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Structure–Activity and Crystallographic Analysis of Benzophenone Derivatives—the Potential Anticancer Agents

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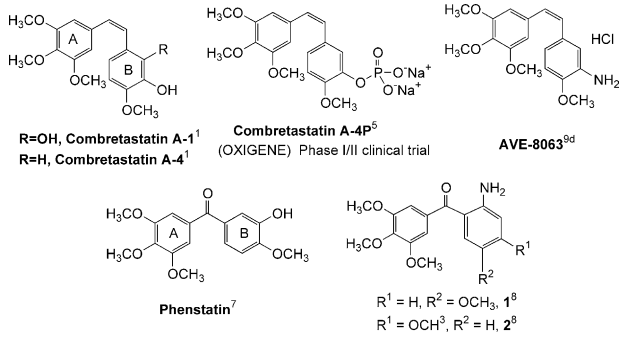
This paper is dedicated to Prof. Chun-Chen Liao, National Tsing Hua University, Taiwan, on the occasion of his 60th birthday, for the celebration of his achievements in masked *o*-benzoquinones in organic synthetic applications.

Abstract—Compounds **1**–**5**, structurally related to combretastatin A-4 showed excellent cytotoxic activities against a panel of human cancer cell lines including multi-drug resistant cell lines. The X-ray three-dimensional structural analysis shows that proton donor in B ring may be required for cytotoxic activity, with intermolecular hydrogen bonding playing an important role.

Combretastatin A-4 (CA-4), a tubulin binding agent isolated from *Combretum caffrum*, has been shown to be a potential anticancer agent.[1] It is active against various cell lines including those that express the MDR phenotypes.[2,3] CA-4 and its phosphate salt damage the tumor vasculature by starving tumors of nutrients.[4] Currently the phosphate salt of CA-4 is in phase-I/II clinical trials.[5] CA-4 has higher affinity for β-tubulin than colchicine at or near colchicine binding site and causes destabilization of tubulin cytoskeleton.[6] A number of CA-4 analogues have been synthesized and evaluated for their structure–activity relationships (SARs).[2,5,7–11] The SAR studies reveal that *Z*-configuration of CA-4 and related analogues is an essential structural requirement for cytotoxic activity.[12–14] Furthermore, the appropriate distance between two aromatic rings is considered to be critical.[15]

Phenstatin, a benzophenone-type CA-4 analogue synthesized by Pettit's group, has been found to be a very strong cytotoxic agent comparable to CA-4 (Fig. 1).[7] Recently we discovered aminobenzophenones **1** and **2**, which showed significantly increased cytotoxicity against many human cancer cell lines compared to phenstatin.[8] SAR information revealed introduction of



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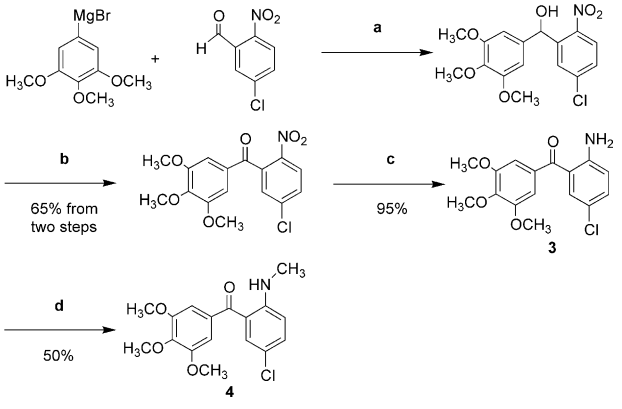
an amino group at the ortho position of the benzophenone ring plays an integral role for increased cytotoxic activity. As a part of our continuing efforts to further examine the importance of three-dimensional spatial arrangements and the physical properties that are critical for activity, we report here our investigation of the cytotoxic potential of two new single crystal compounds **3** and **5** based on a panel of human cancer cell lines including several drug resistant cell lines, cell cycle analysis, confocal microscopy experiments, three-dimensional structural comparison, and molecular orbital calculation.

Chemistry

2-Aminobenzophenone **3** was synthesized as outlined in Scheme 1 in 62% yield from 3, 4, 5-trimethoxybromobenzene and commercially available 4-chloro-2-nitrobenzaldehyde in three steps, Grignard addition, PCC oxidation, and Fe/AcOH reduction.[16] Compound **3** was further mono-*N*-alkylated with methyl iodide to obtain compound **4**. The preparation of 2-hydroxylbenzophenone **5** was achieved from 3, 4, 5-trimethoxybromobenzene and 2-(*tert*-butyldimethylsilanyloxy)-5-chlorobenzaldehyde in three steps, Grignard addition, PCC oxidation, and TBAF deprotection in 65% overall yield as shown in Scheme 2.[17]

