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Study on the Synthesis and Bioactivity of Novel Mahkoside A Derivatives

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Abstract: A series of novel Mahkoside A derivatives was synthesized, and their in vitro cytotoxic activities were evaluated against the human cancer cell line Ec-9706. A Preliminary structure–activity relationship study showed compounds **7** and **8** have obvious cytotoxic activities (IC_{50}: 30.0 and 12.5 $\mu g \cdot mL^{-1}$, respectively).

Keywords: benzophenone, Koenigs–Knorr reaction, Mahkoside A, 2,3,6-tri-O-acetyl-4-chloro-4-deoxy-β-D-galactoside

1 INTRODUCTION

Mahkota Dewa (Phaleria macrocarpa [Scheff.] Boerl) is widely distributed in Indonesia. Empirically, the stems, leaves, and fruits of it are used to control many diseases such as cancer, impotency, hemorrhoids, diabetes mellitus, allergies, liver and heart diseases, kidney disorders, blood diseases, rheumatism, high blood pressure, stroke, migraines, various skin diseases, and acne.[1] There had not been any report about the chemical constituents of the plant before we first separated a new derivative of benzophenone, named Mahkoside A,[2] from *Mahkota Dewa*. It was reported that this class of compounds is usually used as an ultraviolet absorber, photo sensitizer, and medical intermediate;[3] exhibits notable cytotoxicity against the ovarian cancer cell line,[4] the human tumor cell

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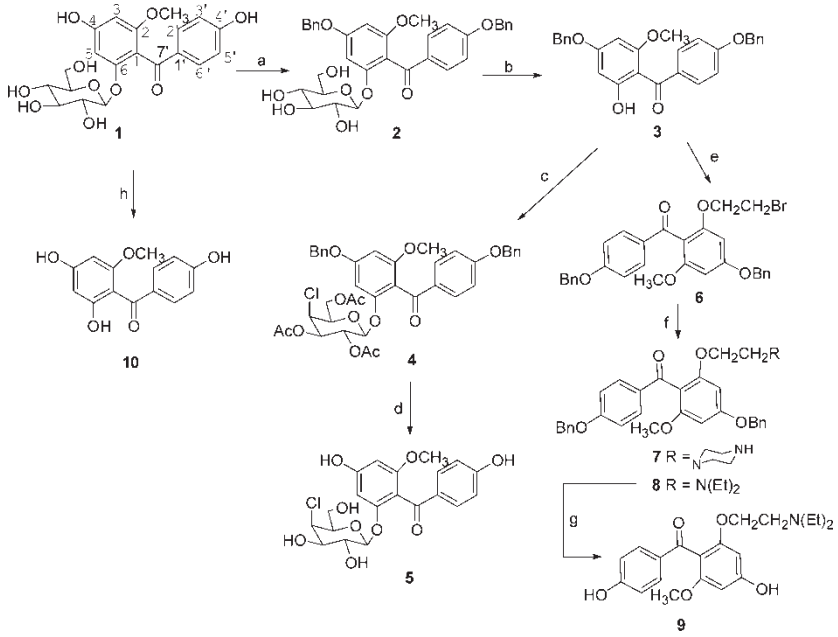
line,[5] the colon cancer cell line,[6] the human submandibular gland adenocarcinoma cell line, and primary human gingival fibroblast;[7] combats the human parasite *Trypanosoma cruzi* (Chagas's disease);[8] and displays significant antioxidant activity.[9] Hence, we attempted to synthesize various Mahkoside A derivatives and evaluate the in vitro cytotoxicity of the product obtained.

