), 7.31 (m, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 10.48 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 60.7, 102.2, 106.8, 118.2, 119.1, 125.6, 126.7, 127.3, 129.2, 133.9, 135.4, 140.8, 152.6, 196.7. MS (EI) *m/z*: 311 (M^+, 100%), 144 (52%), 280 (67%). HRMS (EI) calcd for $C_{18}H_{17}NO_4$ (M^+), 311.1167; found, 311.1162.

1-Propyl-5-(3', 4', 5'-Trimethoxybenzoyl)indole (16). The title compound was obtained in 74% yield from **10** and 1-iodopropane in a manner similar to that described for the preparation of **13**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (t, J = 7.6 Hz, 3H), 1.90 (heptet, J = 7.6 Hz, 2H), 3.87 (s, 6H), 3.96 (s, 3H), 4.13 (t, J = 7.6 Hz, 2H), 6.60 (d, J = 3.2 Hz, 1H), 7.08 (s, 2H), 7.19 (d, J = 3.2 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.78 (dd, J = 8.8, 1.6 Hz, 1H), 8.14 (d, J = 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.4, 23.5, 48.2, 56.2, 60.8, 102.7, 107.4, 109.1, 123.5, 125.2, 127.6, 129.0, 129.4, 134.1, 138.2, 141.0, 152.6, 196.2. MS (EI) *m/z*: 353 (M^+, 100%), 186 (31%). HRMS (EI) calcd for $C_{21}H_{23}NO_4$ (M^+), 353.1634; found, 353.1631.

1-Isopropyl-5-(3', 4', 5'-Trimethoxybenzoyl)indole (17). The title compound was obtained in 65% yield from **10** and 2-bromopropane in a manner similar to that described for the preparation of **13**; mp 89.2-90.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (d, J = 6.8 Hz, 6H), 3.87 (s, 6H), 3.96 (s, 3H), 4.73 (septet, J = 6.8 Hz, 1H), 6.63 (d, J = 3.2 Hz, 1H), 7.09 (s, 2H), 7.31 (d, J = 3.2 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.79 (dd, J = 8.8, 1.6 Hz, 1H), 8.14 (d, J = 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$)

δ 22.7, 47.3, 56.2, 60.8, 103.1, 107.4, 109.1, 123.4, 125.1, 125.2, 127.6, 129.0, 134.1, 137.6, 141.0, 152.6, 196.2. MS (EI) *m/z*: 353 (M^+, 100%), 338 (29%). HRMS (EI) calcd for $C_{21}H_{23}NO_4$ (M^+), 353.1615; found, 353.1621.

[5-(3,4,5-Trimethoxy-benzoyl)-indol-1-yl]-acetic acid (19). A stirred solution of **10** (0.2 g, 0.64 mmol), potassium *tert*-butoxide (0.10g, 0.96 mmol), and methyl bromoacetate (0.1 ml, 0.96 mmol) in CH_3CN (10 ml) was heated at reflux for 4h. The reaction was quenched by water and extracted with EtOAc (15 ml x 3). The combined organic layers were dried over $MgSO_4$ and evaporated in vacuum to give a residue, which was dissolved in methanol (10 ml) and water (1ml). Lithium hydroxide (0.046g, 1.93 mmol) was added to the reaction mixture and heated at reflux. After 3h, the solution was evaporated and extracted with EtOAc (10 ml x 2) and CH_2Cl_2 (10 ml x 2). The combined organic extracts were dried over $MgSO_4$ and evaporated to give a residue, which was chromatographed over silica gel (CH_2Cl_2 : MeOH = 15 : 1) to afford **19**, yield 65%; mp 181.1-182.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (s, 6H), 3.94 (s, 3H), 4.93 (s, 2H), 6.67 (d, *J* = 3.2 Hz, 1H), 7.07 (s, 2H), 7.17 (d, *J* = 3.2 Hz, 1H), 7.31 (d, *J* = 8.8, 1.6 Hz, 1H), 7.79 (dd, *J* = 8.8, 1.6 Hz, 1H), 8.11 (d, *J* = 1.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 48.2, 56.7, 61.4, 104.4, 108.4, 110.0, 124.7, 125.9, 128.9, 130.0, 131.6, 134.9, 140.0, 142.1, 153.5, 171.4, 198.3. MS (EI) *m/z*: 369 (M^+, 100%), 326 (14%), 202 (18%). HRMS (EI) calcd for $C_{20}H_{19}NO_6$ (M^+), 369.1206; found, 369.1209.