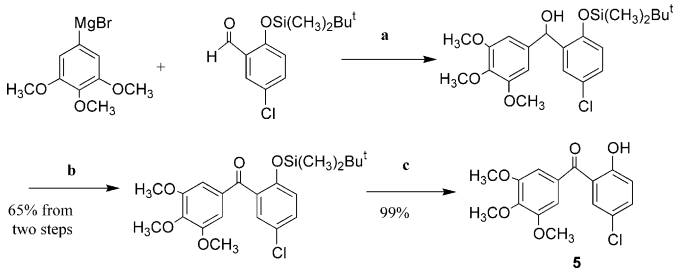


Scheme 1. (a) THF, 0–25 °C; (b) PDC, CH_2Cl_2, 25 °C; (c) Fe, AcOH, EtOH, reflux, (d) CH_3I, CH_2Cl_2, 25 °C.

Biology

CA-4 and related analogues phenstatin and compounds **1**–**5** were evaluated for cell growth inhibition against several human cancer cell lines including drug resistant cell lines using the tetrazolium dye reduction assay (MTT assay).[18] A comparison of IC_{50} values for the tested compounds is provided in Table 1. The IC_{50} values represent the drug concentrations producing 50% decrease in cell growth after 3 days of incubation.

Compounds **1** and **2** with amino and methoxy substituents showed more potent cytotoxicity than phenstatin and slightly less potent than CA-4. Compound **3** with amino and chloro substituents shows good activity against various drug-resistant cell lines, although less active than CA-4. Replacement of the amino group in **3** with a mono-methylated amino group (**4**) decreased cytotoxicity by 50 folds as compared to **3**. At the same time, replacement of the amino group with a hydroxyl group (**5**) also decreased cytotoxicity by 50 folds com-



Scheme 2. (a) THF, 0–25 °C; (b) PDC, CH_2Cl_2, 25 °C; (c) TBAF, THF, 25 °C.

Table 1. IC_{50} values (nM ± SD) of **1**, **2**, **3**, **4**, **5**, CA-4, and phensatatin

Compd	KB[a]	KBs5[b]	KB-Vin10[c]	KB-7D[d]	KB-100[e]	CPT30[f]	Hone-1[g]
1	38.5±3.3	34.7±4.2	48.6±1.6	38.1±2.2	22.5±3.5	41.0±3.4	59.2±10.8
2	25.8±4.0	25.6±3.5	45.8±2.7	29.3±1.6	18.9±2.1	37.3±1.8	35.8±5.6
3	90.4±20.8	71.0±6.9	97.2±9.5	104.0±7.6	156.0±28.2	60.3±8.5	64.5±11.4
4	6080.8±937.7	2293.2±108.5	5854.8±1503.5	5409.8±113.2	7164.1±931.9	5354.9±752.7	5143.4±237.4
5	5462.2±280.5	4550.8±957.2	8336.4±1281.9	5452.1±2342.9	4066.9±2100.4	4997.1±675.7	6112.8±450.7
CA-4	6.2±0.5	7.0±0.6	9.5±0.6	7.9±0.5	7.3±0.6	6.9±0.5	6.5±1.1
Phenstatin	128.7±8.9	183.8±32.7	34.1±0.5	180.2±22.3	34.8±7.5	37.2±1.9	138.7±34.1

[a]KB is a human oral cancer cell line.
[b]KBs5, a taxol-resistant cell line derived from its parental cell line KB at the concentration of 50 nM.
[c]KB-Vin10, a vincristine-resistant cell line derived from its parental cell line KB, showed over expression of p-glycoprotein.
[d]KB-7D, VP16-resistant cell line derived from its parental cell line KB, showed down-regulation of topoisomerase II and overexpression of MRP 1.[19]
[e]KB-100, camptothecin (CPT)-resistant cell line derived from its parental cell line KB, displayed down regulation of topoisomerase I.[20]
[f]CPT30, a CPT-resistant cell line derived from its parental cell line Hone-1, showed quantitative and qualitative change of topoisomerase I.[21]
[g]Hone-1 is a human NPC cancer cell line.

pared to **3**. Based on above cytotoxic activities, several findings are summarized: (a) introduction of an amino group at the ortho position of B ring enhances cytotoxicity; (b) introduction of an *N*-methylated amino group at the ortho position causes cytotoxicity decrease; (c) introduction of a hydroxyl group at the ortho position results in great reduction of cytotoxicity; (d) a methoxy substituent is better than a chloro substituent for increased cytotoxicity; (e) it is interesting to note that no cross-resistance with the CA-4 analogues including CA-4 studied is observed in taxol, vincristine, VP-16, and camptothecin-resistant cell lines.

