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Synthesis and Biological Evaluation of Amide derivatives of Benzophenone Derivatives as Anticancer Agents

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ABSTRACT

A novel series of benzophenone amide derivatives (8a-j) were synthesized, and evaluated for their anticancer activity against human cancer cell lines like MCF-7, MDA MB-231(Breast cancer), A549 (Lung cancer). All the synthesized derivatives are first reported and adriamycin used as reference drug. Among the compounds, compounds 8b, 8h and 8j were showed more potent anticancer activity than control.

Keywords: Combretastatins-A4; Licochalcone A; Anticancer activity

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INTRODUCTION

Combretastatins-A4 (**1**, CA-4) is a tubulin binding agent and was isolated from the bark of *Combretum caffrum* in 1989 [1,2]. It shows anticancer activity against various human cancer cell lines including MDR cancer cell lines [3-6] by binds to the colchicine site of tubulin and inhibit tubulin polymerization [7]. This is the attractive lead natural compound for development of new anticancer drugs for treatment of cancer. Structure-activity relationship studies of combretastatins-A4 have shown that 3,4,5-trimethoxyphenyl ring A, 4-methoxyphenyl ring B and *cis* configured double bond are essential for its anticancer activity [8,9]. Chalcones are α,β-unsaturated ketones were first isolated from flavonoid biosynthesis in plants [10]. Scaffold is containing chalcone as a pharmacophore which is responsible for anticancer activity. Chalcones were showed a variety of pharmacological activities like antitumor,[11] antifungal,[12] anti-inflammatory, [13] antimalarial, [14] antibacterial, [15] antileishmanial, [16] tyrosine kinase inhibitors, [17] anti-HIV,[18] analgesic, [19] and anti-malarial. [20] Licochalcone A (2**,** Figure 1) is a retrochalcone family and was display DNA topoisomerase-I inhibitory activity. [21]
Based on above information and continuous of these efforts, we designed and synthesized a series of amide derivatives benzophenones (8a-j) and their structures confirmed by ^{1}HNMR, ^{13}CNMR and mass spectral analysis. Further, these target compounds were screened for anticancer activity against human cancer cell lines.

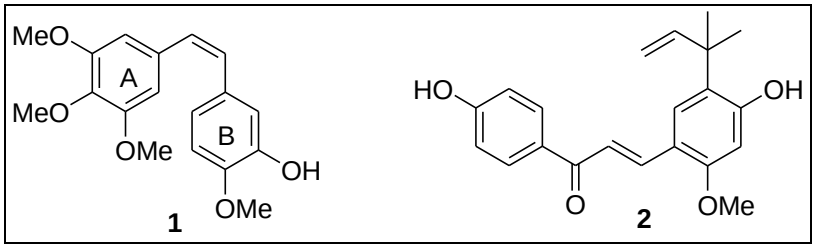


Figure 1

EXPERIMENTAL SECTION

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. ^{1}H NMR and ^{13}CNMR spectra were recorded on Bruker UXNMR/XWIN-NMR and Varian (400 MHz, 300 MHz) instrument. Chemical shifts (d) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an electrothermal melting point apparatus, and are uncorrected.

Ethyl 3,3-bis(3,4,5-trimethoxyphenyl)acrylate (5)
To a suspension of corresponding bis(3,4,5-trimethoxyphenyl)methanone (15 g, 41.3 mmol), ethyl (triphenylphosphoranylidene)acetate (43 g, 124.1 mmol) and 18-crown-6 (10 mg, 0.041 mmol) in anhydrous THF and under nitrogen atmosphere NaH (39 g, 165.2 mmol) were added portion wise carefully. The reaction mixture was stirred till the reaction was completed (monitoring by TLC). Then, the mixture was cooled to 0 ^{o}C and first water and then 10% HCl were carefully added. After 60 minutes stirring at room temperature, the mixture was extracted with ethyl acetate, 10% HCl and water. The combined organic phase was dried over Na_2SO_4 and the solvent was removed in vacuo. The obtained crude product was purified by flash chromatography with ethyl acetate:hexane (2:8) to afford pure compound **5**, 14.6 g with 82% yield. ^{1}H NMR (400 MHz, DMSO-d6): δ 1.57 (t, 3H), 3.87 (s, 12H), 3.92 (s, 6H), 4.67-4.71 (q, 2H), 6.89 (s, 4H), 7.27 (s, 1H); MS (ESI): 433 $[M+H]^+$.