3-[5-(3,4,5-Trimethoxy-benzoyl)-indol-1-yl]-propionic acid (20). Ethyl acrylate (0.12g, 1.44 mmol) was added to a stirred mixture of 10 (0.15, 0.48 mmol) and cesium carbonate (0.31g, 0.96 mmol) in CH_3CN (10 ml) at room temperature, and stirring for 4h. The reaction was evaporated and extracted with EtOAc (15 ml x 3).

The combined organic layer was dried over $MgSO_4$ and then evaporated to give a residue, which was further treated with lithium hydroxide (0.034g, 1.44 mmol) in MeOH (6 ml) and H_2O (1ml), and heated at reflux for 4h. The reaction mixture was evaporated and extracted with EtOAc (15 ml x 2) and CH_2Cl_2 (15 ml x 2). The combined organic layers were dried over $MgSO_4$ and evaporated to give a crude product, which was purified by silica gel chromatography (CH_2Cl_2 : MeOH = 12 : 1) to afford **20**, yield 74%; mp 151.7-153.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.92 (t, *J* = 6.4 Hz, 2H), 3.87 (s, 6H), 3.94 (s, 3H), 4.51 (t, *J* = 6.4 Hz, 2H), 6.60 (d, *J* = 3.2 Hz, 1H), 7.07 (s, 2H), 7.24 (d, *J* = 3.2 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.79 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.12 (d, *J* = 1.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.5, 41.6, 56.2, 60.9, 103.6, 107.6, 108.9, 123.9, 125.3, 127.9, 129.5, 133.9, 137.9, 141.3, 152.7, 175.6, 196.4. MS (EI) *m/z*: 383 (M^+, 100%), 340 (13%), 216 (24%). HRMS (EI) calcd for $C_{21}H_{21}NO_6$ (M^+), 383.1369; found, 383.1396.

1-(*N, N*-Dimethylaminoethyl)-5-(3', 4', 5'-Trimethoxybenzoyl)indole (21). To a stirred solution of **10** (0.15g, 0.48 mmol), potassium *tert*-butoxide (0.27g, 2.41 mmol), KI (0.08g, 0.48 mmol), 2-dimethylaminoethyl chloride hydrochloride (0.14g, 0.96 mmol) in DMF (3.5 ml) was heated at 100-120°C for 16h. The reaction was quenched with water and extracted with EtOAc (15 ml x 2) and CH_2Cl_2 (15 ml x 2). The organic layers was dried over $MgSO_4$ and evaporated to give a residue, which was purified by silica gel chromatography (CH_2Cl_2 : MeOH = 20 : 1) to afford **21**, yield 48%; ^{1}H NMR (500 MHz, CD_3OD) δ 2.31 (s, 6H), 2.77 (t, *J* = 7.1 Hz, 2H), 3.83 (s, 6H), 3.87 (s, 3H), 4.33 (t, *J* = 7.1 Hz, 2H), 6.59 (d, *J* = 3.1 Hz, 1H), 7.04 (s, 2H), 7.32 (d, *J* = 3.1 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.69 (dd, *J* = 8.6, 1.0 Hz, 1H), 8.05 (d, *J* = 1.0 Hz, 1H). MS (EI) *m/z*: 382(M^+, 47%), 324 (100%). HRMS (EI) calcd for $C_{22}H_{26}N_2O_4$ (M^+), 382.1896; found, 382.1894.

References of Supporting Information

1. Finlay, G. J.; Baguley, B. C.; Wilson, W. R. A Semiautomated Microculture Method for Investigating Growth Inhibitory Effects of Cytotoxic Compounds on Exponentially Growing Carcinoma Cells. *Anal. Biochem.* **1984**, *139*, 272-277.
2. Liou, J. P.; Chang, J. Y.; Chang, C. W.; Chang, C. Y.; Mahindroo, N.; Kuo, F. M.; Hsieh, H. P. Synthesis and Structure-Activity Relationships of 3-Aminobenzophenones as Antimitotic Agents. *J. Med. Chem.* **2004**, *47*, 2897-2905.
3. Kuo, C. C.; Hsieh, H. P.; Pan, W. Y.; Chen, C. P.; Liou, J. P.; Lee, S. J.; Chang, Y. L.; Chen, L. T.; Chen, C. T.; Chang, J. Y. BPR0L075, a Novel Synthetic Indole Compound with Antimitotic Activity in Human Cancer Cells, Exerts Effective Antitumoral Activity in Vivo. *Cancer Research* **2004**, *64*, 4621-4628.
4. Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. Epothilones, a New Class of Microtubule-stabilizing Agents with a Taxol-like Mechanism of Action. *Cancer Res.* **1995**, *55*, 2325-2333.
5. Lambier, A.; Engelborghs, Y. A Quantitative Analysis of Tubulin colchicine Binding to Microtubules. *Eur. J. Biochem.* **1980**, *109*, 619-624.