In order to investigate the effects of **3**, **5**, CA-4, and phenstatin on the cell cycle, analysis were performed by flow cytometry against KB human cancer cells after 24 h.[22] The results in Table 2 show that **3** caused significant arrest of the cells at the G_2/M phase at the concentration of 100 nM. The order of cell arrest at the G_2/M phase was CA-4 > phenstatin > **3** > **5**, consistent with their cytotoxic activities against human KB cancer cells.

To study the mode of action of **3**, microtubules of NUGC3 gastric cancer cells treated with **3** were compared with those treated with colchicine and taxol by anti-tubulin immunofluorescence staining followed by confocal microcopy examination. As shown in Figure 2, the treatment of cells with **3** for 20 h resulted in disruption of microtubule cytoskeleton, suggesting that the mode of action of **3** is similar to that of colchicine.

Table 2. Effect of **3**, **5**, CA-4 and phenstatin on cell cycle progression

	Concd (nM)	$SubG_1$	G_0/G_1	S	G_2/M
Control		5.5%	62.2%	14.3%	17.8%
3	25	6.5%	61.7%	14.3%	17.3%
	50	6.4%	61.7%	14.6%	17.3%
	100	13.9%	5.7%	7.1%	73.2%
5	25	5.3%	61.8%	14.6%	18.3%
	50	5.7%	59.4%	15.9%	18.9%
	100	26.5%	45.4%	11.8%	16.0%
CA-4	25	18.3%	8.9%	11.0%	61.6%
	50	16.8%	9.3%	10.6%	63.2%
	100	11.6%	5.2%	10.6%	72.3%
Phenstatin	25	32.9%	17.6%	9.9%	39.6%
	50	17.8%	7.6%	10.5%	63.9%
	100	13.0%	5.6%	7.7%	73.5%

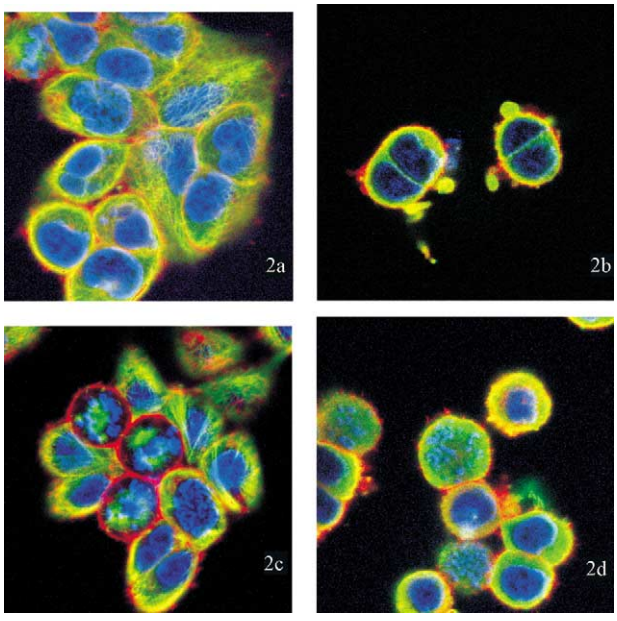


Figure 2. Effect of **3** on microtubule polymerization in NUGC3 Cells. (a) Control, (b) colchicine (1 μM, 20 h), (c) taxol (10 μM, 20 h), and (d) **3** (100 nM, 20 h). Green: β-tubulin, red: F-actin, and blue: nuclear DNA.

X-ray Crystallography and Molecular Orbital Calculation

Compounds **3** and **5** were subjected to single crystal X-ray study[23] and superimposed structures of **3** and **5** are provided in Figure 3. It is interesting to note that the three-dimensional X-ray structures of these two compounds were almost superimposable, but their cytotoxic activities exhibited great differences. In order to further compare the physical properties of **3** and **5**, we carried out the semi-empirical AM1 calculations based on the X-ray structures of compounds **3**, **5**, and phenstatin. The pertinent data are given in Table 3. We observed that although the cytotoxic activities of **3** and **5** showed a 50-fold difference, among the physical parameters of **3**

Table 3. Semi-empirical AM1 calculations based on their X-ray structures

Compd	**3**	**5**	Phenstatin[7]
Dipole (Debye)	3.78	2.50	5.89
HOMO[a]	8.71	9.26	9.10
LUMO[a]	0.56	0.90	0.57
HOMO-LUMO Gap[a]	8.15	8.36	8.53
Two-ring angle(°)[b]	64.4	61.3	63.0
Two-ring distance(Å)[c]	2.58	2.58	2.59

[a]Units of HOMO, LUMO and HOMO-LUMO Gap are eV.
[b]Two-ring angles are calculated by the summation of two adjacent torsion angles between two different aromatic rings.
[c]Two-ring distance is the shortest distance of the carbon atoms between two different aromatic rings.