2 RESULTS AND DISCUSSION

2.1 Chemistry

To find new Mahkoside A derivatives possessing potent bioactivity, we needed a variety of structurally modificated compounds to study the structure–activity relationships of Mahkoside A. Selective protection of Mahkoside A (**1**) with benzyl chloride in the presence of anhydrous K_2CO_3 afforded 4,4′-dibenzyloxy Mahkoside A (**2**) in 80% yield. Acid hydrolysis of **2** with aqueous HCl furnished 6-hydroxy-4,4′-dibenzyloxy-2-methoxy benzophenone (**3**) in 92% yield. In the previous syntheses of flavonol O-glycosides, all the glycosidations employed glycosyl bromides or chlorides under either Koenigs–Knorr conditions (promoted by silver salts) or, later, under phase-transfer-catalyzed (PTC) alkaline conditions.[10] Although the latter glycosidation yield was relatively low (normally lower than 50%), it greatly improved and simplified the workup procedures.[10] We attempted to use the basic PTC conditions (Bu_4NBr, K_2CO_3, $CHCl_3$/H_2O) in the glycosylation of benzophenone **3** with 2,3,6-tri-O-acetyl-4-chloro-4-deoxy-α-D-galactopyranosidyl bromide and successfully gave **4** in 63% yield. This result might be attributed to the decrease of the side reactions of the glycosyl bromide under basic conditions, such as elimination to give the glycal and cleavage of the acyl protecting groups.[10] The 4,4′-dibenzyloxy group on **4** were then completely cleaved by hydrogenolysis in the catalysis of Pd-C under normal pressure, and the acetate groups on the sugar moiety were removed with NaOH in methanol to afford new glycoside **5**.

On the other hand, another method for structural modification of benzophenone **3**, alkylamine of 6-OH, was conducted. Intermediate **3** was reacted with dibromoethane in the refluxing absolute ethanol in the presence of K_2CO_3 to give 6-(2-bromoethoxy)-4,4′-dibenzyloxy-2-methoxybenzophenone **6**. Subsequent substitution of the bromo group with different amino groups led to 6-aminoalkyloxy derivatives **7** and **8** in 74% and 94%, respectively. The final removal of benzyl groups of **8** with catalytic hydrogenolysis afforded **9** in high yield. When a solution of Mahkoside A (**1**) in ethanol was treated with 30% aqueous HCl at reflux temperature, 4,6,4′-trihydroxy-2-methoxybenzophenone (**10**) was obtained in 95% yield. All procedures are shown in Scheme 1.



Scheme 1. BnCl, K_2CO_3, CH_3CH_2OH, reflux; (b) HCl, reflux; (c) K_2CO_3, $CHCl_3$, $(C_4H_9)_4NBr$, 40°C, 2,3,6-tri-O-acetyl-4-chloro-4-deoxy-β-D-galactosidyl bromide; (d) i: H_2, 10% Pd-C, EtOH-EtOAc, 40°C; ii: NaOH, MeOH, rt; (e) $BrCH_2CH_2Br$, K_2CO_3, C_2H_5OH, reflux; (f) RH K_2CO_3, DMF, rt; (g) H_2, 10% Pd-C, EtOH-EtOAc, 40°C; (h) HCl, reflux.

2.2 Biological Activity

The in vitro cytotoxicity of novel Mahkoside A derivatives **2**–**4** and **6**–**10** against human cancer cell line Ec-9706 were evaluated in an assay system. The results are listed in Table 1. The results showed that compounds **7** and **8**

Table 1. In vitro activity of Mahkoside A derivatives

Compound	IC_{50} ($\mu g \cdot mL^{-1}$)	Inhibition (100 $\mu g \cdot mL^{-1}$)
1	>100	18.7
2	>100	19.2
3	>100	6.4
4	>100	17.9
6	>100	7.0
7	30.0	—
8	12.5	—
9	>100	33
10	>100	28.9

exhibit higher antitumor activities (IC_{50} 30 $\mu g \cdot mL^{-1}$ and 12.5 $\mu g \cdot mL^{-1}$), compound **6** does not show any activity, and others are less bioactive. On the basis of this result, we can conclude that the 6-aminoalkyloxy group greatly enhances cytotoxicity and the substitution of two hydroxyl groups at 4,4′-position with two alkyloxy groups increases cytotoxicity also, which are two effective ways to improve the cytotoxicity. The study on the structure–activity relationship indicated that the suitable substitutions at benzene rings greatly improve the bioactivity, but the sugar moiety gives less contribution. The further structural modification of these compounds to improve the bioactivity is in progress.

