Supporting Information

Synthesis and biological evaluation of novel hybrid compounds between chalcone and piperazine as potential antitumor agents

Zewei Mao,[a] Xi Zheng,[b] Mengdi Zhang,[a] Yao Huang,[b] Chunping Wan*[b] and Gaoxiong Rao*[a]

[a] *School of Pharmacy, Yunnan University of Traditional Chinese Medicine, Kunming 650500, PR China*

[b] *Central laboratory, The NO.1 Affiliated Hospital of Yunnan University of Traditional Chinese Medicine, Kunming 650021, PR China*

Contents

1. Experiment

1.1 4-Dimethylamino-4'-fluorochalcone (3): To a solution of EtOH (15mL), 4-dimethyl aminobenzaldehyde (1.49g, 10mmol) and 4-fluoroacetophenone (1.38g, 10mmol), was added 20% KOH (10mL) and left to react for 16 h at rt. The reaction was quenched by the addition of water (30mL) and was extracted with $CHCl_3$ (3×20 mL). The organic layer was dried using anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (10% EtOAc/PE, 2.35g, 87% yield). Yellow solid; m. p 139-141°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01-8.05 (m, 2H), 7.81 (d, *J*=15.3Hz, 1H), 7.55 (d, *J*=8.7Hz, 2H), 7.32 (d, *J*=15.2Hz, 1H), 7.11-7.17 (m, 2H), 6.69 (d, *J*=8.7Hz, 2H), 3.03 (s, 6H); ^{13}C NMR (75MHz，$CDCl_3$) δ：188.9, 166.9, 163.6, 152.1, 146.0, 135.4, 130.9, 130.7, 130.5, 122.5, 116.4, 115.6, 115.3, 111.8, 40.1.

1.2 4-Dimethylamino-4'-(1-piperazinyl) chalcone (**4**). Brown solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.01 (d, *J*=9.0Hz, 2H), 7.80 (d, *J*=15.3Hz, 1H), 7.56 (d, *J*=9.0Hz, 2H), 7.41(d, *J*=15.6Hz, 1H), 6.92 (d, *J*=9.0Hz, 2H), 6.70 (d, *J*=9.0Hz, 2H), 3.33 (t, *J*=4.8Hz, 4H), 3.01-3.04 (m, 10H, NCH_2), 2.12 (s, 1H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.3, 154.2, 151.8, 144.1, 130.3, 130.1, 129.1, 123.0, 116.8, 113.6, 111.8, 48.5, 45.9, 40.2; HRMS: m/z calcd for $C_{21}H_{26}N_3O$ $(M+H)^+$ 336.2070, found 336.2072.

1.3 General procedure for the preparation of chalcone derivatives 5a-5h.

Method A: To a stirred solution of compound **4** (0.5mmol) and pyridine (0.5mL) in dried DCM (10mL), acyl chloride (1.0mmol) was added and reaction mixture was stirred for 2 h at 0°C. After completion of the reaction as indicated by TLC, the reaction was quenched by the addition of saturated $NaHCO_3$ (20mL) and was extracted with $CHCl_3$ (3×10mL). The organic layer was dried using anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (DCM) to afford products.

Method B: To a stirred solution of DCC (1.0mmol) and carboxylic acid (1.0mmol) in dried DCM (10mL), compound **4** (0.5mmol) was added and reaction mixture was stirred for 24 h at rt. After completion of the reaction as indicated by TLC, the mixture was filted and the filtrate was concentrated in vacuo and purified by column chromatography (DCM) to afford compounds.

1.4 General procedure for the preparation of chalcone derivatives 6a-6f: To a stirred solution of compound **4** (0.5mmol) and pyridine (0.5mL) in dried DCM (10mL), sulfonyl chloride (1.0mmol) was added and reaction mixture was stirred for 12 h at rt. After completion of the reaction as indicated by TLC, the reaction was quenched by the addition of 10% NaOH (20mL) and was extracted with $CHCl_3$ (3×10mL). The organic layer was dried using anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (DCM) to afford products.