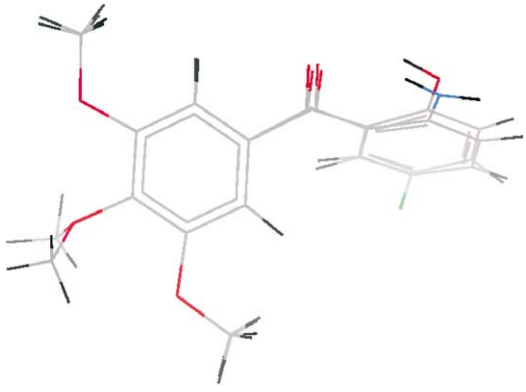


Figure 3. X-ray crystallographic structures of compounds **3** and **5**. Red indicates oxygen atom, blue indicates nitrogen atom, and green indicates chloro atom.

and **5** listed in Table 3, all except for the dipole moment were quite similar.

It is interesting to note that **3**, with an amino group, has an extra available hydrogen as a proton donor that may be important for the higher cytotoxicity for **3** in comparison with **5** having a hydroxyl group. The phenolic hydrogen, present at the ortho position in **5**, is involved in intramolecular hydrogen bonding with the keto group thus decreasing the cytotoxicity greatly. In the same manner, the only remaining anilinic hydrogen in *N*-methylated **4** is also involved in intramolecular hydrogen bonding with the keto group thus diminishing the cytotoxicity dramatically. In summary, compound **3**, with an amino group at the ortho position having an extra hydrogen, and phenstatin, with a hydroxyl group at the meta position, are more cytotoxic due to the presence of an available proton donor in B ring which is apparently important for cytotoxic activity. This hypothesis is supported by the fact that in both compounds **4** and **5** there is substantial loss of activity as both these compounds do not have available free hydrogen to interact with the active site. Combretastatin A-4[1], combretastatin A-1[1] and AVE-8063[9d] (Fig. 1) having an available proton donor in B ring also show good cytotoxic activity, further supporting our proposed hypothesis that available free proton donor in B ring is important for cytotoxic activity of combretastatin analogues.

In conclusion, introduction of an unsubstituted amino group (compounds **1**, **2**, and **3**) at the *ortho* position in benzophenone derivatives is important for increased cytotoxicity. The X-ray three-dimensional structural analysis shows that, although compound **3** and **5** differ drastically in their cytotoxic activities, they have superimposable X-ray structures and similar physical properties. Thus, an available proton donor in B ring, which is not involved in intramolecular hydrogen bonding, plays a crucial role in cytotoxic activity of benzophenone-type combretastatin A-4 analogues.

Acknowledgements

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16. Compound **3**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 6H, 2 × OCH_3), 3.94 (s, 3H, OCH_3), 6.03 (br, 2H, NH_2), 6.70 (d,J = 9.0 Hz, 1H, Ar–$H_{3'}$), 6.90 (s, 2H, Ar–H_2, H_6), 7.23 (dd,J = 9.0 Hz, 2.1 Hz, 1H, Ar–$H_{4'}$), 7.48 (d,J = 2.1 Hz, 1H, Ar–$H_{6'}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.2, 60.8, 106.8, 118.3, 118.7, 119.7, 132.7, 133.8, 134.1, 141.1, 149.1, 152.8, 196.6. MS (EI) m/z: 322 ($M^+ + 1$). HRMS (EI) for $C_{16}H_{16}ClNO_4$ (M^+): calcd, 321.0724; found, 322.0746. Anal. calcd for $C_{16}H_{16}ClNO_4$: C, 59.73; H, 5.01; N, 4.35. Found: C, 59.32; H, 5.23; N, 4.17.
17. Compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$): δ3.91 (s, 6H, 2 × OCH_3), 3.96 (s, 3H, OCH_3), 6.92 (s, 2H, Ar–H_2, H_6), 7.05 (d, J = 9 Hz, 1H, Ar–H_3'), 7.46 (dd, J = 8.7, 2.4 Hz, 1H, Ar–H_4'), 7.65 (d, J = 2.7 Hz, 1H, Ar–H_6'), 11.73 (s, 1H, OH); ^{13}CNMR (75 MHz, $CDCl_3$) δ56.3, 61.0, 107.0, 119.7, 120.0, 123.3, 127.8, 132.1, 135.9, 142.0, 153.1, 161.5, 199.3. MS (EI) m/z: 323 ($M^+ + 1$). HRMS (EI) for $C_{16}H_{15}ClO_5$ (M^+): calcd, 322.0576; found, 322.0592. Anal. calcd for $C_{16}H_{15}ClO_5$: C, 59.54; H, 4.68. Found: C, 59.32; H, 4.34.
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23. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 189015 for compound **3** and 189016 for compound **5**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit @ccdc.cam.ac.uk)