3 EXPERIMENTAL

3.1 General Methods

^1H and ^{13}C NMR spectra were acquired on a Bruker Avance DPX-400 spectrometer with chemical shifts (δ) given in parts per million relative to Me_4Si as an internal standard. Melting points were determined on a WC-1 melting-point apparatus and are uncorrected. IR spectra were obtained on a Thermo Nicolet IR200 unit. High-resolution mass spectra (HRMS) were obtained on a Waters Micromass Q-Tof Micro™ instrument using the ESI technique. Thin-layer chromatography (TLC) was performed on glass plates precoated with silica gel GF_{254} to monitor the reactions and certify the purity of the reaction products. Visualization was accomplished by spraying chromatograms with 10% ethanolic sulfuric acid and charring them on a hot plate. Column chromatography was carried out on silica gel (200–300 mesh).

3.2 Preparation of 2,3,6-tri-O-acetyl-4-chloro-4-deoxy-α-D-galactopyranosidyl Bromide

2,3,6-Tri-O-acetyl-4-chloro-4-deoxy-α-D-galactopyranosidyl bromide was synthesized by following the literature methods.[11,12]

3.3 4,4′-Dibenzyloxy Mahkoside A (2)

To a mixture of Mahkoside A (**1**, 422 mg, 1.00 mmol) and dry K_2CO_3 (276 mg, 2.00 mmol) in dry ethanol (10 mL), benzyl chloride (1 mL) was added. The mixture was refluxed for 3 h (TLC showed the disappearance of Mahkoside A). The resulting mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 10 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was purified by flash chromatography on silica gel (6:1

$CHCl_3$-CH_3OH) to afford **2** (480 mg, 80% yield) as a yellow solid. Mp 86–88°C; IR (KBr): 3402, 1646, 1599, 1454, 1162, 1076, 923 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.72 (s, 3H, CH_3O), 3.43–3.81 (m, 10H), 4.82 (d, $J = 8.4$ Hz, 1H, H-1′-gal), 4.85 (s, 2H, $ArCH_2$), 5.03 (s, 2H, $ArCH_2$), 6.21 (s, 1H, H-3), 6.44 (s, 1H, H-5), 6.87 (d, $J = 8.4$ Hz, 2H, H-3′, H-5′), 7.38–6.89 (m, 10H, Ar-H), 7.75 (d, $J = 8.6$ Hz, 2H, H-2′, H-6′); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 55.6 (CH_3O) 61.9 (C-6′-gal), 69.7 (C-4′-gal), 70.1 ($2ArCH_2$), 73.5 (C-2′-gal), 75.8 (C-3′-gal), 76.0 (C-5′-gal), 94.8 (C-5), 95.7 (C-3), 103.3 (C-1′-gal), 112.8 (C-1), 114.4 (C-3′,5′), 127.5, 127.6, 128.1, 128.2, 128.3, 128.6 (Ar), 131.8 (C-1′), 132.2 (C-2′,6′), 136.1 (Ar), 136.2 (Ar), 157.3 (C-6), 157.7 (C-2), 162.7 (C-4′), 162.9 (C-4), 194.3 (C-7); HRMS (ESI) calcd. for $C_{34}H_{34}O_{10}$: m/z 625.2052 $[M + Na]^+$. Found: 625.2041.

3.4 6-Hydroxy-4,4′-dibenzyloxy-2-methoxybenzophenone (3)

To a solution of **2** (151 mg, 0.25 mmol) in 95% ethanol (10 mL), 30% aq HCl (1 mL) was added. The mixture was refluxed for 2 h, then cooled to room temperature and neutralized with satd. aq. $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2. The organic layer was washed with water and dried over anhydrous Na_2SO_4. Subsequent concentration gave a residue. The residue was purified by flash chromatography on silica gel (4:1 petroleum ether–EtOAc) to afford **3** (101 mg, 92% yield) as a yellow solid. Mp 110–112°C; IR (KBr): 3160, 1634, 1605, 1508, 1172 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.85 (s, 3H, CH_3O), 4.83 (s, 2H, Ar-CH_2), 5.01 (s, 2H, $ArCH_2$), 6.04 (d, $J = 2.3$ Hz, 1H, H-3), 6.19 (d, $J = 2.2$ Hz, 1H, H-5), 6.76 (d, $J = 7.0$ Hz, 2H, H-3′, H-5′), 6.85–7.41 (m, 10H, Ar-H), 7.57 (dd, $J = 6.9$ Hz, $J = 1.8$ Hz, 2H, H-2′, H-6′); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 55.6 (CH_3O), 70.1 ($ArCH_2$), 70.0 ($ArCH_2$), 92.4 (C-5), 93.8 (C-3), 106.1 (C-1), 113.8 (C-3′,5′), 126.0, 127.4, 127.5, 128.0, 128.1, 128.6 (Ar), 130.6 (C-2′,6′), 134.4 (C-1′), 135.6 (Ar), 136.4 (Ar), 160.5 (C-6), 161.5 (C-2), 165.2 (C-4′), 165.8 (C-4), 197.9 (C-7). HRMS (ESI) calcd. for $C_{28}H_{24}O_5$: m/z 463.1522 $[M + Na]^+$. Found: 463.1521.