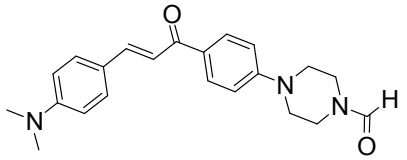
1.5 General procedure for the preparation of chalcone derivatives 7a-7d. To a stirred solution of compound **4** (0.5mmol) and Cs_2CO_3 (1.0g) in dried DCM (10mL), 2-bromoacetophenone (0.6mmol) was added and reaction mixture was stirred for 4 h at rt. After completion of the reaction as indicated by TLC, the reaction was quenched by the addition of 10% NaOH (20mL) and was extracted with $CHCl_3$ (3×10mL). The organic layer was dried using anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (1% Et_3N/DCM) to afford products.

1.6 Antitumor activity: The assay was carried out using the method previously described.

About 1×10^4 cell/well were seeded into 96-well microtiter plates. After twenty-four hours post-seeding, cells were treated with vehicle control or various concentrations of samples for 48 h. 20 μL of MTT solution (5 mg/mL) was added to each well and the tumor cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 air for 4 h. Upon removal of MTT/medium, 150 μL of DMSO was added to each well and the plate was agitated at oscillator for 5 min to dissolve the MTT-formazan. The assay plate was read at a wavelength of 570 nm using a microplate reader.

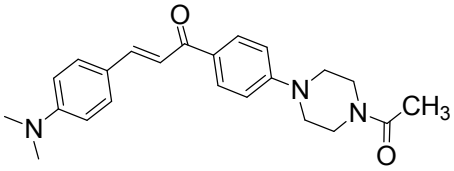
2. Characterization data of compounds 5-7.

Compound 5a



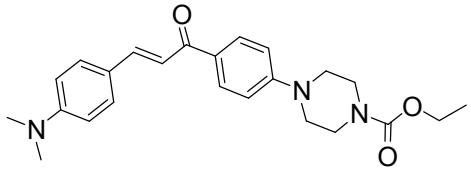
Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.10(s, 1H), 8.00(d, *J*=9.0Hz, 2H), 7.80(d, *J*=15.3Hz, 1H), 7.54(d, *J*=8.7Hz, 2H), 7.37(d, *J*=15.6Hz, 1H), 6.93(d, *J*=9.0Hz, 2H), 6.69(d, *J*=9.0Hz, 2H), 3.71(t, *J*=4.8Hz, 2H), 3.53(t, *J*=4.5Hz, 2H), 3.30-3.37(m, 4H), 3.02(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.5, 160.9, 153.4, 152.0, 144.7, 130.4, 130.3, 123.0, 116.7, 114.7, 111.9, 48.8, 47.7, 45.2, 40.2, 39.8.

Compound 5b



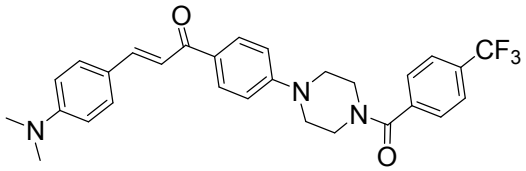
Brown solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.98(d, *J*=9.0Hz, 2H), 7.78(d, *J*=15.6Hz, 1H), 7.53(d, *J*=9.0Hz, 2H), 7.36(d, *J*=15.6Hz, 1H), 6.88(d, *J*=9.0Hz, 2H), 6.74(d, *J*=8.7Hz, 2H), 3.76 (t, *J*=4.8Hz, 2H), 3.60(t, *J*=4.5Hz, 2H), 3.28-3.35(m, 4H), 3.00(s, 6H), 2.10(s, 3H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.3, 169.1, 153.3, 151.9, 144.4, 130.4, 130.2, 129.8, 123.0, 116.7, 114.0, 111.9, 47.7, 5.4, 45.8, 41.0, 40.1, 21.3.

Compound 5c



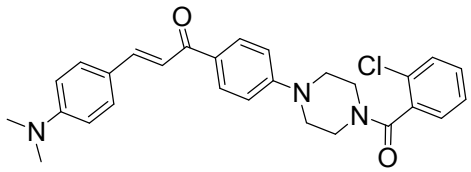
Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.99(d, *J*=8.7Hz, 2H), 7.79(d, *J*=15.3Hz, 1H), 7.54(d, *J*=8.7Hz, 2H), 7.37(d, *J*=15.3Hz, 1H), 6.90(d, *J*=9.0Hz, 2H), 6.68(d, *J*=9.0Hz, 2H), 4.20(q, *J*=7.2Hz, 2H), 3.64(t, *J*=4.8Hz, 4H), 3.33(t, *J*=5.4Hz, 4H), 3.01(s, 6H), 1.30(t, *J*= 7.2 Hz, 3H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.4, 155.5, 153.6, 151.9, 144.4, 130.4, 130.2, 129.8, 123.1, 116.8, 114.1, 111.9, 61.7, 47.6, 43.3, 40.2, 14.8.