3,3-Bis(3,4,5-trimethoxyphenyl)acrylic acid (6)
A solution of 50% NaOH (50 mL) was added to a solution of ethyl 3,3-bis(3,4,5-trimethoxyphenyl)acrylate **(5)** (12 g, 27.7 mmol) in ethanol (60 ml) and the mixture stirred at room temperature for 3 hours. After most of the ethanol has been evaporated the aqueous phase was acidified with 6N HCl to pH 7 and extracted with ethyl acetate (50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford pure compound **6,** with 10.6 g in 95% yield. ^{1}H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.92 (s, 6H), 6.90 (s, 4H), 7.27 (s, 1H), 11.56 (bs, 1H); MS (ESI): 405 $[M+H]^+$.

3,3-Bis(3,4,5-trimethoxyphenyl)-N-phenylacrylamide (8a)
The compound **6** (200 mg, 0.49 mmol) was dissolved in 10 mL of dry CH_2Cl_2, followed by addition of aniline (7a) (0.44 ml, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The reaction mixture was stirred at room temperature for 6 hours. The reaction mixture was washed with saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography with ethyl acetate/hexane (3:7) afford the pure compound 8a, 210 mg in 89% yield. 179-181 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.92 (s, 6H), 6.90 (s, 4H), 7.27 (s, 1H), 737-7.50 (m, 5H), 9.78 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 62.6, 114.5, 121.4, 123.4, 123.9, 130.2, 137.3, 138.6, 138.7, 139.5, 154.3, 155.4, 166.7; MS (ESI): 480 $[M+H]^+$.

N-(3,4-Dimethoxy-5-methylphenyl)-3,3-bis(3,4,5-trimethoxyphenyl)acrylamide (8b)
This compound 8b was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 3,4,5-trimethoxyaniline (7b) (96 mg, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude product was purified by column chromatography with ethyl acetate/hexane (1:1) afford the pure compound 8b, 226 mg in 80% yield. 184-186 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.89 (s, 6H), 3.92 (s, 6H), 3.94 (s, 3H), 6.89 (s, 4H), 7.27 (s, 1H), 7.28 (s, 2H), 9.78 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 58.5, 62.5, 62.9, 101.6, 114.6, 123.5, 137.4, 137.9, 138.4, 138.7, 139.4, 154.3, 155.7, 156.8, 166.8; MS (ESI): 570 $[M+H]^+$.

3,3-Bis(3,4,5-trimethoxyphenyl)-N-(4-methoxyphenyl)acrylamide (8c)
This compound **8c** was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 4-methoxyaniline (7c) (0.6 ml, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude product was purified by column chromatography with ethyl acetate/hexane (4:6) afford the pure compound 8c, 232 mg in 92% yield. 182-184 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.92 (s, 6H), 3.93 (s, 3H), 6.89 (s, 2H), 6.94 (d, 2H, J = 7.89 Hz), 7.27 (s, 1H), 7.45 (d, 2H, J = 7.89 Hz), 9.78 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-

d6): δ 57.6, 57.9, 62.6, 114.5, 120.5, 121.4, 123.7, 134.6, 138.5, 138.6, 139.8, 154.3, 155.6, 156.6, 166.3; MS (ESI): 510 $[M+H]^+$.

N-(4-Chlorophenyl)-3,3-bis(3,4,5-trimethoxyphenyl)acrylamide (8d)

This compound **8d** was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 4-chloroaniline (7d) (0.48 ml, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude product was purified by ethyl acetate/hexane (3:7) afford the pure compound 8d, 224 mg in 89% yield. Mp: 190-192 ^oC, ^1H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.92 (s, 6H), 6.90 (s, 4H), 7.27 (s, 1H), 7.36 (d, 2H, J = 7.90 Hz), 7.50 (d, 2H, J = 7.90 Hz), 9.80 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 62.8, 114.5, 121.4, 123.6, 129.5, 129.8, 138.3, 138.7, 139.6, 139.8, 154.3, 155.6, 166.7; MS (ESI): 514 $[M+H]^+$.

N-(4-Bromophenyl)-3,3-bis(3,4,5-trimethoxyphenyl)acrylamide (8e)

This compound **8e** was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 4-bromoaniline (7e) (84 mg, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude product was purified by column chromatography with ethyl acetate/hexane (3:7) afford the pure compound 8e, 218 mg in 79% yield. Mp: 195-197 ^oC, ^1H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.92 (s, 6H), 6.90 (s, 4H), 7.27 (s, 1H), 7.39 (d, 2H, J = 7.90 Hz), 7.51 (d, 2H, J = 7.90 Hz), 9.80 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 62.8, 112.5, 114.6, 121.5, 123.6, 134.5, 138.5, 138.7, 139.7, 154.5, 155.6, 166.8; MS (ESI): 559 $[M+H]^+$.