3.5 4,4′-Dibenzyloxy-2-methoxybenzophenon-6-yl 2,3,6-tri-O-acetyl-4-chloro-4-deoxy-β-D-galactopyranoside (4)

A mixture of **3** (73 mg, 0.165 mmol), a catalytic amount of Bu_4NBr, and K_2CO_3 (46 mg, 0.33 mmol) in 2:1 $CHCl_3$–H_2O (10 mL) was stirred at 40°C for 1 h. Then, 2,3,6-tri-O-acetyl-4-chloro-4-deoxy-β-D-galactosidyl bromide (128 mg, 0.33 mmol) was added, and stirring continued at 40°C for 1 day. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (10 mL) and brine (10 mL). The organic layer was then dried over

Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (10:1 $CHCl_3$–EtOAc) to afford **4** (78 mg, 63% yield) as a yellow solid. Mp 61–63°C; IR (KBr): 1751, 1660, 1601, 1507, 1231, 1094, 910 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.96 (s, 3H, CH_3CO) 2.08 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 3.79 (s, 3H, CH_3O); 4.06 (t, 1H, $J = 5.9$ Hz, H-5′-gal), 4.24 (dd, 1H, $J = 5.2$ Hz, $J = 11.5$ Hz, H-6′-gal), 4.37 (dd, 1H, $J = 7.0$ Hz, $J = 11.5$ Hz, H-6′a-gal), 4.49 (d, 1H, $J = 3.6$ Hz, H-4′-gal), 4.91 (d, 1H, $J = 8.0$ Hz, H-1′-gal), 4.95 (d, 1H, $J = 4.0$ Hz, H-3′-gal), 4.97 (s, $ArCH_2$, 2H), 5.12 (s, $ArCH_2$, 2H), 5.33 (dd, 1H, $J = 8.0$ Hz, $J = 10.0$ Hz, H-2′-gal), 6.29 (d, 1H, $J = 2.0$ Hz, H-3), 6.49 (d, 1H, $J = 2.0$ Hz, H-5), 6.96 (d, 2H, $J = 8.8$ Hz, 2H, H-3′,5′), 7.77–7.05 (m, 10H, 2Ar-H), 7.78 (d, 2H, $J = 8.8$ Hz, H-2′6′); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 20.5 (CH_3CO), 55.5 (CH_3O), 57.2 (C-4′-gal), 63.2 (C-6′-gal), 67.2 (C-2′-gal), 70.1 ($ArCH_2$), 70.2 ($ArCH_2$), 71.5 (C-3′-gal), 71.8 (C-5′-gal), 94.9 (C-5), 96.8 (C-3), 101.0 (C-1′-gal), 114.1 (C-1), 114.4 (C-3′,5′), 126.7, 127.5, 127.7, 128.2, 128.4, 128.7 (Ar), 131.3 (C-1′), 131.9 (C-2′,6′), 135.6 (Ar), 136.4 (Ar), 155.5 (C-6), 157.1 (C-2), 161.7 (C-4′), 162.8 (C-4), 169.0 (CH_3CO), 170.3 (CH_3CO), 170.4 (CH_3CO), 192.5 (C-7). HRMS (ESI) calcd. for $C_{40}H_{39}ClO_{12}$: m/z 769.2028 $[M + Na]^+$. Found: 769.2014.