Compound 5d



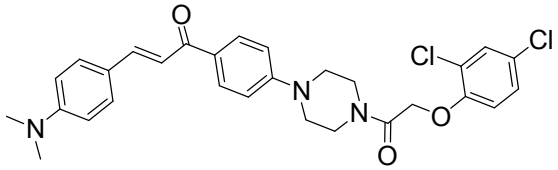
Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.99(d, *J*=8.7Hz, 2H), 7.68-7.79 (m, 3H), 7.54-7.60(m, 2H), 7.53(d, *J*=8.7Hz, 2H), 7.37(d, *J*=15.3Hz, 1H), 6.90(d, *J*=8.7Hz, 2H), 6.67(d, *J*=8.7Hz, 2H), 3.90(s, 2H), 3.57(s, 2H), 3.35(s, 4H), 2.99(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.2, 168.8, 153.2, 151.8, 144.4, 136.2, 130.4, 130.3, 130.2, 130.1, 129.25, 126.8, 124.2, 122.8, 116.5, 114.3, 111.8, 47.7, 40.1; HRMS: m/z calcd for $C_{29}H_{29}F_3N_3O_2$ $(M+H)^+$ 508.2206, found 508.2204.

Compound 5e



Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.00(d, *J*=9.0Hz, 2H), 7.79(d, *J*=15.3Hz, 1H), 7.54(d, *J*=9.0Hz, 2H), 7.30-7.43(m, 5H), 6.91(d, *J*=9.0Hz, 2H), 6.68(d, *J*=9.0Hz, 2H), 3.86-4.04(m, 2H), 3.23-3.46(m, 6H), 3.01(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.4, 167.0, 153.3, 151.9, 144.5, 135.5, 130.5, 130.4, 130.2, 130.1, 129.8, 127.9, 127.4, 123.0, 116.7, 114.3, 111.9, 48.1, 47.6, 46.2, 41.3, 40.2; HRMS: m/z calcd for $C_{28}H_{29}ClN_3O_2$ $(M+H)^+$ 474.1943, found 474.1938.

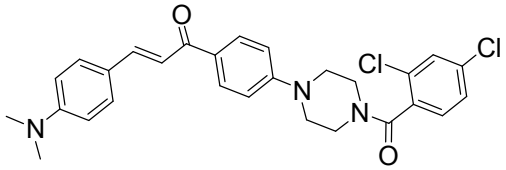
Compound 5f



Brown solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.00(d, *J*=8.7Hz, 2H), 7.79(d, *J*=15.3Hz, 1H), 7.55(d, *J*=8.7Hz, 2H), 7.38(s, 1H), 7.38(d, *J*=15.3Hz, 1H), 7.16-7.20(dd, *J*=2.7Hz, 2.4Hz, 1H), 6.98(d, *J*=8.7Hz, 1H), 6.91(d, *J*=9.0Hz, 2H), 6.70(d, *J*=9.0Hz, 2H), 4.80(s, 2H), 3.75-3.81(m,

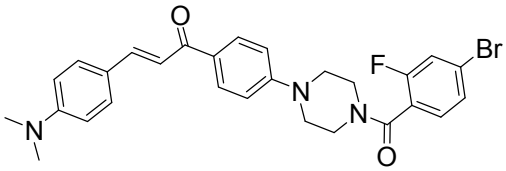
4H), 3.31-3.37(m, 4H), 3.03(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.5, 165.8, 153.3, 152.1, 152.0, 144.7, 130.5, 130.3, 128.0, 127.1, 123.7, 123.1, 116.8, 114.4, 114.4, 112.0, 68.9, 40.3, 34.1, 25.1; HRMS: m/z calcd for $C_{29}H_{30}Cl_2N_3O_3$ $(M+H)^+$ 538.1659, found 538.1653.