N-(4-Fluorophenyl)-3,3-bis(3,4,5-trimethoxyphenyl)acrylamide (8f)

This compound **8f** was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 4-fluoroaniline (7f) (0.53 ml, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude product was purified by column chromatography with ethyl acetate/hexane (3:7) to afford pure compound 8f, 211 mg in 86% yield. Mp: 188-190 ^oC, ^1H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.92 (s, 6H), 6.90 (s, 1H), 7.27 (s, 1H), 7.37 (d, 2H, J = 7.88 Hz), 7.49 (d, 2H, J = 7.88 Hz), 9.80 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.5, 62.8, 114.5, 118.5, 123.5, 124.7, 136.4, 137.7, 138.4, 138.7, 139.7, 154.5, 155.7, 166.7; MS (ESI): 498 $[M+H]^+$.

3,3-Bis(3,4,5-trimethoxyphenyl)-N-(4-nitrophenyl)acrylamide (8g)

This compound **8g** was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 4-nitroaniline (7g) (68 mg, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude product was purified by column chromatography with ethyl acetate/hexane (3:7) afford the pure compound 8g, 230 mg in 89% yield. Mp: 200-202 ^oC, ^1H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.92 (s, 6H), 6.90 (s, 4H), 7.27 (s, 1H), 7.41 (d, 2H, J = 7.91 Hz), 7.53 (d, 2H, J = 7.91 Hz), 9.80 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 62.8, 114.6, 120.6, 123.5, 126.6, 138.4, 138.7, 139.5, 143.4, 144.5, 154.6, 155.7, 166.8; MS (ESI): 525 $[M+H]^+$.

N-(4-Cyanophenyl)-3,3-bis(3,4,5-trimethoxyphenyl)acrylamide (8h)

This compound 8h was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 4-acyanoaniline (7h) (58 mg, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude product was purified by column chromatography with ethyl acetate/hexane (3:7) afford the pure compound 8h, 220 mg in 88% yield. Mp: 205-207 ^oC, ^1H NMR (400 MHz, DMSO-d6): δ 3.87 (s, 12H), 3.92 (s, 6H), 6.90 (s, 4H), 7.27 (s, 1H), 7.43 (d, 2H, J = 7.91 Hz), 7.54 (d, 2H, J = 7.91 Hz), 9.80 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.8, 62.7, 106.5, 114.5, 120.6, 120.8, 123.4, 135.5, 138.5, 138.9, 139.7, 154.3, 155.8, 166.9; MS (ESI): 505 $[M+H]^+$.

3,3-Bis(3,4,5-trimethoxyphenyl)-N-p-tolylacrylamide (8i)

This compound 8i was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 4-methylaniline (7i) (0.5 ml, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude product was purified by column chromatography with ethyl acetate/hexane (3:7) afford the pure compound 8i, 209 mg in 86% yield. Mp: 196-198 ^oC, ^1H NMR (400 MHz, DMSO-d6): δ 2.45 (s, 3H), 3.87 (s, 12H), 3.92 (s, 6H), 6.90 (s, 4H), 7.27 (s, 1H), 7.36 (d, 2H, J = 7.80 Hz), 7.46 (d, 2H, J = 7.80 Hz), 9.80 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 26.5, 57.4, 62.7, 114.7, 121.4, 123.5, 130.6, 134.5, 135.6, 138.5, 138.7, 139.7, 154.5, 155.8, 166.8; MS (ESI): 494 $[M+H]^+$.

3,3-Bis(3,4,5-trimethoxyphenyl)-N-(3,5-dimethoxyphenyl)acrylamide (8j)

This compound 8j was prepared by the method described for 8a, employing 6 (200 mg, 0.49 mmol), 3,5-dimethoxyaniline (7j) (75 mg, 0.49 mmol), EDCI (114 mg, 0.73 mmol) and HOBt (1 mg, 0.098 mmol). The crude

product was purified by column chromatography with ethyl acetate/hexane (4:6) afford the pure compound 8j, 216 mg in 81% yield. Mp: 210-212 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.76 (s, 6H), 3.87 (s, 12H), 3.91 (s, 6H), 6.45 (s, 1H), 6.90 (s, 4H), 7.27 (s, 1H), 7.33 (s, 2H), 9.80 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 58.7, 62.7, 98.5, 101.6, 114.5, 123.6, 138.5, 138.7, 139.5, 140.5, 154.5, 155.7, 158.5, 166.6; MS (ESI): 540 $[M+H]^{+}$.