3.6 4,4′-Dihydroxy-2-methoxybenzophenon-6-yl-4-chloro-4-deoxy-β-D-galactopyranoside (5)

A solution of **4** (60 mg, 0.08 mmol) in 1:1 EtOH–EtOAc (80 mL) was treated with a catalytic amount of 10% Pd-C. After stirring under 1 atm of H_2 at 40°C for 7 h, the reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in dry CH_3OH (10 mL), followed by addition of a catalytic amount of NaOH. After stirring for 1 h at room temperature, the reaction mixture was filtered and solid was washed with CH_3OH (10 mL). The filtrate and washings were combined and concentrated. The residue was purified by flash chromatography on silica gel (4:1 CH_2Cl_2–CH_3OH) to give **5** (21 mg, 61% yield) as a brown solid. Mp 190–192°C; IR (KBr): 3423, 1621, 1600, 1433, 1163, 1081 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ 3.46 (dd, 1H, $J = 7.9$ Hz, $J = 9.2$ Hz, H-2′-gal), 3.65 (dd, 1H, $J = 5.8$ Hz, $J = 11.2$ Hz, H-6′-gal), 3.72 (dd, 1H, $J = 6.8$ Hz, $J = 11.2$ Hz, H-6′-gal), 3.77 (d, 1H, $J = 3.6$ Hz, H-3′-gal), 3.80 (s, 3H,CH_3-O); 3.89 (t, 1H, $J = 6.4$ Hz, H-5′-gal), 4.31 (d, 1H, $J = 3.6$ Hz, H-4′-gal), 4.88 (d, 1H, $J = 7.6$ Hz, H-1′-gal), 6.16 (d, 1H, $J = 2.0$ Hz, H-3), 6.39 (d, 1H, $J = 2.0$ Hz, H-5), 6.78 (d, 2H, $J = 8.4$ Hz, H-3′,5′), 7.68 (d, 2H, $J = 8.8$ Hz, H-2′6′); ^{13}C NMR (CD_3OD, 400 MHz) δ: 55.9 (CH_3O), 62.6 (C-4′-gal), 63.2 (C-6′-gal), 71.6 (C-2′-gal), 73.6 (C-3′-gal), 95.0 (C-5), 96.8 (C-3), 103.1 (C-1′-gal), 111.6 (C-1), 115.9 (C-3′,5′), 131.9 (C-1′), 158.3 (C-6), 159.3 (C-2), 163.8 (C-4′), 164.3 (C-4), 197.1 (C-7); HRMS (ESI) calcd. for $C_{20}H_{21}ClO_9$: m/z 463.0772 $[M + Na]^+$. Found: 463.0756.

3.7 6-(2-Bromoethoxy)-4,4′-dibenzyloxy-2-methoxybenzophenone (6)

A solution of **3** (110 mg, 0.25 mmol), K_2CO_3 (69 mg, 0.5 mmol), and dibromoethane (1 mL) in dry ethanol (10 mL) was refluxed for 6 h. The reaction mixture was filtered, and the solid was washed with ethanol (10 mL). The filtrate and washings were combined and concentrated. The residue was purified by flash chromatography on silica gel (4:1 petroleum ether–EtOAc) to give **6** (88 mg, 65% yield) as a yellow solid. Mp 97–100°C; IR (KBr): 1659, 1602, 1448, 1161 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.38 (t, 2H, $J = 6.8$ Hz, the protons of carbon connected with bromine), 3.80 (s, 3H, CH_3O), 4.19 (t, 2H, $J = 6.8$ Hz, OCH_2), 5.01 (s, 2H, $ArCH_2$), 5.12 (s, 2H, $ArCH_2$), 6.14 (d, 1H, $J = 1.6$ Hz, H-3), 6.21 (d, 1H, $J = 1.6$ Hz, H-5), 6.97 (d, 2H, $J = 8.8$ Hz, H-3′,5′), 7.44–7.09 (m, 10H, 2Ar-H), 7.81 (d, 2H, $J = 8.8$ Hz, H-2′6′); ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 11.1 (2-CH_2CH_3), 47.5 (2CH_2CH_3), 50.9 (C-CH_2-N), 55.5 (CH_3O), 66.7 (O-CH_2-C), 70.1 ($ArCH_2$), 70.3 ($ArCH_2$), 91.8 (C-5), 92.8 (C-3), 112.0 (C-1), 114.4 (C-3′,5′), 126.7, 127.5, 127.6, 128.2, 128.3, 128.7 (Ar), 131.7 (C-2′,6′), 131.9 (C-1′), 136.3 (Ar), 136.6 (Ar), 157.5 (C-6), 157.6 (C-2), 162.1 (C-4′), 162.6 (C-4), 193.3 (C-7). HRMS (ESI) calcd. for $C_{30}H_{27}BrO_5$: m/z 547.1120 $[M + H]^+$. Found: 547.1110.