Compound 5g



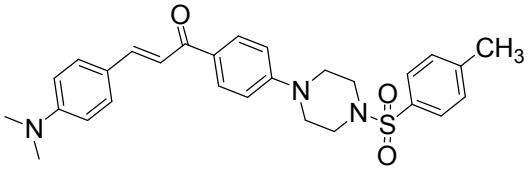
Pale yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.01(d, *J*=8.7Hz, 2H), 7.80(d, *J*=15.3Hz, 1H), 7.50-7.56(m, 4H), 7.38(d, *J*=15.3Hz, 1H), 7.26-7.30(dd, *J*=2.1Hz, 2.4Hz, 1H), 6.93(d, *J* = 9.0Hz, 2H), 6.70(d, *J*=8.7Hz, 2H), 3.87(s, 2H), 3.63(s, 2H), 3.37(s, 4H), 3.03(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.4, 168.1, 153.3, 152.0, 144.7, 135.2, 134.5, 133.2, 130.8, 130.4, 130.3, 129.4, 126.6, 123.0, 116.7, 114.4, 111.9, 40.2, 34.0, 25.0; HRMS: m/z calcd for $C_{28}H_{28}Cl_2N_3O_2$ $(M+H)^+$ 508.1553, found 508.1550.

Compound 5h



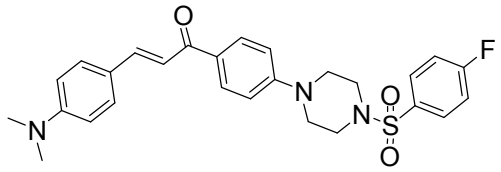
Pale yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 801(d, *J*=8.7Hz, 2H), 7.80(d, *J*=15.3Hz, 1H), 7.56(d, *J*=8.7Hz, 2H), 7.27-7.41(m, 4H), 6.93(d, *J*=9.0Hz, 2H), 7.70(d, *J*=8.7Hz, 2H), 3.95(d, *J*=4.8Hz, 2H), 3.43-3.54(m, 4H), 3.34(d, *J*=5.1Hz, 2H), 3.03(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.4, 164.4, 153.3, 151.9, 144.6, 130.6, 130.4, 130.3, 128.4, 123.0, 119.8, 119.5, 116.7, 114.4, 111.9, 41.9, 40.2, 34.0.

Compound 6a



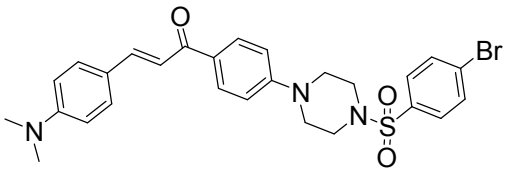
Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.86(d, *J*=8.7Hz, 2H), 7.69(d, *J*=15.3Hz, 1H), 7.57(d, *J*=8.1Hz, 2H), 7.44(d, *J*=9.0Hz, 2H), 7.26((d, *J*=15.3Hz, 1H), 7.24(d, *J*=8.7Hz, 2H), 6.75(d, *J*=9.0Hz, 2H), 6.59(d, *J*=9.0Hz, 2H), 3.31(t, *J*=4.8Hz, 4H), 3.03(t, *J*=5.1Hz, 4H), 2.91(s, 6H), 2.31(s, 3H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.3, 153.0, 151.9, 144.5, 144.0, 132.2, 130.3, 130.2, 130.1, 129.8, 127.9, 122.9, 116.5, 114.4, 111.9, 47.3, 45.73, 40.1, 21.6.

Compound 6b



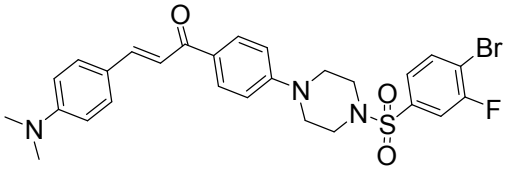
Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.95(d, *J*=8.4Hz, 2H), 7.73-7.80(m, 3H), 7.52(d, *J*=8.4Hz, 2H), 7.35(d, *J*=15.6Hz, 1H), 7.23(t, *J*=8.4Hz, 2H), 6.84(d, *J*=8.7Hz, 2H), 6.68(d, *J*=8.7Hz, 2H), 3.38(d, *J*=4.2Hz, 4H), 3.13(d, *J*=3.9Hz, 4H), 3.00(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.3, 167.1, 163.7, 153.0, 151.9, 144.6, 131.5, 130.6, 130.5, 130.3, 130.2, 122.9, 116.7, 116.6, 116.4, 114.5, 111.9, 47.4, 45.7, 40.1.