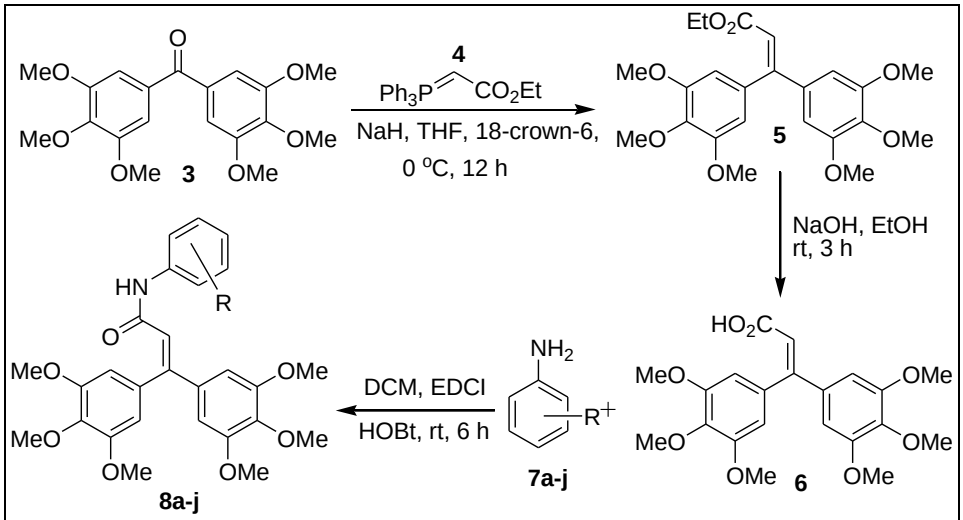
MTT assay

The cytotoxic activity of the compounds was determined using MTT assay. 1×10^{4} cells/well were seeded in 200 ml DMEM, supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 hours at 37 ^{o}C in a CO_2 incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective vehicle control. After 48 hours of incubation, 10 ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/ml) was added to each well and the plates were further incubated for 4 hours. Then the supernatant from each well was carefully removed, formazon crystals were dissolved in 100 ml of DMSO and absorbance at 540 nm wavelength was recorded.

RESULTS AND DISCUSSION

Chemistry

Synthesis of these newly novel compounds (8a-j) was carried out as shown in Scheme 1. Compound bis(3,4,5-trimethoxyphenyl)methanone (3) was reacted with ethyl (triphenylphosphoranylidene)acetate (4) in THF, NaH and 18-crown-6, at 0 ^{o}C for 12 hours to gave pure ethyl 3,3-bis(3,4,5-trimethoxyphenyl)acrylate (5), then this ester was hydrolysized with NaOH, ethanol at room temperature for 3 hours to gave pure 3,3-bis(3,4,5-trimethoxyphenyl)acrylic acid (6) in good yield. After this acid intermediate (6) was coupled with different substituted aryl amines (7a-j) in CH_2Cl_2, EDCI and HOBt at room temperature for 6 hours to afford pure amide compounds (8a-j).



Scheme 1

Biological evaluation

***In Vitro* cytotoxicity**

All these newly compounds (8a-j) were tested *in vitro* against three human cancer cell lines such as MCF-7, MDA MB-231(Breast cancer), A549 (Lung cancer) by MTT assay. All these derivatives exhibit potential cytotoxicity against three human cancer cell lines. These results are summarized in Table 1 and adriamycin used as positive control. Among them, compounds 8b, 8h and 8j were showed more potent anticancer activity than control.

Table 1: Anticancer activity of target compounds (8a-j) (IC_{50} µM)

Compound	MCF-7	A549	MDA MB-231
8a	2.45	3.78	7.89
8b	1.89	0.12	1.1
8c	3.9	12.9	-
8d	5.87	-	-
8e	13.9	23.8	4.78
8f	2.1	3.89	-
8g	8.39	15.8	-
8h	1.33	2.7	8.9
8i	17.4	9.23	10.3
8j	0.45	1.11	2.67
Adriamycin	3.12	2.1	3.41

-**Not active.**

CONCLUSION

In conclusion, we have synthesized a novel series of benzophenone amide (8a-j) derivatives and evaluated for their anticancer activity against different human cancer cell lines include MCF-7, MDA MB-231(Breast cancer), A549 (Lung cancer). Among them, compounds 8b, 8h and 8j were showed more potent anticancer activity than adriamycin.

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