3.8 6-(2-Diethylaminoethoxy)-4,4′-dibenzyloxy-2-methoxybenzophenone (8)

A solution of **6** (60 mg, 0.11 mmol), K_2CO_3 (30 mg, 0.22 mmol), and diethylamine (1 mL) in dry DMF was stirred for 10 h at room temperature. The mixture was filtered. The filtrate was diluted with water (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (4:1 CH_2Cl_2-CH_3OH) to give **8** (56 mg, 94% yield) as a yellow syrup. IR (KBr): 3032, 1661, 1600, 1455, 1250, 1159 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.93 (t, 6H, $J = 7.1$ Hz, 2CH_3); 2.56 (d, 4H, $J = 6.0$ Hz, 2CH_2CH_3), 2.81 (s, 2H, the protons of carbon connected with diethylamino), 3.79 (s, 3H, OCH_3), 4.09 (s, 2H, OCH_2), 4.99 (s, 2H, $ArCH_2$), 5.13 (s, 2H, $ArCH_2$), 6.16 (d, 1H, $J = 1.8$ Hz, H-3), 6.18 (d, 1H, $J = 1.8$ Hz, H-5), 6.96 (d, 2H, $J = 8.8$ Hz, H-3′,5′), 7.44–7.09 (m, 10H, 2Ar-H), 7.81 (d, 2H, $J = 8.8$ Hz, H-2′,6′); ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 11.1 (2CH_2CH_3), 47.5 (2CH_2CH_3), 50.9 (C-CH_2-N), 55.5 (CH_3O), 66.7 (O-CH_2-C), 70.1 ($ArCH_2$), 70.3 ($ArCH_2$), 91.8 (C-5), 92.8 (C-3), 112.0 (C-1), 114.4 (C-3′,5′), 126.7, 127.5, 127.6, 128.2, 128.3, 128.7 (Ar), 131.7 (C-2′,6′), 131.9 (C-1′), 136.3 (Ar), 136.6 (Ar), 157.5 (C-6), 157.6 (C-2), 162.1 (C-4′), 162.6 (C-4), 193.3 (C-7). HRMS (ESI) calcd. for $C_{34}H_{37}NO_5$: m/z 540.2750 $[M + H]^+$. Found: 540.2770.

3.9 6-[2-(Piperid-1-yl)-ethyloxy]-4,4′-dibenzyloxy-2-methoxybenzophenone (7)

A similar procedure as described previously for the preparation of **8** from **6** was used for the preparation of **7**. Yield 74% as a yellow syrup. IR (KBr): 3388, 1659, 1599, 1455, 1251, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.05–2.68 (d, 10H, CH_2NCH_2), 3.78 (s, 3H, OCH_3), 4.02 (s, 2H, OCH_2), 4.98 (s, 2H, $ArCH_2$), 5.14 (s, 2H, $ArCH_2$), 6.12 (s, 1H, H-3), 6.19 (s, 1H, H-5), 6.97 (d, 2H, $J = 7.2$ Hz, H-3′,5′), 7.45–7.08 (m, 10H, 2Ar-H), 7.78 (d, 2H, $J = 7.3$ Hz, H-2′6′); ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 43.2 (NCH_2CH_2N), 49.9 (NCH_2CH_2N), 55.5 (CH_3O), 56.2 ($CH_2NR_1R_2$), 67.2 (O-CH_2-C), 70.2 ($ArCH_2$), 70.3 ($ArCH_2$), 92.2 (C-5), 92.9 (C-3), 112.2 (C-1), 114.5 (C-3′,5′), 126.8, 127.5, 127.7, 128.2, 128.4, 128.7, (Ar), 131.8 (C-2′,6′), 131.9 (C-1′), 131.7 (C-1′), 136.3 (Ar), 136.7 (Ar), 157.4 (C-6), 157.5 (C-2), 162.1 (C-4′), 162.8 (C-4), 193.4 (C-7); HRMS (ESI) calcd. for $C_{34}H_{36}N_2O_5$: m/z 553.2702 $[M + H]^+$. Found: 553.2706.