Compound 6c



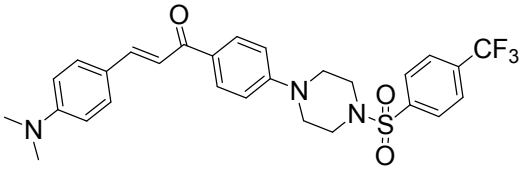
Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.95(d, *J*=8.7Hz, 2H), 7.78(d, *J*=15.3Hz, 1H), 7.68(q, *J*=8.4Hz, 4H), 7.53(d, *J*=8.7Hz, 2H), 7.34(d, *J*=15.3Hz, 1H), 6.84(d, *J*=9.0Hz, 2H), 6.68(d, *J*=9.0Hz, 2H), 3.40(t, *J*=4.5Hz, 4H), 3.14(t, *J*=4.8Hz, 4H), 3.00(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.3, 153.0, 151.9, 144.6, 134.4, 132.6, 130.4, 130.3, 129.3, 128.3, 122.9, 116.5, 114.5, 111.9, 47.4, 45.7, 40.2.

Compound 6d



Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.97(d, *J*=8.7Hz, 2H), 7.79(d, *J*=8.4Hz, 1H), 7.73(s, 1H), 7.54(d, *J*=8.1Hz, 3H), 7.46(d, *J*=7.8Hz, 1H), 7.35(d, *J*=15.6Hz, 1H), 6.88(d, *J*=9.0Hz, 2H), 6.70(d, *J*=8.7Hz, 2H), 3.43(d, *J*=4.5Hz, 4H), 3.21(d, *J*=4.5Hz, 4H), 3.03(s, 6H); ^{1}H NMR (400MHz, $CDCl_3$) δ: 188.6, 153.0, 152.0, 144.8, 134.8, 130.9, 130.5, 130.4, 129.0, 124.5, 123.1, 116.7, 116.2, 115.9, 114.8, 112.0, 47.7, 45.8, 40.3.

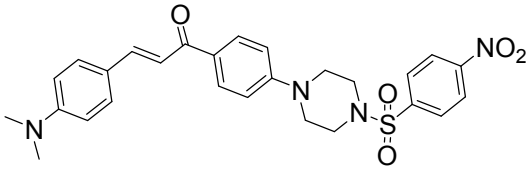
Compound 6e



Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.97(d, *J*=8.7Hz, 2H), 7.94(d, *J*=9.0Hz, 2H), 74-7.84(m, 3H), 7.54(d, *J*=8.4Hz, 2H), 7.35(d, *J*=15.6Hz, 1H), 6.88(d, *J*=8.4Hz, 2H), 6.70(d,

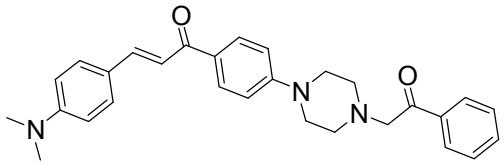
J=8.4Hz, 2H), 3.43(d, *J*=5.1Hz, 4H), 3.21(s, 4H), 3.03(s, 6H); HRMS: m/z calcd for $C_{28}H_{29}F_3N_3O_3S$ $(M+H)^+$ 544.1876, found 544.1875.

Compound 6f



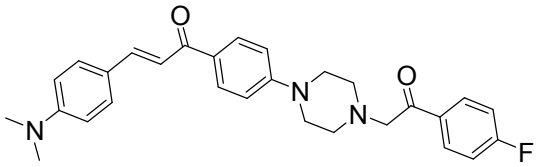
Brown solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.37(d, *J*=8.4Hz, 2H), 7.95(t, *J*=7.2Hz, 4H), 7.72(d, *J*=15.3Hz, 1H), 7.51(d, *J*=8.4Hz, 2H), 7.33(d, *J*=15.3Hz, 1H), 6.85(d, *J*=8.7Hz, 2H), 6.67(d, *J*=8.7Hz, 2H), 3.42(t, *J*=4.5Hz, 4H), 3.20(d, *J*=4.8Hz, 4H), 3.00(s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 188.3, 152.9, 152.0, 150.4, 144.4, 141.5, 130.2, 129.0, 124.5, 122.7, 116.4, 114.5, 111.8, 47.5, 45.7, 40.0.

Compound 7a



Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.99(d, *J*=8.1Hz, 4H), 7.79(d, *J*=15.3Hz, 1H), 7.50-7.54(m, 3H), 7.46(d, *J*=7.5Hz, 2H), 7.39(d, *J*=15.6Hz, 1H), 6.89(d, *J*=8.7Hz, 2H), 6.66(d, *J*=8.7Hz, 2H), 3.84(s, 2H), 3.41(t, *J*=4.2Hz, 4H), 2.97(s, 6H), 2.74(t, *J*=4.5Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 196.1, 188.2, 153.7, 151.7, 144.1, 135.9, 133.4, 130.3, 130.1, 129.1, 128.6, 128.1, 123.0, 116.7, 113.6, 111.8, 64.2, 53.1, 47.3, 40.1; HRMS: m/z calcd for $C_{29}H_{32}N_3O_2$ $(M+H)^+$ 454.2489, found 454.2488.

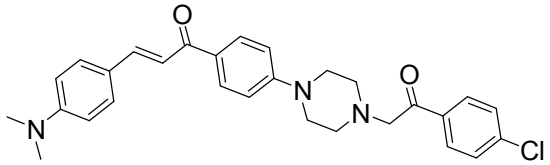
Compound 7b



Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.02-8.07(dd, *J*=5.7Hz, 5.4Hz, 2H), 7.99(d, *J*= 8.7Hz, 2H), 7.79(d, *J*=15.6Hz, 1H), 7.54(d, *J*=8.7Hz, 2H), 7.38(d, *J*=15.3Hz, 1H), 7.14(t, *J*=8.4Hz, 2H), 6.90(d, *J*=8.7Hz, 2H), 6.68(d, *J*=8.7Hz, 2H), 3.81(s, 2H), 3.42(t, *J*=4.2Hz, 4H), 3.00(s, 6H), 2.74(t, *J*=4.8Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 194.76, 188.38, 164.25, 153.77, 151.9, 144.2, 132.4, 131.1, 131.0, 130.4, 130.2, 129.3, 123.1, 116.9, 116.0, 115.7,

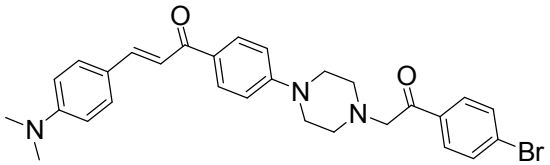
113.8, 111.9, 64.5, 53.2, 47.4, 40.2; HRMS: m/z calcd for $C_{29}H_{31}FN_3O_2$ $(M+H)^+$ 472.2395, found 472.2393.

Compound 7c



Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.99(t, *J*=7.2Hz, 4H), 7.79(d, *J*=15.3Hz, 1H), 7.54(d, *J*=8.4Hz, 2H), 7.44(d, *J*=8.4Hz, 2H), 7.39(d, *J*=15.6Hz, 1H), 6.91(d, *J*=9.0Hz, 2H),, 6.69(d, *J*=8.7Hz, 2H), 3.82(s, 2H), 3.42(t, *J*=4.5Hz, 4H), 3.01(s, 6H), 2.75(t, *J*=4.8Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 195.2, 188.4, 153.8, 151.9, 144.3, 139.9, 134.2, 130.4, 130.2, 129.8, 129.4, 129.0, 123.1, 116.9, 113.8, 111.9, 64.5, 53.2, 47.4, 40.2; HRMS: m/z calcd for $C_{29}H_{31}ClN_3O_2$ $(M+H)^+$ 488.2099, found 488.2100.

Compound 7d



Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.99(d, *J*=8.7Hz, 2H), 7.88(d, *J*=8.7Hz, 2H), 7.79(d, *J*=15.3Hz, 1H), 7.59(d, *J*=8.4Hz, 2H), 7.54(d, *J*=8.7Hz, 2H), 7.39(d, *J*=15.6Hz, 1H), 6.90(d, *J*=9.0Hz, 2H), 6.68(d, *J*=8.7Hz, 2H), 3.80(s, 2H), 3.41(t, *J*=4.8Hz, 4H), 3.00(s, 6H), 2.73(t, *J*=4.8Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 195.3, 188.3, 153.7, 151.8, 144.2, 134.6, 132.0, 130.4, 130.2, 129.8, 129.3, 128.6, 123.0, 116.8, 113.8, 111.9, 64.4, 53.1, 47.4, 40.2; HRMS: m/z calcd for $C_{29}H_{31}BrN_3O_2$ $(M+H)^+$ 532.1594, found 532.1592.