3.10 6-(2-Diethylaminoethoxy)-4,4′-dihydroxy-2-methoxybenzophenone (9)

A suspension of **8** (72 mg, 0.13 mmol) and a catalytic amount of 10% Pd-C in 1:1 EtOH–EtOAc (80 mL) was stirred under 1 atm of H_2 at 40°C for 7 h. The reaction mixture was filtered and concentrated in vacuo. The residue was purified by silica-gel chromatography (2:1 CH_2Cl_2-CH_3OH) to afford **9** (46 mg, 96% yield) as a yellow syrup. IR (KBr): 3436, 1660, 1604, 1432, 1274, 1157 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ: 0.84 (t, 6H, $J = 7.2$ Hz, $2CH_2CH_3$), 1.23 (d, 4H, $J = 4.6$ Hz, $2CH_2CH_3$), 2.70 (s, 2H, the protons of carbon connected with diethylamino), 3.74 (s, 3H, OCH_3), 3.99 (t, 2H, $J = 5.2$ Hz, OCH_2), 6.12 (s, 1H, H-3), 6.14 (s, 1H, H-5), 6.80 (d, 2H, $J = 8.8$ Hz, H-3′,5′), 7.55 (d, 2H, $J = 8.4$ Hz, H-2′6′); ^{13}C NMR (CD_3COCD_3, 400 MHz) δ: 8.2 ($2CH_2CH_3$) 47.1 ($2CH_2CH_3$), 49.6 (C-CH_2-N), 55.2 (CH_3O), 63.6 (O-CH_2-C), 91.7 (C-5), 94.6 (C-3), 107.8 (C-1), 115.0 (C-3′,5′), 131.5 (C-2′,6′), 131.7 (C-1′), 158.6 (C-6), 160.8 (C-2), 161.9(C-4′), 164.1 (C-4), 195.0 (C-7). HRMS (ESI) calcd. for $C_{20}H_{25}NO_5$: m/z 360.1811$[M + H]^+$. Found: 360.1813.

3.11 4,4′,6-Trihydroxy-2-methoxybenzophenone (10)

A similar procedure as described previously for the preparation of **3** from **2** was used for the preparation of **10** from **1**. Yield 95% as a yellow solid. Mp 83–85°C; IR (KBr): 3347, 1634, 1608, 1438, 1162; ^1H NMR (CD_3COCD_3,

400 MHz) δ: 3.80 (s, 3H,OCH_3), 6.04 (s, 2H, H-3,5), 6.85 (d, 2H, $J = 8.4$ Hz, H-3′,5′), 7.61 (d, 2H, $J = 8.8$ Hz, H-2′6′); ^{13}C NMR (CD_3COCD_3, 400 MHz) δ: 55.6 (CH_3O), 94.3 (C-5), 94.3 (C-3), 115.0 (C-1), 115.1 (C-3′,5′), 132.3 (C-2′,6′), 132.9 (C-1′), 161.8 (C-6), 162.1 (C-2), 162.4 (C-4′), 165.9 (C-4), 197.6 (C-7). HRMS (ESI) calcd. for $C_{14}H_{12}O_5$: m/z 283.0583 $[M + Na]^+$. Found: 283.0583.

3.12 Biological Activity Assays

In vitro cytotoxicity study: The Ec-9706 cell line was kindly presented by the Henan Institute of Medical Sciences, China. The culture medium was RPMI-1640 supplemented with 10% (v/v) fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Sensitivity test: Cancer cells, growing in monolayer cultures at 37°C under 5% CO_2, were trypsinized, rinsed with PBS (w/o Ca^{2+} and Mg^{2+}), and plated into 96-well plates (7×10^3 cells/well). The next day, test substances were freshly dissolved in DMSO, resulting in 10-mg/mL stock solutions. Stock solutions were diluted in the culture medium and added (200 μL/well) at various concentrations to the wells, resulting in five final concentrations between 6.25 and 100 μg/mL. The MTT-test was performed 48 h later.

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